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Lack of Mutagenic Activity of Crude and Refined Oils in the Unicellular Alga *Chlamydomonas reinhardtii*

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Over the past several years, an increasing number of studies have presented evidence for the mutagenicity and/or carcinogenic potential of petroleum-derived hydrocarbons (e.g. Felton and Nebert 1975; Huberman 1975; Coombs et al. 1976). These most usually were obtained with individual hydrocarbons, and using either specialized bacterial strains (e.g. Ames' strains) or mammalian tissue preparations. While providing important insights into mutagenic mechanisms involving xenobiotic compounds, the relevance of these studies to the natural aquatic environment is not always evident. This applies especially to the mutagenic potential of water-soluble fractions of hydrocarbon mixtures, as in whole oils or in complex distillate fractions, and involving typical marine biota.

Accordingly, we have examined the mutagenic potential of the water-soluble fractions of four oils (two crude oils and two refined oils) using the unicellular haploid alga Chlamydomonas reinhardtii.

MATERIALS AND METHODS

Although rapid tests for mutagenic activity have been developed using specific bacterial strains (Ames et al. 1973a, 1973b), the genetic complement of these is significantly different from that in either phytoplankton or higher invertebrate or vertebrate materials. For that reason we chose C. reinhardtii because of its closer genetic relationship to marine phytoplankton and because considerable data already exists on its mutation potential. As well, its generation time is relatively short (ca. 2 to 3 generations/24h under our conditions). The algae used in this study, the wild-type strain 137c(mt+) of C. reinhardtii, were obtained originally from N.W. Gilham and J.E. Boynton of Duke University, N.C., USA. The experimental approach used consisted simply of culturing algal innocula on oil-impregnated agar medium for three weeks, in the light. The level of mutagenic activity that occurred over this period was then estimated by the recovery of streptomycin resistant mutants.

The algae were plated onto solid HS agar media (Sueoka et al. 1967), previously combined with equal aliquots of aqueous extracts of the various test oils. To prepare the media, 200 ml of auto-

claved liquid HS medium was shaken with 10 ml of the appropriate autoclaved test oil in sterile glass containers, on a Burrell Wrist Action Shaker (10 min, max. setting). The resulting oil: medium mixture was allowed to separate for five minutes in a glass separatory funnel. The lower aqueous phase was then drawn off, to be used in preparing the solid media. Concentrations of oil in HS medium prepared in this way were as follows: Kuwait crude oil 3.05 $\pm$ 1.7 ppt (gm/L); Saran Gach crude oil, 5.51 $\pm$ 1.80 ppt (gm/L); Diesel 25, 4.60 $\pm$ 2.95 ppt (gm/L); Bunker C, 0.70 $\pm$ 0.07 ppt (gm/L) (UV-fluorescence determination by the method of Gordon and Keizer, 1974). To prepare the solid media, the aqueous phase was then mixed in a one to one ratio with double strength (30%) agar in HS medium, to yield an oil-impregnated 15% agar/oiled-HS medium. Oil concentrations in this final solid medium were assumed to be half of those determined in the aqueous extracts.

The plates were innoculated and cultured for three weeks, in the light, after which the cells were resuspended in HS medium in concentrations of approximately 5×10^6 cells/mL in the medium, and streptomycin resistant colonies were recovered by the method of Lee and Jones (1973). Plates containing only oil-free solid HS medium (15% agar) served as controls.

RESULTS AND DISCUSSION

During the initial three-week oil-exposure period all cells appeared to grown normally, those cultured on oiled medium being indistinguishable morphologically from control cultures on oil-free medium. Of over 700 colonies examined, there was no apparent change noted in either colony forming ability or in colony morphology.

Table 1. Incidence of streptomycin resistant mutants in cultures of *Chlamydomonas reinhardtii*, wild-type strain 137c(mt+), grown on agar impregnated with water-soluble fractions of various fuel oils.

Test condition	N	Revertants per 10^6 viable cells plated ($x \pm$ S.D.)
Control plates	19	2.23 $\pm$ 2.26
Kuwait crude oil	10	3.14 $\pm$ 1.60
Saran Gach crude oil	10	0.71 $\pm$ 0.57
Diesel 25 fuel oil	10	1.12 $\pm$ 1.02
Bunker C fuel oil	10	1.73 $\pm$ 1.32

With respect to mutagenic activity, no significant difference was noted in the number of spontaneous streptomycin-resistant mutants in the control plates and those including Kuwait crude, Diesel 25 or Bunker C (Table 1). Those cultured on Saran Gach showed a

slightly lower mutation incidence, but not significantly so. Although the standard deviations appeared high, these were well within the range of values normally obtained in this assay.

The failure to obtain enhanced mutation levels with these oils is somewhat surprising, especially after about forty to fifty generations (three-weeks incubation), because of the high susceptibility of this particular strain of C. reinhardtii to very low dosages of known mutagenic agents. For example, exposure to low doses of UV light (Lee, unpublished observations) and to low concentrations of MNNG (Lee and Jones, 1973) and MMS (Hawkes and Lee, 1976) will increase mutation incidence over five-fold, with a higher than 80% survival. Higher doses of these agents further increase incidence of these mutants. Therefore the failure to obtain enhanced mutations in the present study is particularly surprising, because various polycyclic aromatics, common to the oils that were tested here, are known mutagens (e.g. 3-methyl cholanthrene, 7,12-dimethylbenz(a)anthracene, 7-Me-benz(a)anthracene, Huberman, 1975).

Two possible explanations suggest themselves immediately. First, there is of course the possibility that the test oils lost some of their mutagenic fractions by evaporation during the preparative steps, either during the sterilization (autoclaving) step, or during the cooling step. While possible, this seems unlikely because the temperatures during the preparative stages were always below 47 oC, which is well below the boiling point of many of the larger molecular weight hydrocarbons.

More likely is that the total concentration of mutagenic components in these test oils and in the oiled media available to the cells was in fact far less than normally used in laboratory mutagenesis studies. In other words, concentrations of compounds used to elicit enhanced mutation levels and to standardize mutagenic responses frequently far exceed concentrations found either in whole oils or in environmental samples under spill or pollution conditions. A further compounding factor of complex mixtures, such as these whole oils, is the presence of toxic or inhibiting fractions which may potentially mask any mutagenic activity, especially at low mutagen concentrations (Petrilli et al. 1980; Haugen and Peak 1983; Vandermeulen et al. 1985).

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