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Toxicity Studies of Mild Gasification Products

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Contractor:

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DISCLAIMER

Toxicity Studies of Mild Gasification Products

CONTRACT INFORMATION

Cooperative Agreement	DE-AI21-89MC26018
Contractor	National Institute for Occupational Safety and Health 944 Chestnut Ridge Road Morgantown, WV 26505 (304) 291-4516
Contract Project Manager	Ms. Barbara H. Brown
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METC Project Manager	Mr. Wu K. Lan
Period of Performance	September, 1990 to May, 1993

Schedule and Milestones

Month	0	4	8	12	16	20	24	28	32	36
1. Test Plan Development	———									
2. Genotoxicity Testing										
a. Ames Salmonella Assay		———	———							
b. Mammalian Cell In-vitro Assays					———	———				
3. Aromaticity Determination						———				
4. Characterization of Fractions										
a. Chemical Fractionation		———	———	———						
b. Chemical Characterization						———	———			
c. Mutagenicity Assay								———		
5. Data Analysis and Preparation of the Final Report									———	———

OBJECTIVES

The objectives of this project are: (1) to perform mutagenicity studies with the Ames Salmonella/microsomal assay system on coal liquids produced by mild gasification from different coals and/or processing conditions, (2) to determine whether coal liquids which are mutagenic to bacteria are also genotoxic to mammalian cells, (3) to establish correlations between mutagenicity, aromaticity, and boiling point range of coal liquids, and (4) to identify the chemical classes which are likely to be responsible for the mutagenic activity of gasification products.

BACKGROUND INFORMATION

Cancer is the second leading cause of death from disease in the United States (U.S.D.C., 1986). Only cardiovascular disease takes more lives each year in this country. Recent evidence indicates that a high percentage of human cancers of known origin results from exposure to carcinogenic agents in our environment (Friedberg, 1986). By reducing these carcinogenic agents both at home and in the occupational setting, many incidents of cancer could be prevented. Thus, the identification, detection, and regulation of carcinogenic agents are important in ensuring human health and safety.

A substance may be labelled as a human carcinogen only after sufficient human epidemiological evidence has been accumulated. This type of study, of course, can only be performed when a human population has had substantial, long-term exposure to a particular agent. An agent may, however, be tested for its carcinogenic potential in laboratory assays. After inducing tumors in animals in life-time bioassays, an agent may be described as a potential or suspected human carcinogen. These assays are not always practical because they can last over two years and cost 500,000 to 1 million dollars per compound.

A more cost-effective and less time-consuming approach to screening new agents involves the use of bacterial and/or mammalian cell cultures. These assays detect damage to the DNA, which may be involved in the initiation phase of cancer development. The Ames Salmonella/microsomal assay is a frequently used bacterial mutagenicity test that takes less than a week to perform. Mammalian cell assays utilize cultured cell lines from mammalian sources and include a battery of tests that take a few months to complete. The decreased time requirement, combined with the fact that fewer animals need to be cared for and sacrificed, makes these assays much less expensive than animal studies. An extensive database exists for these assays that indicates a positive correlation between short-term test results and the carcinogenicity of certain chemical classes. The availability of these short-term tests allows toxicologists to screen genotoxic agents and potential carcinogens rapidly and inexpensively.

Many coal-derived materials and/or conversion products have been shown to be genotoxic in short-term assays (NIOSH, 1981 and Gray et al., 1988). Most of the genotoxicity research in this field, however, has centered on the analysis of coal liquefaction products and processes, with less information being available about coal gasification products and processes. If coal gasification products are to be used in industry or the home, the human health hazards need to be detailed. It has been demonstrated that both the coal type used and the process conditions (such as catalyst quality, reaction severity, and residence time) affect the genotoxicity of coal-derived materials (Gray et al., 1988). The effects of process conditions are expressed predominantly through their influence on the boiling point and aromaticity of the coal

products. Therefore, data generated with regard to genotoxicity and these chemical characteristics may provide useful information when planning modifications to the process conditions which may reduce the genotoxic potential of the products.

