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Survey of the mutagenicity of surface water, sediments, and drinking water from the Penobscot Indian Nation

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HIGHLIGHTS

- Penobscot Indian Nation, Maine, U.S., drinking water mutagenicity was 337 rev (L-eq)$^{-1}$.
- The Penobscot River water mutagenicity was 177 rev (L-eq)$^{-1}$.
- The river sediment mutagenicity was 244 rev (g-eq)$^{-1}$.
- Most samples of river water/sediment and drinking water were not mutagenic.
- The aquatic environment of the Penobscot Indian Nation has no or low mutagenicity.

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ABSTRACT

U.S. Environmental Protection Agency (US EPA) Regional Applied Research Effort (RARE) projects address the effects of environmental pollutants in a particular region on the health of the population in that region. This report is part of a RARE project that addresses this for the Penobscot Indian Nation (PIN), Penobscot Island, Maine, U.S., where the Penobscot River has had fish advisories for many years due to high levels of mercury. We used the *Salmonella* mutagenicity assay with strains TA100, TA98, YG1041, and YG1042 with and without metabolic activation to assess the mutagenic potencies of organic extracts of the Penobscot River water and sediment, as well as drinking-water samples, all collected by the PIN Department of Natural Resources. The source water for the PIN drinking water is gravel-packed groundwater wells adjacent to the Penobscot River. Most samples of all extracts were either not mutagenic or had low to moderate mutagenic potencies. The average mutagenic potencies (revertants/L-equivalent) were 337 for the drinking-water extracts and 177 for the river-water extracts; the average mutagenic potency for the river-sediment extracts was 244 revertants (g-equivalent)$^{-1}$. This part of the RARE project showed that extracts of the Penobscot River water and sediments and Penobscot drinking water have little to no mutagenic activity that might be due to the classes of compounds that the *Salmonella* mutagenicity assay detects, such as polycyclic aromatic hydrocarbons (PAHs), nitro-PAHs (nitroarenes), and aromatic amines. This study is the first to examine the mutagenicity of environmental samples from a tribal nation in the U.S.

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1. Introduction

The U.S. Environmental Protection Agency (U.S. EPA) has responsibility for protecting the nation's surface water and groundwater and for ensuring that the supply of drinking water is safe for public consumption. The Clean Water Act (U.S. EPA, 2011) sets national standards for drinking water to protect against health risks, considering available technology and cost. This act regulates point–source and nonpoint–source discharges of pollutants to the waters of the United States, including tribal nations.

The U.S. EPA works with tribes on a government-to-government basis when U.S. EPA actions and decisions may affect tribal interests (Ruckelshaus, 1984). In this context, the U.S. EPA sponsors Regional Applied Research Effort (RARE) projects to address the

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effects of environmental pollutants in a particular region on the health of the population in that region. The present report is part of such a RARE study that was designed to help address this for the Penobscot Indian Nation (PIN), Indian Island, Maine, U.S., where the Penobscot River has had fish advisories for many years due to high levels of mercury (ATSDR, 2014). The PIN RARE project involved two parts: (1) an analysis of flora and fauna for dioxins, PCBs, and mercury, which is published (ATSDR, 2014), and (2) a survey of the mutagenic potencies of organic extracts of the drinking water and the Penobscot River water and sediments from the Penobscot River using the *Salmonella* mutagenicity assay, which is reported here.

As a riverine tribe, the Penobscot culture and traditions are tied inextricably to the Penobscot River watershed. However, members of the PIN are concerned whether the ecosystem that supports the flora and fauna they use to sustain their cultural practices is contaminated. This concern is not unfounded as evidenced by health advisories regarding levels of mercury and other contaminants in fish (State of Maine, 2011; Penobscot Indian Nation, 2012). Therefore, we determined the mutagenic activity in aquatic components of the PIN.

