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Comparison of the Ames *Salmonella* Assay and Mutatox Genotoxicity Assay for Assessing the Mutagenicity of Polycyclic Aromatic Compounds in Porewater from Athabasca Oil Sands Mature Fine Tailings

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The oil sands in the Athabasca region of northeastern Alberta, Canada, represent a significant hydrocarbon resource that is currently exploited by mining, followed by separation of bitumen from sand using hot water flotation. This process generates large quantities of bitumen-contaminated tailings. Current research involves an assessment of whether the tailings ponds can ultimately be converted to biologically productive lakes, with one unresolved issue being the toxicity of the polycyclic aromatic compounds (PACs) that might be released from the tailings. In this paper, we have identified several polycyclic aromatic hydrocarbons in the porewater from oil sands mature fine tailings and have compared the responses of 17 PACs in the Ames and Mutatox genotoxicity assays. The Mutatox assay was unsuitable as a surrogate for the Ames test in this application; poor (50%) concordance between the two assays occurred because the mechanism of light emission in the Mutatox assay is uncertain, leading to positive responses that could not be unambiguously associated with genotoxicity. Benzo[*a*]pyrene equivalency factors (BEFs) in the Ames assay were determined for a large number of PACs, from this work and from literature data, to express the genotoxic potencies of environmental mixtures in terms of benzo[*a*]pyrene equivalent concentrations (BEQs). In the case of porewater samples obtained from the mature fine tailings, even extracts concentrated 10,000-fold were below the detection limit of 1 μg/L BEQ, consistent with the value of 0.14 μg/L calculated using BEFs of PACs identified in the porewater.

Introduction

Biological assays are an indispensable tool to signal and predict toxicological problems caused by the environmental impact of chemicals (*1*−*3*) because, unlike chemical analysis, they allow the evaluation of integrated toxic effects such as mutagenicity. Polycyclic aromatic compounds (PACs), which enter the environment from both natural and anthropogenic sources, include several known mutagens and animal and/or human carcinogens (*4, 5*). The sources of PACs relevant to the present research are the synthetic crude oil operations in northeastern Alberta, Canada. Bitumen is extracted from the oil sands by a hot water extraction process, which generates large volumes of fluid waste (tailings) that are contained in storage ponds (*6*). The tailings contain relatively high concentrations of unrecovered bitumen (*6*) that comprises a complex mixture of high molecular weight organic compounds (*7*); the PAC fraction of this mixture is the subject of this investigation.

The Alberta Environmental Protection Act prohibits the release of known carcinogens, mutagens, and other toxic compounds into the environment and requires the oil sands companies to remediate their leases to a state approximating the environment present prior to the beginning of mining operations (*8*). One proposed method of achieving this objective is the "wet landscape option", which involves disposal of the fluid tailings into a mined-out pit and capping them with a layer of clean water, so that a self-sustaining aquatic ecosystem can develop (*6*). An issue of concern is that as the tailings consolidate, they will release water (porewater) containing organic compounds derived from unrecovered bitumen into the capping water and then into the wider environment. For the purposes of this paper, porewater is operationally defined as the water released from the fine tailings by centrifugation, recognizing that it is composed of both interstitial water (true porewater) and process affected waters. The PACs in porewater comprise only a small fraction of the total PACs in fine tailings and an even smaller fraction of the extractable organic material (*6*) but are a high priority for study because of their aqueous solubility and their mutagenic or carcinogenic potential.

Many in vitro bioassays are available to measure the genotoxic effects of single chemicals and environmental mixtures, including PACs (*1, 2, 9, 10*), of which the most widely used is the Ames *Salmonella typhimurium* assay. This is based on histidine-requiring (*his*$^-$) mutants that revert to the *his*$^+$ phenotype by either base pair or frameshift mutations (*2, 11, 12*). The use of the extracellular fraction S9 of "induced" rat liver allows the Ames assay to be applied to promutagens such as PACs that require metabolic phase I bioactivation (*1, 2*).

Recently, Azur Environmental has commercialized the Mutatox (trademark of Azur Environmental) assay (*13*), which employs a dark mutant variant (strain M169) of the normally bioluminescent bacterium *Vibrio fischeri*. Exposure to sublethal concentrations of genotoxic agents restores light production in a dose-dependent fashion (*14*−*16*). As with the Ames assay, the addition of S9 allows for the detection of promutagens (*16*).

