

MUTGEN 1973

Mutagenicity of mild gasification products in *Salmonella typhimurium*

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(Received 22 January 1993)

(Revision received 12 August 1993)

(Accepted 18 August 1993)

Keywords: Mild gasification; Salmonella pre-incubation assay; Solvent interaction; Aromatic amines; Coal-conversion products

Summary

Mild gasification is a coal-conversion technology that is currently under development in order to help meet future energy needs. 7 products from this process were assayed for mutagenic activity in the pre-incubation variant of the Salmonella assay (Ames test) using both DMSO and Tween 80 as sample solvents. Significant mutagenic activity was detected only in the wide-boiling-point composite materials, and the amount of this activity was found to be dependent on the solvent utilized. The highest number of revertants detected were on TA98 and its *O*-acetyltransferase over-producing derivative, YG1024, in the presence of the S9 microsomal fraction. Aromatic amines were suggested as a possible source of the mutagenic activity elicited. An examination of the liquid and tar phases of one composite material (MG-120) indicated that the mutagenic activity was restricted to the tar phase.

Mild gasification is an emerging coal-conversion process that is currently being developed by the United States Department of Energy (DOE) and private industry as a means to up-grade lower rank coals. As this technology evolves and its commercialization becomes more viable, an understanding of the safety of products from this synthetic fuel process becomes imperative.

Previous studies have indicated that certain coal-derived materials contain components with high specific mutagenic activities when tested in the Salmonella assay (Gray et al., 1988). Factors

associated with differences in the mutagenic activity of coal-conversion products include: content of high molecular weight compounds, boiling-point range, the processing conditions (especially with regard to the catalyst quality, reaction severity and residence time), and the type of coal processed. Although polycyclic nuclear aromatic compounds (PNAs) are generally present in these materials and have been implicated in their activity, often only a small subclass of these compounds (such as aromatic amines) account for the majority of the mutagenicity detected (Guerin et al., 1980). Furthermore, relationships between cancers induced in dermal skin-painting assays and mutagenic activity in the Salmonella assay

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have been demonstrated between the 2–5-ring polycyclic aromatic hydrocarbons and aromatic amines found in coal-conversion compounds (Later, 1985). These studies and others indicate that the Salmonella assay, when coupled with chemical analysis, can be a sensitive tool for use in pre-screening, isolating, and identifying the potential biohazards of coal-conversion materials.

Numerous studies have shown that varying genotoxic responses can be obtained in mutagenicity assays depending upon the solvent system utilized; this suggests that potentially hazardous compounds may go undetected due to mutagen/solvent interactions (Mori et al., 1985). These findings indicate that it is preferable for materials to be dissolved and tested in more than one solvent system in order to avoid possible artifactual results, especially when they have not been previously examined for their genotoxic potential (Nestmann et al., 1985). For example, although DMSO has been used with great success for the solvation and testing of numerous substances (including coal-derived materials), it has also been shown to depress the genotoxic activity of several test agents including aromatic amines (DeMarini et al., 1991) and nitrosamines (Brodberg et al., 1987). Thus, it may be very important to use a second solvent system to ensure more comprehensive test results, especially when the materials assayed are complex mixtures which may have antagonistic interactions or are pure chemicals of low mutagenic potential. One such alternative solvent system that has been used with success in a limited number of mutagenicity studies on both coal-derived and petroleum samples is Tween 80 emulsification (Ma et al., 1983). Tween 80 (polyoxyethylene–sorbitan monooleate) is a non-ionic detergent that forms an emulsion when mixed with water and is compatible for dissolving both polar and nonpolar chemicals for mutagenicity testing. This solvent system has low cytotoxicity to bacterial cells and is non-volatile.

This study was concerned with the first tier screening of 7 mild gasification products utilizing, primarily, the bacterial assay strains TA98 and TA100 with DMSO and Tween 80 as solvents. Additionally, in direct comparison experiments, the products found to be mutagenic with TA98

were subjected to dual solvent, comparison testing using both TA98 and YG1024. YG1024 is a TA98 derivative with enhanced sensitivity to aromatic amines/nitroarenes (Watanabe et al., 1990). Thus, the study was also aimed at assaying these coal-derived materials biologically for the presence of mutagenic aromatic amines and ascertaining whether one of the two solvent systems suppressed the observed activity. Finally, in order to examine the phase source of the mutagenic activity in one composite sample (MG-120), this material was partitioned into its liquid and tar phases and assayed on both TA98 and YG1024.

