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EXPLORATORY RESEARCH ON MUTAGENIC ACTIVITY OF COAL-RELATED MATERIALS

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Progress Report

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Abstract

Samples ETTM-01 through ETTM-18 were tested for mutagenicity in Salmonella strain TA1537. Samples previously shown to be non-mutagenic or marginally for TA1538, TA98 and TA100 were not mutagenic for TA1537. Those samples mutagenic for TA1538, TA100 and TA98 were also mutagenic for TA1537 but to a much lesser degree.

This report covers the testing of the following samples for mutagenicity with Salmonella strain 1537:

ETTM-01	ETTM-10
ETTM-02	ETTM-11
ETTM-03	ETTM-12
ETTM-04	ETTM-13
ETTM-05	ETTM-14
ETTM-06	ETTM-15
ETTM-07	ETTM-16
ETTM-08	ETTM-17
ETTM-09	ETTM-18

1. Preparation for Mutagenicity Assay

All samples were stored in the dark at 5°C. Samples were prepared for assay by weighing 50-200 mg and adding dimethylsulfoxide (DMSO) so as to obtain a presumptive concentration of 10 mg/ml. Samples ETTM-06, ETTM-07, ETTM-13 and ETTM-14 dispersed completely in the DMSO. None of the other samples (ETTM-01,02, 03,04,05,08,09,10,11,12,15,16,17,18) completely dissolved. The amount of the 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. In these two samples, therefore, one ml of the 100% solution was blown down under nitrogen and the remaining residue was taken as the concentration of the solution.

Sample solutions were routinely filter sterilized and applied as 0.1 ml alequots. Dilutions were made in DMSO so that these percentages of the original solutions were tested: 100%, 50%, 10%, 5%, 1%. Limited mutagenicity (2-4 dose point response with S-9) was done with TA98 to determine if the newly prepared samples were similar in bioactivity to the previous sets of samples for which there are extensive data.

2. Results of Mutagenicity Testing

Samples which have been previously shown to be non-mutagenic or marginally mutagenic (ETTM-03,04,05,06,07,12,13,14) for strains TA1538, TA98 and TA100 were not mutagenic for TA1537 (Tables 1 and 2). Those samples which were mutagenic for TA1538, TA98 and TA100 (ETTM-01,02,08,09,10,11,15,16,17,18) were also mutagenic for TA1537, but to a much lesser degree. None of the samples were mutagenic for any of the strains tested in the absence of 50 µl/plate S-9. The -1 frameshift lesion in TA1538 and TA98 is apparently more susceptible to the mutagenic activity of our samples than the +1 frameshift (TA1537).

Sample ETTM-13, which had been previously reported as very slightly mutagenic for TA1537, in these repeat assays showed no activity. TA98 mutagenicity of this and all other samples (except ETTM-01) was shown to be the same level as that reported in the past.

3. Analytical Work

Samples of polycyclic aromatic hydrocarbons, aromatic amines and amino-acridine compounds have been obtained. These will be used to prepare standard mixtures for HPLC analysis method development.

4. Publication

The manuscript entitled "Mutagenicity of Products from Coal Gasification and Liquefaction in the Salmonella/Microsome Assay" by Rita Schoeny, David Warshawsky, Lois Hollingsworth, Mary Hund, and George Moore, has been accepted for publication in Environmental Mutagenesis. A second manuscript, "Evaluation of Coal Gasification and Liquefaction Technologies by Salmonella Mutagenesis" by David Warshawsky, Rita Schoeny, and George Moore has been submitted to Science and is under review.

