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Cancer Risk Assessment of Polycyclic Aromatic Hydrocarbon Contaminated Soils Determined Using Bioassay-Derived Levels of Benzo[*a*]pyrene Equivalents

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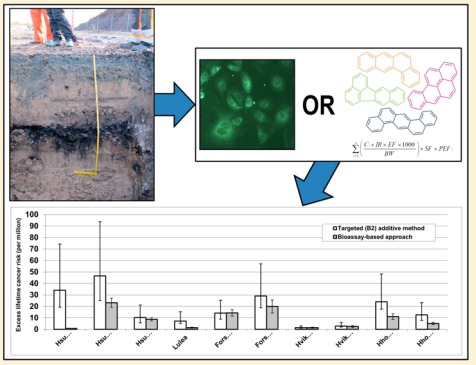
ABSTRACT: Here we evaluate the excess lifetime cancer risk (ELCR) posed by 10 PAH-contaminated soils using (i) the currently advocated, targeted chemical-specific approach that assumes dose additivity for carcinogenic PAHs and (ii) a bioassay-based approach that employs the in vitro mutagenic activity of the soil fractions to determine levels of benzo[*a*]pyrene equivalents and, by extension, ELCR. Mutagenic activity results are presented in our companion paper.[1] The results show that ELCR values for the PAH-containing fractions, determined using the chemical-specific approach, are generally (i.e., 8 out of 10) greater than those calculated using the bioassay-based approach; most are less than 5-fold greater. Only two chemical-specific ELCR estimates are less than their corresponding bioassay-derived values; differences are less than 10%. The bioassay-based approach, which permits estimation of ELCR without a priori knowledge of mixture composition, proved to be a useful tool to evaluate the chemical-specific approach. The results suggest that ELCR estimates for complex PAH mixtures determined using a targeted, chemical-specific approach are reasonable, albeit conservative. Calculated risk estimates still depend on contentious PEFs and cancer slope factors. Follow-up in vivo mutagenicity assessments will be required to validate the results and their relevance for human health risk assessment of PAH-contaminated soils.



INTRODUCTION

Industries involved in the production of manufactured gas (also known as coal gas or town gas), coking operations, and wood preservation facilities generate or use coal tar and/or coal-tar creosote. Improper disposal and release of coal tar and creosote has resulted in an abundance of contaminated land at or nearby these industrial sites. The contaminated areas contain complex mixtures of hundreds of chemicals, including a range of polycyclic aromatic hydrocarbons (PAHs) and related polycyclic aromatic compounds (PACs) (e.g., oxygenated PAHs, and O-, N- and S- heterocyclic compounds).[2] Several PAHs are known or suspected human carcinogens,[3] thus human health risk assessment (HHRA) of PAH-contaminated sites generally includes an assessment of excess lifetime cancer risk (ELCR) for a given level of exposure. The results of these risk assessments drive custodial decisions and risk management activities, including access restriction, prioritization for remediation, and determination of the suitability of the sites for subsequent agricultural, residential, commercial, or industrial use. The primary route of exposure for PAHs at contaminated sites is generally assumed to be via nondietary ingestion of PAHs adsorbed to soil particles.[4]

Complex mixture risk assessment is by no means a simple task. Some researchers and governmental agencies argue that insufficient knowledge regarding the interaction(s) of PAHs in mixtures, coupled with the presence of hitherto unidentified hazardous compounds in the mixtures necessitate the development and implementation of HHRA methodologies that involve biological (i.e., hazard) assessment of the whole mixture. However, difficulties related to toxicity assessment of complex materials such as contaminated soil have led to the development of HHRA methodologies that focus on a small number of targeted substances that have been highlighted by governmental regulatory agencies for concern and control.

In 2000, the United States Environmental Protection Agency (U.S. EPA) published a guidance document for conducting HHRAs of chemical mixtures,[5] and in 2010, Health Canada published an analogous "Guidance on Human Health Detailed Quantitative Risk Assessment for Chemicals (DQRA)".[6] These documents provide guidance for HHRA of contaminated sites, including federally owned contaminated sites in Canada, most of which contain complex mixtures of toxic substances. Both

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Table 1. Characteristics and Location of Contaminated Sites Investigated (Reproduced with Permission from Lemieux et al., 2008,[13] SETAC Press)

location	industry	period of operation	known pollutants	sampling depth	soil type	LOI[a](%)
Holmsund-1	wood preservation	1943−1983	creosote, CCA[b], zinc	20−30 cm	sandy till	9.8
Holmsund-2	wood preservation	1943−1983	creosote, CCA, zinc	10−20 cm	sandy till	6.9
Holmsund-3	wood preservation	1943−1983	creosote, CCA, zinc	10−20 cm	sandy till	2.7
Luleå	coke production	[c]	PAH, arsenic	top soil	sediment	13
Forsmo-1	wood preservation	1933−1950	creosote, CCA	2−18 cm	fine sand	2.6
Forsmo-2	wood preservation	1933−1950	creosote, CCA	0−10 cm	fine sand	13
Hässleholm-1	wood preservation	1946−1965	creosote, CCA, zinc	40 cm	coarse sand	6.8
Hässleholm-2	wood preservation	1946−1965	creosote, CCA, zinc	40−60 cm	coarse sand	2.2
Husarviken-1	gas work	1893−1972	coal tar, heavy metals, cyanide	see footnote[d]	sand	19
Husarviken-2	gas work	1893−1972	coal tar, heavy metals, cyanide	see footnote[d]	sand	12