PROJECT DESCRIPTION

Ames Salmonella/microsomal Assay

Approximately twenty coal-derived mild gasification products will be examined in this study. The samples are being collected from different coal sources and processing conditions. The products and/or their extracts will be tested for genotoxicity, as stated in the objectives. The overall design of the project is outlined in the flow chart in Figure 1. The Ames Salmonella/microsomal assay (Figure 2) detects the reverse mutation of bacteria from histidine dependence to histidine independence (Maron and Ames, 1983). Each sample of mild gasification product will be tested on bacterial tester strains TA98 and TA100 in the presence and absence of a rat liver preparation, S9. The use of the two tester strains allows the determination of the type (frameshift or basepair substitution) of chemical mutagen(s) detected. The S9 shows whether these mutagens cause genetic damage directly or whether they require activation by metabolic enzymes produced in mammalian livers. All the samples will be tested in the standard plate incorporation assay of the Ames test. If no activity is detected, a modified protocol, the pre-incubation variation, which is often more sensitive than the standard assay will be employed in testing the coal liquids to verify the negative results. The modification requires that the bacteria, the sample, and the S9 be incubated together prior to plating. Experiments will be performed using at least two replicates, and dose-adjusted confirmatory tests will be run and repeated as necessary. 2-aminoanthracene, a known mutagen with well characterized properties is to be used as a positive control. The solvent will be used as a negative control. A positive response is defined as a doubling of the background (solvent control) mutation frequency, in a dose-dependent manner.

Subfraction Studies. Six of the coal-derived products that are found to be mutagenic to bacteria in the Ames test will be selected for chemical fractionation by organic solvent extraction (Whong et al., 1981) into acidic, basic, nonpolar neutral, and polar neutral subfractions (Figure 3). In order to identify the chemical fraction(s) responsible for the mutagenic activity found in the sample, the subfractions will be tested in the microsuspension variant of the Ames Salmonella test, which can be used when the amount of sample is limited. Each subfraction will be characterized to determine the H:C ratio and the aromatic content, so that correlations may be made between mutagenicity and chemical character.

Mammalian Cell Studies

These studies include assays for the induction of gene mutation, sister chromatid exchange and micronuclei in cultured Chinese hamster lung (V79) cells. These tests take considerably longer to conduct than the Ames assay, but it is important to know if coal gasification products which are genotoxic to bacteria are also genotoxic to mammalian cells.

RESULTS

NIOSH has, to date, received and tested seven mild gasification products. One coal tar, SHELL#830331, came from Shell Oil Company. Western Research Institute (WRI) sent five samples. Three of them are from the same coal/production source but were distilled at different boiling point ranges; MG-122 IBP-

