• Mutagenicity of Complex Mixtures

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The effect of coal-derived complex chemical mixtures on the mutagenicity of 6-aminochrysene (6-AC) was determined with *Salmonella typhimurium* TA98. Previous results suggested that the mutagenic potency of 6-AC for TA98 in the standard microsomal activation (Ames) assay increased if it was presented to the cells mixed with high-boiling coal liquids (CL) from the solvent refined coal (SRC) process. In this year's work, the apparent mutational synergism of CL and 6-AC was independently verified in a fluctuation bioassay which allowed quantitation of mutational frequencies and cell viability. The results of this assay system were similar to those in the Ames assay. Moreover, the fluctation assay revealed that mutagenesis and cellular toxicity induced by 6-AC were both strongly enhanced if 6-AC was presented to the cells mixed in a high-boiling CL.

The fluctation mutagenicity bioassay system, which allows direct measurements of both mutagenesis and viability, was used to determine the effect of a CL on the genetic potency of 6-AC for S. typhimurium TA98. As shown in Figure 1, the mutational frequencies for 6-AC plus an EDS 850+°F boiling-point coal distillate (designated 040) were from 10 to >100 times greater than those for 6-AC alone. These measurements were based on the number of revertants of TA98/viable cell, and they are independent, over at least several orders of magnitude, of cell killing. These data strongly support our interpretation of the standard Ames assay data that synergistic effects between 6-AC and CL enhance the potency of 6-AC.

Additional support is given to this theory by the fact that the mixture of 6-AC plus 040 appeared to induce extensive killing of TA98 (Figure 2), ranging from ~50% (at 0.005 μg 6-AC/ml exposure medium) to >95% (0.01 to 0.02 μg 6-AC/ml exposure medium). This is the effect anticipated, since enhanced DNA damage leading to mutation (Figure 3) would likely cause cell death, especially at higher levels of 6-AC.

In contrast to results for 6-AC, the mutagenic potency of benzo[a]pyrene or 1-nitropyrene plus 040 did not appear significantly greater than for these chemical mutagens alone (Figure 4).

Finally, the enhanced mutagenesis of 6-AC plus 040 versus that of 6-AC alone was strongly dependent, in the fluctuation assay, on metabolic activation by S9. However, these data do not show whether the effects leading to enhanced mutagenesis are at the level of the activating enzymes or at the target cell (TA98) level, or both. Future work will be directed to investigating possible mechanisms by which CL enhances the mutagenic potency of 6-AC and other amino polycyclic aromatic hydrocarbons.

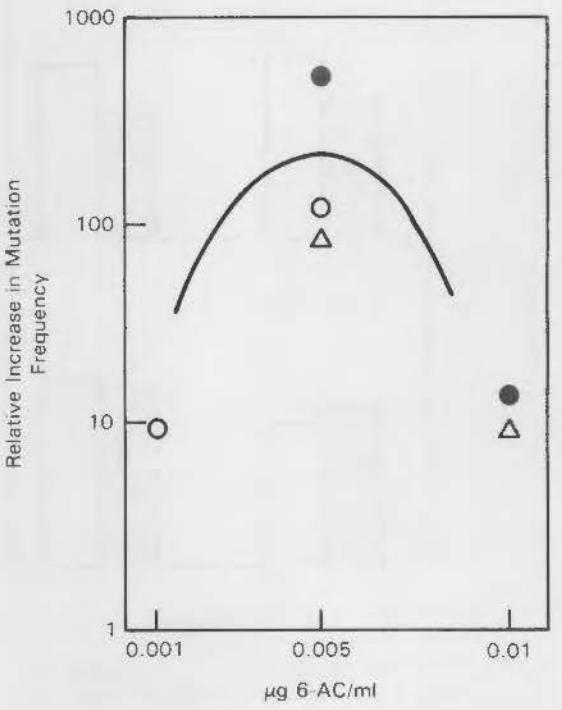

FIGURE 1. Relative Increase in Mutation Frequency in Cells of *S. typhimurium* TA98 Exposed to 6-AC Plus the EDS 850°+F (040) Coal Distillate Versus that for Cells Exposed to 6-AC Alone. Concentrations of 6-AC are as indicated; concentration of EDS 850°+F (040), when present, was 5 μg/ml exposure medium. Relative mutation frequencies are expressed as the ratio of revertants TA98/viable cell for cells exposed to 6-AC plus 040 to revertants TA98/viable cell for 6-AC alone. All assays were carried out in the presence of Aroclor-induced rat liver microsomes (S9). All responses were significant at $P < 10^{-3}$, based on chi-square statistical tests. Symbols identify three different experiments.