[a]Loss on ignition (measure of total organic content), determined by heating samples at 130 °C overnight and then at 550 °C for 2 h. [b]Chromated copper arsenate. [c]Facility still in operation at the time of sampling, start unknown. [d]Not known.

agencies currently advocate and employ similar strategies for estimating the ELCR attributable to complex PAH mixtures, including (i) evaluation of the risk attributable to the actual mixture of concern (i.e., the PAH-contaminated soil), (ii) evaluation of the risk attributable to a *sufficiently similar* mixture, or (iii) evaluation of the risk attributable to a small number of targeted PAHs in the mixture (e.g., the 16 PAHs highlighted by the U.S. EPA[7]), and application of an assumption of additivity to calculate total risk as the sum of the incremental contributions from each targeted PAH.[5,6] Methods currently advocated by Health Canada and the U.S. EPA focus on only 7−8 PAHs that have been highlighted as known, probable, or possible human carcinogens (i.e., BaP, dibenz[*a*,*h*]anthracene, benz[*a*]-anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]-fluoranthene, benzo[*ghi*]perylene, indeno[*1*,*2*,*3-c*,*d*]pyrene[3,4,8]). Analogous approaches are recommended by several other countries (e.g., Sweden, the Netherlands, the U.K.), as well as the World Health Organization's International Programme on Chemical Safety (WHO/IPCS).[9] Some jurisdictions advocate, where possible, inclusion of a broader range of PAHs and PACs in HHRAs and concomitant regulatory decisions.[10−12]

Although contaminated site risk assessment, and any subsequent regulatory decisions, should be based on the risk attributable to the actual mixture of concern, assessment of the actual PAH-contaminated material, or even a sufficiently similar mixture of targeted PAHs in the material, is rarely practical. Thus, the aforementioned targeted, chemical-specific approach (iii, above) is most often employed; the incremental contributions of each known (i.e., targeted) carcinogen in the mixture are assumed to be additive, and the total risk is equal to the sum of the incremental risks. Such an approach does not require any direct measurements of hazard for the actual material being evaluated (i.e., the PAH-contaminated soil), but rather, the approach employs chemical analyses to determine the concentrations of the targeted PAHs, applies potency equivalency factors (PEFs) to convert the concentrations of each of the targeted PAHs in the mixture to an equivalent amount of the reference carcinogen benzo[*a*]pyrene (i.e., BaP equivalents), and calculates the total quantity of BaP equivalents in the material as the sum of the contributions from each targeted, carcinogenic PAH. The oral cancer slope factor for BaP can then be employed to calculate the total estimated ELCR posed by the contaminated material at a given site. The choice of PEF for each of the targeted PAHs, the cancer slope factor, and the assumptions regarding the frequency and duration of exposure, all of which may vary across jurisdictions, all influence the magnitude of the calculated ELCR.

In an earlier study,[13] we employed the Salmonella reverse mutation assay (i.e., Ames test) to assess the mutagenic activity of organic extracts of PAH-contaminated soils, and moreover, we used the calculated mutagenic potencies for each soil and a novel bioassay-based approach (i.e., the mutagenic potency ratio or MPR method) to derive an estimate of ELCR. This *bioassay-based approach* to HHRA does not assume additivity of targeted PAHs but rather estimates risk using bioassay-derived levels of BaP-equivalents, which are calculated using the measured mutagenic potency of the mixture and its ratio to BaP potency. A comparison of risk estimates derived using the bioassay-based approach to those derived using the targeted, chemical-specific approach suggested that current risk assessment methods may be underestimating the risks posed by the PAH-containing fraction of contaminated soils. Moreover, mutagenic activity assessment of semipolar aromatic fractions of soil organic extracts suggested that more polar compounds, which remain largely unidentified and are not ordinarily included in the risk assessment process, may pose additional risk. Although interesting and pertinent to regulatory evaluations and decision-making, these results were based on measurements obtained using the Salmonella reverse mutation assay. The assay system permits reductive bacterial metabolism and employs an exogenous metabolic activation system derived from the livers of Aroclor-induced rats for oxidative metabolism. As such, it is renowned for its sensitivity to some PACs. Thus, our evaluation of targeted, chemical-specific HHRA methods for PAH-contaminated soils based solely on Salmonella mutagenic potency may not be generally applicable to the determination of risk for mammalian systems.

We have now assessed the mutagenic activity of organic extracts from the same PAH-contaminated soils using a transgenic mammalian cell line (i.e., the Muta^{TM}Mouse FE1 cell line) that has an endogenous capacity to convert PAHs such as BaP to DNA-reactive metabolites,[14,15] and the reader is referred to the companion paper[1] for details of the mutagenic activity results. The current study continues our evaluation of the assumption of additivity routinely used for risk assessment of complex PAH mixtures. Specifically, we (i) employ mutagenic activity data from the *lacZ* transgene mutation assay in Muta^{TM}Mouse FE1 cells, and the aforementioned bioassay-based approach, to derive estimates of ELCR for nondietary ingestion of PAH in contaminated soils, and (ii) compare the bioassay-based risk estimates to those calculated using the targeted, chemical-specific approach currently advocated by the U.S. EPA and Health Canada.

