MUTAGENICITY OF PULP AND PAPER MILL EFFLUENT: A COMPREHENSIVE STUDY OF COMPLEX MIXTURES

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INTRODUCTION

Pulp and paper mill effluents are complex mixtures of dissolved lignin and cellulose degradation products and other substances extracted during the pulping process. The toxicity of these effluents to fish has been documented (Howard and Walden, 1971; Wande, 1976; Walden and Howard, 1977). A number of investigators have shown that effluents and process streams from pulp and paper mills are mutagenic in Salmonella (Ander et al., 1977; Eriksson et al., 1979; Douglas et al., 1980) and cause chromosome damage in mammalian cells (Douglas et al., 1980). Over 300 compounds have been identified in studies on various pulp and paper mill effluents (reviewed in CPAR, 1978). Because of the extensive use of chlorine in the bleaching process, many of these compounds contain chlorine substitutions. Since first chlorination-stage liquors are consistently among the most mutagenic by-products of the pulping process, it has been suggested that chlorinated substances are responsible for a major portion of the mutagenicity found (Bjørseth et al., 1979; Nestmann et al., 1979; Nestmann et al., 1980; McKague et al., 1981; Rapson et al., 1980).
In our laboratories, we have begun a coordinated program of investigation, consisting of three basic components: 1) From the list of known chemical constituents, selected compounds were tested using the Salmonella/mammalian-microsome assay and in Saccharomyces cerevisiae. Compounds identified as mutagens in this initial screen are being tested further in a battery of in vitro mammalian cell assays. 2) In addition to the study of known constituents, studies are in progress on first chlorination-stage effluent as a model complex mixture using a battery of microbial and mammalian in vitro and in vivo mutagenicity tests. 3) In order to provide new data on the components responsible for the mutagenicity, fractions of the above effluent were tested for mutagenicity in Salmonella and the most mutagenic fractions subjected to chemical analysis. Compounds so identified were then tested for determination of their mutagenicity in Salmonella. These approaches, taken together, are designed to better characterize the course of the mutagenicity in pulp and paper effluents and to gain insight into the mechanisms involved in the mutagenicity of mixtures.

MATERIALS AND METHODS

Mutagenicity Assays

The Salmonella/mammalian-microsome assay was carried out according to the method recommended by Ames et al. (1975), and assays for reverse mutation and gene conversion in strains of Saccharomyces cerevisiae followed the method of von Borstel et al. (1981) for treating exponential-phase cultures. In vitro mammalian cell assays in wild-type Chinese hamster ovary (CHO) cells were performed as described by Douglas et al. (1981) to detect the following end points: cytotoxicity, detected as survival of colony-forming ability; DNA damage, detected by alkaline sucrose gradient (ASC) sedimentation; chromosome aberrations; and sister-chromatid exchanges (SCE). In addition, the CHO/HGPRT forward mutation assay was performed essentially as described by O'Neill and Hsie (1979). For all in vitro assays, Aroclor 1254-induced rat-liver homogenate (S9) mix was prepared according to the method published by Ames et al. (1975). For the detection of in vivo genetic effects, the mouse bone marrow micronucleus assay was carried out according to the revised procedure described by Salamone et al. (1980).