The first objective of the present study was to compare the responses of the Mutatox and Ames assays toward model PACs related to those present in oil sands fine tailings porewater and to evaluate the mutagenic response of porewater extracts. The second was to determine whether these assays could be placed on a quantitative basis by determining analytical parameters such as detection limits, calibration sensitivities, and limits of quantification. Because most environmental samples that contain PACs are complex mixtures whose components are present at varied concentration and having different individual toxic potencies (*9, 17,*

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18), we propose the use of benzo[*a*]pyrene equivalency factors (BEFs) to assess the relative mutagenic potencies of PACs in complex environmental mixtures such as the oil sands tailings porewater.

Methods

Chemicals. Phenanthrene, fluorene, fluoranthene, pyrene, benzo[*a*]pyrene, and anthracene were obtained from Supelco Canada, Mississauga, ON; carbazole, acridine, 3,6-dimethylphenanthrene, 9-phenanthrol, phenanthrenequinone, and dimethyl sulfoxide were obtained from Aldrich Chemical, Milwaukee, WI; dibenzothiophene, 2-methylanthracene, 9-methylanthracene, and 1-methylphenanthrene were acquired from Chem Service, West Chester, PA; 5,6-benzoquinoline was acquired from AccuStandard, New Haven, CT; and 7,8-benzoquinoline was obtained from ICN Biomedicals, Aurora, OH.

Methanol (distilled in glass) and phenol were obtained from Caledon Laboratories Inc., Georgetown, ON, and dichloromethane, pentane, and 2-propanol (all distilled in glass) were obtained from Burdick and Jackson, Muskegon, MI. Reagent water was prepared with a Milli-Q system from Millipore Corporation, Bedford, MA.

Ampicillin was obtained from Fisher Scientific, Fairlawn, NJ, and the *S. typhimurium* strains were graciously donated by Dr. Bruce Ames, University of California, Berkeley, CA. The Aroclor 1254-induced mouse liver (male Sprague−Dawley rat) fraction was obtained from Molecular Toxicology Inc., Boone, NC, and 4-nitroquinoline-*N*-oxide was obtained from Sigma-Aldrich, Oakville, ON.

Lyophilized Mutatox reagent (dark mutant strain of *V. fischeri*), growth media with and without S9 fraction, reconstitution solutions, vials, and cuvette holders were purchased from Azur Environmental, Carlsbad, CA. Media and reagent were stored at −30 °C prior to use.

Tailings Porewater Fractions. Oil sands tailings were supplied by Dr. M. MacKinnon (Syncrude Canada Ltd) and collected at a depth of 14 m from the south end of the Mildred Lake Settling Basin near Ft. McMurray, AB. They were centrifuged at 30000*g* (1 h) and then filtered through a combusted (450 °C) Whatman GF/F glass fiber filter to produce the porewater. The porewater was then acid−base separated (*19*) and extracted with dichloromethane (DCM). Fractions were passed through combusted Na_2SO_4 and evaporated before further fractionation. The acid fraction (fraction 2) was rehydrated with reagent water and then back-extracted with DCM to recover any lost base/neutral organic compounds.

The base/neutral organic extract (fraction 1) was further separated by silica gel chromatography (5 g, cf. ref *20*). Four subfractions were obtained: fractions A1 (eluting with 15 mL of pentane), A2 (15 mL of 1:1 DCM:pentane), A3 (15 mL of DCM), and A4 (15 mL of 1:9 isopropyl alcohol:DCM). Fraction A2 contained most of the PACs (*20*). Fractions 1 and 2 from 1 L of porewater were solvent-exchanged to 100 μL in DMSO (10,000× concentration of the sample). The four subfractions (A1−A4) of fraction 1 were solvent-exchanged to 1 mL in DMSO (1,000× concentration).

Ames Assay. The preincubation modified plate incorporation test was performed as described in Maron and Ames (*11*) with or without S9 metabolic activation. *Salmonella* strains TA100 and TA98 were grown in Oxoid nutrient broth no. 2 in a Gyrotory water bath shaker (New Brunswick Scientific Co. Inc., Edison, NJ) shaker/incubator (180 rpm, 37 °C) for 12 h. Each assay tube contained 25 μg/mL ampicillin. Test PAC or sample fraction(s) (0.1 mL) in DMSO were added to sterile, single-use 5-mL plastic test tube(s). Negative controls contained DMSO (0.1 mL). Benzo[*a*]pyrene (5 μg/plate) and 4-nitroquinoline-*N*-oxide (0.1 μg/plate) were used as positive controls for the S9-dependent and -independent Ames *Salmonella* assays, respectively. A three-log dose dilution series was initially tested for each compound; those showing a positive response were then studied using a dilution series ±1 log unit around the positive dose level.