Materials and methods

Mild gasification samples

Shell No. 830331 is a low-temperature coal tar which was obtained from Shell Oil Company, Houston, TA. MG-122 ([Initial Boiling Point-] IBP-420°F), MG-122 (420–720°F), and MG-122 (720°F⁺) are samples with different boiling-point ranges derived from the same production/coal source. MG-122 (IBP-420°F) and MG-122 (420–720°F) are liquid samples, whereas MG-122 (720°F⁺) is a solid, but brittle residue material. These samples were obtained from Western Research Institute (WRI), Laramie, WY. MG-119 and MG-120 are composite materials that have a liquid/tar consistency. These samples contain compounds from a wide range of boiling points and were also obtained from WRI. CTC No. 11 is a composite coal tar which was obtained from Coal Technology Corporation, Bristol, VA.

Reference material and chemicals

SRC-II HD, a coal-conversion reference material, is a heavy distillate from the Solvent Refined Coal-II coal-liquefaction process (sample No. 2445, boiling-point range 550–850°F). This material was obtained from the Pittsburg and Midway Coal Mining Company, Fort Lewis, WA. 2-Aminoanthracene (2AA), used as a positive control, was purchased from Aldrich Chemical Company, Milwaukee, WI. The solvent controls utilized in this study were dimethyl sulfoxide (DMSO) and Tween 80. DMSO (sterile, spectrophotometric grade from lot No. 7209) was purchased from EM Science, Cherry Hill, NJ. Tween

80 used in the assay system in a 4% w/v solution suspended in triple distilled, deionized water was obtained from Sigma Chemical Company, St. Louis, MO.

DMSO solvation

The mild gasification products were dissolved using the DMSO slurry method of sample preparation (Ma et al., 1983). The MG-122 (720°F⁺) material required additional preparation due to its solid nature. This sample was first ground to a fine powder in a mortar and pestle prior to its solvation. Mild heating to 50°C was a further requirement in order to obtain the solvation of the two higher boiling-point products and the composite materials. Sonication and vortexing were also performed on the samples in order to obtain homogeneous mixtures. The test substances were dissolved to their upper solubility end-point (usually 100 mg/ml) and diluted with DMSO.

Tween 80 solvation

The mild gasification materials were prepared for mutagenicity testing using methodologies similar to those described by Ma et al. (1983). Approximately 200 mg of each sample was placed in a sterile vial (20-ml capacity) and 400 mg of Tween 80 was added. This mixture was warmed to 50°C and sonicated in a Branson model 3200 water bath/sonicator for 30 min in order to facilitate homogenization. Triple distilled, deionized water (5 ml) was then added dropwise and the mixture warmed, sonicated, and vortexed until a homogeneous emulsion was obtained. Triple distilled, deionized water was then added to the emulsified sample until a final sample concentration of 20 mg/ml (4% Tween 80) was reached. Lower concentrations of products utilized in testing were obtained by serial dilution of the original 20 mg/ml emulsion in sterile, 4% Tween 80. The solid material, MG-122 (720°F⁺), required further sonication using a probe type sonicator (Heat Systems XL at a power setting of 10–15% for 3 min) in order to break up the small particles present in this sample. The positive controls and the reference material were prepared in a similar manner as the coal-derived compounds in order to ensure consistent experimental design.

Phase separation

Simple phase separation of the MG-120 composite material was accomplished by cooling this liquid/tar blend to 3°C and allowing the lighter liquid phase to partition from the heavier tar phase. The liquid phase was then carefully decanted and saved, and the remaining tars were allowed to dry overnight in a vented chemical safety hood prior to their collection.

Mutagenicity assay

Initial testing of the mild gasification products used the standard Salmonella plate-incorporation assay with DMSO as the sample solvent (Ames et al., 1975). These assays failed to demonstrate positive mutagenic activity for the initial samples; thus further testing utilized the often more sensitive pre-incubation modification of the Salmonella assay (Yagahi et al., 1975) with DMSO and Tween 80 as dual sample solvents. In order to achieve homogenization, each test tube was vortexed prior to and after the 30-min period of pre-incubation used in these assays. The *Salmonella typhimurium* strains TA98 and TA100 were obtained from Dr. Bruce N. Ames of the University of California, Berkeley, and the *O*-acetyltransferase over-producing derivative of TA98, YG1024 (pYG219), was obtained from Dr. Takehiko Nohmi of the National Institute of Hygienic Sciences, Tokyo, Japan. Overnight cultures of TA98 and TA100 were grown in Oxoid Nutrient Broth No. 2 in the presence of 25 µg/ml ampicillin, and YG1024 was grown under similar conditions but with both ampicillin and 6.25 µg/ml of tetracycline in the media. The criteria used to determine a positive mutagenic response for the test materials included: (1) a doubling of the number of revertants detected above those of the solvent control plates, and (2) an accompanying dose-related response in two or more of the concentrations examined. The assays were repeated at least once in order to confirm the reproducibility of the results, and adjustments were made in the concentrations of samples examined in subsequent tests. Because the growth of the YG1024 strain is slower than the conventional Ames strains, the plates containing this bacteria were incubated 72 h instead of the 48 h used for TA98 and TA100 in order to reach comparable colony sizes as