In this part of the RARE study, we used the *Salmonella* mutagenicity assay to screen surface water, sediment, and drinking water from the PIN for mutagenicity. The *Salmonella* mutagenicity assay has been used extensively to identify genotoxic substances in environmental samples (Chen and White, 2004; Claxton, 1985; Claxton et al., 1998, 2004, 2010; Claxton and George, 2002; Claxton and Woodall, 2007; Lemos et al., 2009; Maertens et al., 2004; Ohe et al., 2003, 2004; Richardson et al., 2007; Vargas et al., 1993; White and Claxton, 2004; Zwiener et al., 2007). The assay is useful in the present context because of its ability to identify mutagenic activity in surface waters (Ohe et al., 2003, 2004), sediments (Chen and White, 2004), and drinking waters (Richardson et al., 2007).

The assay determines the mutagenicity, but not specifically the carcinogenicity, of compounds and complex mixtures (Zeiger, 1998; Mortelmans and Zeiger, 2000). However, because many carcinogens act by mutagenic mechanisms, most organic carcinogens that are mutagens are positive in the *Salmonella* assay (Zeiger, 1998). Conversely, the assay has identified some mutagens that have not been shown to be carcinogens. The *Salmonella* assay is the assay used most widely to identify mutagenic activity in environmental samples and for comparing locations, identifying sources, and identifying the potential carcinogens in complex environmental mixtures (Claxton et al., 2010).

Surveying sediments, surface waters, and drinking water with the *Salmonella* assay is not without precedent. For example, the regulatory body in São Paulo State, Brazil, routinely has monitored its rivers with the *Salmonella* assay for more than 20 years (Umbuzeiro et al., 2001). Investigators in Japan have used the *Salmonella* assay to monitor the seasonal fluctuation of the mutagenicity of river water in Fukui, Japan (Watanabe et al., 2002). Our study was conducted to determine the level of mutagenic activity in the Penobscot River water, sediment, and PIN drinking water.

2. Materials and methods

2.1. General study design

We designed the study to be a survey of the water sources impacting the PIN. Therefore, three types of samples were collected on various dates at various locations within PIN Territory: drinking (tap) water from Indian Island, surface water from the Penobscot River, and sediments from the Penobscot River; sampling sites are shown in Fig. 1. As noted in the figure, the sites at which we collected river water and sediments were (a) Salmon Stream Lake, which was ~50 km upstream of the outfall; (b) Lincoln Outfall: the river-water samples were taken in the immediate downstream vicinity of an industrial-effluent outfall (Lincoln Paper and Tissue Mill), and the sediment sample was taken from slightly downstream (~300 m) of the outfall; and (c) West Enfield, which was a site ~20 km downstream from the outfall. The Lincoln Outfall site is also located ~0.5 km downstream of the town of Lincoln's municipal wastewater treatment plant. We obtained a fourth sediment sample from a site adjacent to Indian Island. We collected drinking-water samples at the PIN Department of Natural Resources, Water Quality Monitoring Laboratory on Indian Island, ME. The source water for the PIN drinking water is gravel-packed groundwater wells adjacent to the Penobscot River; the water is chlorinated.

At the time of sampling and for many years prior, the Lincoln Paper and Tissue Mill produced kraft pulp and specialty dyed paper products. The mill used oxygen delignification and chlorine dioxide for bleaching, and its waste received secondary treatment on site. (The mill is no longer a pulping facility.) Tables 1 and 2 give details about the sites and samples taken. After we shipped the samples to the U.S. EPA in Research Triangle Park, NC, we extracted, concentrated, and tested the samples using the *Salmonella* mutagenicity assay as described below.

2.2. Sampling

We obtained samples of water from the Penobscot River by collecting a composite of 5 sequentially filled 2.5-L bottles at each location for each sampling event. We collected the samples in amber bottles that were pre-cleaned and had a Teflon™ cap (Cat. No. #293680, Sci Spec, Hanover, MD) by submerging the capped bottle within 0.3 m of the river's surface, uncapping the bottle until it was filled, and recapping the bottle under the water. We took care to avoid disturbing bottom sediments to keep them from entering the sample bottle. We placed the samples in a cooler with ice in the field and then stored them at 4 °C in the dark until they were shipped. To keep samples cooled, we shipped them in coolers containing frozen Blue Ice®.