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MATERIALS AND METHODS

Soils. Ten soil samples obtained from PAH-contaminated sites in Sweden were analyzed (Table 1). These sites include three wood preservation sites (Holmsund, Forsmo and Hässleholm), one manufactured gas plant site (Husarviken), and one coke oven site (Luleå). Detailed site information and the results of chemical analyses can be found in Lemieux et al. (2008).[13]

Organic pollutants were extracted from each of the 10 contaminated soils using accelerated solvent extraction on an ASE200 (Dionex, Sunnyvale, CA), and the extracts were fractionated into nonpolar neutral and semipolar aromatic soil fractions on open silica gel as described in the companion paper[1] and in Lemieux et al. (2008).[13] Prior validation of the fractionation protocol confirmed that homocyclic, unsubstituted PAHs (including the U.S. EPA priority PAHs), alkyl-PAHs, and O- and S-heterocyclic compounds are contained in the nonpolar neutral soil fraction, whereas the semipolar aromatic soil fraction contains oxygenated PAHs, nitroarenes, and aromatic amines, including N-heterocyclic compounds.[16] Chemical analyses of selected PAHs and PACs were carried out by gas chromatography−mass spectrometry as previously described.[13,16]

Cancer Risk Assessment. The ELCR for an adult exposed to each of 10 PAH-contaminated soils was evaluated using two methods: (1) the targeted, chemical-specific approach currently advocated by regulatory agencies such as Health Canada and the U.S. EPA[5,6,10] and, (2) a bioassay-based approach, originally described in Lemieux et al. (2008).[13] Note that in our previous publication this approach is referred to as the mutagenic potency ratio (MPR) approach.

Targeted, Chemical-Specific Approach. The targeted, chemical-specific approach (i.e., B2 additive) employs the soil concentrations of a targeted group of known PAHs, a soil ingestion rate, exposure factors, body weight, an oral slope factor for BaP, and PEFs to calculate risk according to eq 1. The targeted PAHs included are those currently identified by the U.S. EPA as probable human carcinogens (i.e., B2 carcinogens BaP, dibenz[*a*,*h*]anthracene, benz[*a*]anthracene, chrysene, benzo[*b*]-fluoranthene, benzo[*k*]fluoranthene, benzo[*ghi*]perylene, indeno[*1*,*2*,*3-c*,*d*]pyrene).[8] This *B2 Additive* approach assumes dose additivity to determine the total ELCR for a given soil. This method only allows for risk estimations that relate to selected components of the nonpolar neutral fractions of organic soil extracts (i.e., compounds for which PEFs have been assigned). Contributions related to components of the semipolar aromatic soil fractions are rarely incorporated into risk estimation because the chemical composition of the fraction remains largely unknown, and, with few exceptions, PEFs for PACs have not been determined.[11,12,17]

$$\text{Total Excess Lifetime Cancer Risk} = \left(\sum_{i=1}^{n}\left(\frac{C_i \times \text{IR} \times \text{EF} \times 1000}{\text{BW}}\right) \times PEF_i\right) \times SF \quad \text{for PAHs 1 through } n \tag{1}$$

where C_i refers to the soil concentration of each targeted PAH (μg PAH/g soil) (available in companion work[1]), and IR refers to the soil ingestion rate (mg soil/day). Adults were assumed to consume 20 mg soil per day during the exposure period.[10] The exposure factor (EF) was calculated according to Health Canada recommendations[10] (i.e., 5 days/week, 48 weeks/year, 35 years of exposure, life expectancy of 75 years), and a body weight (BW) of 70.7 kg was assumed.[10] The cancer slope factor (SF) for BaP employed for these analyses was 2.3 per mg BaP/kg BW/day.[18] The PEFs or potency equivalency factors are from CCME (2010).[4] Upper and lower limits of risk estimates were calculated using highest and lowest PEF values, respectively, from the scientific literature.[4,19−27] Excess lifetime cancer risk is expressed as the number of expected cases in excess of 1 in a million (i.e., 10^{-6}).

Bioassay-Based Approach. The bioassay-based approach, which we described previously,[13] employs the experimentally determined mutagenic potencies of each of the soil fractions, as well as the mutagenic potency of BaP to derive risk estimates according to eq 2. Mutagenic potencies are defined as the slope of the linear portion of the concentration−response functions for the induction of *lacZ* transgene mutations in FE1 cells, and they are reported in the companion paper.[1] Rather than using the concentrations of targeted PAHs, and the associated PEFs, this method determines the equivalent concentration of BaP required to elicit the observed response for a selected fraction of a given soil sample and uses this concentration of BaP equivalents and the soil ingestion rate to determine the daily dose of BaP equivalents. Because the method employs a bioassay response for the complex soil fractions to provide a BaP equivalent dose, PEFs and an assumption of additivity are not required.