TABLE 1
RESULTS OF THE SALMONELLA ASSAY FOR DMSO-SOLVATED MILD GASIFICATION PRODUCTS

Sample	Conc. ($\mu\text{g}/$ plate)	Average number of revertant colonies per plate ^a				Sample	Conc. ($\mu\text{g}/$ plate)	Average number of revertant colonies per plate ^a			
		TA98		TA100				TA98		TA100	
		-S9	+S9	-S9	+S9			-S9	+S9	-S9	+S9
MG-122 IBP-420°F	0 ^b	19 ± 4	31 ± 4	85 ± 27	77 ± 1	MG-120	0 ^b	38 ± 7	55 ± 11	74 ± 9	74 ± 11
	25	23 ± 6	35 ± 5	88 ± 11	91 ± 13	Composite	12.5	37 ± 9	-	87 ± 2	-
	50	19 ± 3	32 ± 13	94 ± 1	89 ± 25		25	36 ± 7	-	94 ± 3	-
	100	16 ± 5	31 ± 6	85 ± 9	73 ± 21		50	41 ± 17	84 ± 10	92 ± 15	90 ± 5
	200	19 ± 11	32 ± 16	75 ± 20	69 ± 11		100	34 ± 2	67 ± 2	84 ± 5	116 ± 8
MG-122 420-720°F	0 ^b	19 ± 4	31 ± 4	85 ± 27	77 ± 1		200	-	71 ± 8	-	112 ± 12
	6.2	22 ± 9	-	84 ± 18	-		400	-	50 ± 7	-	105 ± 11
	12.5	19 ± 7	-	91 ± 4	90 ± 1	Shell	0 ^b	19 ± 4	31 ± 4	85 ± 27	77 ± 1
	25	22 ± 4	20 ± 2	95 ± 4	93 ± 11	No. 830331	25	18 ± 5	44 ± 7	87 ± 18	109 ± 11
	50	25 ± 12	26 ± 1	62 ± 0	104 ± 21		50	19 ± 2	40 ± 2	82 ± 3	124 ± 14
MG-122 720°F+	100	-	35 ± 4	-	113 ± 10		100	20 ± 1	41 ± 9	79 ± 1	118 ± 8
	200	-	26 ± 4	-	-		200	19 ± 3	38 ± 10	65 ± 28	114 ± 1
	0 ^b	38 ± 7	55 ± 11	74 ± 9	74 ± 11	CTC No. 11	0 ^b	22 ± 4	35 ± 5	101 ± 15	86 ± 16
	12.5	-	-	94 ± 5	-	Composite	6.2	30 ± 2	-	171 ± 26	-
	25	39 ± 4	-	100 ± 20	-		12.5	35 ± 2	365 ± 53 *	183 ± 10	328 ± 39 *
MG-119 Composite	50	39 ± 5	65 ± 5	96 ± 6	117 ± 16		25	75 ± 6 *	679 ± 154 *	239 ± 24 *	321 ± 62 *
	100	44 ± 10	62 ± 6	91 ± 5	113 ± 15		50	64 ± 7 *	787 ± 87 *	218 ± 17 *	363 ± 72 *
	200	50 ± 4	59 ± 6	-	122 ± 9		100	-	1289 ± 21 *	-	350 ± 6 *
	400	-	44 ± 5	-	88 ± 9 ^c						
	0 ^b	38 ± 7	55 ± 11	74 ± 9	74 ± 11	SRC-II HD ^d	0 ^b	38 ± 7	55 ± 11	74 ± 9	74 ± 11
MG-119 Composite	12.5	-	-	69 ± 11	-		31.2	33 ± 4	-	84 ± 11	-
	25	38 ± 10	-	87 ± 8	-		62.5	34 ± 9	-	71 ± 8	-
	50	42 ± 3	61 ± 14	84 ± 9	71 ± 16		125	-	260 ± 84 *	-	156 ± 26 *
	100	37 ± 3	62 ± 3	67 ± 14	82 ± 21		250	-	407 ± 34 *	-	193 ± 17 *
	200	10 ± 2 ^c	59 ± 2	-	79 ± 13	2AA ^d	0 ^b	38 ± 7	55 ± 11	74 ± 9	74 ± 11
400	-	41 ± 9	-	72 ± 3		2.5	39 ± 7	879 ± 113 *	96 ± 3	1072 ± 39 *	

^a Each datum reflects the mean of three plates.

^b DMSO solvent control.