We used the following criteria as evidence of genotoxicity: a 3-fold increase in the number of revertants detected in treated plates as compared to the solvent controls; dose−response behavior; and replication of the results in at least two independent experiments.

Mutatox Assay. Stock solutions of PACs were made up in methanol at a concentration of 1 mg/mL except for anthracene and 2-methylanthracene (0.5 mg/mL). For all tests, positive and negative controls were used with each vial of reagent bacteria. Phenol (10 mg/mL in reconstitution solution) was the positive control for the direct assay, and benzo[*a*]pyrene (0.5 mg/mL) was the positive control for the indirect (S9 fraction) assay. For the PAC stock solutions, methanol was the negative control.

The Mutatox test was carried out according to the standard protocol (*13*). Each lyophilized vial of medium was reconstituted with 15 mL of reconstitution solution and kept on ice prior to use. Ten samples were prepared as follows. Cuvette 1 contained 500 μL of reconstitution medium; all other cuvettes contained 250 μL of medium. The sample (10 μL of 1 mg/mL of PAC stock solution or 10 μL of porewater fraction) was added to cuvette 1; 1:2 serial dilutions were made by transferring 250 μL from cuvette to cuvette with mixing after each transfer; 250 μL was discarded from cuvette 10. The concentrations therefore ranged from 20 μg/mL (5 μg/tube) in tube 1 to 0.04 μg/mL (0.01 μg/tube) in tube 10.

To each cuvette, 10 μL of bacterial suspension (rehydrated with 1.1 mL of reconstitution solution per vial of *V. fischeri* reagent) was added. Test solutions in the indirect assay were incubated at 35 °C for 45 min; test solutions in the direct assay were held at 22 °C for 45 min, after which the solutions were then incubated for 24 h at 27 °C. Bioluminescence determinations were made with an Azur Environmental M500 toxicity analyzer set in the Mutatox mode. Light readings were taken for each cuvette at 14, 18, and 22 h and recorded as arbitrary light units using the Mutatox Data Capture Software.

A positive response in the Mutatox assay was defined as a 2-fold increase in light output as compared with the solvent and/or media negative controls when this increased response was observed in a series of three or more dilutions and when the whole experiment was replicated at least twice.

Results and Discussion

Porewater samples were analyzed for PACs by GC−MS in both single-ion monitoring and full-scan modes. Details of these analyses are available elsewhere (*19*). The results are summarized in Table 1; concentrations of individual compounds were all <500 ng/L (back-calculated to the original porewater) with a total concentration of identified PACs of 2.6 μg/L.

Ames Assays. Table 2 shows the results from 17 PACs tested in the Ames assay. No PAC gave a positive response in the absence of metabolic activation.

When the Ames assay was applied to the porewater samples, none of the extracts tested reverted TA98 or TA100 with or without S9, even at a concentration of porewater base/neutral organic extract (fraction 3) that was 10,000× higher than that of the raw porewater. This is a concentration factor similar to the bioconcentration factors (BCFs) of PACs for many fish and crustaceans (*21*). In other words, the extract contained as much PACs as would be expected inside biota living in the water cap. The acid organic extract (fraction 2) was acutely toxic to TA98 and TA100 at 10,000× and 1,000× the original tailings porewater concentration but showed no reversion of TA98 or TA100 at lower concentrations.

TABLE 1. Tentative Identification and Concentrations of Low Molecular Weight PACs from Fraction 1 (Base/Neutral Organic Extract) of Tailings Porewater

compd/group[a]	concn of PACs or groups of PAC congeners (ng/L of porewater)
naphthalene	101 ± 27
2-methylnaphthalene	28 ± 8
1-methylnaphthalene	33 ± 9
C2-naphthalene	61 ± 16
C3-naphthalene	200 ± 55
acenaphthylene	91 ± 25
acenaphthene	210 ± 56
fluorene	120 ± 32
C1-fluorene	230 ± 63
phenanthrene	330 ± 90
C1-phenanthrene/anthracene	390 ± 110
1-methylphenanthrene	89 ± 24
2-methylphenanthrene	130 ± 36
C2-phenanthrene/anthracene	200 ± 54
3,6-dimethylphenanthrene	45 ± 12
fluoranthene	10 ± 3
pyrene	15 ± 4
C1-fluoranthene/pyrene	6 ± 1
chrysene	12 ± 3
dibenzothiophene	37 ± 10
C1-dibenzothiophene	240 ± 65
4-methyldibenzothiophene	77 ± 21
2/3-methyldibenzothiophene[b]	56 ± 15
1-methyldibenzothiophene	59 ± 16
C2-dibenzothiophene	150 ± 41
2,8-dimethyldibenzothiophene	24 ± 7
C3-dibenzothiophene	98 ± 26
2,4-dimethylquinoline	24 ± 6
7,8-benzoquinoline	63 ± 17
5,6-benzoquinoline	120 ± 33
acridine	31 ± 8

[a] C1 (methyl), C2 (dimethyl/ethyl), C3 (trimethyl/methyl + ethyl/propyl/isopropyl). [b] 2- and 3-methyldibenzothiophene cannot be discriminated by retention time or GC–MS SIM.

