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TITLE: GENOTOXICITY INDUCED BY A SHALE OIL BYPRODUCT IN CHINESE HAMSTER CELLS FOLLOWING METABOLIC ACTIVATION



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# GENOTOXICITY INDUCED BY A SHALE OIL BYPRODUCT IN CHINESE HAMSTER CELLS FOLLOWING METABOLIC ACTIVATION

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## INTRODUCTION

A process water obtained from a holding tank during the surface retorting of oil shale has been shown to induce a linear dose response of ~100 histidine revertants/$\mu$l in the Ames/Salmonella test. The complex mixture has also previously been shown to induce genotoxicity in mammalian cells following activation by near ultraviolet light and natural sunlight (Strniste and Brake, 1980; Strniste and Chen, 1981; and Strniste et al., 1982). This report focuses on the effects of a particular oil shale retort process water on cultured Chinese hamster cells following metabolic activation by either rat liver homogenate or lethally irradiated but metabolically competent Syrian hamster embryonic cells. Cytotoxic and mutagenic responses induced by the process water and a fractionated sample from it containing the majority of the mutagenic activity (as assessed by the Salmonella test) were measured under conditions designed to optimally measure the mutagenic potency of the promutagen, benzo(a)pyrene. These results suggest a possible discrepancy in the genotoxic potential of this complex mixture when various methods are utilized to measure its potential.

## MATERIALS AND METHODS

### Retort Process Water

The water used in this study was obtained from a holding tank used in an above ground retorting facility (Paraho) utilizing oil shale deposits from the Green River Formation at Anvil Points, Colorado. Prior to use the water was filtered through Whatman #42 paper and a Millipore 0.2 μ Swinnex unit. The acid/base extraction procedure from which the base/neutral (B/N) sample was derived is described elsewhere in these proceedings (Strniste, et al.).

### Cell Culture and Mutation Assays

Chinese hamster ovary cells (AA8-4) were obtained from Dr. L. Thompson (Lawrence Livermore Laboratories) and were cultured under conditions described previously (Strniste and Chen, 1981). S9 activation, cytotoxicity measurements, and mutagenicity at the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus have also been described previously (Okinaka et al., 1981; Strniste and Chen, 1981). Primary Syrian hamster embryo (SHE) cells were obtained using the protocols described by Pienta et al. (1978). Metabolic activation utilizing SHE cells (lethally-irradiated [4000 r x-rays] at $2x10^6$/60mm dish) involved their co-incubation with CHO cells ($3x10^5$ per 60 mm dish) in α MEM medium supplemented with 10% fetal calf serum. Following 48 hours of incubation in the presence of the insulting agent the CHO cells were trypsinized and replated for cytotoxic measurements or maximal mutation expression periods in fresh medium. The plating efficiencies for non-treated CHO cells were regularly between 80-90%. The observed mutation frequency is expressed as the ratio of mutant colonies per dish to the number of viable cells plated per dish.

### Ames/Salmonella Bioassay

Standard plate assays for determining Salmonella histidine revertants were performed with strains TA98 and TA100 essentially as described by Ames et al. (1975). S9 was prepared as described earlier (Okinaka et al. 1981). Linear regression analysis was used to determine the number of revertants per μl of sample.

## RESULTS AND DISCUSSION

The process water used throughout this study has been shown to induce a linear dose response in the bacterial mutation assay developed by Ames et al. (1975). Increases in histidine

revertants for both strains TA98 and TA100 as a function of process water concentrations are illustrated in Figure 1. Strain