^c Cytotoxic.

^d SRC-II HD is a coal-derived reference material; 2AA (2-aminoanthracene) is the positive control.

-, not tested.

* Positive response.

TABLE 2
RESULTS OF THE SALMONELLA ASSAY FOR TWEEN 80-SOLVATED MILD GASIFICATION PRODUCTS

Sample	Conc. ($\mu\text{g}/$ plate)	Average number of revertant colonies per plate ^a				Sample	Conc. ($\mu\text{g}/$ plate)	Average number of revertant colonies per plate ^a			
		TA98		TA100				TA98		TA100	
		-S9	+S9	-S9	+S9			-S9	+S9	-S9	+S9
MG-122 IBP-420°F	0 ^b	32 ± 3	26 ± 7	100 ± 11	104 ± 9	MG-120	0 ^b	32 ± 3	26 ± 7	100 ± 11	104 ± 9
	250	33 ± 5	32 ± 6	126 ± 13	115 ± 18	Composite	62.5	25 ± 5	-	-	-
	500	36 ± 6	34 ± 8	134 ± 9	111 ± 7		125	24 ± 5	-	-	-
	1000	30 ± 1	51 ± 3	106 ± 25	113 ± 17	250	27 ± 4	55 ± 12 *	123 ± 15	154 ± 4	
	2000	35 ± 6 ^c	39 ± 7 ^c	63 ± 51 ^c	135 ± 68 ^c	500	29 ± 1	61 ± 9 *	135 ± 26	183 ± 9	
MG-122 420-720°F	0 ^b	32 ± 3	26 ± 7	100 ± 11	104 ± 9	1000	-	91 ± 5 *	81 ± 36	91 ± 6	
	250	29 ± 7	45 ± 4	144 ± 16	124 ± 17	2000	-	103 ± 12 *	46 ± 2 ^c	173 ± 37	
	500	24 ± 11	49 ± 8	121 ± 3	150 ± 7	Shell No. 830331	0 ^b	32 ± 3	26 ± 7	100 ± 11	104 ± 9
	1000	18 ± 9 ^c	35 ± 8	71 ± 13 ^c	123 ± 17		250	33 ± 10	51 ± 8	119 ± 4	125 ± 13
	2000	- ^c	25 ± 8	- ^c	59 ± 3 ^c	500	45 ± 2	46 ± 7	111 ± 15	140 ± 3	
MG-122 720°F ⁺	0 ^b	32 ± 3	26 ± 7	100 ± 11	104 ± 9	1000	45 ± 7	51 ± 7	125 ± 10	113 ± 29	
	250	37 ± 7	39 ± 7	115 ± 10	101 ± 12	2000	28 ± 3 ^c	49 ± 13	105 ± 20	117 ± 13	
	500	26 ± 4	39 ± 9	148 ± 8	118 ± 16	CTC No. 11 Composite	0 ^b	23 ± 12	25 ± 8	98 ± 3	83 ± 9
	1000	44 ± 17	34 ± 12	126 ± 10	104 ± 12		12.5	34 ± 11	141 ± 13 *	-	-
	2000	62 ± 10 ^c	50 ± 4 ^c	131 ± 16	126 ± 11	25	39 ± 4	270 ± 59 *	184 ± 13	172 ± 30 *	
MG-119 Composite	0 ^b	32 ± 3	26 ± 7	100 ± 11	104 ± 9	50	58 ± 9 *	443 ± 98 *	225 ± 20 *	234 ± 28 *	
	62.5	17 ± 1	-	-	-	100	60 ± 12 *	589 ± 170 *	254 ± 28 *	252 ± 25 *	
	125	21 ± 6	-	-	-	200	-	-	244 ± 14	304 ± 45 *	
	250	29 ± 1	48 ± 12	156 ± 31	173 ± 37	SRC-II HD ^d	0 ^b	32 ± 3	26 ± 7	100 ± 11	104 ± 9
	500	23 ± 4	61 ± 14 *	152 ± 21	191 ± 18		125	36 ± 5	192 ± 12 *	127 ± 5	207 ± 51
1000	-	73 ± 14 *	114 ± 13	176 ± 59	2AAA ^d	0 ^b	32 ± 3	26 ± 7	100 ± 11	104 ± 9	
2000	-	94 ± 18 *	64 ± 13 ^c	156 ± 14	2.5	31 ± 3	980 ± 208 *	117 ± 13	1464 ± 59 *		

^a Each datum reflects the mean of three plates.

^b Tween 80 solvent control.

^c Cytotoxic.

^d SRC-II HD is a coal-derived reference material; 2AA (2-aminoanthracene) is the positive control.

-, not tested.

* Positive response.

recommended by Dr. Nohmi (personal communication).