Mutatox Assays. Most of the 17 PACs gave dose-dependent positive responses in the Mutatox assay with or without S9 metabolic activation (Table 2), with sensitivities that were consistent with other work on PACs (*14, 16, 22*). The whole base/neutral organic extract (fraction 1) of tailings porewater produced positive responses in the direct Mutatox assay (Table 3) but not in the indirect (S9) assay. In the direct assay, subfractions A2 (aromatics), A3 (semipolar aromatics), and A4 (polar compounds) were positive, but fraction A1 (saturates) showed no detectable response. The acid extract (fraction 2) also produced a positive response in the direct assay; it was acutely toxic to *V. fischeri* in the indirect assay but did not give a genotoxic response after dilution.

Comparison between Ames and Mutatox Assays. The present results with a subset of PACs contrast with the concordance often reported between the Mutatox and Ames assays (*16, 22, 23*), although Sun and Stahr (*15*) also found significant discordance between the two assays. Heteroatom-substituted PACs probably induce light re-emission in the direct Mutatox assay by the same (unknown) mechanism as phenol, which is used in this protocol as a positive control, but which is neither a positive genotoxin in the Ames assay nor a carcinogen in animals (*4, 5*). Potential mechanisms for light re-emission without DNA damage include inactivation of the lux system's repressor protein, inactivation of the corresponding repressor gene, or blockage of polymerase III (*24*) allowing initiation of the SOS response with unrepressed transcription of the luciferase operon (*2, 14*). Phenol and other nongenotoxic chemicals may be able to activate the SOS-response system in bacterial cells and hence stimulate light re-emission without DNA modification (*2, 25*). Another speculation is that the luminescence machinery of *V. fischeri* is a multi-enzyme complex that includes an isoform of cytochrome P450 (*26*). This might allow *V. fischeri* to detoxify hydrophobic compounds by hydroxylation in a manner parallel to that of microsomes and might also explain why S9 activation is not needed for a positive response by low molecular weight PACs.

TABLE 2. Comparison of the Mutatox (*V. fischeri*) and Ames (*S. typhimurium*) Assays Responses to Selected Model PACs with Four or Fewer Aromatic Rings

		Mutatox		
compound	S9	D–R[a]	LDC[b] (μg/tube)	Ames
fluorene	+	3	1.3	–
	–	4	0.63	–
anthracene	+	4	0.31	+
	–	NR[c]	–	–
2-methylanthracene	+	3	0.63	+
	–	5	0.31	–
9-methylanthracene	+	4	0.38	–
	–	5	0.02	–
phenanthrene	+	3	1.3	+
	–	5	0.38	–
1-methylphenanthrene	+	NR	–	+
	–	4	1.4	–
3,6-dimethylphenanthrene	+	6	0.08	+
	–	NR	–	–
9-phenanthrol	+	NR	–	–
	–	3	0.06	–
phenanthrenequinone	+	NR	–	–
	–	NR	–	–
dibenzothiophene	+	NR	–	–
	–	5	0.38	–
fluoranthene	+	4	0.73	+
	–	4	1.4	–
pyrene	+	4	0.63	+
	–	6	0.16	–
carbazole	+	4	0.63	–
	–	7	0.16	–
acridine	+	9	0.63	–
	–	6	0.16	–
5,6-benzoquinoline	+	4	0.63	+
	–	6	0.08	–
7,8-benzoquinoline	+	4	0.63	+
	–	5	0.31	–
benzo[*a*]pyrene	+	4	0.31	+
	–	NR	–	–

[a] Dose–response (i.e., the number of positive light-producing tubes per dilution series). [b] Lowest detectable concentration (i.e., the lowest light output from *V. fischeri* greater than 2× the control). [c] NR (no response in the Mutatox assay).