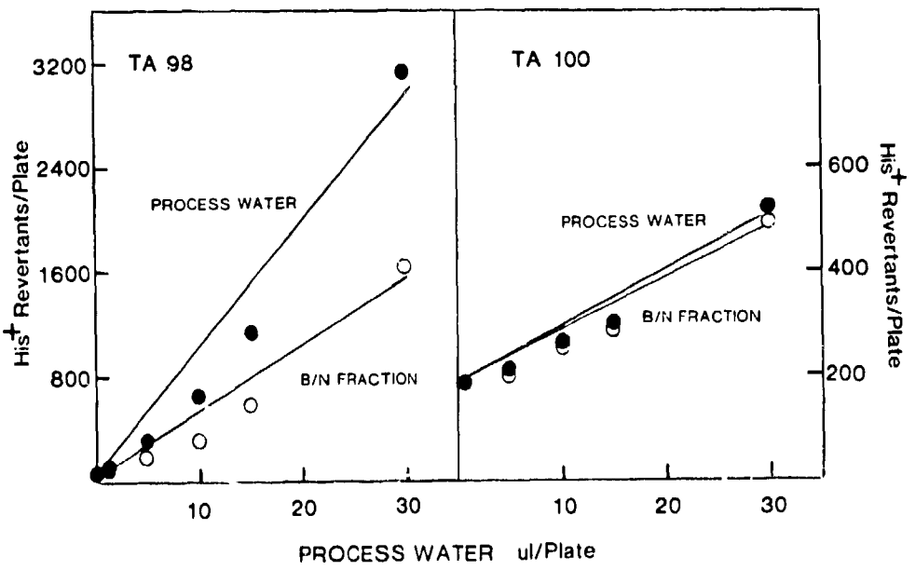


Figure 1. Histidine revertants induced in Salmonella by an oil shale retort process water and a base/neutral fractionated sample from it. S9 concentration equalled 15 µl/plate (465 µg protein). More details on the Ames/Salmonella bioassay can be found in Strniste et al., these proceedings.

TA98 (a frameshift detector) is clearly more susceptible to the mutagenic properties of the process water than is the base substitution strain TA100. Following acid/base fractionation (see Strniste et al. in these proceedings for details on the fractionation of this complex mixture), a reduced but substantial level of the mutagenic activity appears to reside in the base-neutral (B/N) fraction when strain TA98 is used in the assays. These results coupled with positive photoactivation results in Chinese hamster cells (Strniste and Chen, 1981: Strniste et al., these proceedings) suggest that this process water would be suited for studies incorporating Chinese hamster cells as targets with metabolic activation by various sources.

Attempts to determine the genotoxic properties of this complex mixture in mammalian cells, however, is complicated by components which appear to mask the mutagenic potential of the mixture. Unlike most indirect carcinogens, shale oil process waters (and other complex mixtures) possess moieties which are extremely cytotoxic to mammalian cells even before metabolic activation (Figure 2, open squares). Concentrations of the process water

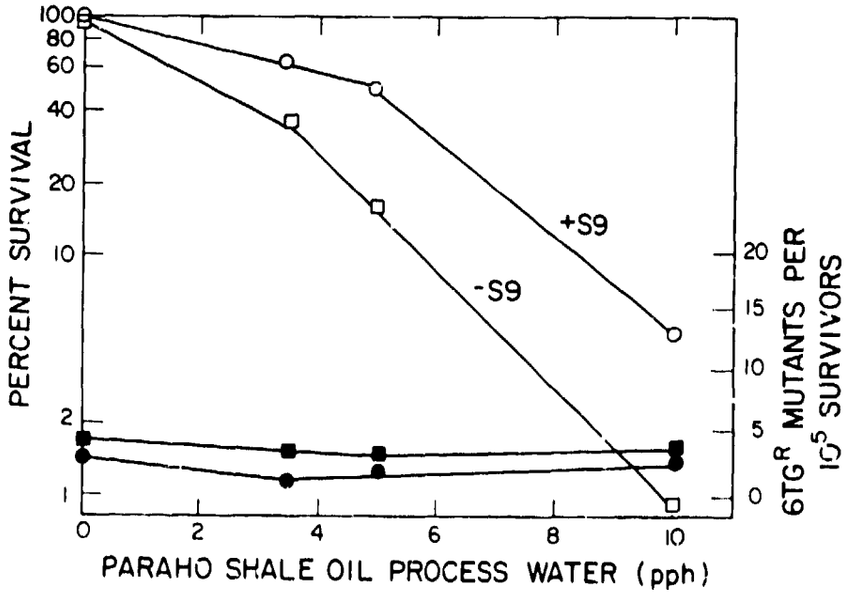


Figure 2. Cytotoxic and mutagenic responses induced by shale oil retort process water in CHO cells. Exposures were for 2h at 37°C either with or without the addition of S9. Open symbols represent plating efficiencies after treatment. Closed symbols represent mutation frequencies measured as described earlier.