We obtained drinking-water samples by collecting a composite of 5 sequentially filled 2.5-L bottles for each sampling event from a convenient tap on Indian Island at the PIN Water Quality Monitoring Laboratory. We took one composite sample on each of the days that we also sampled the river water. We ran water for 10 min from a drinking-water tap, and then we collected the samples in amber bottles and stored them at 4 °C. We shipped the samples in the same manner as the river-water samples.

We obtained river-sediment samples at approximately the same sites as we sampled the river water; thus, 3 river-sediment samples, one at each site, were taken. At each location we took a composite of 3–5 grab samples of the top sediment (<15 cm) using a Ponar dredge. We placed the grab samples in a stainless-steel container and mixed them together until homogenous. We then divided the composite sample and transferred it into 3 pre-cleaned amber jars with Teflon™ caps, each containing ~500 g wet weight of sediment. We cleaned the dredge and sampling equipment with Alconox, deionized water, and methanol before and between sampling at each site. We placed each jar in a plastic bag, sealed the bag, and placed each in a cooler with ice for transport from the field until the bag was transferred to a dark 4 °C refrigerator. We shipped sediment samples in coolers with Blue Ice®.

2.3. Extraction and concentration of samples

We extracted the river-water samples by open-column chromatography using a 50:50 layer (50 g each) of XAD-2/XAD-8 resin with the XAD-2 on the bottom; organics were eluted with ethyl