TABLE 1

Sample Number	Type	Revertant Colonies/µg[a]				
		TA1537[b]	TA1538	TA98 I	TA98 II[b]	TA100
01	Liquefaction A Vehicle Oil	0.98	30.12	18.54	10.16	6.89
02	Gasification Tar	0.82	11.16	6.75	5.35	6.49
03	Gasification Cyclone Dust	-[c]	N.D.	-	-	-
04	Gasification Bottom Ash	-	N.D.	-	-	-
05	B1 Powdered Coal	-	±	±	0.21	±
06	Liquefaction B1 Untreated Water	-	N.D.	-	-	-
07	Liquefaction B1 Light Oil	0.01	N.D.	-	0.31	-
08	Liquefaction B1 Separator Bottoms	1.54	3.30	17.17	12.90	8.38
09	Liquefaction B1 Filtered Liquid	1.40	7.03	11.42	13.37	6.76
10	Liquefaction B1 Vacuum Overhead	0.21	7.30	10.88	6.77	2.10
11	Liquefaction B1 Vacuum Bottoms	2.44	27.92	27.03	21.09	11.36
12	B2 Powdered Coal	-	±	±	±	-
13	Liquefaction B2 Untreated Water	-	-	-	-	-
14	Liquefaction B2 Light Oils	-	0.08	0.10	0.05	-
15	Liquefaction B2 Separator Bottoms	0.12	2.39	3.68	2.67	1.78
16	Liquefaction B2 Filtered Liquid	0.10	1.37	2.56	2.50	1.80
17	Liquefaction B2 Vacuum Overhead	0.04	0.79	1.54	1.19	0.86
18	Liquefaction B2 Vacuum Bottoms	2.42	3.18	13.95	13.46	8.66

[a] Data on TA1537 and TA98II from this quarterly report. Data on TA1538, TA98I and TA100 from DOE Contract #EW-78-S-22-0222 and Final Report DOE #ET-00222-4.

[b]

Sample	TA1537	TA98
01	n = 38 r = 0.8748	n = 22 r = 0.8685
02	n = 30 r = 0.9229	n = 26 r = 0.9269
05		n = 22 r = 0.7902
07	n = 54 r = 0.6445	n = 30 r = 0.9131
08	n = 25 r = 0.9222	n = 22 r = 0.9282
09	n = 26 r = 0.9434	n = 21 r = 0.9710
10	n = 34 r = 0.9610	n = 22 r = 0.9727
11	n = 46 r = 0.9693	n = 22 r = 0.9120
14		n = 30 r = 0.6124
15	n = 50 r = 0.9107	n = 22 r = 0.9451
16	n = 50 r = 0.9034	n = 26 r = 0.9584
17	n = 45 r = 0.7592	n = 26 r = 0.9308
18	n = 46 r = 0.9385	n = 30 r = 0.7942

(continued)

TABLE 1 (continued)

[c] N.D. = Not Done

\- = No Dose Response

$\overset{+}{-}$ = Marginally response, see report #ET-00222-6

TABLE 2

Mutagenicity of ETTM Samples in the Presence of
50 µl/Plate Aroclor 1254-Induced S-9

Sample	Amount/Plate	Revertant Colonies/Plate[a] TA1537	TA98
DMSO[b]	0.1 ml	8±3	22±8
MNNG	spot	31±18	
9AA	100 µg	293±230	
ACNA	150 µg		2392±734
DMSO[c]	0.1 ml	8±4	29±8
2AA	5 µg		1259±196
ETTM-01	7.9 µg	10	
	9.7	18	80
	39.6	49	432
	48.7	56	
	79.1	88	
	97.4	94	742
	395.5	146	1706
	487.0	206	
	791.0	167	
	974.0	372	
ETTM-02	6.8 µg	12	
	9.2	16	78
	33.9	39	188
	46.5	44	
	67.7	57	
	92.4	66	528
	339.0	93	735
	464.5	142	
	677.0	100	
	924.0	164	

TABLE 2 (Continued)

Mutagenicity of ETTM Samples in the Presence of
50 µl/Plate Aroclor 1254-Induced S-9