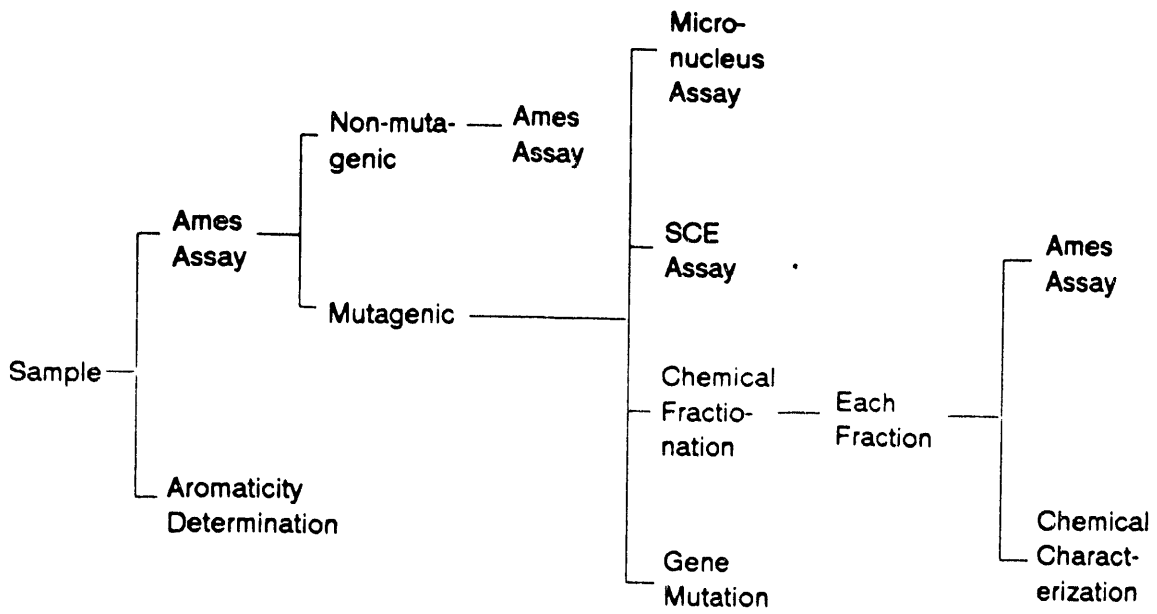

Figure 1. Project Flow Chart

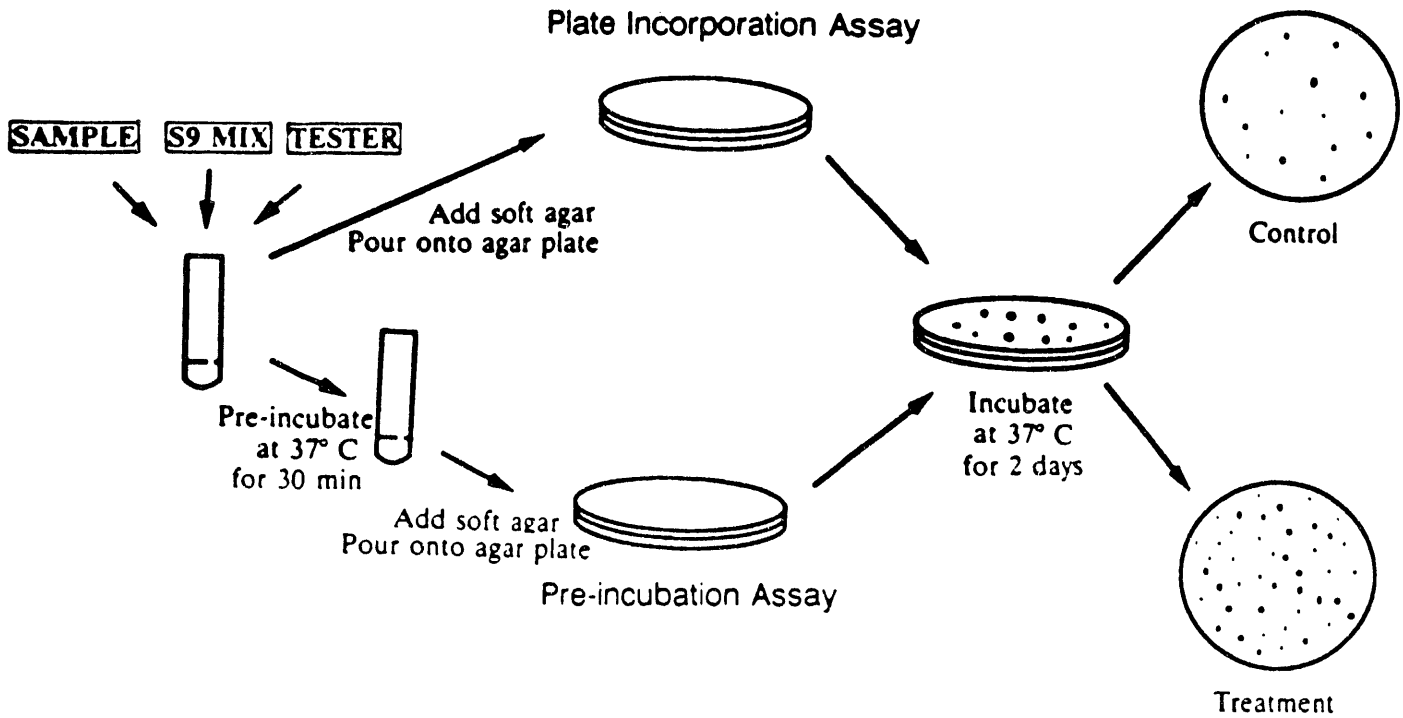

Figure 2. Ames Salmonella Microsomal Assay System

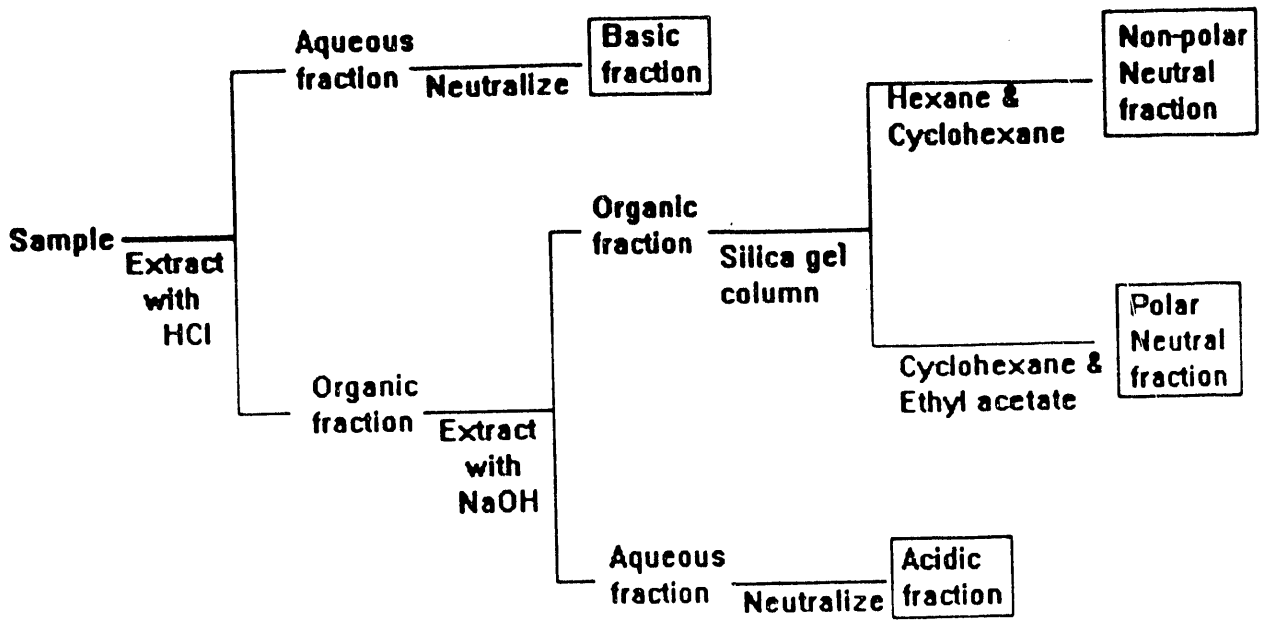

Figure 3. Procedure for Chemical Fractionation

420°F, MG-122 420-720°F, and MG-122 720°F+. The other two are composites from different coal/production sources containing materials from a wide range of boiling points, MG-119 and MG-120. CTC#11, another composite material, was received from Coal Technology Corporation.

Due to reports that solvent/mutagen interactions may occur causing false positive or negative results in genotoxicity tests, two solvents with different properties, DMSO and Tween 80, were used for each sample. Tween 80 allows testing at higher concentrations without toxicity, but DMSO has been used more frequently in testing coal conversion products. Testing started at the upper solubility endpoint with concentrations decreasing in full logarithmic steps. The initial tests define the appropriate range of concentrations for mutagenicity testing (between the cytotoxic level, determined by microscopic inspection of the bacterial lawn, and a low dose that has no effect) within which the test concentrations were increased by doubling, rather than logarithmically.