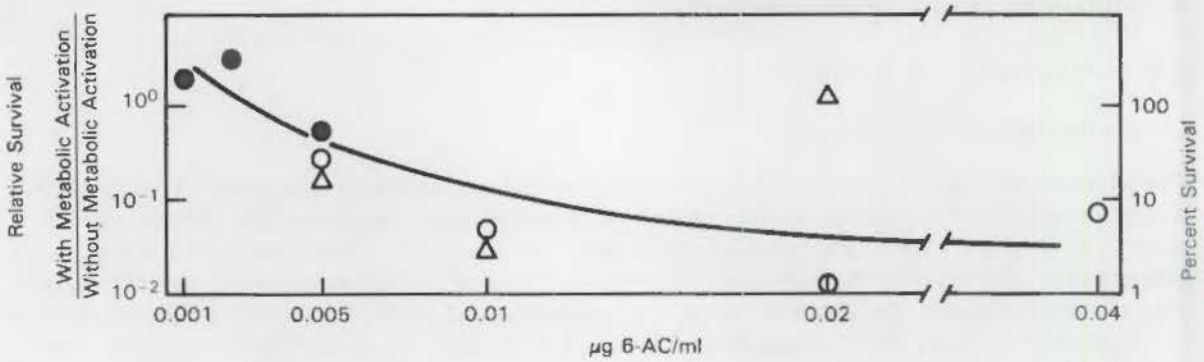

FIGURE 2. Relative Survival of *S. typhimurium* TA98 Exposed to 6-AC Plus EDS 850°+F Coal Distillate (040) Versus that of Cells Exposed to 6-AC Alone. Survival is expressed as the ratio of viable cells TA98 exposed to 6-AC plus 040 to viable cells TA98 exposed to 6-AC alone. Levels of 6-AC are as indicated; concentration of 040 (when present) was 5 μg/ml. All assays were carried out in the presence of Aroclor-induced rat liver microsomes (S9). Symbols identify three different experiments.

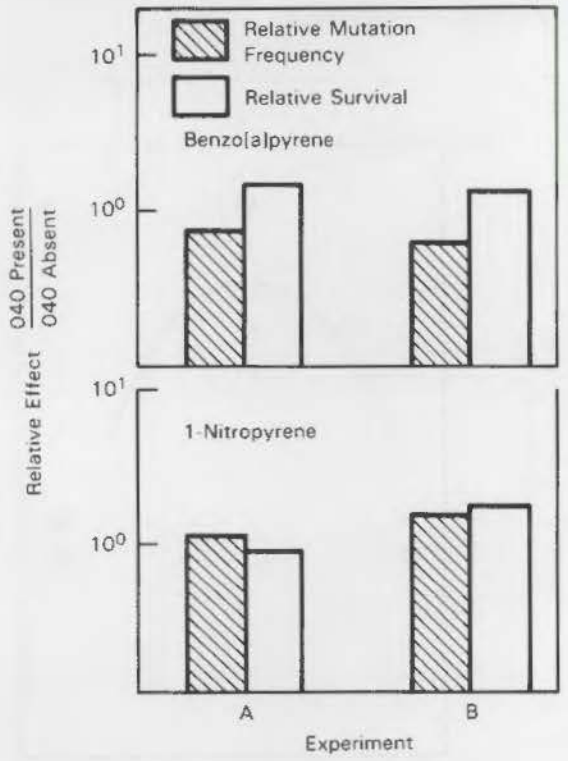

FIGURE 3. Relative Changes in Mutation Frequency and Survival for Cells of *S. typhimurium* TA98 Exposed to Benzo[a]-pyrene Plus or Minus EDS 850°+F Distillate (040) and 1-Nitro-pyrene Plus or Minus 040. Relative mutation frequency is expressed as the ratio of revertants TA98/viable cell plus 040 to revertants TA98/viable cell minus 040. Similarly, relative survival is expressed as the ratio of viable cells TA98 exposed to 040 (plus chemical) to viable cells TA98 exposed to chemical alone. Benzo[a]pyrene was present in the exposure medium at 10 μg/ml; 1-nitropyrene at 0.2 μg/ml and 040 at 5 μg/ml. All assays were carried out in the presence of Aroclor-induced rat liver microsomes (S9). All mutational responses (plus or minus 040) were significant at $P < 10^{-3}$, based on chi-square statistical tests. Viable count for cells exposed to benzo[a]-pyrene was not significantly different ($P > 0.05$) from that of solvent controls.

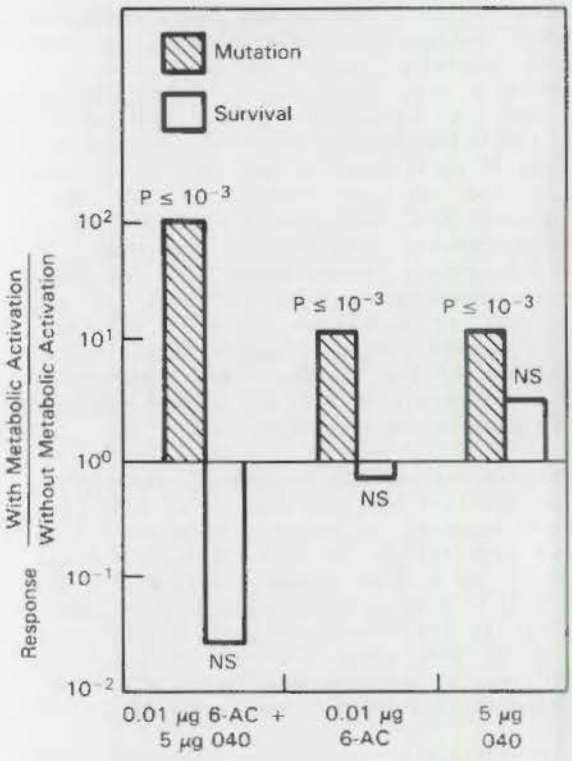

FIGURE 4. Dependency of Mutagenesis and Cell Survival on Metabolic Activation. Metabolic activation supplied by Aroclor-induced rat liver microsomes (S9). P-value followed by a number indicates the level of significance, based on chi-square test, or NS (not significant) if $P > 0.05$.