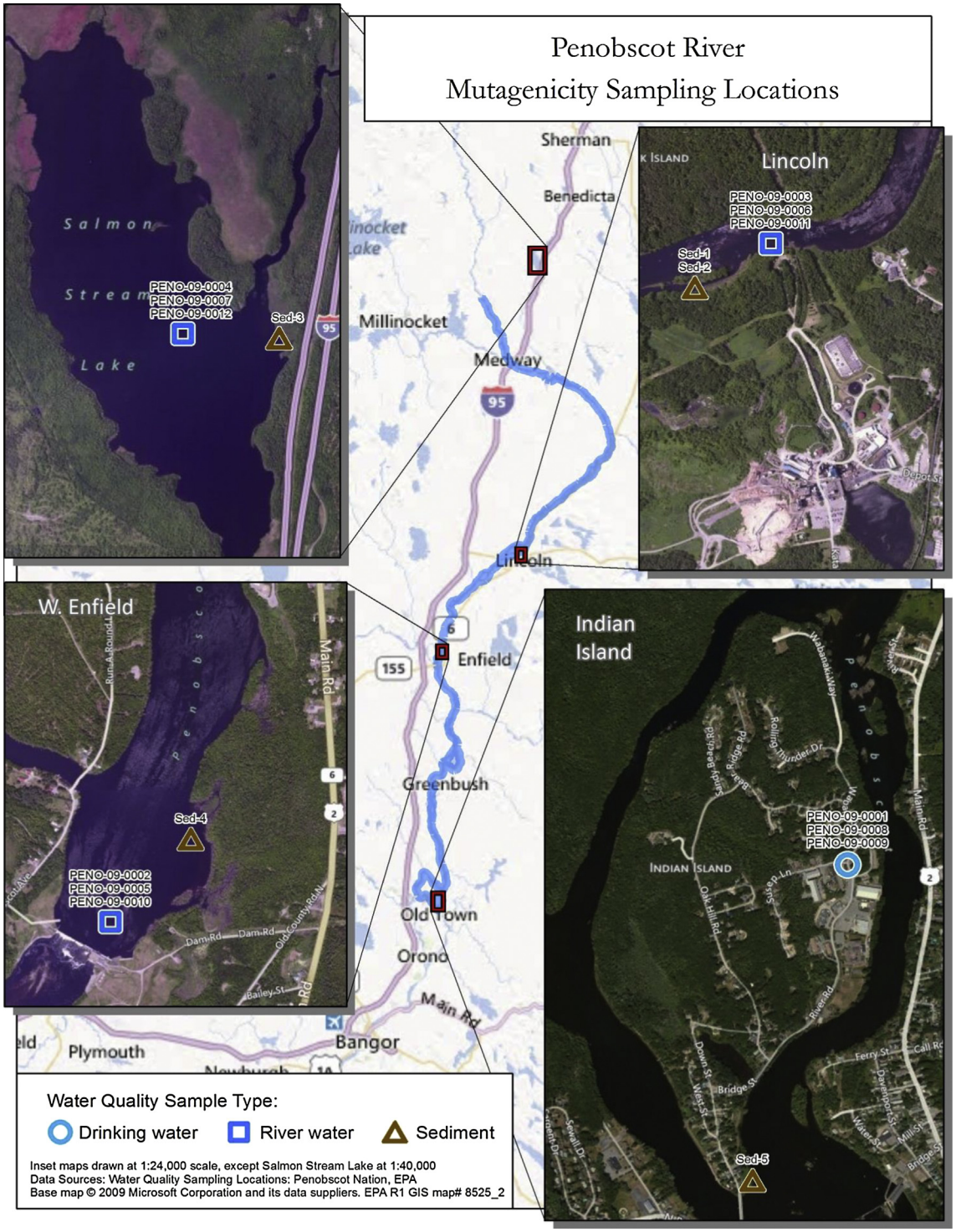

Fig. 1. Location of sample-collection sites for mutagenicity studies of drinking water, river water, and river sediments.

acetate. Prior to use, we washed the XAD resin sequentially with 0.1-M NaOH, water, and methanol, followed by sequential 24-h soxhlet extractions with methanol, ethyl acetate, and methanol. We stored the resin in methanol until use. We dried the extracts over sodium sulfate, concentrated them, filtered them across a 0.45-µm PTFE-syringe filter, and solvent-exchanged them into dimethyl sulfoxide (DMSO) at 5000× for the bioassay (unless this was too thick, at which point more DMSO was added to make the concentrate at 1000×). We processed the drinking-water samples as above except that the water was first acidified to pH 2 prior to extraction; we then prepared 10000× concentrates in DMSO. We also prepared blanks in the same way with XAD and evaluated these for mutagenicity. We processed river-sediment samples by first air-drying the samples and then extracting 100 g dry-weight of each sample by Accelerated Solvent Extraction (ASE) with a 50:50 mix of dichloromethane/methanol using an ASE 350 (Dionex Corp, Sunnyvale, CA). We filtered the extracts across 0.45-µm Teflon™ laminated-filter disks, concentrated them, and solvent-exchanged them into 1 mL of DMSO to make 100× concentrates (w/v).

Table 1
Sampling information for river sediments.

Site	Location	Date/time	Conductivity (μS)	Description[a]
Salmon Stream Lake (Upstream/ Control)	N45°43′27.5″W068°28′34.8″ ± 15 ft water depth 0.75 m; ~50 km; upstream of outfall	07/29/09 12:45 pm	80	Fine-grained mixed w/fibrous material
Lincoln (Outfall)	N45°22′27.4″W068°30′58.4″ ± 13 ft water depth 1–2 m; ~300 m downstream of outfall	07/29/09 09:50 am	159	Fine-grained
W. Enfield Impoundment (Downstream)	N45°15′13.2″W068°38′38.4″ ± 11 ft water depth 2 m; ~40 m from shore on east side directly across river from boat landing and ~20 km downstream of outfall	07/29/09 03:14 pm	55	Fine-grained

[a] Grain size and total organic carbon (TOC) of sediment samples collected in 2008 (one year prior to our collection) from the same sites as those used for this mutagenicity study, have the following characteristics: percentage sand, silt, clay and TOC for Salmon Stream Lake/Control = 37.9, 57.6, 4.5 and 5; Lincoln Outfall = 84.6, 11.5, 3.8 and 5.3; and West Enfield = 31.4, 59.1, 9.6, and 5.8, respectively.