Results

Data from the Salmonella pre-incubation testing of the 7 mild gasification products utilizing the 2 solvent systems are summarized in Tables 1 and 2. When DMSO was used as the sample solvent, the highest concentrations testable without considerable cytotoxicity were in the range of 100–400 $\mu\text{g}/\text{plate}$ (Table 1). Considerably higher

concentrations (up to 2000 $\mu\text{g}/\text{plate}$) of test materials could be examined when solvated in Tween 80, with the exception of CTC No. 11 (Table 2). Only the CTC No. 11 mild gasification material showed a positive response on the conventional Salmonella tester strains when solvated and tested in DMSO (Table 1), although some dose-related trends in the data were present throughout testing with this solvent system for the other 6 products. The CTC No. 11 sample elicited moderate amounts of mutagenic activity on TA98 and TA100, both in the presence and absence of the

TABLE 3

COMPARATIVE SALMONELLA ASSAY ACTIVITY OF THE MILD GASIFICATION COMPOSITE MATERIALS IN TWEEN 80 AND DMSO

Sample	Concentration ($\mu\text{g}/\text{plate}$)	Average number of revertant colonies per plate ^a			
		TA98 (+S9)		YG1024 (+S9)	
		Tween 80	DMSO	Tween 80	DMSO
MG-119	0 ^b	18 ± 2	24 ± 2	50 ± 0	110 ± 4
Composite	15.6	35 ± 6	25 ± 1	168 ± 12 *	127 ± 15
	62.5	39 ± 7 *	28 ± 2	269 ± 10 *	131 ± 6
	250	42 ± 13 *	28 ± 3	287 ± 6 *	156 ± 23
	1000	57 ± 8 *	35 ± 2 ^c	251 ± 9 *	292 ± 38 ^{*c}
MG-120	0 ^b	18 ± 2	24 ± 2	50 ± 0	110 ± 4
Composite	15.6	34 ± 7	25 ± 3	214 ± 6 *	108 ± 13
	62.5	41 ± 23 *	28 ± 3	315 ± 41 *	111 ± 5
	250	52 ± 12 *	31 ± 2	340 ± 19 *	114 ± 9
	1000	62 ± 7 *	42 ± 6 ^c	379 ± 29 *	272 ± 40 ^{*c}
CTC No. 11	0 ^b	32 ± 6	36 ± 7	75 ± 11	123 ± 2
Composite	0.7	31 ± 3	43 ± 7	108 ± 27	237 ± 14
	3.1	53 ± 8	57 ± 10	284 ± 50 *	492 ± 19 *
	12.5	243 ± 46 *	511 ± 104 *	1441 ± 333 *	2486 ± 374 *
	50	593 ± 98 *	1202 ± 50 *	2594 ± 354 *	2341 ± 149 *
SRC-II HD ^d	0 ^b	18 ± 2	24 ± 2	50 ± 0	110 ± 4
Composite	15.6	139 ± 11 *	115 ± 11 *	988 ± 183 *	1501 ± 21 *
	31.2	148 ± 6 *	151 ± 14 *	1252 ± 205 *	1975 ± 91 *
	62.5	207 ± 7 *	209 ± 6 *	1792 ± 137 *	2134 ± 198 *
	2AA ^d	0 ^b	18 ± 2	24 ± 2	50 ± 0
Composite	0.07	22 ± 7	57 ± 5 *	145 ± 7 *	1417 ± 179 *
	0.25	58 ± 11 *	162 ± 17 *	1713 ± 60 *	1885 ± 42 *
	0.70	329 ± 44 *	337 ± 30 *	– ^c	– ^c
	2.50	1554 ± 81 *	920 ± 10 *	– ^c	– ^c

^a Each datum reflects the mean of three plates.

^b Tween 80 and DMSO solvent controls.

^c Cytotoxic.

^d SRC-II HD is a coal-derived reference material; 2AA (2-aminoanthracene) is the positive control.

–, not tested.

* Positive response.

S9 microsomal fraction. Using DMSO solvation, CTC No. 11 elicited the greatest mutagenic activity in tester strain TA98 with S9.

All three composite materials (MG-119, MG-120 and CTC No. 11) had positive activity when assayed using the Tween 80 solvent system (Table 2). In agreement with the DMSO-solvated testing, no activity was detected in the other 4 mild gasification samples when dissolved and assayed in Tween 80. The mutagenic activity of composites MG-119 and MG-120 in this solvent system was low but significant, and restricted to tester strain TA98 in the presence of the S9 microsomal fraction. The CTC No. 11 product again showed positive activity on both tester strains with and without the S9. However, the activity of CTC No. 11 in the presence of the S9 fraction was considerably lower when assayed and dissolved in Tween 80 as opposed to DMSO. Thus, the mutagenicity of the CTC No. 11 material was greatest on TA98 in the presence of the S9 fraction, when DMSO was used as the sample solvent. As expected, the SRC-II HD coal-liquefaction reference material and the positive control, 2AA, showed obvious mutagenic activities in both solvent systems in the presence of the S9 microsomal fraction. Each demonstrated similar mutagenic responses independent of the solvent utilized.