$$\text{Total Excess Lifetime Cancer Risk} = \left[\left(\frac{\text{Activity}_{\text{soil}}}{\text{Activity}_{\text{BaP}}} \times \text{IR} \times \text{EF}\right) \times \text{BW}^{-1}\right] \times \text{SF} \tag{2}$$

where $\text{Activity}_{\text{soil}}$ is the mutagenic potency of the nonpolar neutral (i.e., PAH-containing) or semipolar aromatic soil fractions measured using the in vitro transgenic mutation assay in FE1 cells (mutant frequency $\times 10^{-5}$/mg dry soil eq./ml), and $\text{Activity}_{\text{BaP}}$ is the mutagenic potency of BaP as measured using the same assay (mutant frequency $\times 10^{-5}$/mg BaP/ml). IR refers to the soil ingestion rate (mg soil/day). Adults were assumed to consume 20 mg soil per day during the exposure period.[10] The exposure factor (EF) was calculated according to Health Canada recommendations[10] (i.e., 5 days/week, 48 weeks/year, 35 years of exposure, life expectancy of 75 years), and a body weight (BW) of 70.7 kg was assumed.[18] For the bioassay-based risk calculations, the aforementioned Health Canada cancer slope factor for BaP was used (i.e., 2.3 per mg BaP/kg BW/day).[18] The 95% confidence intervals of bioassay-derived risk estimates were determined using propagated error values calculated from the standard errors of the mutagenic potencies for BaP and the soil fractions examined, and the appropriate critical values of the *t* distribution (two-sided, $p = 0.05$).[28]

RESULTS

Total measured PAH levels (i.e., ΣPAH24) ranged from 72−9256 μg PAH/g dry soil, with priority PAH levels (i.e., ΣPAH16) from 60 to 8823 μg/g. The levels of carcinogenic (i.e., B2) PAHs ranged from 29 to 1707 μg PAH/g dry soil, which accounted for 18−60% and 19−67% of the total and priority PAHs, respectively. Detailed chemical characterization of PAHs and PACs in the soils evaluated in this study can be found in Lemieux et al. (2008)[13] and the companion paper.[1] As noted, the targeted, chemical-specific approach for risk assessment employs the concentrations of targeted PAHs, PEF values, and a BaP cancer slope factor to calculate estimates of cancer risk according to eq 1. Using this method, the calculated risk (per million) ranged from

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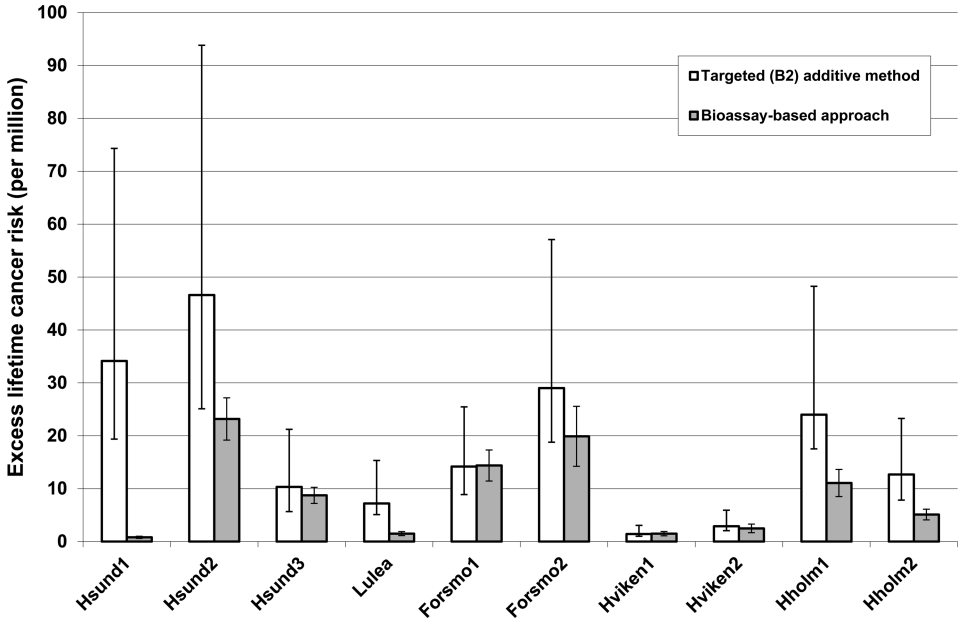

Figure 1. Excess lifetime cancer risk associated with nondietary ingestion of PAH-contaminated soils by a typical adult. Risk was calculated using the targeted, chemical-specific approach (i.e., B2 additive method), as well as the bioassay-based approach described in the text (nonpolar neutral fraction only). Error bars associated with B2 additive values represent minimums and maximums calculated using the lowest and highest PEF values for B2 PAHs, respectively, from the scientific literature.[4,19−27] The error bars for the bioassay-based values represent the composite standard error calculated from the standard errors of the mutagenic potencies for BaP and the nonpolar neutral soil fractions. All risk calculations employed the slope factor for BaP recommended by Health Canada.[16] Hsund = Holmsund, Hviken = Husarviken, Hholm = Häsleholm.

1.4 for Husarviken-1, the least contaminated soil sample, to 46.6 for Holmsund-2, the most contaminated soil sample. In contrast, the bioassay-based approach calculates risk using bioassay response data, and, as such, can be employed for both of the soil fractions investigated (i.e., nonpolar neutral and semipolar aromatic soil fractions). Mutagenic potency values for the nonpolar neutral soil fraction (i.e., the PAH-containing fraction) ranged from 16 to 463 × 10^{-5} mutants/mg soil eq./ml, whereas those of the semipolar aromatic fractions ranged from not detected (i.e., Forsmo-2 and Holmsund-3) to 217 × 10^{-5} mutants/mg soil eq./ml (i.e., Forsmo-1). Details regarding the mutagenic potency of the soil fractions can be found in the companion paper.[1] The mutagenic potency of BaP was 3989 ± 257 mutants/μg BaP/ml, and the bioassay-based ELCR values for the nonpolar neutral (i.e., PAH-containing) fractions (per million) ranged from 0.8 for Holmsund-1 to 23.2 for Holmsund-2.