2.4. Mutagenicity assays

We tested the sample extracts in the *Salmonella* mutagenicity assay with and without metabolic activation (Aroclor 1254-induced Sprague–Dawley rat-liver S9, Moltox Inc., Boone, NC) following the procedures of Maron and Ames (1983) with modifications from Claxton et al. (1987). We used the frameshift strain TA98 and the base-substitution strain TA100, which were provided by Dr. B.N. Ames, Children's Hospital Oakland Research Institute, Oakland, CA. We also used strain YG1041 (derived from TA98) and strain YG1042 (derived from TA100), which over-express acetyltransferase and nitroreductase, enhancing the sensitivity of the strains to aromatic amines and nitroarenes (Hagiwara et al., 1993). These YG strains were kindly provided by Dr. T. Nohmi, National Institute of Health Sciences, Tokyo, Japan.

We prepared all extracts in DMSO as well as the direct-acting controls 2-nitrofluorene and sodium azide (3.0 μg plate^{-1}, Sigma, St. Louis, MO) and the indirect-acting control 2-aminoanthracene (0.5 μg plate^{-1}, Sigma). Due to limited amounts of samples, we first tested each sample in the plate-incorporation assay at one plate per dose (5–7 doses) and then repeated the experiment if the volume of extract permitted. We incubated the plates for 72 h at 37 °C, and we counted colonies with an AccuCount™ 1000 automatic colony counter (Biologics, Inc., Manassas, VA). We entered data in the GeneTox Manager statistical analysis program (Claxton et al., 1995) to determine mutagenic potencies using the Bernstein method (Bernstein et al., 1982). We calculated the mutagenic potencies as revertants (rev) per liter-equivalent (L-eq) for the river and drinking waters and as rev per gram-equivalent (g-eq) for the sediment samples. We defined a positive result as one in which the extract produced a dose-related increase of at least twofold over the DMSO number of rev/plate; the DMSO controls were used in the potency calculations from the dose–response curves.

We tested river-water samples for mutagenicity in strains YG1041 and YG1042 with and without S9 because these strains have enhanced metabolic capabilities that may be useful for detecting mutagenic activity in river sediments (Ohe et al., 2003, 2004; Umbuzeiro et al., 2001). We performed the first experiments with eight doses (10–500 mL-eq/plate) using YG1041. We then performed a repeat test using a dose range of 100–500 mL-eq/plate with YG1041; we performed a single experiment with YG1042 with this dose range (with and without S9) due to limited sample.

We performed the first experiments with the drinking-water samples in strains TA98 and TA100 without S9 using the same doses used for the river-water samples; we did not use some of the lower doses for the repeat experiments. We performed a final experiment in TA100 using a dose range of 300–1000 mL-eq/plate without S9 because this strain is the most sensitive to the mutagenic activity of drinking water, which is due primarily to disinfection by-products (Richardson et al., 2007).

We tested river-sediment samples in strains TA98, TA100, YG1041, and YG1042 with and without S9 because these strains have been shown to be the most sensitive for detecting mutagenic activity in aquatic sediments (Chen and White, 2004; Umbuzeiro et al., 2004; Oliveira et al., 2006; Aouadene et al., 2008). We performed the first experiments using a dose range of 0.2–10 g-eq/plate, and we performed repeat experiments using a dose range of 0.1–1 g-eq/plate.

3. Results

For those experiments with river-water samples that showed positive results, we calculated linear regressions, and the slopes (mutagenic potencies) are shown in Table 3. We did not detect mutagenic activity in the majority of the river-water samples tested in YG1041 and YG1042 with or without S9 (Table 3). The Lincoln Outfall sample, which was derived by pooling 5, 2.37-L captures, was mutagenic in both YG1041 +/−S9 and YG1042 −S9 only on the third day of sampling. The resulting average mutagenic potencies for YG1041 were 144 rev (L-eq)$^{-1}$ −S9 and

Table 2
Sampling information for river water.