The results of the comparative study of the three composite materials assayed in the dual solvents on both TA98 and YG1024 with S9 are shown in Table 3. As in the earlier studies utilizing TA98 (Tables 1 and 2), MG-119 and MG-120 showed low amounts of mutagenic activity when solvated and assayed in Tween 80 but no activity in DMSO. These samples also demonstrated similar, weak activities at each concentration tested on YG1024 when solvated in Tween 80; however, only the highest concentration was active in the DMSO-solvated materials assayed on this tester strain. The mutagenicity of these products was up to 5–10-fold higher on YG1024 than on TA98. As noted previously, sample CTC No. 11 was more mutagenic on TA98 when dissolved in DMSO as opposed to Tween 80. This material showed a similar pattern of higher activity for the varying DMSO-solvated concentrations plated on YG1024, except for the highest concentration tested where the activities were nearly equivalent.

Like the MG-119 and MG-120 composite materials, greater mutagenic activity was observed for CTC No. 11 on tester strain YG1024 than on TA98.

The SRC-II reference material showed similar responses with each of the two solvents when tested on TA98 (Tables 1 and 2); however, slightly higher amounts of activity were demonstrated on YG1024 when DMSO was used as the sample solvent. As with the mild gasification composite products, the mutagenic activity was much greater on tester strain YG1024 as compared to TA98 for this complex mixture. The positive control, 2AA (an aromatic amine) was tested at several concentrations in the two solvents and yielded concentration-dependent data trends. This chemical showed higher responses on both strains for the two lowest concentrations utilized when dissolved in DMSO, but the trend evened and finally reversed at the highest concentration plated on TA98. As expected for an aromatic amine, 2AA was more mutagenic on strain YG1024 than on

TABLE 4
RESULTS OF THE SALMONELLA ASSAY FOR MG-120 COMPOSITE AND PHASE-SEPARATED MATERIALS

Sample per plate ^a (+ S9)	Concentration (μg/plate)	Average number of revertant colonies	
		TA98	YG1024
MG-120 (Whole sample)	0 ^b	24 ± 2	110 ± 4
	31.2	25 ± 2	111 ± 4
	62.5	28 ± 3	111 ± 5
	125	24 ± 6	109 ± 7
MG-120 (Liquid phase)	0 ^b	24 ± 2	110 ± 4
	31.2	31 ± 10	122 ± 34
	62.5	28 ± 5	108 ± 5
	125	29 ± 3	113 ± 25
MG-120 (Tar phase)	0 ^b	24 ± 2	110 ± 4
	31.2	45 ± 9	460 ± 69 *
	62.5	63 ± 16 *	611 ± 37 *
	125	81 ± 16 *	715 ± 71 *
2AA ^c	0 ^b	24 ± 2	110 ± 4
	0.25	162 ± 17 *	1885 ± 42 *

^a Each datum reflects the mean of three plates.

^b DMSO solvent control.

^c 2AA (2-aminoanthracene) is the positive control.

* Positive response.

strain TA98. Assays using YG1024 had 10–40-fold higher numbers of revertants than assays using TA98, regardless of the solvent utilized.

The mutagenic activity of MG-120 was confined to the tar phase (Table 4). Both the liquid phase and the whole composite materials showed no activity when tested on TA98 or YG1024 using DMSO as the sample solvent; however, the tar phase of this composite product indicated positive mutagenic activity on both tester strains. Similar to the previous testing of the coal-derived reference material, the mild gasification samples, and the 2AA positive control, the tar phase showed greatly enhanced mutagenic activity on YG1024 when compared to the activity elicited on TA98.

Discussion

While the mutagenic/carcinogenic activity of coal-gasification materials appears similar to the activity of coal-liquefaction materials, adequate information to confirm this inference does not exist. An inadequate number of processes and a paucity of physical property information is present for the coal-gasification samples examined for toxicological effects (Craun and Fillo, 1988). However, the limited database does suggest that sample properties for coal-gasification materials relevant to their genotoxicity are similar to those for coal-liquefaction and hydrotreated products. In agreement with the findings of previous studies of coal-liquefaction materials, we have shown that the full-boiling-range (composite) mild gasification products MG-119, MG-120 and CTC No. 11 possessed mutagenic activity in the Salmonella assay on TA98 with S9. This activity indicated the presence of an indirect-acting frameshift mutagen in these complex mixtures. Since these samples were many times more mutagenic on the *O*-acetyltransferase over-producing strain YG1024 (with S9) than on TA98, the presence of aromatic amines was suggested (Table 3). In particular, the CTC No. 11 sample demonstrated the largest comparative (YG1024/TA98) fold increases of the three composite materials tested, and higher amounts of activity on YG1024 than any other test substance including the 2AA-positive control and the SRC-II HD reference material. Also

