DOE-ET-00222-5

EXPLORATORY RESEARCH ON MUTAGENIC ACTIVITY OF COAL-RELATED MATERIALS

Progress Report

for

Period March 1, 1980 - June 1, 1980

D. Warshawsky and R. Schoeny

University of Cincinnati Medical College
Kettering Laboratory
Department of Environmental Health
3223 Eden Avenue
Cincinnati, Ohio 45267

Prepared for U.S. Department of Energy
PITTSBURGH ENERGY TECHNOLOGY CENTER
Under Contract No. DE-AS-22-78ET00222

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Abstract

Samples ETTM-05 and ETTM-12 (powdered coal) were not mutagenic for any of 4 Salmonella strains. ETTM-18 (solid residual) was mutagenic for strains TA1538, TA98 and TA100 when assayed with Aroclor-induced S-9.

This report covers data on the following samples:

ETTM-05 Coal

ETTM-12 Coal

ETTM-18 Liquefaction Solid Residual

The testing of organic solvent fractions of ETTM-15 - ETTM-17, which was not covered in the last quarterly report, has been described in AS22-78-ET00222 final report, #DOE-ET-00222-4.

1. Preparation for Assay

ETTM-05 and ETTM-12 are black powders; ETTM-18 is a black solid easily reduced to powder. Test materials were stored at 5°C in the dark. They were prepared for assay by weighing 20-70 mg and adding dimethylsulfoxide (DMSO) so as to obtain a presumptive concentration of 10 mg/ml. For no sample did all material dissolve. For ETTM-18 the amount of insoluble sample was subtracted from the total to determine the adjusted concentration used in calculating mutagenic activities. Samples ETTM-05 and ETTM-12 retained the solvent after evaporation to apparent dryness, making it impossible to ascertain the weight of insoluble material. Data from assays of these samples are expressed as function of sample dilution rather than ug sample/plate.

Sample solutions were routinely filter sterilized and applied as 0.1 ml aliquots. Dilutions were made in DMSO so that these percentages of the original solution were tested: 100%, 50%, 10%, 5%, 1%.

Organic solvent fractionation of all three samples were done as previously reported (#DOE-ET-00222-4 final report). One set of fractions from each of the three samples has been prepared and assayed. These data, as well as those generated from assay of the repeat extractions, will be presented in a subsequent report.

2. Chemical analysis of mutagenic fractions.

A new set of organic solvent fractions has been prepared from the following coal-related materials:

ETTM-01
ETTM-02
ETTM-08
ETTM-09
ETTM-10
ETTM-11
ETTM-15
ETTM-16

All of these samples have previously been shown to be mutagenic for Salmonella and have three or more mutagenic organic solvent fractions. Limited mutagenicity testing (TA98 only, 4 point dose response with S9) has been done to determine if this new set of fractions is similar in bio-activity to those two sets for which there are extensive data. These data are still being analyzed. Samples will be selected from this fraction set for determination of PAH and aromatic amine content by high performance liquid chromatography and GC-MS.

3. Results of Mutagenicity Testing

Quantitative dose response assays were done with ETTM-05, ETTM-12 and ETTM-18 using four Salmonella strains. None of these were mutagenic when no Aroclor-induced S9 extract was included on the test plates. No overt bacterial toxicity was observed for any of these samples at the concentrations tested.

Data from assay of ETTM-05 and ETTM-12 are given in Table 1. While there are instances of colony counts on test plates 2 x the spontaneous counts, there is no evidence of dose dependent mutagenesis in the presence of S9. Accurate determination of concentrations of sample tested could not be made due to the tendency of these materials to retain the solvent after evaporation to apparent dryness.

Data in Table 2 show ETTM-18 to be mutagenic for TA1538, TA98 and TA100 but not for TA1535. Table 3 ranks all of the mutagenic samples by their specific activity for strain TA98 (colonies/ug values were determined by regression analyses of the linear portions of dose response curves). ETTM-18 can be seen to be one of the more mutagenic samples of this set. It has approximately 60% the activity of ETTM-11 which was presumably sampled at the same process point from an earlier identical liquefaction run.