Site	Location	Date/time	Conductivity (μS)	pH	Velocity (ft/s)
Salmon Stream Lake (Upstream)	N45°43′28.4″W068°29′0.7″ ± 9 ft water depth 2.9 m	07/30/09 02:50 pm	74	No data	None
	N45°43′28.4″W068°29′0.7″ ± 9 ft water depth 3.0 m	08/03/09 11:01 am	73	3.78	None
	N45°43′28.4″W068°29′0.7″ ± 9 ft water depth 3.0 m	08/05/09 10:58 am	78	7.63	None
Lincoln (Outfall)	N45°22′32.6″W068°30′46.1″ ± 11 ft water depth 2.9 m	07/30/09 01:15 pm	~400	6.46	0.79
	N45°22′32.1″ W 068° 30′ 45.9″ ± 11 ft water depth 3.1 m	08/03/09 09:30 am	~270	4.26	1.20
	N45°22′32.1″W068°30′45.9″ ± 11 ft water depth 3.0 m immediately downstream of outfall of Lincoln Paper and Tissue Mill	08/05/09 09:26 am	~370	7.13	Slack
W. Enfield (Downstream)	N45°15′03.6″W068°38′51.4″ ± 14 ft water depth 6 m	07/30/09 09:55 am	46	6.57	0.87
	N45°15′03.6″W068°38′51.4″ ± 14 ft water depth 6 m	08/03/09 08:27 am	45.5	6.44	1.25
	N45°15′03.6″W068°38′51.4″ ± 14 ft water depth 6.1 m	08/05/09 08:33 am	45.5	6.75	1.19

Table 3
Summary of mutagenicity (rev/L-eq) of 3 samples of river water from each of 3 sites.

Strain	Experiment	S9	Sampling date and site								
			Salmon Stream Lake (upstream)			Lincoln (outfall)			W. Enfield (downstream)		
			7/30/09	8/03/09	8/05/09	7/30/09	8/03/09	8/05/09	7/30/09	8/03/09	8/05/09
YG1041	1	–	N[a]	N	N	N	N	180	N	N	N
		+	N	N	N	N	N	227	N	N	N
	2	–	N	N	N	N	N	108	N	N	N
		+	N	N	N	N	N	192	N	N	N
YG1042	1	–	N	N	N	N	N	179	N	N	N
		+	N	N	N	N	N	N	N	N	N

[a] N = not mutagenic.

Table 4
Summary of mutagenicity (rev/g-eq) of composite-river sediments from 4 sites.

Strain	Experiment	S9	Sampling site			
			Salmon Stream Lake (upstream)	Lincoln (outfall)	W. Enfield (downstream)	Indian Island
TA98	1	–	N[a]	N	N	N
	2	+	N	N	N	N
		+	N	N	N	N
TA100	1	–	N	N	N	N
	2	–	N	N	N	N
		+	N	N	N	N
YG1041	1	–	N	N	N	96
		+	276	N	N	N
	2	–	N	N	N	203
YG1042	1	–	N	N	N	N
		+	N	N	N	N
	2	–	333	N	N	314

[a] N = not mutagenic.

210 rev (L-eq)$^{-1}$ +S9. The same pooled sample was mutagenic in strain YG1042. The other two sampling days from the Outfall and the other sampling sites (Salmon Stream Lake and W. Enfield) were negative in both strains and S9 conditions. Blank XAD samples were not mutagenic (data not shown).

For those experiments with the river sediments that showed positive results, we calculated linear regressions, and the slopes (mutagenic potencies) are shown in Table 4. Results from sediment samples tested in TA98, TA100, YG1041, and YG1042 with and without S9 were mostly negative (Table 4). Positive results for this group of samples were found for Salmon Stream Lake (Upstream/Control) samples in YG1041 +S9 and YG1042 −S9, which gave an average of 276 and 166 rev (g-eq)$^{-1}$, respectively. The Indian Island sample was mutagenic in YG1041 −S9 (149 rev (g-eq)$^{-1}$) and YG1042 −S9 (157 rev (g-eq)$^{-1}$). Mutagenic potencies for the sediment samples in all strains ranged from 95.5 to 332.5 rev (g-eq)$^{-1}$ (Table 4). Solvent blanks were not mutagenic (data not shown).