Figure 1 compares the bioassay-based risk estimates for the nonpolar neutral fractions with those derived using the targeted, chemical-specific approach. The figure shows that the targeted, chemical-specific approach yields risk estimates that are often (i.e., for 8 of 10 soils) higher than those calculated using the bioassay-based approach. The chemical-specific ELCR values range from 1.2-fold greater than the corresponding bioassay-derived values for Holmsund-3 and Husarviken-2, to 42.6-fold greater for Holmsund-1. With the exception of Holmsund-1, all chemical-specific ELCR values are less than 5-fold greater than their corresponding bioassay-derived values, with the geometric mean ratio of chemical-specific ELCR to bioassay-derived ELCR equal to 2.9. More detailed scrutiny of the data used to generate Figure 1 indicates that although the chemical-specific ELCR value generally exceeds the corresponding bioassay-derived value, chemical-specific ELCR values calculated using the aforementioned CCME PEF values[4] exceed the upper 95% confidence limit of the corresponding bioassay-derived values for only five soils (i.e., Holmsund-1, Holmsund-2, Luleå, Häsleholm-1, Häsleholm-2). Moreover, for 4 of these 5 soils (i.e., Holmsund-1, Luleå, Häsleholm-1, Häsleholm-2), even the lower limit of the calculated chemical-specific ELCR values (i.e., determined using the lowest published PEF values) exceeds the upper 95% confidence limit for the bioassay-derived value. In contrast, only 2 of the 10 soils investigated (i.e., Forsmo-1, Husarviken-1) yielded bioassay-based ELCR values that were greater than their corresponding chemical-specific values; moreover, the increases are only 1.5 and 5.4%, respectively. Thus, the chemical-specific ELCR estimates are generally greater than their corresponding bioassay-derived values, and often significantly greater; whereas, for the few instances where the bioassay-derived value is greater, the differences are small (i.e., <10%).

In principle, the bioassay-based approach can be employed for any soil fraction that induces a response in the bioassay employed to assess mutagenic activity. However, because the identities of the components in the semipolar aromatic fraction are not known, and there is no evidence that the putative mutagens in this fraction are mutagenic carcinogens, the use of BaP as a reference compound cannot be systematically defended. Nevertheless, since 8 of the 10 semipolar aromatic fractions yielded a significant mutagenic response in the FE1 assay,[1] and, as noted, several PACs are mutagenic carcinogens, it is reasonable to assert that components in the fraction can contribute to total ELCR. Guarded calculations of BaP equivalents for the semipolar aromatic fractions provides ELCR values greater than 10^{-6} for only two sites (Holmsund-1, Forsmo-1). The values associated with this fraction surpass that of the nonpolar neutral fraction for only one site (i.e., Holmsund-1).

DISCUSSION

This study compares estimates of ELCR calculated using a targeted, chemical-specific approach that focuses on a small

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number of PAHs with those based on an approach that employs a biological response to complex soil fractions (i.e., FE1 mutagenic potency for the nonpolar neutral and semipolar aromatic fractions). This bioassay-based approach, first described in Lemieux et al. (2008),[13] uses the mutagenic potency of complex soil extracts/fractions (i.e., ability to induce *lacZ* transgene mutations in Muta™Mouse FE1 cells) calculate a BaP-equivalent dose and thus does not rely on chemical-specific PEF values or an assumption of additivity.

Our companion paper[1] showed that all 10 nonpolar neutral fractions derived from the sites examined, and 8 of the 10 corresponding polar aromatic fractions induced a significant response in the *lacZ* transgene mutation assay in FE1 cells exposed in vitro. This bioassay response, and an assumption of an empirical relationship between carcinogenic potency and mutagenic potency (discussed below), permitted the use of the bioassay-derived method for estimation of ELCR values for the nonpolar neutral fraction. Estimates for each soils were then compared with ELCR values calculated using the targeted, chemical-specific approach based on the additive responses of seven targeted PAHs (i.e., the U.S. EPA B2 PAHs). The results (Figure 1) show that, if the PEFs and slope factor recommended by Health Canada[4,18] (2.3 per mg BaP/kg BW/day) are employed, then the traditional additive ELCR estimates are generally greater than those derived using the bioassay-based approach (i.e., 8 out of 10 soils). Moreover, even when the bioassay-derived ELCR values were greater, the differences are small (i.e., <10%). This suggests that, despite the fact that the complex soil fractions contain a mixture of known and hitherto unknown PAHs and related compounds, risk estimates based on a small subset of PAHs may yield conservative estimates of ELCR. However, it is important to reiterate that the relationship between the bioassay-derived estimates and the traditional additive estimates will depend on the PEF values employed to determine the concentration of BaP equivalents. Use of the lowest and highest PEF values presented in the scientific literature provided a means for more rigorous comparisons of traditional additive and bioassay-derived ELCR values. The results obtained indicate that for half of the eight soils that yielded higher chemical-specific ELCR values, even ELCR estimates based on the lowest available PEF values were above the upper 95% upper confidence limit of the bioassay-derived values. Thus, the traditional additive ELCR values are generally greater, and for 40% of the soils examined, they are significantly greater.