Four of the seven samples tested so far failed to demonstrate any mutagenic activity under any conditions tested. Those samples were SHELL#830331, MG-122 IBP-420°F, MG-122 420-720°F, and MG-122 720°F+. Table 1 summarizes the results from all samples tested in DMSO and Tween 80.

When solvated in DMSO, MG-119 and MG-120 composite materials displayed slight, but ultimately insignificant, genotoxic activity on TA98 and TA100 in the presence of S9. When Tween 80 was used as the solvent, MG-119 and MG-120 displayed slight, but significant, geno-

Table 1. Mutagenicity of Seven Mild Gasification Products

Sample	Solvated in	Mutagenic Activity			
		TA 98		TA 100	
		-S9	+S9	-S9	+S9
Shell #830331	DMSO	-[a]	-	-	-
	Tween-80	-	-	-	-
MG-122 IBP-420°F	DMSO	-	-	-	-
	Tween-80	-	-	-	-
MG-122 420-720°F	DMSO	-	-	-	-
	Tween-80	-	-	-	-
MG-122 720°F+	DMSO	-	-	-	-
	Tween-80	-	-	-	-
MG-119 Composite	DMSO	-	-	-	-
	Tween-80	-	+[b]	-	-
MG-120 Composite	DMSO	-	-	-	-
	Tween-80	-	+	-	-
CTC#11	DMSO	+	+	+	+
	Tween-80	+	+	+	+

a "-" is no activity b "+" is positive activity

toxic activity on TA98 with S9 (Figure 4). CTC#11 in DMSO displayed significant genotoxic activity on both TA98 and TA100 with and without S9. The activity was higher on TA98 than TA100, and higher with S9 than without, primarily indicating the presence of indirect-acting frameshift mutagen. The results of the testing on CTC#11 were similar for both solvents, DMSO and Tween 80 (Table 2).

The samples that showed positive responses have been fractionated and tested in the microsuspension variant of the Ames assay. Despite being solvated in DMSO, and not in Tween 80, the nonpolar neutral subfractions of MG-119 and MG-120 displayed weak and moderate significantly genotoxic activity, respectively (Tables 3 and 4). The fact that the activity was apparent when the sample had been fractionated, but not when it was a complex mixture, may be due to chemical masking. The inhibition of mutagens by nonmutagenic components of a complex mixture has been proposed by Gray et al. (1988) as an explanation of a phenomenon similar to what was noted here. It is also possible, although unlikely, that the fractionation procedure, with its acid/base treatments, altered the chemical properties of the compounds in the mixture, thereby activating them.

All of the four subfractions of CTC#11 displayed positive genotoxic activity in the microsuspension Ames assay (Table 5). The highest activity was shown in the basic fraction on TA98 with S9 activation. That fraction's activity on TA98 without S9 was low, but significant. These results indicate the presence of