TABLE 3. Mutagenic Response of the Base/Neutral Organic Fraction (1) of Tailings Porewater, in DMSO, in the Direct-Acting Mutatox (*V. fischeri*) Assay

		Mutatox	
sample fractions	concn factor	D–R[a]	LDT[b]
fraction 1	10,000×	4	7
fraction A1	1,000×	ND[c]	
fraction A2	1,000×	3	6
fraction A3	1,000×	4	4
fraction A4	1,000×	3	4
negative control		ND	

[a] Number of positive light-producing tubes per dilution series. [b] Lowest detectable tube: lowest dilution with light output greater than 2× solvent standard (higher number = lower concentration of sample). [c] Not detectable as a mutagen in the Mutatox assay.

Quantitative Measurement of Genotoxicity. Spontaneous revertant yields in the Ames assay were consistent from experiment to experiment; *Salmonella* strain TA100 averaged

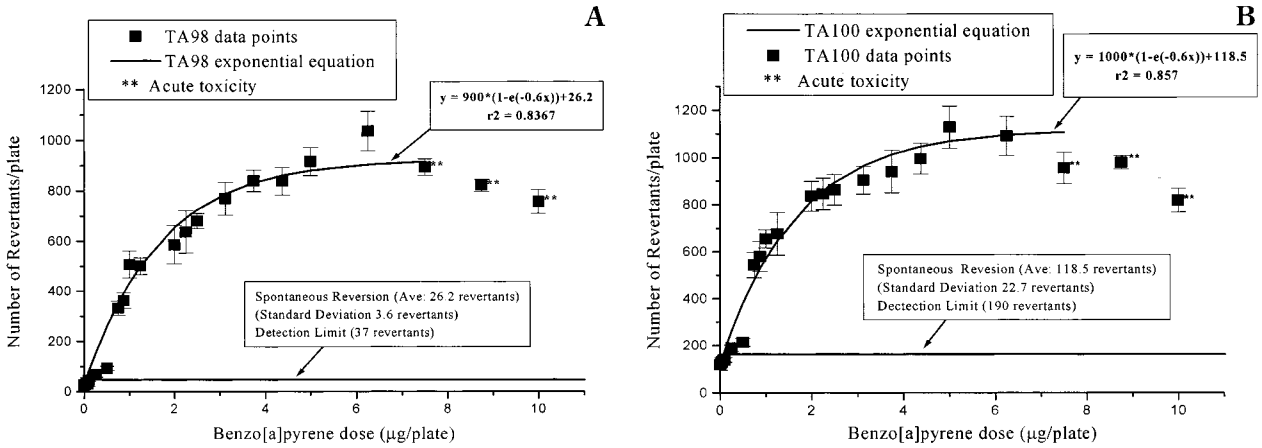

FIGURE 1. (A) Dose−response curve for benzo[*a*]pyrene mutagenicity in the presence of S9 fraction (metabolic activation) in *S. typhimurium* strain TA98. (B) Dose−response curve for benzo[*a*]pyrene mutagenicity in the presence of S9 fraction (metabolic activation) in *S. typhimurium* strain TA100.

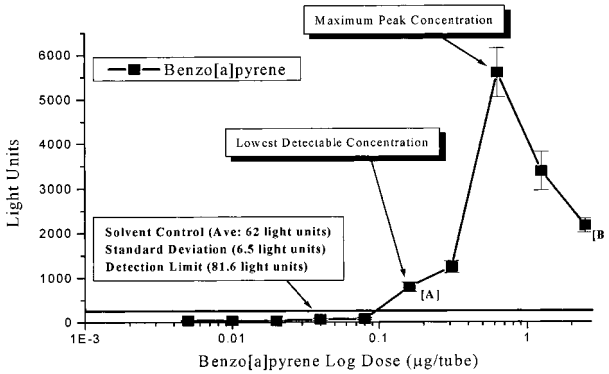

FIGURE 2. Typical concentration response obtained for benzo[*a*]-pyrene with metabolic activation in the Mutatox (*V. fischeri* rat hepatic S9 fraction) assay.

118 ± 23 revertants per plate (rpp) and strain TA98 averaged 26.3 ± 3.6 rpp, yielding detection limits of 187 and 37 rpp, respectively, at three standard deviations. The Mutatox assay had a spontaneous reversion rate of 62 ± 6.5 light units, giving a detection limit of 85 light units at 3 SD.

Most mutagenic PACs were detected in the Ames assay at doses of 20−200 μg/plate. Dose response curves for B[a]P followed empirical exponential relationships (Figure 1A,B; *27*, *28*). No attempt was made to fit the data beyond the maximum response. The curves deviated from linearity at lower concentration than suggested by previous work (*11*). The detection limits were 0.090 μg of B[a]P for TA98 and 0.570 μg of B[a]P for TA100, with limiting calibration sensitivities (slopes) of 430 revertants/μg of B[a]P for TA98 and 570 revertants/μg of B[a]P for TA100. The upper limit is ~5 μg of B[a]P/plate, only about 1 order of magnitude above the detection limit; this limits the use of Ames assays as a quantitative tool. Dilution series must be prepared over a logarithmic dose range in a preliminary experiment to bring the sample's concentration into the range suitable for quantitation.

