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TITLE: DETERMINATION OF DIRECT-ACTING MUTAGENS AND CLASTOGENS IN OIL-SHALE RETORT PROCESS WATER

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Determination of Direct-Acting Mutagens and Clastogens in Oil Shale Retort Process Water

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INTRODUCTION

Shale oil products contain various metabolically active and photoactive genotoxic components (Strniste and Chen, 1981; Okinaka et al., 1981). In addition our preliminary observations indicated that retort process water contain direct-acting mutagens which cause significant increases in 6-thioguanine resistance ($6TG^R$) mutants in Chinese hamster ovary (CHO) cells (Chen et al., 1981). However, we have been unable to demonstrate the occurrence of direct-acting mutagens in these process waters when tested in the standard Ames/Salmonella assay (Nickols and Strniste, 1981). In this report we present results concerning the dose response of direct-acting mutagenicity in an above ground retort process (ARP) water and various fractions from it. Since many mutagenic agents are also clastogenic, we have compared the cytogenetic and mutagenic effects of this process water under the same experimental conditions.

MATERIALS AND METHODS

Test Materials.

The shale oil process (ARP) water used in this study was obtained from a holding tank at an above ground retort facility which utilized oil shale deposits located in the western U.S. in the Green River formation. The product water was filtered before use as described elsewhere (Strniste and Chen, 1981). ARP water

was further fractionated into base/neutral (B/N), base tar precipitate (BTP), acid (A), acid tar precipitate (ATP) and residual water (RW) fractions according to the acid-base extraction procedure described by Strniste et al., (1982) in these proceedings.

Cytotoxicity and Mutagenicity.

CHO cells, line AA8-4, were maintained in suspension culture as previously described (Strniste and Chen, 1981). Between $2x10^5$ and $1x10^6$ AA8-4 cells were plated into 100 mm dishes containing 12 ml alpha-MEM media with 10% FCS and incubated at 37°C for 18 h before treatment. The plated cells were exposed to various dilutions of the ARP water in medium for 48 h. For cytotoxicity measurements, the cells were rinsed twice with serum free alpha-MEM medium and 200 - 2000 cells from each treatment dose were plated into 60 mm dishes with alpha-MEM medium containing 10% FCS. Cell colonies developed after incubating dishes for 6 days and they were fixed in ethanol, stained with 10% solution of crystal violet and counted. In addition to cytotoxicity measurements, treated cells were replated in 100 mm dishes with 12 ml alpha-MEM medium containing 10% FCS and subcultured every two days for an expression period of eight days. Selection protocols used for measuring mutagenicity at the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus using this cell line have been reported elsewhere (Strniste and Chen, 1981; Chen and Strniste, 1982). Plating efficiencies for non-treated cells were between 80-90%.

Chromosome Preparation and Analysis.

Cultures treated as indicated above were rinsed twice with serum-free alpha-MEM, refed with fresh medium containing 10% FCS and incubated at 37°C for 24h. Two hours before harvest colcemid (0.1 µg/ml) was added to each culture. The cells were removed with trypsin, treated with 0.075M KCl for 10 min and fixed in 3:1 methanol-acetic acid. Slides were prepared by flame-drying and stained in 2% Giemsa for 10 min. The percentage of cells with at least one break or exchange per 100 metaphase cells was determined. Achromatic lesions (gaps) were not scored. The results are the mean of two experiments.

RESULTS AND DISCUSSION

In order to avoid cell density effects, cytotoxicity was determined by replating treated cells as described in "Materials and Methods". Cytotoxicity induced in CHO cells after exposure to ARP water or various fractions from it for 48h in the dark (37°C)

is shown in Figure 1. The ARP water is extremely toxic to CHO cells under the described treatment conditions. The concentration of ARP water necessary for inactivation to 37% surviving fraction CHO was 0.7% (v/v). The various acid/base extracted fractions and resulting precipitates were diluted to the equivalent volume of the original process water. The most toxic of these various fractions was the BTP followed in order of decreasing toxicity by B/N, ATP, A and RW fractions. However, the magnitude of the toxic effect for each individual fraction was less than the original process water.