which produce acute cytotoxic responses after 2 hour exposures, are insufficient to induce a significant number of mutations at the HGPRT locus in CHO cells (closed squares). The addition of Aroclor 1254-induced rat liver homogenates (S9 preparations), at levels which maximally induce mutations with benzo(a)pyrene (Chen et al., 1981; and Figure 4), also does not increase mutations to 6-thioguanine resistance (closed circles). Instead, the addition of the S9 complex causes a detoxification of the cytotoxic

responses produced by the process water itself. These results would indicate that toxic, non-mutagenic components may interfere with the measurement of metabolic activation responses.

The toxic effects of complex mixtures can be eliminated by simply assessing the mixtures at dilutions which are not toxic to the cells. The results depicted in Figure 3 illustrate the

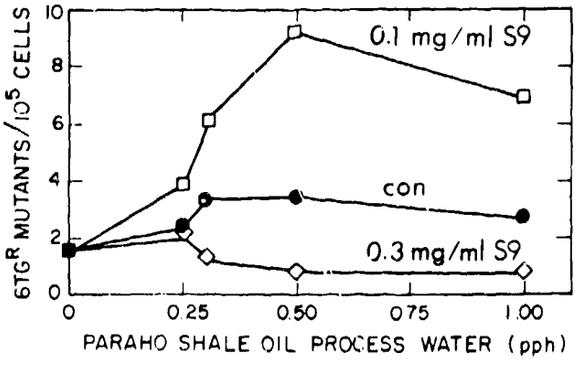


Figure 3. Mutation induction by subtoxic doses of an oil shale retort process water in CHO cells with S9 activation. Exposures were as described in Figure 2.

mutagenic responses produced by subtoxic doses of the Paraho process water in combination with metabolic activation enzymes. When 0.1 mg/ml of S9 protein is used in the activation mixtures, a linear dose response is observed with up to 0.5 parts per hundred concentration of the process water. However, a higher S9 concentration (0.3 mg/ml) results in a decrease in mutagenicity when compared to non-activated samples. These results are not surprising since other studies with polycyclic aromatic hydrocarbons have indicated that S9 preparations used in excess can exhibit detoxifying and deactivating capacities in addition to activating potential (DeFlora, 1978; Thornton, et al. 1981; Chen et al., 1982a).

By comparison to the mutagenicity induced in Salmonella TA98 (Fig. 1) the genotoxic response reported here for CHO can only be described as marginal. However, this response is not quantitatively that different in magnitude from the results obtained with TA100. It can also be argued that dilution of the process waters to subtoxic levels may in effect lower the active mutagen concentration below detectable limits. This dilution

effect was circumvented by a fractionation scheme which eliminated some of the toxic components from the mutagenic fractions (described earlier as the B/N fraction). In the experiment depicted in Figure 4 the mutagenic properties of this B/N fraction were measured in CHO cells following metabolic activation with

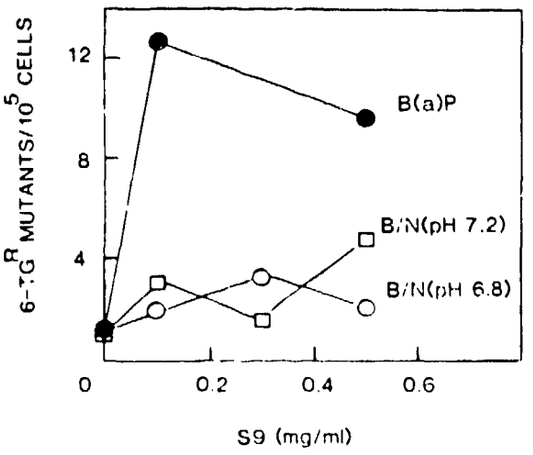


Figure 4. Mutation induction by the base/neutral fraction in CHO with S9 activation. B/N concentration equalled 10% (v/v). B(a)P concentration equalled 2 μg/ml. pH's represent final values in reaction mixtures.

