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Project Summary

Bioassay and Chemical Analysis for Hazardous Materials in Residual Oils

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Residual fuel oils have been considered as an energy source for the chemically active fluidized-bed (CAFB) process, designed to produce a low-sulfur, low-Btu fuel gas suitable for power-plant utilization in a conventional steam generator. This project was designed to ascertain the presence of potentially bio-hazardous substances in residual fuel oils.

In spite of the length of time residual fuel oil has been available, there has been little definitive study of what would be typical compositions for such materials (or whether even a composition can be "typical"). There has been less effort to assay this product for bio-hazard, and there has been no real effort to associate possible bio-hazard with composition. The very nature of refinery processes leads to residual oils which will contain polycyclic materials, nitrogen, oxygen, and sulfur heteroatoms, and be appended with diverse functional groups. All these types of materials would be suspect as potential carcinogens if water soluble or present in high enough concentrations.

In fulfillment of this contract, bioassay and chemical analyses were performed on residual fuel oils. The bioassay work would have been simplified if the contract wording had been followed in performing bioassays only on as-received oils. However, Westinghouse (in the spirit of the contract and with project officer approval) applied the bioassay to particular oil fractions in an effort to more deeply probe mutagenicity and potential carcinogenicity of this type of material. It was determined early that mutagenicity (i.e., the accepted measure of potential carcinogenicity) was not a serious concern with the 26 as-received residual oils.

The analyses sought the maximum information for material characterization compatible within the time-cost constraints of the contract. The major difficulties associated with these chemical analyses were sample complexity, low sample volatility, and lack of reference data.

This Project Summary was developed by EPA's Industrial Environmental Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

This project produced several new, specific chemical and bioassay questions which need to be considered and answered when using fuel oil for power generation. Of particular relevance to environmental impact analyses is the discovered novel fluorescent property of fractionated sample and its associated mutagenicity.

The data bank for the report was derived from the following completed tasks:

41 Residual fuel oil samples received.
26 As-received samples bioassayed.
32 As-received samples pre-fractionated by column chromatography into seven fractions each.
23 Samples examined by combined gas chromatography/mass spectroscopy/data system as seven fractions each.
6 Samples bioassayed as seven fractions each.
1 Sample partitioned into dimethylsulfoxide solubles and insolubles and then pre-fractioned by column chromatography into seven fractions each.
1 Sample, partitioned and pre-fractioned as above, examined by bioassay and gas chromatography/mass spectroscopy/data system.

Direct insertion mass spectrometry, ultraviolet fluorescence, infra-red analyses, and elemental composition studies have been performed within the time-cost framework.

Bioassay of the as-received fuel oils required solubilization in DMSO or p-dioxane to introduce the material into the bacterial test system. Water solubility is negligible and whatever part is in solution does not elicit mutagenic response in the Ames test. The two-solvent-extracted residual oil part was generally non-mutagenic, although some oils (19%) exhibited a very weak, positive test result. Residual fuel oils can be fractionated by column chromatography so that, within the scope of this effort, a particular fraction is consistently mutagenic. Even an oil that is not mutagenic as-received may be positive in this specific fraction. As with as-received oils, this particular fraction will show varying degrees of mutagenicity. The most powerful tool, discovered in this project, for isolating mutagenicity of a residual oil is the dimethylsulfoxide partitioning of the as-received sample followed by column chromatography fractionation of the dimethylsulfoxide soluble portion. In a single test run, each of the seven fractions obtained was mutagenic.

Four major factors affect the analyses for organic compounds in residual fuel oil:

1. The sheer complexity of the sample in terms of the hundreds of compounds it contains.
2. The inconsistent source, treatment, and post-treatment refinery blending from sample to sample.
3. The essentially non-volatile nature of residual fuel oils; this precludes complete sample analyses by the most powerful analytical tool, combined gas chromatography/mass spectroscopy/data system.
4. The lack of signal reference data to tie the sample components to known materials.

The organic analyses obtained in this program suggest that:

1. The most mutagenic components of a residual fuel oil reside in the carbazole-type compounds class.
2. The second most mutagenic agents of a residual fuel oil are associated with polycyclic ketones such as anthraquinone and benzanthrone. Concentration-related toxic effects appear to prevent these substances from being bioassayed in bacterial systems as the most mutagenic agents.
3. Major mutagenic substances in residual fuel oils are not associated with "classical" organic classes, such as polycyclic aromatics (e.g., benzopyrene).
4. The unusual, orange fluorescence effect under ultraviolet irradiation of the more mutagenic sample fractions suggests that this phenomenon should be studied further for routine bioassay application in fossil fuel analyses.

The elemental composition of residual oils is elucidated to some extent in this report. The nature of inorganic material occurrence is such that it does not appear that these materials can be solubilized as required for performance of bioassay testing.

Based on the work that has been performed, it is recommended that:

1. Because there is little analytical reference data to connect materials of interest in residual fuel oils to known compounds, efforts should be continued to identify mutagens in residual fuel oils that will add to the known list of such bio-active materials.
2. Residual fuel oils should be examined by second-tier bioassay such as Syrian Hamster Embryo cells, Chinese Hamster Ovary, Sister Chromatid Exchange, or Mouse Lymphoma System. It appears that mutagenicity of residual fuel oils does not principally reside in the "classical" polycyclic aromatic hydrocarbon portion. It is therefore possible that accepted "relationships" between the Ames test and potential carcinogenicity of residual oil components may not be reflected in the literature and may not follow expected trends.
3. A test plan should be formulated and carried out to evaluate the potential of usage/environment concentration of mutagenic factors, and to check undesirable environmental alterations which residual fuel oil can undergo. Any mutagenicity of an as-received residual fuel oil studied in this work has been very weak. Fractions from mutagenic oil as obtained from dimethylsulfoxide partitioning/column chromatography can be very strong, due to active agent concentration.
4. Further chemical identification of mutagenic species should be carried out. This would certainly include use of the dimethylsulfoxide partitioning/column chromatography technique. It would also include re-evaluation of the well known "separation by classes" technique of Fuson and Shriner which could now be effective since presumed obstructive and inconsequential matrix (i.e., dimethylsulfoxide insolubles) is removed from the sample. This effort should also include attempts to upgrade the column chromatography method.
5. The feasibility of developing dimethylsulfoxide partitioning and/or the phenomenon of ultraviolet fluorescence as a "field test" for mutagenicity should be explored.
6. Each of the above recommendations should be applied for chemical analysis and genetic toxicology examination of materials, product, and waste from other energy conversion processes such as coal gasification, coal liquefaction, and shale oil extraction.
7. The 15 as-received oils not examined should be bioassayed by Ames test. The collection of 41 residual fuel oil samples assembled here is unique and probably could never be duplicated. Given the "individuality" of composition of such samples, all 41 should be subjected to DMSO partitioning followed by column chromatography. The final fractions should be bioassayed and examined by organic chemical analysis.

8. Any of the above work should be performed in serial rather than parallel fashion consisting of: sample separation, bioassay, and chemical analysis. Results of a prior operation should determine if there will be a subsequent operation.

In summary, environmental affects of residual oil use in power generation is minimal.

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The complete report is in two volumes, entitled "Bioassay and Chemical Analysis for Hazardous Materials in Residual Oils":
Volume 1. (Order No. PB 82-117 078; Cost: $24.00, subject to change)
Volume 2. (Order No. PB 81-190 944; Cost: $34.50, subject to change)
will be available only from:
National Technical Information Service
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