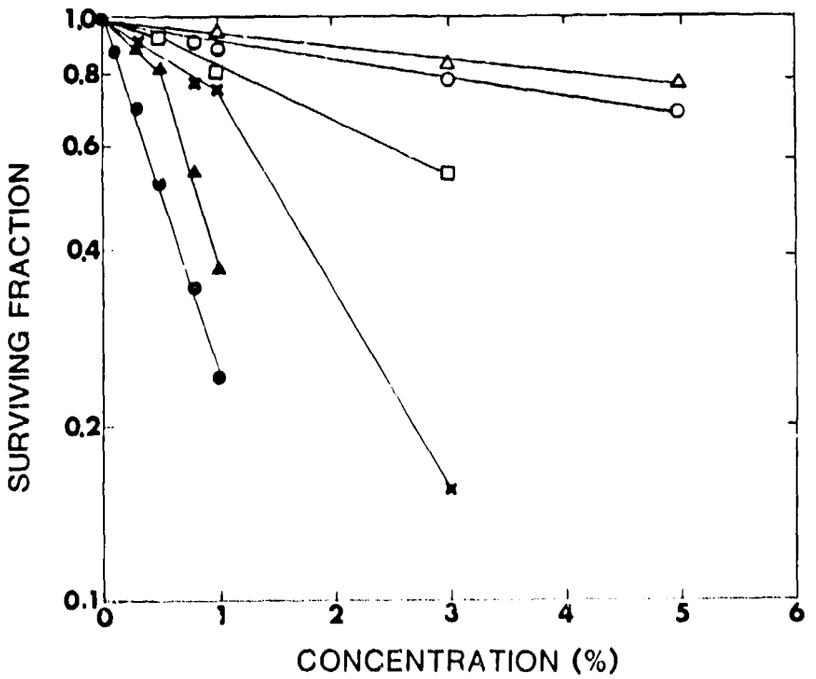

Figure 1. Cytotoxicity in CHO cells treated with oil shale retort process water and various fractions from it for 48 h at 37°C in dark. Samples used are: (●) ARP water, (▲) base tar precipitate, (✖) base/neutral fraction, (□) acid tar precipitate, (○) acid fraction and (△) residual water.

In Figure 2 we show mutagenicity in CHO cells treated with dilution of the ARP water or various fractions from it. The data