noted was the alterations in the trends of the data elicited for CTC No. 11, SRC-II HD and 2AA (especially on YG1024) in the two solvent vehicles at the highest concentrations utilized. These similarities may be indicative of the presence of a common class of mutagen in these test materials, or at least some common mode of solvent-dependent changes in the expressed mutagenic activity. In fact, aromatic amines have been shown to be prevalent indirect-acting chemical mutagens in many coal-derived materials including the SRC-II HD (Pelroy and Wilson, 1981). Thus, the biological detection of these agents in this study is not surprising. Preliminary studies using YG1024, without the S9 fraction, did not show any increases in the mutagenic responses above those of TA98 (data not shown). Hence, there is no evidence for the detection of nitroarenes in any of the composite samples. Additionally, sample CTC No. 11, the only overtly mutagenic product on both TA98 and YG1024, showed positive responses under all test conditions except for the lowest sample concentrations examined on TA100 in both solvent systems. Hence, the presence of low concentrations of a direct-acting chemical mutagen is also indicated for this material.

The lower boiling-point product, MG-122 (IBP-420°F), was not found to possess mutagenic activity in this assay. Since the boiling points of coal-conversion materials correlate with the molecular weight of their components, we believe the lack of high molecular weight compounds in this product contributed to the absence of detectable mutagenic activity.

Somewhat contrary to previous information on other coal-conversion materials, the high-boiling-point sample, MG-122 (420–720°F); the coal tar product, Shell No. 830331; and the residue material, MG-122 (720°F⁺) show no mutagenic activity. The lack of mutagenic activity in the residue material may result from secondary reactions occurring at high temperatures which degrade mutagenic PNAs into lower molecular weight, inactive, compounds. The lack of mutagenic activity in the high-boiling-point sample, the tar product, and the low mutagenicity of the composite materials MG-119 and MG-120 may be related to the less severe processing conditions of the mild gasi-

fication technique. In fact, the Shell product is described as a low-temperature coal tar, which implies milder processing conditions that may avoid the formation of significant amounts of mutagenic/carcinogenic PNAs. Furthermore, the MG-122 (420–720°F) sample was noted to contain a yellow, non-polar, wax-like substance, which may be significant in that lipophilic hydrocarbons can reduce the mutagenic activity elicited (Ma et al., 1983; Blackburn et al., 1986). It may be of interest to re-examine this material after dewaxing via cyclohexane/DMSO partitioning in order to explore this possibility. Obviously, other factors such as the coal stock utilized, the antagonistic effects of components present in the complex mixtures, and solvent-related effects may play a role, but the processing differences between the moderately mutagenic CTC No. 11 sample and the other less-to-non-mutagenic samples must be compared in order to detail their differences and to ensure the production of the safest coal-conversion materials.

The simplest explanation for the finding of significant amounts of mutagenic activity in the MG-119 and MG-120 composite products, when solvated and assayed in Tween 80 versus DMSO, is that initially higher doses of test materials were examined in Tween 80 (Tables 1 and 2). Indeed, since the lower molecular weight components in coal-conversion materials have been cited as having the greatest cytotoxic potential (Gray et al., 1988), there may be some interactions occurring between these substances and the Tween 80 emulsion which reduced their cytotoxicity, allowing generally higher concentrations of mild gasification materials to be examined with this solvent system. However, this explanation does not completely account for the solvent-related differences observed, since the comparative study (Table 3) which used matching, non-cytotoxic concentrations of these composite compounds showed low but significant amounts of mutagenic activities only in the Tween 80 solvent system when assayed on TA98 with S9. Differential sample permeabilities imparted by the two solvent systems may also explain the variable cytotoxicities observed and could, in part, be responsible for the varying amounts of mutagenic activity elicited. For example, DMSO may be a better solvent for

conducting the mutagenically active components of the CTC No. 11 product into the bacterial cells yielding higher sample responses than Tween 80, while the opposite situation may be true for MG-119 and MG-120.

