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Qualitative Assessment of the Mutagenicity of Road Coating Asphalt

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Asphalt, a thick viscous residue formed by the distillation of petroleum, is primarily composed of oily materials, resins, asphaltenes, carbenes and carboids (Zakar 1971). Carcinogenic polycyclic aromatic hydrocarbons (PAH) such as anthanthene, benzo(a)pyrene and indeno(1,2,3-cd)pyrene have also been found in asphalt in concentrations ranging up to 4,000 ppm. The concentration of these PAHs depends upon the source of the asphalt and its manufacturing process (Wallcave et al. 1971). In view of the widespread use of asphalt (27 million tons in 1975, Sittig, 1979), the potential health problems due to asphalt exposure are poorly understood and should be investigated. Due to the expense and time required for animal experiments, this study utilized a widely accepted mutagenicity test, the Salmonella/microsomal assay, to make a preliminary determination of the mutagenic activity of the extracts of whole petroleum asphalts used in the road paving process.

Using the Salmonella/microsomal assay, Monarca et al. (1987) were unable to detect mutagenic activity in the asphaltene fraction of asphalt samples collected during road-paving operations. Examination of the asphaltene fraction, as well as samples of urine from exposed workers showed no increase in mutation rates. In the above study sample testing was performed on the basis of PAH content (137.2 - 218.1 ug/g) and information on the actual asphalt concentration of the sample was not given.

Relatively few studies have reported on the carcinogenic or mutagenic aspects of asphalt exposure. In 1959, Simmers et al. applied an unspecified concentration of pooled asphalt dissolved in benzene to the interscapular region of C57 black mice. The solution was applied twice weekly for the life of the mouse. Twelve epidermoidal carcinomas were found in 68 experimental animals compared to no skin tumors in the benzene-treated controls. Additional animal studies (Simmers 1965a, 1965b and Hueper and Payne 1960) have suggested that cancer can result from asphalt exposure, but these early reports do not account for experimental variables such as the carcinogenic potential or

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synergistic effects of the applications of hot asphalt. A dose-response relationship can not be ascertained from these studies due to the lack of information on the actual dose absorbed by the animals.

A review of the literature fails to provide clear evidence of increased genotoxic risk associated with human exposure to asphalt. Epidemiological studies are difficult to interpret due to employee exposure to other known carcinogenic substances such as coal tar, roofing tar and petroleum based products. Among the few reports to specifically address asphalt, Mommsen and Agard (1984) discovered a significant increase in bladder cancer among men who were occupationally exposed. In a review of the carcinogenic risk posed by asphaltic bitumens, the International Agency for Research on Cancer (IARC) (1985) concluded that insufficient evidence existed to declare these compounds to be carcinogens or suspected carcinogens. No data were available on such basic aspects as the absorption, distribution, excretion and metabolism of asphalt in laboratory animals or humans. Data were also unavailable on the reproductive and prenatal effects of asphalt exposure.

MATERIALS AND METHODS

Five whole asphalt samples were obtained from actual road-paving operations in Oklahoma. These samples were taken prior to mixing with the gravel aggregate to eliminate the necessity of extracting the asphalt from the road gravel matrix. Extractions were performed according to the method of Penalva et al. (1983). Briefly the asphalt was warmed to approximately 60°C to allow a less viscous flow. Samples were then placed into a pre-weighed flask and extractant added to yield a concentration of 200 mg asphalt/mL of extractant. Two extractants were used for each asphalt sample, thus giving a total of 10 extractions (2 per asphalt sample). The first extractant, dimethyl sulfoxide (DMSO), is a nonpolar apoitic solvent that gives a negative response in the Salmonella/microsomal assay and is used to extract polycyclic organic material (Natusch and Tomkins 1978). DMSO has the ability to penetrate the asphaltic material to a greater extent than other non-mutagenic organic solvents. The second extractant was distilled water adjusted to pH of 5.0. This extraction was performed to simulate roadway leachate after a rainfall. Following addition of the extractant, the samples were shaken for 18 hours prior to analysis.

The Salmonella/microsomal assay (Maron & Ames 1983) was used to determine the mutagenic activity of both extracts. Strains TA98 and TA100 were tested at 0.05, 0.1,0.5, 1.0, 5.0 and 10 mg of extract per plate with and without microsomal activation. S-9 was purchased from Organon Teckniks, Dallas Texas (0.1 mL rate liver enzyme/mL S-9). Positive controls consisted of benzo(a)pyrene (BaP) at 5 ug/plate and 2-nitrofluorene (2NF) at 25 ug/plate. Negative controls consisted of DMSO and distilled water with an adjusted pH

of 5.0. All samples were tested on duplicated plates in two independent experiments.