Quantitative analysis was not practical with the Mutatox assay. As shown in Figure 2, the calibration curve for B[a]P exhibited a concentration range from the lowest detectable concentration (about 0.2 μg/tube) to maximal response of only half an order of magnitude. The lowest detectable quantity was lower than TA100 but higher than TA98 in the Ames assay. Mutatox gave some positive responses with the porewater samples but only in the absence of metabolic activation (Table 3). However, there is ambiguity as to whether a positive Mutatox test represents genotoxicity because many PAC standards that are not mutagenic in the Ames assay respond positively in the Mutatox assay. This is unfortunate because the Mutatox assay has the advantage of small sample requirements that allowed replicate testing at high concentration even for the porewater samples, which were difficult to prepare. It also required minimal training, used low volumes of consumables, and produced little waste.

Benzo[*a*]pyrene Equivalency Factors. Polycyclic aromatic compounds occur in the environment as complex mixtures of compounds with widely varying toxic potencies (*17*, *18*, *21*). B[a]P is often used as an indicator of risk for PAC mixtures, due to its high mutagenic potency, ubiquitous presence in the environment and extensive physical and chemical characterization (*18*, *21*). Toxic equivalency factors (TEFs) have been developed for a limited number of polycyclic aromatic hydrocarbons (PAHs) (*17*, *29*) as an aid to determining the human health risk associated with airborne mixtures of PAHs, a concept similar to that used for assessing mixtures of dioxins (*30*). The benzo[*a*]pyrene equivalency factor (BEF) of the reference compound B[a]P is set to 1.0, and those of other mutagens are assigned so that their BEFs parallel their relative potencies, similar to work done by Anderson et al. (*31*) and Petry et al. (*17*). The total potency or benzo[*a*]pyrene equivalence (BEQ) is defined by

$$\text{BEQ} = \Sigma(\text{concentration of component } i) \times (\text{BEF of component } i) \quad (1)$$

We have searched the literature in order to determine tentative BEFs for PACs in aqueous media (Table 4) using Ames assay data obtained in the presence of metabolic activation. These were available as rpp/μg of PAC (*4*, *32*−*43*). Because data were usually insufficient to draw dose−response curves, we report in Table 4 the largest reported ratio rpp/μg of PAC to give the highest (most conservative) BEF. We likewise record the highest BEF for PACs that revert more than one tester strain. Compounds causing amounts of reversion below the detection limit were assigned a BEF of zero. B[a]P is the most potent PAC quantitated to date.

The BEQ concept allows the potency of an environmental sample to be expressed in terms of the concentration of B[a]P that would give the same response (rpp) in the assay. Before this approach can be accepted, it should be demonstrated that mixtures of compounds behave additively in the assay. Synergism among compounds (a rare event) makes the mixture appear more potent than the sum of its components, whereas antagonism makes the mixture appear less potent. Antagonism, or masking of the effects of one toxicant by another, has the undesirable effect of producing false

TABLE 4. Literature and Experimental Genotoxic Responses of Model PACs on the Ames *Salmonella* (Strains TA98 and TA100) Assay (with Rat Hepatic S9 Fraction)[a]