Figure 4 . Mutagenicity of MG-119 and MG-120 in TA98 with S9

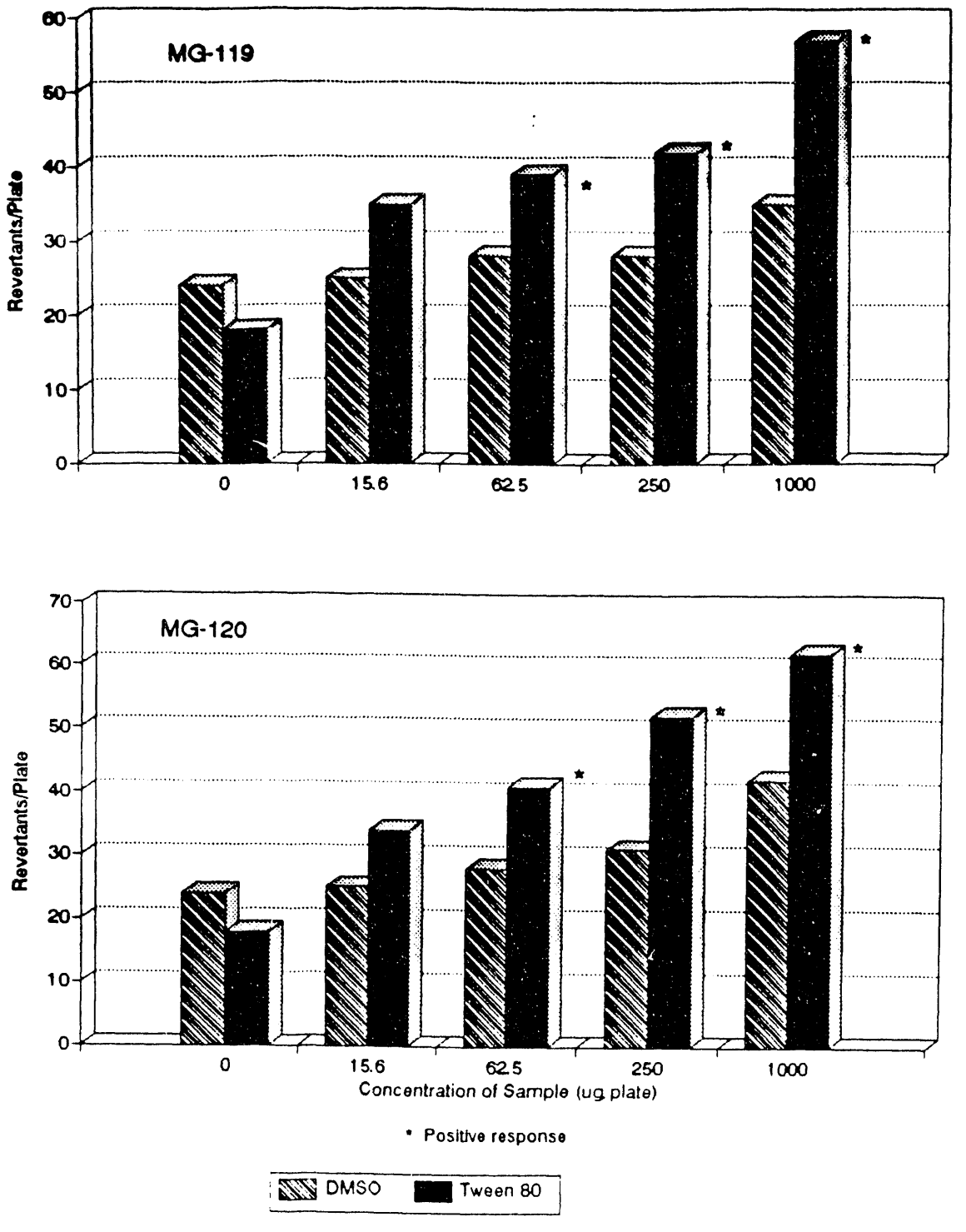

Table 2. Comparison of the Mutagenicity of CTC#11 Solvated in DMSO or Tween 80

Conc.	DMSO				Tween 80			
	TA98		TA100		TA98		TA100	
ug/plate	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
0	22	27	101	86	18	19	98	83
12.5	73*	347*	171	196*	-	-	-	-
25	55*	663*	183	328*	35	59*	184	172*
50	138*	853*	239*	321*	46*	71*	225*	234*
100	87*	1035*	218*	363*	54*	101*	254*	252*
200	53*	1145*	194	560*	105*	171*	244*	304*
400	-	-	-	-	173*	228*	224*	301*

The zero dose represents the solvent control.