Sample	Amount/Plate	Revertant Colonies/Plate[a] TA1537	TA98
ETTM-03	1.4 µg	8	
	7.0	5	26
	14.0	7	
	70.0	6	21
	140.0	6	
ETTM-04	2.0 µg	6	
	10.0	8	25
	20.0	6	
	100.0	7	29
	200.0	9	
ETTM-05	2.6 µg	6	
	13.0	4	32
	26.0	4	
	130.0	7	56
	260.0	11	
ETTM-06	10.0 µg	7	
	50.0	8	34
	100.0	8	
	500.0	7	38
	1000.0	7	
ETTM-07	10.0 µg	8	
	50.0	9	40
	100.0	9	
	500.0	12	93
	1000.0	21	

TABLE 2 (Continued)

Mutagenicity of ETTM Samples in the Presence of
50 µl/Plate Aroclor 1254-Induced S-9

Sample	Amount/Plate	Revertant Colonies/Plate[a]	
		TA1537	TA98
ETTM-08	9.3 µg	18	
	9.8	8	60
	46.4	80	626
	49.0	55	
	92.8	95	
	97.9	85	499
	464.0	153	1973
	489.5	204	
	928.0	276	
	979.0	326	
ETTM-09	9.2 µg	16	
	9.9	15	69
	45.9	73	647
	49.6	40	
	91.8	101	
	99.3	83	709
	459.0	169	1674
	496.5	148	
	918.0	213	
	993.0	238	
ETTM-10	9.8 µg	16	84
	10.0	12	
	49.0	40	
	50.0	36	367
	97.9	22	476
	99.9	36	
	489.5	132	
	499.5	106	1267
	979.0	228	
	999.0	159	

TABLE 2 (Continued)

Mutagenicity of ETTM Samples in the Presence of
50 µl/Plate Aroclor 1254-Induced S-9

Sample	Amount/Plate	Revertant Colonies/Plate[a] TA1537	TA98
ETTM-11	2.7 µg	10	
	5.1	13	47
	13.4	56	327
	25.7	60	
	26.8	115	
	51.4	195	647
	134.0	398	2676
	257.0	610	
	268.0	522	
	514.0	783	
ETTM-12	1.6 µg	5	
	8.0	5	28
	16.0	7	
	80.0	6	41
	160.0	13	
ETTM-13	10.0 µg	9	
	50.0	8	43
	100.0	9	
	500.0	8	32
	1000.0	6	
ETTM-14	10.0 µg	6	
	50.0	6	42
	100.0	10	
	500.0	10	55
	1000.0	12	
ETTM-15	8.8 µg	8	44
	9.6	25	
	44.0	28	
	47.8	28	156
	88.0	34	220

TABLE 2 (Continued)

Mutagenicity of ETTM Samples in the Presence of
50 µl/Plate Aroclor 1254-Induced S-9

Sample	Amount/Plate	Revertant Colonies/Plate[a] TA1537	TA98
ETTM-15 (cont.)	95.5 µg	36	
	440.0	83	
	477.5	75	788
	880.0	119	
	955.0	84	
ETTM-16	9.9 µg	12	52
	10.0	11	
	49.4	26	
	49.8	32	171
	98.8	36	270
	99.7	36	
	494.0	67	
	498.5	71	673
	988.0	114	
	997.0	95	
ETTM-17	8.5 µg	10	46
	9.9	6	
	42.4	15	
	49.6	19	82
	84.8	19	134
	99.1	19	
	424.0	27	
	495.5	32	326
	848.0	30	
	991.0	46	

TABLE 2 (Continued)

Mutagenicity of ETTM Samples in the Presence of
50 µl/Plate Aroclor 1254-Induced S-9

Sample	Amount/Plate	Revertant Colonies/Plate[a] TA1537	TA98
ETTM-18	0.9 µg	9	
	3.7	10	36
	4.4	17	46
	8.9	33	
	18.7	18	
	37.4	49	197
	44.5	119	874
	89.0	235	
	187.0	195	
	347.0	278	

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

[b]DMSO = dimethylsulfoxide

MNNG = methyl methanesulfonate

9AA = 9-aminoacridine

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

2AA = 2-aminoanthracene

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

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