compound[b]	strain[c]	genotoxic results (+S9)[d]	no. of revertants/ μg of PAC	% BEF[e]	literature ref
anthracene[3]	TA100	+	1.1	0.2	46
2-methylanthracene	TA100	+	10	1.8	32
9-methylanthracene	TA98	+	2.1	0.5	37, 39
2,9-dimethylanthracene	TA100	+	187	29	37
9,10-dimethylanthracene[f]	TA100	+	122	21	37
2,9,10-trimethylanthracene	TA100	+	207	32	37
2,3,9,10-tetramethylanthracene	TA100	+	147	23	37
benzo[*a*]anthracene[2A]	TA98	+	52	12	4
7,12-dimethylbenzo[*a*]anthracene	TA100	+	73	11	33
phenanthrene[3]	TA100	+	18	2.8	4
1-methylphenanthrene[3]	TA100	+	56	9.8	32
2-methylphenanthrene	TA100	+	16	2.7	32
9-methylphenanthrene	TA100	+	229	36	35
1,4-dimethylphenanthrene[3]	TA100	+	185	29	35
3,6-dimethylphenanthrene	TA100	+	11	2	32
4,10-dimethylphenanthrene	TA100	+	214	33	35
9-methylfluorene	TA98	+	125	20	34
1,9-dimethylfluorene	TA98	+	300	47	34
2,9-dimethylfluorene	TA100	+	106	17	34
3,9-dimethylfluorene	TA100	+	138	22	34
4,9-dimethylfluorene	TA100	+	129	20	34
2,3,9-trimethylfluorene	TA98	+	135	21	34
11-methylbenzo[*a*]fluorene	TA98	+	112	3.3	34
11-methylbenzo[*b*]fluorene	TA98	+	285	44	34
7-methylbenzo[*c*]fluorene	TA98	+	190	30	34
3-methylbenzo[*b*]naphtho[1,2-*d*]thiophene	TA98	+	36	8.0	47
6-methylbenzo[*b*]naphtho[2,1-*d*]thiophene	TA98	+	21	4.7	47
4-methylbenzo[*b*]naphtho[2,3-*d*]thiophene	TA98	+	39	8.5	47
fluoranthene[3]	TA98	+	15	3.3	4
benzo[*b*]fluoranthene[2B]	TA98	+	69	15	4
pyrene[3]	TA100	+	26	4.6	4
benzo[*a*]pyrene[2A]	TA98/100	+/+	432/570	100/100	4, 20, 32, 37, 50
benzo[*e*]pyrene[3]	TA100	+	39	7	38
chrysene	TA100	+	166	25	38
4-methylchrysene[3]	TA100	+	108	17	38
5-methylchrysene[2B]	TA100	+	240	37	38
1,6-dimethylpyrenequinone	TA100	+	34	6.0	32
1,8-dimethylpyrenequinone	TA100	+	27	4.8	32

[a] The following compounds all gave no response in the Ames assay (BEF = 0): naphthalene (*33*); 1-methylanthracene (*37*); acridine (*41*, *42*); 3-methylphenanthrene, 4-methylphenanthrene, 1,9-dimethylphenanthrene, 2,7-dimethylphenanthrene, 4,5-dimethylphenanthrene, 4,9-dimethylphenanthrene, 2-ethylphenanthrene, 3-ethylphenanthrene, 9-ethylphenanthrene, 9-*n*-propylphenanthrene, 9-isopropylphenanthrene, 2,4,5,7-tetramethylphenanthrene, and 3,4,5,6-tetramethylphenanthrene (*35*); 9-phenanthrol (*19*); fluorene (*50*); 1-methylfluorene, 2-methylfluorene, 3-methylfluorene, and 4-methylfluorene (*34*, *43*); 2,3-dimethylfluorene, 9,9-dimethylfluorene, 2,7-dimethylfluorene, 2,7,9-trimethylfluorene, 9-hydroxymethylfluorene, benzo[*a*]fluorene[3], benzo[*b*]fluorene[3], and benzo[*c*]fluorene[3] (*34*); carbazole (*4*); dibenzothiophene (*19*, *40*); 1-methyldibenzothiophene, 2-methyldibenzothiophene, 3-methyldibenzothiophene, 4-methyldibenzothiophene, benzo[*b*]naphtho[1,2-*d*]thiophene, 1-methylbenzo[*b*]naphtho[1,2-*d*]thiophene, 2-methylbenzo[*b*]naphtho[1,2-*d*]thiophene, 4-methylbenzo[*b*]naphtho[1,2-*d*]thiophene, 5-methylbenzo[*b*]naphtho[1,2-*d*]thiophene, 6-methylbenzo[*b*]naphtho[1,2-*d*]thiophene, benzo[*b*]naphtho[2,1-*d*]thiophene, 1-methylbenzo[*b*]naphtho[2,1-*d*]thiophene, 2-methylbenzo[*b*]naphtho[2,1-*d*]thiophene, 3-methylbenzo[*b*]naphtho[2,1-*d*]thiophene, 4-methylbenzo[*b*]naphtho[2,1-*d*]thiophene, benzo[*b*]naphtho[2,3-*d*]thiophene, 1-methylbenzo[*b*]naphtho[2,3-*d*]thiophene, 2-methylbenzo[*b*]naphtho[2,3-*d*]thiophene, 3-methylbenzo[*b*]naphtho[2,3-*d*]thiophene, 5-methylbenzo[*b*]naphtho[2,3-*d*]thiophene, and 6-methylbenzo[*b*]naphtho[2,3-*d*]thiophene (*40*); 1-methylchrysene[3], 2-methylchrysene[3], 3-methylchrysene[3], and 6-methylchrysene[3] (*38*); naphthoquinone, anthraquinone, 2-methylanthraquinone, 1,6-dimethylpyrenequinone, and 1,8-dimethylpyrenequinone (*32*); and phenanthrenequinone (*19*, *32*). [b] IARC, 1987. Notation for the classification of PAHs under the IARC guidelines for the determination of human carcinogenicity upon exposure to the PAH(s). Compounds without IARC numbers have not been assessed for their carcinogenic potential in humans. These classifications do not imply that a higher % BEF mutagenic potency on the Ames assay is a probable or possible human carcinogen or that a lower % BEF mutagenic potency is a nonhuman carcinogen. The classifications by IARC are presented as information on the small quantity of reliable epidemiological data on PAHs and human carcinogenicity as compared with the large quantity of data on mutagenic compounds in the Ames assay. 2A, probable human carcinogen. 2B, possible human carcinogen. 3, not classifiable as to its carcinogenic potential in humans. [c] Ames *Salmonella* strain(s) used to detect genotoxicity of PAHs. If a PAH was positively detected as a genotoxin on more than one strain, the strain with the largest number of revertants/μg of PAH was chosen. [d] All presented genotoxic results are in the presence of the S9 metabolic fraction. [e] Percent of B[a]P equivalence factors. The percent dose of B[a]P required to elicit the same genotoxic potency as the given PAC (see discussion in text). A nondetectable (ND) mutagen was given a B[a]P percent equivalent of 0%. [f] 9,10-Dimethylanthracene was the only PAH detected as a mutagen in the presence and absence of metabolic activation on strain TA98 (*36*).

