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Preliminary Toxicological Analysis of the Effect of Coal Slurry Impoundment Water on Human Liver Cells

By Joseph E. Bunnell

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Preliminary Toxicological Analysis of the Effect of Coal Slurry Impoundment Water on Human Liver Cells

By Joseph E. Bunnell[1]

Background and experiment

Coal is usually "washed" with water and a variety of chemicals to reduce its content of sulfur and mineral matter. The "washings" or "coal slurry" derived from this process is a viscous black liquid containing fine particles of coal, mineral matter, and other dissolved and particulate substances. Coal slurry may be stored in impoundments or in abandoned underground mines.

Human health and environmental effects potentially resulting from leakage of chemical substances from coal slurry into drinking water supplies or aquatic ecosystems have not been systematically examined. Impoundments are semipermeable, presenting the possibility that inorganic and organic substances, some of which may be toxic, may contaminate ground or surface water. The Agency for Toxic Substances and Disease Registry, part of the Centers for Disease Control and Prevention, has concluded that well water in Mingo County, West Virginia, constitutes a public health hazard (ATSDR, 2005). Residents of the Williamson area (Mingo County), especially children, have been subjected to chronic exposure to certain inorganic compounds from their private wells (ATSDR, 2005). These elements may have been mobilized to their well water from nearby coal extraction activities. The presence of and exposure to organic compounds, some of which are known genotoxins, carcinogens, and teratogens, that may also be mobilized from mining sites to ground water, were not, however, covered under the scope of the ATSDR report.

As a first step in examining the potential toxicity of coal slurry impoundment water to human liver tissue, a preliminary experiment using slurry impoundment water collected in 2006 from Mingo County, West Virginia, was conducted to determine its effect on HepG2 cells (ATCC, Manassas, VA). Low passage number cells were maintained and grown at 37° C and 5% CO_2, and experiments were conducted in 96-well plates. Twelve replicates of each slurry concentration (1%, 5%, and 10%) were included in the experiment, and 24 untreated controls were included in the analysis. The outer perimeter of wells was not treated or included in the analysis to minimize variability and provide thermal insulation for the assayed wells. Optical density measurements from the study for all treatments and controls are reported in Tables 1 and 2, for measurement at 570 nm and 630 nm, respectively. Concentration-dependent effects were observed, with 1%, 5%, and 10% exposures resulting in 13.5%, 14.0%, and 15.4% inhibition of cell viability, respectively, as compared to untreated controls using the AlamarBlue (Biosource International, Carlsbad, CA) oxidation-reduction colorimetric indicator. This cytotoxicity assay was selected because it has been demonstrated to have relatively low sensitivity with HepG2 cells, generating relatively conservative results (e.g., Jondeau and others, 2006).

[1] USGS Eastern Energy Resources Team, Reston, VA.

References Cited

Adeline Jondeau, Laurence Dahbi, Marie-Hélène Bani-Estivals and Marie-Christine Chagnon, 2006, Evaluation of the sensitivity of three sublethal cytotoxicity assays in human HepG2 cell line using water contaminants: Toxicology, v. 226, p. 218-228.

ATSDR, 2005, Health consultation: Private well water quality, Williamson WV sites (aka Williamson Area): Atlanta, Ga., Agency for Toxic Substances and Disease Registry, Public Health Service, U.S. Department of Health and Human Services, 31 p.

Table 1. HepG2 cell viability assay 4-13-07, optical density measurements @ 570 nm

[Rows B – G, Columns 4 and 5 were treated with 1.0 μL impoundment water; Rows B – G, Columns 6 and 7 were treated with 5.0 μL impoundment water; and Rows B – G, Columns 8 and 9 were treated with 10.0 μL impoundment water; all other wells were untreated]

	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8	Column 9	Column 10	Column 11	Column 12
Row A												
Sample ID	T1	T9	T17	T25	T33	T41	T49	T57	T65	T73	T81	T89
Position	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
Well Label	T1	T9	T17	T25	T33	T41	T49	T57	T65	T73	T81	T89
OD Results	0.653	0.609	0.593	0.585	0.583	0.582	0.575	0.56	0.569	0.563	0.56	0.59

	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8	Column 9	Column 10	Column 11	Column 12
Row B												
Sample ID	T2	T10	T18	T26	T34	T42	T50	T58	T66	T74	T82	T90
Position	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
Well Label	T2	T10	T18	T26	T34	T42	T50	T58	T66	T74	T82	T90
OD Results	0.665	0.581	0.546	0.531	0.529	0.537	0.512	0.481	0.463	0.491	0.515	0.553

	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8	Column 9	Column 10	Column 11	Column 12
Row C												
Sample ID	T3	T11	T19	T27	T35	T43	T51	T59	T67	T75	T83	T91
Position	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
Well Label	T3	T11	T19	T27	T35	T43	T51	T59	T67	T75	T83	T91
OD Results	0.658	0.542	0.54	0.523	0.528	0.51	0.523	0.522	0.517	0.522	0.533	0.601

	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8	Column 9	Column 10	Column 11	Column 12
Row D												
Sample ID	T4	T12	T20	T28	T36	T44	T52	T60	T68	T76	T84	T92

Position	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
Well Label	T4	T12	T20	T28	T36	T44	T52	T60	T68	T76	T84	T92
OD Results	0.603	0.533	0.53	0.517	0.522	0.528	0.516	0.515	0.521	0.524	0.528	0.587