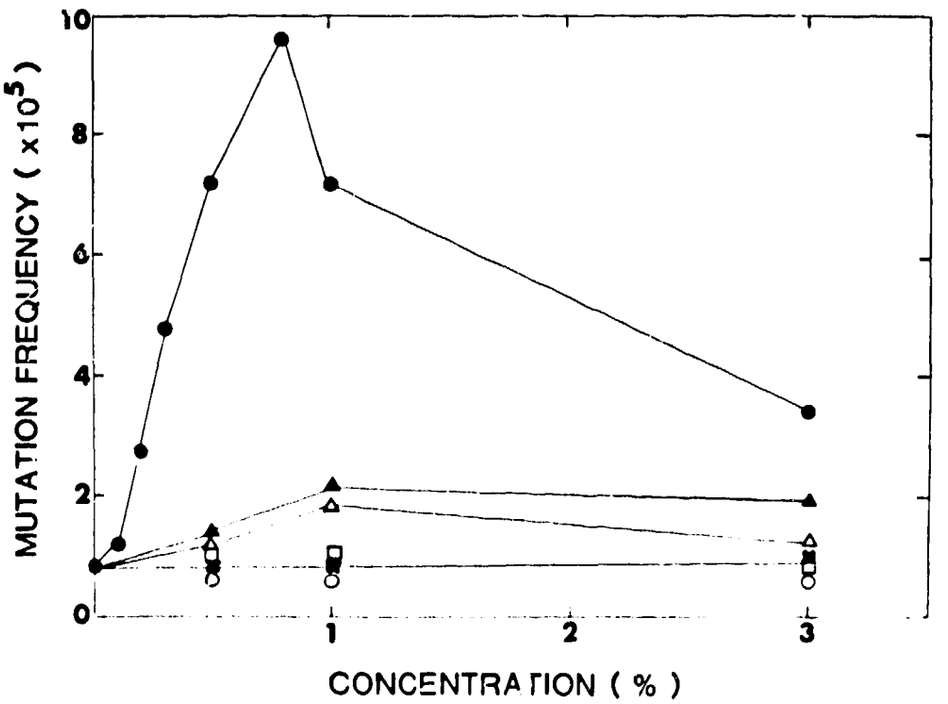

Figure 2. Mutagenicity in CHO cells treated with ARP water and various fractions for 48 h at 37°C in dark. Samples used are: (●) ARP water, (▲) base tar precipitate, (✖) base/neutral fraction, (□) acid tar precipitate, (○) acid fraction and (△) residual water.

indicate significant mutagenic activity for the ARP water when treated in CHO cells without metabolic activation. A 10-fold increase in $6TG^R$ mutants was seen for cells exposed to a 0.8% solution of the process water for 48 h in the dark. Due to the extreme cytotoxic effects (Fig. 1) increased ARP concentrations resulted in a decrease in mutation frequency. When various fractions were tested for direct-acting mutagenic potential, negative results were obtained.

Table 1 summarizes the data on the cytogenetic effects observed in cells treated with ARP water. There was a significant, dose-related increase in the percentage of cells with chromosomal aberrations. The aberrations were primarily chromatid breaks and exchanges. At concentrations of 0.3-1.0% ARP water the

Table 1

Cytogenetic Effects of an Oil Shale Retort Process Water in CHO Cells

ARP Water (%)	% Cells With Chromosomal Aberrations	% Tetraploid Cells	% Endoreduplicated Cells
0	0.5	0.2	0
0.1	2.5	5.5	0
0.3	6.0	11.5	1.0
0.5	13.0	5.0	6.0
0.8	26.0	4.0	9.5
1.0	39.5	1.0	12.0

Figure 3. An endoreduplicated CHO metaphase cell observed after oil shale retort process water treatment.

frequency of cells demonstrating tetraploidy and endoreduplication (diplochromosomes) ranged from 11-13% (see Fig. 3). Tetraploid cells are often the result of endoreduplication during a previous cell division (Sutou and Tokuyaman, 1974). Therefore, the apparent dose-related increase of endoreduplicated cells and the concurrent decrease in tetraploid cells is probably due to proliferative delay at the higher concentration of the ARP water.

The cytogenetic analysis of the various fractions at concentrations of 0.1-3.0% indicated that only the B/N fraction contained components that induce chromosomal aberrations and endoreduplication. The 3% concentration of the B/N fraction induced chromosomal aberrations in 16% of the cells, tetraploidy in 11% of the cells, and 28.5% of the metaphases were endoreduplicated. The 0.1-1.0% concentrations of this fraction did not induce increased frequencies of any of these parameters.

The lack of mutagenic effect in various ARP fractions indicate that components which were responsible for the mutagenic activity may have been selectively lost during the extraction procedure. Alternatively, direct-acting mutagenic components may be equally distributed among the various fractions diluting their activity (~5-fold) to non-detectable levels in this particular assay. Comparing cytotoxic and mutagenic activities of the ARP water and various fractions from it indicate that measurements of cytotoxicity are not indicators of mutagenic potential for these complex mixtures.

The induction of endoreduplication in CHO cells following treatment with shale-derived oils has been previously reported by Deaven and Nock (1979). In addition to mutagenicity, the induction of chromosomal breaks, endoreduplication and tetraploidy by ARP water indicate that shale-derived byproducts contain direct-acting genotoxins. Furthermore, the results of this study suggest that employment of mammalian assays in a testing strategy should eliminate false negative responses observed in the Ames test for some of these direct-acting components in complex mixtures.

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