The choice of cancer slope factor used for the calculation of ELCR can also significantly affect the outcome of the risk assessment and subsequent risk management decisions, and the effect of slope factor choice will affect both the chemical-specific and bioassay-derived ELCR estimates. Cancer slope factors routinely used by various jurisdictions vary by approximately 20-fold (Table 2), and this variability can be attributed to differences in the data used to derive the value (e.g., epidemiological, animal bioassay), and the model used for dose/species extrapolation (e.g., differences in allometric scaling methods).[29] Unfortunately, the studies used to derive cancer slope factors are often older studies that may contain critical weaknesses. This study employed the oral slope factor for BaP recommended by Health Canada (i.e., 2.3 mg BaP/kg BW/day); however, this and several other BaP slope factors are based on the Neal and Rigdon study from 1967,[30] which does not contain sufficient information on exposure duration. Consequently, some jurisdictions (e.g., the Netherlands) are considering methods that do not rely on dose–response analysis and derivation of cancer slope factors. For example, Kroese et al. (2001) discusses the *virtually safe dose* approach, where linear extrapolation from a point-of-departure such as the LOAEL (i.e., the lowest dose level associated with significant tumor response) is used to determine an acceptable dose.[31] Although interesting, this approach yields acceptable substance-specific dose levels, and as such, it cannot be applied to mixtures.

Table 2. Summary of BaP Cancer Slope Factor Values Employed by Different Regulatory Agencies for Assessment of Excess Lifetime Cancer Risk[a]

jurisdiction	cancer slope factor (per mg/kg bw/day)	key study	reference
U.S.A., New Zealand	7.3	(30, 34)	(32, 35)
Canada	2.3	(30)	(18)
WHO Drinking-water Quality	0.46	(36)	(37, 38)
California	9.03	(30)	(33)

[a]All values derived from dose-response data for gastric tumors in mice or rats. For details, see New Zealand Ministry for the Environment (2011)[32] and California Environmental Protection Agency (1997).[33]

The bioassay-based approach employs bioassay-derived potency data for a complex soil component (i.e., non-polar neutral fraction); knowledge of the mixture composition is not required. Thus, in principle, the bioassay-based approach can evaluate the potential health risks posed by chemicals that are not typically included in the targeted, chemical-specific approach used by many governmental organizations. For the soils studied here, both nonpolar neutral and semipolar aromatic soil fractions were mutagenic in the Salmonella reverse mutation assay[13] and in FE1 cells,[1] and the bioassay-based approach can provide an estimate of ELCR posed by mutagenic compounds found in both fractions. However, lack of information regarding the identity, physical-chemical properties, and carcinogenicity of mutagenic components in the semipolar aromatic fraction impedes convincing use of BaP as a reference compound, and by extension, realistic estimation of human ELCR. Nevertheless, it is useful to reemphasize that the components of the semipolar aromatic soil fraction would not be monitored in a conventional risk assessment, despite the fact that this fraction could be expected to contain PACs such as oxygenated PAHs, nitroarenes, and aromatic amines, including N-heterocyclic compounds[39] and nitro-PAH derivatives, some of which are known mutagens and possible human carcinogens (e.g., 3-nitrobenzanthrone[40−42]).

The bioassay-based approach assumes that BaP is an appropriate reference compound for conversion of mutagenic activity values for complex PAH-containing mixtures to chemical equivalents that can be employed for HHRA. Our companion work noted a significant empirical relationship between the mutagenic potency of the nonpolar neutral fractions, and by extension, the calculated level of BaP equivalents and the concentrations of both total measured and priority PAHs. The lack of a correlation between the bioassay-derived levels of BaP equivalents for the semipolar aromatic fraction and PAH level (not shown) confirms that, although interesting, estimation of ELCR for the semipolar fraction cannot be mechanistically defended, and must be interpreted with caution.

The bioassay-based approach also assumes a correlation between mutagenic and carcinogenic potency (i.e., an assumption that the ratio of mutagenic potency values for BaP and the soil extracts is equivalent to the ratio of the

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corresponding carcinogenic potency value). In practice, validating this assumption is not practical because it would require evaluations of the carcinogenicity of each soil extract in a two-year rodent cancer study. Nevertheless, analysis of the empirical relationship between the relative mutagenic potency of five targeted PAHs (i.e., relative to BaP), measured using the *lacZ* mutation assay in Muta^{TM}Mouse FE1 cells, and the corresponding relative rodent carcinogenic potency value, revealed a highly significant relationship (r^2 = 0.98, F ratio = 77.0, $p < 0.004$). In addition, the results published by Hernández et al. (2011) support the contention that mutagenic potency is indeed empirically related to carcinogenic potency when a mutagenic mode of action has been documented. In that study, the authors revealed a strong correlation between mutagenic and carcinogenic potency for 18 carcinogens with a mutagenic mode of action (including BaP).[43] More specifically, the authors examined the correlation between benchmark dose (BMD_{10}) values derived from the in vivo micronucleus assay and in vivo transgenic rodent mutation assay, and those derived from murine carcinogenicity studies.