Variations in the mutagenic responses of crude oils and coal-derived materials in different solvent systems have been noted previously (Hermann et al., 1980; Ma et al., 1983; Marino, 1987). Hermann et al. (1980) showed that Tween 80 gave more reliable results for the mutagenic activity of oils than DMSO in the Salmonella assay, when sample solubility for the latter solvent was a problem. Ma et al. (1983) found comparable mutagenic response values in 3 of 4 oils solvated and tested in the Salmonella assay in both the DMSO and Tween 80 systems. One product, coal oil CRM1 (a gasifier tar), showed higher responses when solvated and tested in DMSO as compared to Tween 80 — similar to our findings with the CTC No. 11 mild gasification sample. However, in the forementioned study, all of the materials submitted for DMSO-solvated testing were dewaxed by cyclohexane/DMSO partitioning, whereas whole, unfractionated samples were used in the Tween 80-solvated testing. Since DMSO has been shown to not only have antagonistic, but also synergistic effects on the assaying of test articles (Nestmann et al., 1985), and has been implicated in the formation of cytotoxic test substances (Vaughan et al., 1989), the genotoxicity data from DMSO-solvated materials must be interpreted with caution. DMSO is a potent antioxidant which can quench free-radicals *in vitro* (Hrelia et al., 1990) reducing levels of demonstrable mutagenic activity. This is particularly important for coal-gasification products since they generally contain more oxidative products than coal-liquefaction materials (Wilson et al., 1988). In addition, DMSO and other organic solvents have been shown to interfere with microsomal enzymes (Wolff, 1977). Since the DMSO concentration is higher during the period of pre-incubation (prior to plating), the antagonistic interaction of DMSO with metabolic enzymes is quite plausible for this study and others using the pre-incubation Ames test variant. In our study, the lowered number of revertants yielded as the DMSO-solvated, 2AA concentration increased may be

reflective of a decreasing amount of metabolic enzyme activity (Table 3), and may be applicable to the solvent-related observations for CTC No. 11 and the SRC-II HD. Recent investigations by Ayrton et al. (1992) show that the enzymes of the hepatic cytosol are responsible for the activation of 2AA and similar aromatic amines, whose presence is suggested in the mild gasification samples by the activity expressed on YG1024. These enzymes may be particularly sensitive to organic solvents such as DMSO, since they are not protected by localization in the cell membrane.

The use of Tween 80 as a sample solvent is technically complex. The preparation of the emulsion for the materials at their initial, high concentration is more involved than the simple dissolution of these compounds in DMSO. Frequent vortexing of the sample/assay tubes is often needed to maintain a homogeneous suspension prior to plating and can cause vesicle formation, producing small bubbles in the overlays of the assay plates. In most of the previous mutagenicity studies of coal-conversion products and other materials, DMSO is the solvent vehicle, complicating the comparison of results when Tween 80 is the sole solvent. Hence, neither Tween 80 nor DMSO appear to be ideal solvents for genotoxicity assays on these materials, and if time and funds permit, dual solvents are more desirable.

In addition to their direct effects on mutagenic activity, solvents may also be able to reduce chemical masking. PNA-enriched complex mixtures have been implicated, via a mechanical trapping effect, in the suppression of the mutagenicity of indirect-acting mutagens such as benzo(a)pyrene, 2-aminoanthracene, benzo[a]-anthracene, and 7,9-dimethylbenz[*c*]acridine (Petrilli et al., 1980). While Petrilli et al. (1980) were unable to relieve the mechanical trapping of benzo[a]pyrene by crude oil PNAs with oil dispersants, the presence of the masking or mechanical trapping of chemical mutagens in complex mixtures such as the mild gasification samples must be considered. In a study of 42 coal-liquid materials analyzed in the Salmonella assay, there were 7 cases which gave no detectable response on unfractionated samples, but the testing of chemically fractionated examples of these com-

pounds indicated a positive weighted-sum response (Fillo and Craun, 1985).

The detection of mutagenic activity in only the tar portion of the MG-120 composite material is in agreement with the findings of Warshawsky (1979) for products from the Chapman low-Btu gasifier at the Holston Munition Plant in Tennessee. Also, Schoeny et al. (1981) showed positive mutagenic activity for products from a pilot plant fixed-bed, air-blown gasifier. The separated tar material for MG-120 demonstrated higher numbers of revertants than previously detected in the whole, unpartitioned sample for TA98 and especially YG1024. Again, the fold differences in the activities expressed between strains suggested the presence of aromatic amines in this composite material. Since the partitioning study (Table 4) was more qualitative than quantitative, the absence of activity in the whole sample versus the partitioned tar cannot be related. However, these results further indicate the greater mutagenicity of higher molecular weight components and especially aromatic amines in the genotoxicity of gasification materials.

Acknowledgements

This study was performed and funded under an interagency agreement between NIOSH and METC. We wish to gratefully acknowledge E. Dawn Bryant for providing technical support, and Richard Johnson of the Morgantown Energy Technology Center for providing the samples used in this study.

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