For those experiments with drinking water that showed positive results, we calculated linear regressions, and the slopes (mutagenic potencies) are shown in Table 5. The drinking-water samples from all 3 sampling days were mutagenic in TA98 −S9, with an average mutagenic potency in TA98 −S9 of 198 rev (L-eq)$^{-1}$. Samples from day 8/03/09 were positive in TA100 −S9. The average mutagenic potency for TA100 −S9 was 476 rev (L-eq)$^{-1}$. Blank XAD samples were not mutagenic (data not shown).

Table 5
Summary of mutagenicity (rev/L-eq) of 3 samples of drinking water.

Strain	S9	Experiment	Sampling date		
			7/30/09	8/03/09	8/05/09
TA98	–	1	217	195	182
TA100	–	1	N[a]	425	N
	–	2	IS[b]	793	N
	–	3	IS	211	IS

[a] N = not mutagenic.
[b] IS – insufficient sample to test.

4. Discussion and conclusions

The purpose of this portion of the RARE Penobscot River Exposure Assessment study was to determine the mutagenic activity in the Penobscot River water, the river sediment, and the municipal drinking water of Indian Island, Maine, U.S.A. As discussed in the Introduction, the *Salmonella* mutagenicity assay is the bioassay of choice for determining the mutagenicity of environmental samples (Claxton et al., 2010). A positive result suggests the possibility that the water or sediment may contain potential carcinogens; a negative result less so (Zeiger, 1998).

We chose the strains and metabolic activation condition (with or without S9) based on the sensitivities of the strain/S9 combinations for each type of extract as indicated from previous studies. Strain TA100 −S9, which detects direct-acting mutagens that induce base-substitution mutations in DNA, has been used frequently when testing chlorinated drinking water and is the most sensitive strain for this purpose (DeMarini et al., 1995). Strain TA98 detects agents that induce frameshift mutations and has been used with river water and sediments (Chen and White, 2004; Ohe et al., 2003, 2004). Surface-water samples and sediments were also tested with YG1041 and YG1042, which express elevated levels of both nitroreductase and acetyltransferase activity and are used for the detection of mutagenicity in environmental samples when nitroarenes and aromatic amines may be present (Claxton et al., 2010).

Our Penobscot River-water samples were not mutagenic other than those at the outfall on one sampling date (Table 4). This suggests the possibility that the observed mutagenicity may have been due to mutagens in the outfall on that day. We captured a second set of samples (data not shown) when river-water levels were lower than the initial sampling period to see if the river volume was affecting the results. That sample set consisted of surface water from the outfall and downstream locations and were tested in YG1041 and YG1042 with and without S9; all were negative (data not shown).

We compared our river-water results to the rankings identified in a compilation of surface-water-quality monitoring (Umbuzeiro et al., 2001) and a review of surface-water-mutagenicity studies (Ohe et al., 2004). Ohe et al. (2004) developed a ranking scheme based on data from the non-metabolically enhanced TA strains and concluded that surface-water samples with potencies <150 rev (L-eq)$^{-1}$ had low mutagenic potency; they suggested that samples with potencies >5000 rev (L-eq)$^{-1}$ would be considered as having high mutagenic potencies. Our results were negative in the

TA strains, and our positive results in the metabolically enhanced YG strains (average 177 rev $(L\text{-eq})^{-1}$) suggested to us that these samples had low mutagenic potencies. Although untreated effluent from paper-dye manufacturing can be highly mutagenic and produce mutagenic activity in the receiving river (Umbuzeiro et al., 2004), we found that river-water samples taken on only one day at the outfall were mutagenic, and that mutagenicity was low.