TABLE 1

MUTAGENICITY OF ETTM-05 AND ETTM-12 IN THE PRESENCE OF 50 UL/PLATE AROCLOR-1254 INDUCED S9

Compound	Amount/Plate	Colonies/Plate[a] TA1535	TA1538	TA98	TA100
DMSO[b]	0.1 ml	16 ± 5	9 ± 3	18 ± 4	120 ± 24
MNNG	spot	+4[c]			
ACNA	150 ug		1167 ± 260	3583 ± 581	
MMS	13.3 mg				1597 ± 510
DMSO[d]	0.1 ml	26 ± 13	20 ± 6	31 ± 10	142 ± 24
2AA	5 ug				1388 ± 85
ETTM-05 10 mg/ml[e]	0.1 ml of 1%			27	123
	0.1 ml of 5%			27	130
	0.1 ml of 10%			30	139
	0.1 ml of 50%			47	156
	0.1 ml of 100%			46	151
50 mg/ml[e]	0.1 ml of 1%	20	32	48	181
	0.1 ml of 5%	28	42	66	168
	0.1 ml of 10%	36	43	54	183
	0.1 ml of 50%	38	44	52	147
	0.1 ml of 100%	30	41	64	249
ETTM-12 10 mg/ml[e]	0.1 ml of 1%			30	138
	0.1 ml of 5%			26	134
	0.1 ml of 10%			30	131
	0.1 ml of 50%			40	137
	0.1 ml of 100%			43	181
50 mg/ml[e]	0.1 ml of 1%	30	34	38	136
	0.1 ml of 5%	28	41	59	163
	0.1 ml of 10%	40	42	40	178
	0.1 ml of 50%	42	54	84	214
	0.1 ml of 100%	35	37	79	181

[a] Numbers are means of colony counts, n ≥ 4. Standard deviations are included for control plates. Underlined numbers are 2 x the spontaneous rate.

[b] DMSO = dimethylsulfoxide

MNNG = N-methyl-N'-nitro-N-nitrosoguanidine

ACNA = 1-amino-2-carboxy-4-nitroanthraquinone

MMS = methyl methanesulfonate

2AA = 2-aminoanthracene

[c] +4 = Ring of revertant colonies too numerous to count.

[d] These and subsequent plates received 50 ul/plate Aroclor-induced S-9.

[e] Starting concentrations.

TABLE 2

DOSE DEPENDENT MUTAGENICITY OF ETTM-18 IN THE PRESENCE OF AROCLOR 1254-INDUCED S-9

Compound	Amount/Plate	Colonies/Plate[a] TA1535	TA1538	TA98	TA100
DMSO[b]	0.1 ml	16 ± 5	9 ± 3	18 ± 4	120 ± 24
MNNG	spot	+4[c]			
ACNA	150 ug		1167 ± 260	3583 ± 581	
MMS	13.3 mg				1597 ± 510
DMSO[d]	0.1 ml	26 ± 13	20 ± 6	31 ± 10	142 ± 24
2AA	5 ug				1388 ± 85
ETTM-18	1.50 ug			36	134
	4.46 ug	29	36	47	163
	8.0 ug			155	173
	15.0 ug			436	307
	22.3 ug	27	94	126	190
	44.6 ug	31	179	602	264
	74.7 ug			1599	918
	149. ug			2274	996
	223. ug	41	733	1373	648
	446. ug	45	1111	2170	1114

[a] Numbers are means of colony counts, $n \geq 4$. Standard deviations are included for control plates. Underlined numbers are 2 x the spontaneous rate.

[b] DMSO = dimethylsulfoxide

MNNG = N-methyl-N'-nitro-N-nitrosoguanidine

ACNA = 1-amino-2-carboxy-4-nitroanthraquinone

MMS = methyl methanesulfonate

2AA = 2-aminoanthracene

[c] +4 = Ring of revertant colonies too numerous to count.

[d] These and subsequent plates received 50 ul/plate Aroclor-induced S-9.

TABLE 3

RELATIVE MUTAGENIC ACTIVITIES OF COAL-RELATED MATERIALS

Sample Number	Sample Type	Revertant Colonies/ug[a]		
		TA98	TA1538	TA100
ETTM-11	Liquefaction Solid Residual Run 1	27.03	27.92	11.36
ETTM-01	Vehicle Oil	18.54	30.12	6.89
ETTM-08	Liquefaction Heavy Liquid Run 1	17.17	3.30	8.38
ETTM-18	Liquefaction Solid Residual Run 2	15.98	3.18	6.40
ETTM-09	Liquefaction Product Run 1	11.42	7.03	6.76
ETTM-10	Liquefaction Distillate Oils Run 1	10.88	7.30	2.10
ETTM-02	Gasification Tar	6.75	11.16	6.49
ETTM-15	Liquefaction Heavy Liquid Run 2	3.68	2.39	1.78
ETTM-16	Liquefaction Product Run 2	2.56	1.37	1.80
ETTM-17	Liquefaction Distillate Oils Run 2	1.54	0.79	0.86
ETTM-14	Liquefaction Light Oils Run 2	0.10	0.08	-[b]

[a] Numbers are slopes of least square lines, n = 6-78. All data from mutagenicity assays with 50 ul/plate S9 from Aroclor-induced Sprague-Dawley rats.

[b] - = No dose dependent mutagenicity.