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MUTAGENICITY TESTING OF EXTRACTS FROM PETROLEUM AND COAL TAR PITCHES

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Summary

Dimethylsulfoxide extracts of Ashland-240 petroleum pitch and Barrett M-30 coal tar pitch were tested for mutagenicity using Salmonella histidine reversion assay (Ames test). Four strains of Salmonella were employed. The strains sensitive to frameshift mutations were reverted, with the coal tar pitch indicating a three fold greater mutagenic activity than the petroleum pitch. Metabolic activation with rat liver extracts was required.

As a prescreen to aid investigators in ordering their priorities, the short-term tests appear to be a valid approach to testing the large number of hazardous compounds and complex mixtures that man encounters in his environment.

Introduction

The enormous amount of industrial and technological activity carried out in the modern world creates a large number of chemical pollutants. The developing fossil fuel industries serve as examples that could have significant environmental impact on man. In addition to obvious toxic effects, these chemical pollutants might possess carcinogenic, mutagenic, or teratogenic effects. The research effort on the health effects of chemicals in the environment is being carried out, but it has become obvious that methods to cut down the research time and expense are necessary to confront the large number of potentially hazardous substances that man encounters.

A simple, quantitative, and sensitive bacterial assay system to determine the ability of chemicals to revert hisitidine auxotrophs of Salmonella typhimurium to histidine prototrophy is being extensively used for the detection of mutagenic agents [1]. The use of rat liver microsomal fractions in this assay extends the scope of this system by including the mammalian metabolic enzymes that are required for the conversion of certain promutagens into their ultimate active form. A high degree of correlation between carcinogenicity of compounds and their mutagenic potential [1] not only supports the somatic mutation theory for cancer, but also helps in determining the biohazard (mutagenic/carcinogenic damage) due to various chemical pollutants. This rapid and inexpensive test system has been effectively utilized to detect mutagenic activity of petroleum and coal tar pitch samples.

Mutagenicity Testing

The Salmonella tester series (obtained through the courtesy of Dr. Bruce N. Ames, see Table 1) is composed of histidine mutants that revert after treatment with mutagens to the wild-type state or growth independent of histidine. Both missense mutants and frameshift mutants comprise the set and their reversion characteristics with a potential chemical mutagen qualify the mechanism of action. In addition, the detection scheme yields the highest resolution possible by the inclusion of other mutations: (a) the deep rough mutation, rfa, which affects the lipopolysaccharide coat, making the bacteria more permeable; and (b) the deletion of the uvrB region, eliminating the excision repair system.

Generalized testing of compounds was accomplished using the standard tester strains, TA1535 and TA1537, in combination with the R factor strains TA100 and TA98. Briefly, the compound to be tested was dissolved in dimethylsulfoxide (DMSO). Concentration was varied over a range of µg→mg added per plate except with highly toxic compounds. The various Salmonella strains were treated using a plate incorporation assay.

Screening of Complex Mixtures

Recently we have extended the assays, specifically with the Salmonella system, to a study of the feasibility of investigating crude industrial products and effluents [2]. By using the genetic results as estimates of biohazards, we can establish priorities for further testing. Our initial efforts argue for the applicability of the testing but only when coupled with analytical work and fractionation of the crude mixtures.

To rapidly determine the potential biohazards (mutagenicity/carcinogenicity) of various crude and complex test materials derived from various industrial activities, we have used short-term genetic assays to predict, isolate, and identify the chemical hazards.

As an example of the screening of complex mixtures, we applied the mutagenicity testing to a set of exemplary pitches: Ashland-240 petroleum pitch and Barrett M-30 coal tar pitch. The samples were ground, stirred with DMSO and the DMSO extract was separated by filteration before subjecting to biological studies.

The qualitative bioassay assay results are shown in Table 2. Both pitches (extracted with dimethylsulfoxide) showed mutagenicity in the Salmonella system. Metabolic activation was required.

Mutagenic index was determined form the linear portion of dose response curves. Quantitatively, the M-30 coal tar pitch shows a higher (2-3X) mutagenic activity than the A-240 petroleum pitch as measured with the frame-shift strain TA98 including metabolic activation with an Aroclor-induced rat liver enzyme preparation (Table 3). Both of these responses argue for polyaromatic hydrocarbons as the major contributors to the activity. The frameshift mechanism of reversion of the TA98 strain is typical of PAH's and Aroclor induces the family of liver enzymes (aryl hydroxylases) necessary for activation of PAH. Thus, we assume that the bulk of the activity originates from this class. Further fractionation coupled with biotesting will clarify this point. Upon

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chemical analysis, the A-240 Polyaromatic hydrocarbons are seen to be far more complex in nature than those of the M-30, most likely from greater concentrations of alkylated aromatics. This is consistent with the petroleum origin of the A-240 pitch. In contrast, the M-30 aromatics appear to consist almost exclusively of the parent, unsubstituted PAHs - in keeping with the high-termperature history of this coal tar pitch and the lesser stability of alkyl substituent bonds versus aromatic carbon-carbon bonds. These parent PAHs are approximately 2-3X as concentrated as those in the A-240 aromatic fraction; this observation is consistent with the greater microbial mutagenic activity observed for the M-30 DMSO extract in this work.

In this initial feasibility study, the point in question is not whether these results reflect a relative biohazard for comparison with other materials or processes. The results simply show that biological testing - the Ames histidine reversion assay in this study - can be carried out with the newly developed tester strains and crude materials. The working hypothesis is that sensitive detection of potential mutagens in fractionated complex mixtures could be used to isolate and identify the biohazard. In addition, the information could be helpful in establishing priorities for further testing, either with other genetic assays or with carcinogenic assays. Finally, the procedures might show utility in monitoring plant processes, effluents or personnel early in the formation of the engineering and environmental technology that will eventually evolve in, perhaps, the ceramic industry. The approach and preliminary results showed that the coupled chemical-biological scheme is a feasible research mechanism and is applicable to the ascertainment of potential human health hazard of a wide variety of environmental exposures, either occupationally or to the population in general.

References

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2. Epler, J. L., Young, J. A., Hardigree, A. A., Rao, T. K., Guerin, M. R., Rubin, I. B., Ho, C.-h. and Clark, B. R., Mutation Res. 57:265-276 (1978).

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Table 1. Genotype of Salmonella Strains Used for Testing Mutagens.

Additional Mutations in		Histidine Mutation in Strain		
LPS	Repair	hisG46 (missense)	hisC3076 (frameshift)	hisD3052 (frameshift)
rfa	ΔuvrB	TA1535	TA1537	TA1538
rfa	ΔuvrB	TA100*	--	TA98*

*Plus R factor (sensitive strains containing a resistance transfer factor).

Abbreviations: his = histidine; LPS = lipopolysaccharide; rfa = deep rough derivative of tester strain, defective in LPS; ΔuvrB = deletion in repair system.

Table 2. Mutagenicity of Petroleum and Coal Tar Pitch Samples with Tester Strains of Salmonella typhimurium[a]

	Salmonella ($his^- \rightarrow his^+$)			
	Assay TA1535	TA1537	TA98	TA100
DMSO	-	-	-	-
A-240	-	+	+	+
M-30	-	+	+	+

[a]Metabolic activation with Aroclor induced rat liver homogenate was induced.

Table 3. Mutagenic Index of Petroleum and Coal Tar Pitch Samples

	Salmonella typhimurium his^+ Rev/Plate[a]			
	TA98		TA100	
DMSO Extract From	-ACT	Ar S-9	-ACT	Ar S-9
A-240	30	480	0	400
M-30	90	1360	0	1400

[a]Determined from the linear portion of a dose response curve.