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Effect based monitoring of seasonal ambient air exposures in Australia sampled by PUF passive air samplers

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Abstract

There has been relatively little bioanalytical effect based monitoring conducted using samples derived from polyurethane foam (PUF) passive air samplers. Combining these techniques may provide a more convenient and cost effective means of monitoring the potential for biological effects resulting from exposure to complex mixtures in a range of scenarios. Seasonal polycyclic aromatic hydrocarbon (PAH) levels were monitored at sites around Australia using direct chemical analysis. In addition, both indirect acting genotoxicity (umuC assay) and aryl hydrocarbon receptor (AhR) activity (chemically activated fluorescent gene expression [CAFLUX assay]), which are effects potentially relevant to subsequent carcinogenesis for these compounds, were measured. The levels of PAHs as well as genotoxicity and AhR activity were all higher in winter compared to summer and for sites in urban capital cities compared to other locations. Statistically significant relationships were found between the levels of PAHs and both genotoxicity and AhR activity. The dominant contributors to the total AhR activity, were found to be for compounds which are not resistant to H_2SO_4/silica gel treatment and were relatively rapidly metabolised that is consistent with a PAH type response. Relative potency estimates for individual PAHs determined for the first time on the CAFLUX assay were used to estimate the proportion of total AhR activity (≤ 3.0%) accounted by PAHs monitored. Observed responses are thus largely due to non-quantified AhR active compounds.

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Supporting Material **Available:** Information on CAFLUX ASSAY and *umu*C ASSAY, Description of sampling sites (Table S1), A summary of parameters used in this study to quantify either exposure or effect (Table S2), Seasonal sampling rates, volume of air sampled and volume of air dosed for effect assessment (Table S3), Average seasonal PAH levels (C_{AIR}, ng m^{-3}), average seasonal ratios and benzo[*a*]pyrene toxic equivalent air concentrations in urban capital cities, regional centres and one rural background site estimated using PUF passive air samplers (Table S4), CAFLUX derived AhR potencies expressed as TCDD equivalent air concentrations (pg m^{-3}) at the 50% TCDD max effect level after 48 and 72 hour incubation periods in summer and winter (Table S5), CAFLUX derived relative potency estimates for individual PAH with respect to TCDD for the 5 and 20% effect level in different incubation periods (Table S6), Location of passive air sampling sites (Figure S1), Dose response curves for the growth and induction ratios of 6-nitrochrysene without metabolic activation (-S9) on the *umu*C genotoxicity assay (Figure S2), Seasonal direct acting genotoxicity (-S9) expressed as 6-nitrochrysene equivalent air concentrations (6-nCHY Eq_{BIO}, ng m^{-3}) derived from PUF passive air samplers for urban capitals, regional centres and a rural background location (Figure S3). This information is available free of charge via the Internet at http://www.atmospolres.com.

Keywords

Passive air sampling; Polyurethane foam (PUF); Polycyclic aromatic hydrocarbons; Aryl hydrocarbon receptor activity; Genotoxicity

1. Introduction

Passive air samplers consisting of polyurethane foam (PUF) (Shoeib and Harner 2002) have been used to monitor a broad range of semivolatile organic chemicals in ambient air. Polycyclic aromatic hydrocarbons (PAHs) are a class of hazardous air pollutants which can be monitored using these PUF samplers (Jaward et al., 2004). Higher levels of PAHs are typically observed in urban areas during winter since the dominant sources for these compounds are incomplete combustion processes (vehicular emissions and wood smoke) (Prevedouros et al., 2004). Carcinogenicity is the critical health endpoint for assessing the risk of exposure to PAHs in air (NEPC, 2003). While approximately five hundred individual PAHs have been identified in ambient air, relatively few of these compounds are routinely monitored and standard or guideline values are typically only available for specific marker compounds such as benzo[a]pyrene (Bostrom et al., 2002).

A range of studies have demonstrated relationships between PAH exposures and effects such as genotoxicity (Binkovda et al., 2003; Brits et al., 2004; Skarek et al., 2007a; Skarek et al., 2007b), aryl hydrocarbon receptor (AhR) activity (Arrieta et al., 2003; Skarek et al., 2007b) and AhR inducible cytochrome P450-1A1 activity (Arrieta et al., 2003; Brown et al., 2005; Cavanagh et al., 2009). Both AhR activity and genotoxicity are potentially related and mechanistically relevant for carcinogenesis. For certain carcinogenic PAHs, metabolic activation by PAH-inducible AhR-responsive cytochrome P450s (i.e. CYP1A1/1B1) to a more DNA reactive form(s) results in PAH-DNA adduct formation (DNA damage) (Shimizu et al., 2000; Matsumoto et al., 2007). Accordingly, many PAHs may be considered complete carcinogens in the sense that a role in both initiation (DNA damage fixed as a mutation) and promotion of carcinogenesis (increased cell growth and proliferation) is possible for these compounds (Bostrom et al., 2002).

The AhR is a ligand-dependent transcription factor whose activation by structurally diverse ligands, including PAHs, dioxin-like halogenated aromatic hydrocarbons (HAHs) and other chemicals, result in a range of effects in vivo including carcinogenesis (Safe, 2001; Janosek et al., 2006). Although there are a wide variety of species- and tissue-specific biological/toxicological effects mediated by AhR ligands, they can be divided into those effects that are either dioxin-like or non-dioxin-like. Dioxin-like effects are exhibited by high affinity HAH ligands which, when compared to PAHs, are extremely resistant to metabolic degradation and as such they produce sustained induction of gene expression that lead to toxicity (Denison and Heath-Pagliuso, 1998). The dominant sources for dioxins in Australian air according to the National Pollutant Inventory are combustion processes (i.e., backyard incinerators and bushfires).