RESULTS AND DISCUSSION

Table 1 shows the results of the DMSO extracted samples. Data are given for both TA98 and TA100 strains tested with and without metabolic activation. A weak mutagenic response occurred for Sample 1 at 5 and 10 mg DMSO extract per plate in strain TA100 with metabolic activation. A positive response was defined as a doubling of the number of revertants over a minimum of two dose levels (Chu et al. 1981). the water extract from Sample 10 induced a doubling of revertant colonies in strain TA98 with metabolic activation but only at the highest dose (Table 2). It should be noted that although only Sample 1 induced a clearly recognizable mutagenic response, both DMSO and water extracts produced a slight increase in the number of revertants over that of the controls in TA100 with metabolic activation.

Our results demonstrated that asphalt has the potential to induce a detectable mutagenic response in the Salmonella/microsomal assay. Considering the extremely variable chemical composition of petroleum asphalt, it is not surprising that only one out of the five asphalt samples tested was positive. The DMSO extracts of all asphalt samples produced a slight increase in the number of revertants in strain TA100 with metabolic activation. This response was not of sufficient magnitude to be considered positive in the Salmonella/microsomal assay using the criteria of Chu et al. (1981).

Previous studies conducted on bitumens are difficult to interpret due to the nomenclature used, which prohibits differentiation between experiments using petroleum, coal, or tar sand products. Penalva et al. (1983) used the Salmonella assay to ascertain the mutagenicity of road coating tar. Although their results were positive, road coating tar is coal-derived and cannot be directly compared to petroleum-derived products. Monarca et al. (1987) examined the asphaltene fraction of asphalt in the Salmonella assay and found it to be negative. If the asphaltene fraction is not mutagenic then it can be hypothesized that either the resin or oil fractions of asphalt are responsible for the increased number of revertants in the DMSO extracts.

The absence of a strong mutagenic response produced by the water extracts of asphalt my be due to the presences of oils. The literature does suggest that the presence of oil or similar substances produces an inhibitory effect on carcinogens in the Salmonella assay. The negative results obtained from the DMSO extractions may be a result of the inhibitory effect.

The results of our study indicate the need for further analysis of the ability of road coating asphalts to produce mutations. Research in this area should include a wider variety of asphalts and detailed chemical analysis of each

sample in order to correlate asphalt composition with mutagenicity. In addition, it would be valuable to investigate extraction with alternate solvents and possibly HPLC fraction to isolate the various components of this complex mixture.

Table 1. Mutagenic activity of asphalt DMSO extracts

		Total Number of Revertants			
		TA 98		TA 100	
		-S9	+S9	-S9	+S9
Sample	mg/plate	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.
1	0.05	15 ± 1.41	29 ± 3.54	85 ± 12.73	106 ± 14.85
	0.10	21 ± 4.95	30 ± 8.49	114 ± 11.31	105 ± 3.44
	0.50	24 ± 7.37	34 ± 1.29	81 ± 5.56	120 ± 5.06
	1.00	22 ± 6.19	29 ± 6.46	98 ± 14.39	151 ± 27.54
	5.00	30 ± 4.95	34 ± 13.44	94 ± 0.71	226 ± 0.71
	10.00	40 ± 4.24	35 ± 2.12	101 ± 6.36	230 ± 4.24
4	0.05	19 ± 4.95	25 ± 10.61	101 ± 6.36	142 ± 7.07
	0.10	26 ± 2.12	22 ± 6.36	95 ± 15.56	139 ± 6.36
	0.50	21 ± 4.43	30 ± 3.51	97 ± 5.69	121 ± 25.84
	1.00	30 ± 4.50	35 ± 9.64	95 ± 9.22	126 ± 16.79
	5.00	25 ± 2.12	31 ± 6.36	100 ± 5.66	117 ± 0.71
	10.00	19 ± 4.24	37 ± 14.85	84 ± 12.02	140 ± 5.66
5	0.05	16 ± 0.71	32 ± 7.78	86 ± 0.71	135 ± 11.31
	0.10	25 ± 3.54	34 ± 2.12	90 ± 23.33	135 ± 41.72
	0.50	27 ± 8.88	25 ± 1.41	91 ± 13.43	115 ± 9.54
	1.00	24 ± 1.50	35 ± 9.13	76 ± 14.03	120 ± 12.78
	5.00	16 ± 0.71	30 ± 3.54	90 ± 7.07	120 ± 6.36
	10.00	27 ± 0.71		84 ± 12.73	119 ± 9.86
10	0.05	23 ± 0.71	31 ± 7.07	71 ± 5.66	135 ± 0.00
	0.10	20 ± 7.07	34 ± 0.71	89 ± 5.66	121 ± 19.09
	0.50	21 ± 4.99	46 ± 11.62	84 ± 6.16	130 ± 35.68
	1.00	22 ± 4.57	43 ± 18.40	90 ± 18.71	122 ± 4.96
	5.00	20 ± 8.49	51 ± 7.07	71 ± 7.07	126 ± 0.71
	10.00	16 ± 1.41	56 ± 11.31	87 ± 2.12	130 ± 5.65
12	0.05	24 ± 0.00	35 ± 3.54	76 ± 2.12	145 ± 26.87
	0.10	24 ± 2.83	25 ± 3.54	84 ± 2.83	156 ± 15.56
	0.50	23 ± 4.66	31 ± 3.59	92 ± 12.34	133 ± 20.89
	1.00	23 ± 4.08	30 ± 5.72	82 ± 22.46	134 ± 14.84
	5.00	26 ± 2.12	29 ± 6.36	101 ± 9.19	117 ± 13.44
	10.00	24 ± 2.83	31 ± 9.19	76 ± 0.71	120 ± 3.54
Control		30 ± 4.17	30 ± 5.67	98 ± 24.00	80 ± 9.36
BAP		238 ± 5.66	45 ± 12.75	60 ± 5.66	173 ± 68.93
2NF		2248 ± 162.0		755 ± 13.44	