The traditional (i.e., targeted, chemical-specific) approach to PAH HHRA inherently assumes that the cancer risk of all PAHs present in a mixture are additive. The convention of assuming risk additivity for PAHs is largely accepted because it is understood that carcinogenic PAHs generally act via a common mode of action (i.e., the formation of bulky DNA adducts that contribute to the establishment of mutations and cancer initiation).[5,10] Several studies support the contention that PAHs in mixtures can exhibit additive effects;[13,44] however, there is also evidence to support subadditive phenomena, particularly at higher concentrations or doses[1,45,46] where competition for enzymatic catalysis may be expected. Supra-additive or synergistic effects are also possible, and these are most likely due to metabolic augmentation (e.g., upregulation of CYP isozymes by mutagenic and nonmutagenic PAHs[47,48]). For a more comprehensive discussion regarding factors that control the generation of activated metabolites and their influence on the (geno)toxicity and carcinogenicity of PAH mixtures, the reader is referred to our companion paper.[1] Without comprehensive information about the chemical composition of a mixture, and of the interactions of all components, neither of which are feasible, the assumption of response additivity for a subset of targeted PAHs in a complex mixture is a pragmatic approach for routine assessment of ELCR. Interestingly, although the targeted, chemical-specific approach only focuses on a small subset of PAHs that might be expected in a contaminated soil, the ELCR estimates presented here are generally higher, and largely within an order of magnitude, of those determined using a bioassay-based approach. The chemical-specific approach yielded a value more than an order of magnitude above the corresponding bioassay-derived value for only one site (i.e., Holmsund-1), and the geometric mean ratio of chemical-specific to bioassay-derived ELCR suggests that the former are generally about 3-fold greater than the latter. Moreover, for the two soils where the bioassay-derived ELCR values are greater than their corresponding chemical-specific values, the difference is very small (i.e., 1.5 and 5.4%). It is therefore reasonable to contend that ELCR estimates calculated using the targeted, chemical-specific approach are pragmatic, realistic, and somewhat conservative. Thus, despite the fact that they only focus on a small subset of PAHs, they can be used for sound custodial decisions (i.e., access restriction, site remediation, and reclamation).

The convention of assuming response additivity for PAHs in mixtures is exemplified by the widespread use of PEFs for complex PAH mixture risk assessment. Nevertheless, PEFs, or the related toxic equivalency factors (TEFs), which are broadly used for the risk assessment of polychlorinated dioxin-, dibenzofuran-, biphenyl-containing mixtures,[49] do have their limitations. First, PEFs are usually only available for a small subset of carcinogenic PAHs, restricting mixture risk assessments to effects attributable to those PAHs. Indeed, it is now recognized that some carcinogenic PAHs (e.g., dibenz(*a,l*)pyrene, DB*al*P) are more mutagenic and carcinogenic than BaP,[50−53] and even a small amount of these potent substances in a PAH-contaminated soil could contribute to a substantial increase in estimated cancer risk. However, potent PAHs such as DB*al*P are not included in the U.S. EPA's priority PAH list and, as such, have not traditionally been monitored at contaminated sites or incorporated into risk calculations. Some jurisdictions (e.g., California Environmental Protection Agency, Minnesota Department of Health, Health Canada) are now recommending, where possible, the use of PEFs for other PAHs and PACs, including DB*al*P and potent alkylated PAHs, thus expanding the scope of ELCR assessments for PAH-contaminated sites.[10−12,17] Nevertheless, it should be noted that it may not be practical or desirable to derive and apply PEF values for an extensive list of components identified in complex PAH-containing mixtures. The analyses conducted herein indicates that ELCR values based on only a small subset of PAHs are already conservative; addition of risks attributable to additional PAHs such as those mentioned above will only serve to enlarge the gap between chemical-specific and bioassay-derived values. Moreover, determining the identity of the toxicologically relevant components, and routine quantification of these components in complex matrices undergoing regulatory evaluation, constitutes a significant analytical challenge.

Derivation of PEFs for an extended series of compounds necessitates the use of inadequate bioassay results, adoption of several assumptions, and extrapolation to the suspected target tissue(s) in humans. For example, PEFs are often derived from the results of dermal (skin painting), and/or intrapulmonary installation studies;[54] however, they are routinely employed for HHRA calculations that assume oral exposure (i.e., ingestion as the primary route for contaminated soils). This raises the concern that toxicokinetic differences may affect the suitability of the PEFs for contaminated site risk assessment. Moreover, differences in the PEF values for PAHs recommended by different governmental agencies (for reviews, see Delistraty, 1997[55] and WHO, 1998[56]) only serve to further hamper a generalized interpretation of HHRAs for PAH mixtures. Interestingly, the current Health Canada guidance document for quantitative risk assessment of federal contaminated sites lists 44 PEF values for PAHs and alkylated PAHs;[6,17] however, few site assessments quantify an extensive range of PAHs and related compounds (e.g., alkylated PAHs). The aforementioned recommendations of the Minnesota Department of Health includes PEFs for an extended list of only 19 PAHs, indicating that monitoring of additional PAHs that could constitute a carcinogenic hazard is problematic due to analytical challenges and/or toxicological uncertainty. The U.S. EPA's IRIS (Integrated Risk Information System) Program is currently engaged in an extensive review of the RPF (relative potency factor) approach for PAH mixtures (see http://cfpub.epa.gov/ncea/iris_drafts/recordisplay.cfm?deid=194584); however, the results have yet to be released.