*, positive mutagenic activity

-, not tested or too much cytotoxicity

indirect-acting and direct-acting, frameshift mutagens. The acidic fraction displayed a pattern of mutagenicity similar to, but much lower than, the basic fraction. Indirect-acting, frameshift mutagens were also indicated in the polar neutral fraction; however, the activity in that case was very weak. In the nonpolar neutral fraction, the activity appeared higher on TA100 than TA98 and higher without than with S9. In this fraction, the results suggest the presence of direct-acting frameshift mutagens.

Results are not yet available for the mammalian cell assays.

FUTURE WORK

The Ames Salmonella/microsomal assay will be performed on the remaining samples yet to be received. Up to six samples will be selected for testing of their ability to induce SCE, gene mutation, and micronucleus formation in cultured mammalian (V79) cells. Those selected samples will also be fractionated into basic, acidic, nonpolar neutral, and polar neutral fractions. The mutagenic activity of each fraction will be determined by the microsuspension variation of the Ames assay. The H:C ratio will also be determined for those samples.

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Table 3. Mutagenicity of Fractionated MG-119

Fraction	Conc. ug/plate	TA98 -S9	TA98 +S9	TA100 -S9	TA100 +S9
Basic	0	33	29	88	86
	38	38	40	106	133
	75	36	45	115	120
	150	39	35	124	127
	300	37	30	121	126
Acidic	0	33	29	88	86
	625	32	28	108	114
	1250	32	33	118	105
	2500	32	28	127	117
	5000	32	30	113	100
Nonpolar Neutral	0	27	24	86	84
	187.5	41	58*	126	146
	375	42	72*	140	179*
	750	39	74*	154	221*
	1500	61*	69*	206*	193*
Polar Neutral	0	27	24	86	84
	16	29	-	104	-
	31	36	37	106	-
	63	25	36	104	107
	125	0	43	82	110
	250	-	29	-	87

The zero dose represents the solvent control.

*, positive mutagenic activity

-, not tested or too much cytotoxicity

Table 4. Mutagenicity of Fractionated MG-120

Fraction	Conc. ug/plate	TA98 -S9	TA98 +S9	TA100 -S9	TA100 +S9
Basic	0	29	34	111	122
	48	37	32	105	104
	95	35	38	92	120
	190	30	41	101	127
	380	40	57	87	139
Acidic	0	29	34	111	122
	659	24	34	105	256
	1318	31	37	116	189
	2635	30	22	90	283
	5270	17	38	89	324
Nonpolar Neutral	0	29	34	111	122
	94	66*	65	246*	256
	188	80*	66	352*	189
	375	141*	96*	505*	283*
	750	196*	108*	617*	324*
Polar Neutral	0	29	34	111	122
	15	36	50	120	112
	30	42	55	98	121
	60	30	46	107	122
	120	13	43	59	103

The zero dose represents the solvent control.

*, positive mutagenic activity

-, not tested or too much cytotoxicity

Table 5. Mutagenicity of Fractionated CTC#11

Fraction	Conc. ug/plate	TA98 -S9	TA98 +S9	TA100 -S9	TA100 +S9
Basic	0	26	29	115	99
	4.7	56	565*	160	277*
	9.4	90*	685*	181	361*
	18.75	113*	642*	162	367*
	37.5	120*	923*	195	423*
Acidic	0	26	29	115	99
	37.5	37	57	161	122
	75	46	74*	174	166
	150	68*	200*	173	200*
	300	69*	274*	190	234*
Nonpolar	0	26	29	115	99
Neutral	9.4	156*	147*	-	-
	18.75	227*	243*	639*	442*
	37.5	345*	293*	686*	545*
	75	520*	430*	823*	619*
	150	-	-	913*	717*
Polar	0	26	29	115	99
Neutral	1.2	32	48	-	-
	2.4	28	53	158	164
	4.7	38	72*	180	167
	9.4	152	147*	188	193
	18.75	-	-	224*	186

The zero dose represents the solvent control.

*, positive mutagenic activity

-, not tested or too much cytotoxicity

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