increasing concentrations of S9 mixtures. It is clear that the B/N fraction is not highly mutagenic despite the use of conditions which are optimum for the S9 mediated-activation of a known promutagen, benzo(a)pyrene (B(a)P). The fractionated sample induces maximally only 4-5 fold 6-thioguanine resistant (6TG$^R$) mutants above background levels.

It has previously been shown that activation of certain promutagens (eg., B(a)P) by lethally irradiated but metabolically proficient cells, such as primary Syrian hamster cells, can lead to a greatly enhanced mutagenic response in target CHO cells (Carver et al., 1980; Bradley et al., 1981; Chen et al., 1982b). Despite these findings a SHE cell-mediated activation system does not appear to significantly increase the mutagenic response of CHO cells to the effects of either the base/neutral fraction or the parental process water (Fig. 5). Conditions which were optimal for B(a)P metabolism greatly enhanced the mutagenicity of this promutagen (Fig. 5, closed circles) in CHO cells in comparison to

the S9 induced response (Fig. 4). However, the maximal response to the base/neutral fraction following cell-mediated activation remained only 4-5 fold above background.

These results are perplexing since both the process water and the fractionated B/N fraction are highly mutagenic in (1) the Ames standard plating assay with S9 activation and in (2) the CHO

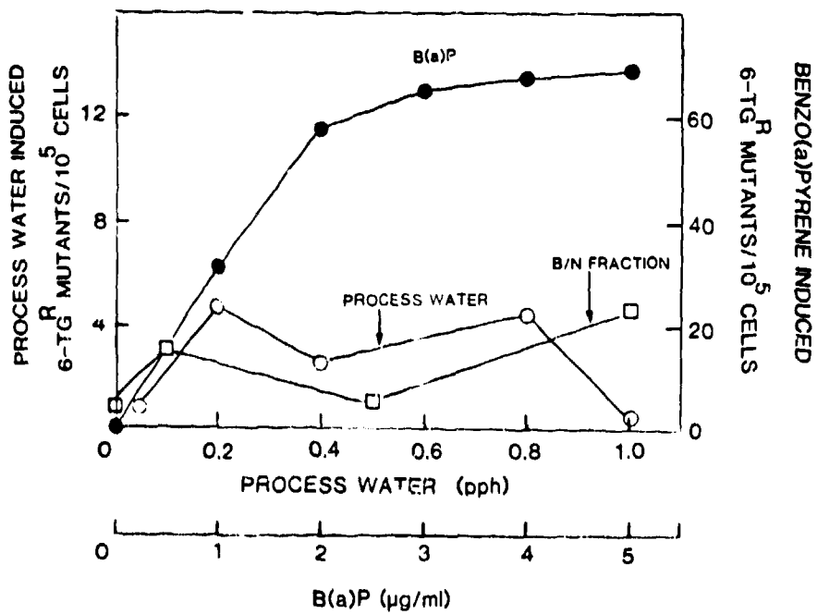


Figure 5. Mutation induction by the base/neutral fraction in CHO with SHE cell-mediated activation.

mutation assay following light activation (Strniste and Chen, 1981 and Strniste et al. these proceedings). The CHO/HGPRT system may in part reflect the mutagenic variation which can be demonstrated in the two different strains of Salmonella (Fig. 1). Some preliminary results suggest that locus specificity may contribute to the minimal response observed in these present experiments. Alternatively, conditions for assessing the mutagenic potential of complex mixtures in mammalian cells may require further refinement to optimize metabolic activation. The reasons for this apparent discrepancy between the mutagenic responses induced in Salmonella and CHO following metabolic activation however remains unknown.

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