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It is important to note that only the nondietary ingestion route was evaluated in the current study, because it is considered the primary route of exposure for soil-bound PAHs and other PACs at contaminated sites. In an actual risk assessment, other routes of exposure would also be considered (e.g., inhalation of airborne soil particles); however, such an evaluation is beyond the scope of the current study. Moreover, the analyses only assessed risk for a typical adult, and other receptors with different exposure factors (e.g., construction workers) would also be considered in a more comprehensive assessment.[6] Assumptions include exposure levels (e.g., soil ingestion rates) for various receptors, the degree to which contaminants are absorbed by the human gastrointestinal tract (i.e., bioaccessibility), and the assumption that the risks posed by mixture components are additive.[6] Moreover, different international jurisdictions base regulatory decisions on different thresholds for acceptable risk. Even provincial jurisdictions within Canada differ in their HHRA methods and the levels of acceptable cancer risk.[57] It should also be noted that the current study only evaluated ELCR attributable to targeted PAHs or mutagens in the organic fractions examined. It is not unreasonable to assert that other components in the contaminated soils could contribute to carcinogenic risk. Although it would prove interesting to conduct an analogous in vivo study that investigated the effects of unaltered soils ingested by experimental animals, rodents would not be physiologically appropriate for studies to be interpreted in an HHRA context. Porcine models have been offered as physiologically relevant alternatives (Bode et al. 2010); however, the costs of porcine studies are generally prohibitive.[58]

In contrast to the results of this study, our previous analyses showed that bioassay-based risk estimates obtained using Salmonella reverse mutation assay results for the nonpolar neutral fractions were much *higher* than estimates obtained using the targeted, chemical-specific approach.[13] In light of the current results, and the aforementioned sensitivity of the Salmonella-based mutagenicity assessment system, it is reasonable to contend that the earlier results may not be generally applicable to mammalian systems. Oversensitivity of the bacterial test system, at least for the complex soil fractions examined here and in our earlier study, can likely be attributed to the inherent ability of Salmonella to metabolically activate selected PACs (e.g., nitroarenes and aromatic amines), as well as the extraordinarily high P450-mediated enzymatic capacity of the exogenous activation system commonly employed (i.e., CYP1A1, CYP1A2, CYP2B1 activities). The enhanced sensitivity will contribute to an inflated measure of mutagenic potency relative to BaP, a concomitant higher level of BaP equivalents, and ultimately, a higher estimate of risk. The bioassay-based risk estimates derived here, which are based on results from metabolically competent mammalian cells, are indeed 1−2 orders of magnitude lower than those calculated using the Salmonella assay results. We contend that the current risk estimates represent a more realistic evaluation of human health risk compared to those derived in our previous study.

The results of the current study show that for complex mixtures of chemicals present at PAH-contaminated sites, estimates of ELCR determined using existing risk assessment strategies (i.e., a targeted chemical-specific approach) are generally greater than those determined using a bioassay-based approach. Although the assumption of dose additivity inherently assumed by the targeted chemical-specific approach may be simplistic in light of the known toxicokinetic and toxicodynamic complexities for different groups of substances, different dose levels, and different routes of exposure, for the soils evaluated in this study, it does appear to provide risk estimates that are conservative relative to those derived using an approach based on the biological activity of the complex mixture of PAHs in the soil matrix. It is important to note that it is not our intention to suggest that the bioassay-based approach supplant the traditional, chemical-specific additive approach that relies on well-recognized, accurate methods for chemical analyses. Rather, the bioassay-based approach employed herein, which does not require a priori knowledge regarding the identity and levels of the putative soil toxicants or assumptions regarding the toxicological behavior of these compounds in a mixture, provided a convenient means to evaluate the chemical-specific approach. Although it has proved to be a useful tool and, indeed, has revealed that chemical-specific assessments based on only seven B2 PAHs are likely conservative, routine application of a bioassay-based approach for evaluation of complex PAH-contaminated matrices (e.g., soil) would likely be impractical.

In this work, we have employed a mammalian cell bioassay-based approach to critically evaluate the HHRA method for PAH-contaminated matrices based on additivity of a small number of components, and we have shown that the targeted chemical-specific approach provides higher oral risk estimates that are generally well within an order of magnitude of those determined using a bioassay-based approach. Moreover, consistent with our previous work,[13] preliminary calculations suggest that additional hazard and risk may be attributable to more polar compounds, such as oxygenated PAHs and nitroaromatic compounds that are known to be present in PAH-contaminated soils. Lundstedt et al. (2007) recommend monitoring of oxygenated PAHs at contaminated sites such as those studied here;[59] however, incorporation of these compounds into a risk assessment would require compound-specific PEF values, and these are generally not available. Although the current work employed a metabolically competent mammalian cell line, rigorous evaluation of the additive, chemical-specific approach for HHRA of complex PAH-containing mixtures will require in vivo analyses in selected tissues following oral exposure. Indeed, follow-up investigations, which are already underway, are employing in vivo mutagenicity assessment in selected Muta^{TM}Mouse tissues to extend the current evaluation of the bioassay-based approach. Additional assessments of nonpriority PAHs, which are also underway, will contribute to a comprehensive, objective evaluation of cancer risk assessment methods that are routinely applied to mixtures of PAHs and related compounds.

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Notes

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