Table 2. Mutagenic activity of asphalt water extracts

		Total Number of Revertants			
		TA 98		TA 100	
		-S9	+S9	-S9	+S9
Sample	mg/plate	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.
1	0.05	26 ± 0.00	30 ± 4.95	79 ± 8.49	135 ± 12.02
	0.01	26 ± 14.10	31 ± 6.36	96 ± 12.02	131 ± 4.95
	0.50	23 ± 4.32	33 ± 1.53	95 ± 28.15	113 ± 36.32
	1.00	22 ± 3.56	37 ± 9.54	92 ± 16.16	110 ± 25.98
	5.00	31 ± 7.07	51 ± 8.49	82 ± 7.78	85 ± 13.44
	10.00	27 ± 0.71	44 ± 0.71	77 ± 0.71	85 ± 13.44
4	0.05	20 ± 7.78	30 ± 4.95	109 ± 9.19	168 ± 8.49
	0.10	21 ± 5.07	33 ± 1.53	105 ± 3.54	126 ± 14.85
	0.50	22 ± 4.24	37 ± 9.53	109 ± 23.57	115 ± 31.31
	1.00	20 ± 6.36	51 ± 8.49	104 ± 30.69	107 ± 16.37
	5.00	30 ± 3.54	44 ± 0.71	92 ± 21.21	85 ± 2.83
	10.00			126 ± 0.71	101 ± 7.07
5	0.05	20 ± 6.36	36 ± 0.71	126 ± 12.73	137 ± 14.85
	0.10	20 ± 7.07	34 ± 2.12	102 ± 7.07	139 ± 4.24
	0.50	23 ± 5.26	30 ± 5.89	99 ± 12.29	120 ± 45.59
	1.00	21 ± 4.44	35 ± 8.60	87 ± 16.75	114 ± 38.19
	5.00	20 ± 6.36	51 ± 8.49	84 ± 12.02	101 ± 4.95
	10.00	30 ± 3.54	44 ± 0.71	91 ± 4.95	101 ± 5.66
10	0.05	21 ± 7.10		70 ± 7.78	135 ± 3.54
	0.10	28 ± 6.36	29 ± 4.24	65 ± 13.44	120 ± 4.95
	0.50	27 ± 4.50	31 ± 3.59	83 ± 16.69	127 ± 32.70
	1.00	21 ± 9.32	35 ± 7.81	97 ± 13.05	121 ± 26.40
	5.00	15 ± 0.00	41 ± 5.65	109 ± 20.51	119 ± 7.07
	10.00	20 ± 5.66	61 ± 4.95		116 ± 2.12
12	0.05	25 ± 2.83	34 ± 1.41	73 ± 1.41	146 ± 16.26
	0.10	21 ± 5.66	37 ± 0.71	99 ± 8.49	127 ± 0.71
	0.50	25 ± 1.50	36 ± 8.18	101 ± 21.47	112 ± 25.41
	1.00	24 ± 5.35	39 ± 5.19	99 ± 16.00	126 ± 22.23
	5.00	25 ± 0.71	40 ± 4.24	101 ± 9.19	94 ± 2.83
	10.00	27 ± 0.00	35 ± 13.44	107 ± 0.00	87 ± 13.44
Control		20 ± 4.17	30 ± 5.66	87 ± 11.70	97 ± 10.99

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