The mutagenic potencies for the sediment samples tested with TA98, TA100, and YG1041 with and without S9 were also negative or low relative to other sediments (Umbuzeiro et al., 2004; Chen and White, 2004; Aouadene et al., 2008) for the classes of compounds that this assay detects. As noted in Table 4, the positive sediment samples were from Salmon Stream Lake (upstream) and Indian Island. The average mutagenic potencies of the sediment extracts (244 rev $(g\text{-eq})^{-1}$) in metabolically enhanced strains (YG1041 and YG1042) were less than those reported with these strains for sediments from Brazil (Umbuzeiro et al., 2004) or France (Aouadene et al., 2008); those studies reported rev/g-eq values of 2900–51 000 from Brazil and 98–842 from France. The average mutagenic potency of the PIN sediments (244 rev $(g\text{-eq})^{-1}$) in metabolically enhanced strains (YG1041 and YG1042) was typical of those from urban/industrial areas based on data from non-metabolically enhanced strains (TA98 and TA100), which average ~150 rev $(g\text{-eq})^{-1}$ (Chen and White, 2004). For comparison, sediments from remote regions or heavily contaminated regions have potency values in non-metabolically enhanced strains of 10 or >10 000 rev $(g\text{-eq})^{-1}$, respectively (Chen and White, 2004). Thus, the river-sediment samples from the PIN, which were negative in TA98 and TA100 and had low potencies in YG1041 and YG1042, had a mutagenic potency typical of that from urban/industrial areas as described in the literature.

The drinking-water samples exhibited negative or low mutagenic potencies for the classes of compounds that this assay detects relative to drinking-water samples from Ohio and Louisiana (DeMarini et al., 1995; Schenck et al., 1998) and Japan (Takanashi et al., 2009). Currently there is no comprehensive review of the mutagenic potencies of drinking waters from around the world other than from Japan (Takanashi et al., 2009). Nonetheless, compared to samples from Japan and elsewhere in the U.S., the PIN drinking water had a low mutagenic potency. The average mutagenic potency for the positive drinking-water samples in this study was 337 rev $(L\text{-eq})^{-1}$, with most samples giving negative results. For comparison, Takanashi et al. (2009) found an average mutagenicity of 1,100 rev $(L\text{-eq})^{-1}$ among 179 water samples from 17 sampling sites located from Hokkaido to Kagoshima Prefecture, Japan. Compared to the potencies reported in other studies (DeMarini et al., 1995; Schenck et al., 1998; Takanashi et al., 2009), the average mutagenic potency of the drinking-water samples (337 rev $(L\text{-eq})^{-1}$) was lower than typical drinking waters described in the papers above, which are ~1000 rev $(L\text{-eq})^{-1}$. As noted earlier, the drinking water of the PIN is chlorinated. However, because groundwater generally has lower levels of organics than does surface waters, then drinking water made from ground water might be expected to have low mutagenic activity compared with drinking water made from surface water.

The Penobscot River is a valuable resource to the Penobscot Indian Nation and has played a major role in their cultural traditions of hunting and fishing. Any threat of contamination to the river is a concern for tribal members. There have been improvements to the water quality as shown in an Agency for Toxic Substances and Disease Registry's (ATSDR) review of tissue samples from fish caught in the Penobscot River near the town of Lincoln, Maine (upstream from Indian Island) spanning 1988–2003 (Williams and Cseh, 2007). These samples showed a slight decrease in the toxic-equivalency-quotient concentrations of dioxins and furans, but a slight increase in the levels of methyl mercury (Williams and Cseh, 2007). This may be due in part to some changes in the processes of the paper and tissue mill (U.S. EPA, 2007). However, there are fish advisories in place for the Penobscot River near Lincoln for dioxins and PCBs and throughout the river for mercury regarding fish consumption limits based on findings by the Maine Bureau of Health and the PIN. The ATSDR (2014) analysis performed as part of this RARE study was in agreement with these advisories.

Our findings in this survey study of the PIN show that the surface water, sediment, and drinking-water samples evaluated here are either not mutagenic or have low mutagenic potencies. Thus, the environmental samples evaluated here can be inferred to contain no or low levels of environmental mutagens such as PAHs, aromatic amines, heterocyclic amines, or nitroarenes, which are readily detectable by the strains of *Salmonella* used here. However, the ATSDR (2014) survey showed that other contaminants, such as mercury and dioxins, are elevated in some aquatic species, limiting the advisable amounts that these species should be consumed per month. This study is the first to evaluate the mutagenicity of environmental media in a tribal nation in the U.S., and the results indicated that the river water, river sediment, and drinking water had little or no mutagenic activity.

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