negatives in environmental monitoring programs, causing a contaminated sample to appear "clean".

Previously, Herman (*44*) and others (*45*−*49*) have found that other PAHs could behave either additively (at low concentrations) or antagonistically (at high concentrations) to the mutagenic potency of B[a]P in the Ames assay. This so-called target molecule antagonism is typical of toxicants competing for a limited supply of a target molecule (in this case, presumably CYP 1A1 in the S9 mix); at high total PAH concentrations, the other PAHs inhibit the metabolism of B[a]P. The recent work of Rodriguez et al. (*50*) can be explained similarly. These workers reported nonadditive formation of B[a]P−DNA adducts in infant mice when B[a]P was administered in conjunction with the PAC fraction of a manufactured gas plant mixture as compared with B[a]P alone.

As already noted, the porewater base/neutral extracts tested negative in *Salmonella* strains TA98 and TA100. The

detection limits of 0.09 μg of B[a]P for TA98 and 0.57 μg of B[a]P for TA100 correspond to 0.9 μg of B[a]P equiv/L of porewater for TA98 and 5.7 μg of B[a]P equiv/L of porewater for TA100. Combining the calculated BEFs with chemical analysis of the porewater samples yielded a mutagenic potential for the porewater samples as 0.14 μg/L of BEQ, which is below the detection limit of mutagenic response in both TA98 and TA100. We are confident that our data refer to the "additive" region of the dose−response curve because in the "antagonistic" region, we would have seen a higher response as we diluted the porewater extracts.

We therefore conclude, in the context of the Athabasca oil sands "wet landscape option", that most of the PACs present in porewater should have low mutagenic potential based on their BEFs. The whole porewater showed no genotoxicity toward the two standard PAC-detecting strains of *S. typhimurium*, even when concentrated 1,000- and 10,-000-fold. The BEQ of the porewater was below the detection limit of 0.9 μg/L, but we cannot state by how much. The positive responses obtained from the concentrated porewater samples by the Mutatox assay suggested a BEQ of 0.05 μg/L. Given the tendency of the Mutatox assay toward false positives, this should be an overestimate. Since, however, we cannot positively ascribe these responses to genotoxicity, we do not wish to propose substituting the Mutatox test for the well-validated Ames assay for environmental monitoring and risk assessment of PACs in these environmental samples.

The BEQ of a given porewater sample will be dependent on the fine tailings sample from which it was prepared. The sample supplied by Syncrude Ltd for this study was considered "representative", in terms of hydrocarbon content and composition, of the fine tailings that will be transferred to wet landscape lakes (Dr. M. MacKinnon, Syncrude Ltd, personal communication). However, given ongoing process changes in bitumen recovery with time, it is inevitable that contaminant levels in the fine tailings will vary at different locations in the tailings ponds. Should a more complete assessment be required, fine tailings from a variety of locations within the Mildred Lake Settling Basin could be collected, processed, extracted, and analyzed to determine BEQs using the protocol developed in this study.

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