Row E	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8	Column 9	Column 10	Column 11	Column 12
Sample ID	T5	T13	T21	T29	T37	T45	T53	T61	T69	T77	T85	T93
Position	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
Well Label	T5	T13	T21	T29	T37	T45	T53	T61	T69	T77	T85	T93
OD Results	0.637	0.535	0.53	0.534	0.517	0.526	0.528	0.535	0.523	0.528	0.546	0.605

Row F	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8	Column 9	Column 10	Column 11	Column 12
Sample ID	T6	T14	T22	T30	T38	T46	T54	T62	T70	T78	T86	T94
Position	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Well Label	T6	T14	T22	T30	T38	T46	T54	T62	T70	T78	T86	T94
OD Results	0.672	0.563	0.531	0.527	0.539	0.519	0.516	0.534	0.523	0.521	0.526	0.587

Row G	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8	Column 9	Column 10	Column 11	Column 12
Sample ID	T7	T15	T23	T31	T39	T47	T55	T63	T71	T79	T87	T95
Position	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
Well Label	T7	T15	T23	T31	T39	T47	T55	T63	T71	T79	T87	T95
OD Results	0.593	0.53	0.54	0.528	0.526	0.538	0.542	0.605	0.525	0.533	0.544	0.556

Row H	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8	Column 9	Column 10	Column 11	Column 12
Sample ID	T8	T16	T24	T32	T40	T48	T56	T64	T72	T80	T88	T96

Position	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12
Well Label	T8	T16	T24	T32	T40	T48	T56	T64	T72	T80	T88	T96
OD Results	0.581	0.599	0.598	0.605	0.595	0.582	0.599	0.593	0.578	0.589	0.599	0.599

Table 2. HepG2 cell viability assay 4-13-07, optical density measurements @ 630 nm

[Rows B – G, Columns 4 and 5 were treated with 1.0 μL impoundment water; Rows B – G, Columns 6 and 7 were treated with 5.0 μL impoundment water; and Rows B – G, Columns 8 and 9 were treated with 10.0 μL impoundment water; all other wells were untreated]

	Column	Column	Column	Column	Column	Column	Column	Column	Column	Column	Column	Column
Row A	1	2	3	4	5	6	7	8	9	10	11	12
Sample ID	T1	T9	T17	T25	T33	T41	T49	T57	T65	T73	T81	T89
Position	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
Well Label	T1	T9	T17	T25	T33	T41	T49	T57	T65	T73	T81	T89
OD Results	0.118	0.129	0.12	0.135	0.12	0.124	0.125	0.123	0.126	0.125	0.126	0.125

	Column	Column	Column	Column	Column	Column	Column	Column	Column	Column	Column	Column
Row B	1	2	3	4	5	6	7	8	9	10	11	12
Sample ID	T2	T10	T18	T26	T34	T42	T50	T58	T66	T74	T82	T90
Position	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
Well Label	T2	T10	T18	T26	T34	T42	T50	T58	T66	T74	T82	T90
OD Results	0.118	0.131	0.126	0.133	0.125	0.145	0.125	0.122	0.12	0.133	0.137	0.129

	Column	Column	Column	Column	Column	Column	Column	Column	Column	Column	Column	Column
Row C	1	2	3	4	5	6	7	8	9	10	11	12
Sample ID	T3	T11	T19	T27	T35	T43	T51	T59	T67	T75	T83	T91

Position	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
Well Label	T3	T11	T19	T27	T35	T43	T51	T59	T67	T75	T83	T91
OD Results	0.128	0.127	0.127	0.133	0.128	0.126	0.127	0.136	0.135	0.133	0.138	0.135

Row D	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8	Column 9	Column 10	Column 11	Column 12
Sample ID	T4	T12	T20	T28	T36	T44	T52	T60	T68	T76	T84	T92
Position	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
Well Label	T4	T12	T20	T28	T36	T44	T52	T60	T68	T76	T84	T92
OD Results	0.124	0.131	0.131	0.128	0.127	0.133	0.124	0.135	0.132	0.129	0.138	0.133

Row E	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8	Column 9	Column 10	Column 11	Column 12
Sample ID	T5	T13	T21	T29	T37	T45	T53	T61	T69	T77	T85	T93
Position	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
Well Label	T5	T13	T21	T29	T37	T45	T53	T61	T69	T77	T85	T93
OD Results	0.13	0.129	0.128	0.139	0.128	0.139	0.128	0.138	0.143	0.147	0.145	0.138

Row F	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8	Column 9	Column 10	Column 11	Column 12
Sample ID	T6	T14	T22	T30	T38	T46	T54	T62	T70	T78	T86	T94
Position	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Well Label	T6	T14	T22	T30	T38	T46	T54	T62	T70	T78	T86	T94
OD Results	0.126	0.138	0.129	0.132	0.132	0.131	0.125	0.136	0.141	0.14	0.15	0.134

Row G	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8	Column 9	Column 10	Column 11	Column 12
Sample ID	T7	T15	T23	T31	T39	T47	T55	T63	T71	T79	T87	T95

Position	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
Well Label	T7	T15	T23	T31	T39	T47	T55	T63	T71	T79	T87	T95
OD Results	0.126	0.129	0.126	0.132	0.122	0.131	0.129	0.159	0.143	0.136	0.16	0.136

Row H	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8	Column 9	Column 10	Column 11	Column 12
Sample ID	T8	T16	T24	T32	T40	T48	T56	T64	T72	T80	T88	T96
Position	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12
Well Label	T8	T16	T24	T32	T40	T48	T56	T64	T72	T80	T88	T96
OD Results	0.134	0.141	0.137	0.143	0.132	0.134	0.129	0.136	0.141	0.137	0.153	0.143