While relatively few studies (Isidori et al., 2003; Cupr et al., 2006; Slapsyte et al., 2006; Bonetta et al., 2009; Kennedy et al., 2009) have combined passive air sampling with effect based techniques, these samplers potentially provide a more convenient and cost effective means of monitoring the potential for biological effects resulting from exposure to complex mixtures in a range of scenarios. Since the compounds which will be sampled by these PUF samplers are those present predominantly in the vapour phase and in respirable particle size ranges (Chaemfa et al., 2009a), it is likely that effect based estimates derived from these samples will be particularly relevant from an inhalation toxicology perspective.

The main aim of this study was to monitor the seasonal levels of PAHs in Australia as sampled by PUF passive air samplers using effect based monitoring strategies: genotoxicity (umuC assay) and AhR activity (chemically activated fluorescent gene expression [CAFLUX assay]), potentially relevant to subsequent carcinogenesis for these compounds. A concomitant chemical analysis of the levels of predominantly priority pollutant PAHs in ambient air was undertaken. Given the number and differential toxicity of PAH analytes, benzo[*a*]pyrene (B[*a*]P) concentrations and the ambient concentrations of sampled PAHs converted to B[*a*]P toxic equivalent concentrations, were employed as markers of exposure to PAHs.

The significance of non-dioxin like chemicals to total AhR activity was assessed by: testing equivalent proportions of samples which were either treated or not treated with H_2SO_4/silica gel; and also through a comparison of the kinetics of response exhibited by both individual PAHs and the samples with respect to the sustained induction exhibited by the most potent HAH ligand for the AhR (2,3,7,8-tetrachlorodibenzo-*p*-dioxin, TCDD). In order to do this, relative potency estimates were determined for the first time for individual PAHs with respect to TCDD on the CAFLUX assay. Subsequently, the proportion of AhR response which could be accounted for by the specific PAH levels monitored in this study was then quantified.

2. Experimental Methods

2.1. Passive air sampling-seasonal deployments

The PUF samplers and deployment of these samplers in the field have been described in detail previously (Kennedy et al., 2010). Two replicate samples were deployed at each site for either: chemical analysis (CHEM-PUF) that included performance reference compounds (PRCs); or for bioanalytical effect assessments (BIO-PUF), which did not contain PRCs to avoid interference with effect based assessments. Three replicate field blanks were prepared for both CHEM- and BIO-PUF samples. The deployment sites were located at Melville and Bunbury in Western Australia; Gladstone, Mutdapilly and Woolloongabba in Queensland; and Gawler, Hindmarsh and Mt. Gambier in South Australia. Three sites (Melville, Woolloongabba, and Hindmarsh) were located within urban capital cities (Perth, Brisbane and Adelaide, respectively) in each state. Four sites were smaller regional centres within each state (Bunbury, Gladstone, Gawler, Mt. Gambier). One site of low population density (Mutdapilly) in a rural area was also included as a background site. Site locations and descriptions for each location are provided in Supporting Material (SM) Figure S1 and Table S1, respectively.

2.2. PUF sampler processing

PUFs were pre-extracted and extracted (dichloromethane) using accelerated solvent extraction and post-extraction were subjected to gel permeation chromatography (GPC) as described previously (Kennedy et al., 2010). CHEM-PUF samples only were spiked (100 ng sample^{-1}) prior to extraction with a deuterated 7-PAH internal standard mix: D_{10}-fluorene, D_{12}-fluoranthene, D_{12}-benz[*a*]anthracene, D_{12}-benzo[*b*]fluoranthene, D_{12}-benzo[*a*]pyrene, D_{12}-indeno[*1,2,3-c,d*]pyrene, D_{12}-benzo[*g,h,i*]perylene.

Post-GPC, the BIO-PUF extracts were then gently evaporated under nitrogen to a final volume of 120 μL in dimethyl sulfoxide (DMSO) for effect assessment with the bioassays. For the winter BIO-PUF samples only, 10% of the extract was solvent exchanged to hexane and treated overnight with H_2SO_4/silica gel (Kennedy et al., 2009) before rotary evaporation/solvent exchange to ethyl acetate and then evaporation/solvent exchange to a final volume of 12 μL in DMSO for assessment on the CAFLUX. This treatment step was

incorporated in order to quantify the proportion of "total" AhR activity (determined by testing of remaining untreated proportion of each extract in a final volume of 108 μL) that is potentially accounted for by dioxin-like HAHs, which are resistant to this treatment.

CHEM-PUF extracts post GPC were spiked with 20 μL of nonane as a keeper prior to being gently evaporated under purified nitrogen to 100 μL in hexane for chemical analysis. A recovery standard (20 ng D_{12}-benzo[*e*]pyrene), was added prior to chemical analysis. Chemical analysis for PAHs (GC-MS SIM, Kennedy et al., 2007) was performed by Queensland Health Forensic and Scientific Services (QHFSS). Detection limits for PAHs were determined either from the QHFSS reporting limit provided (2 ng) or if detectable amounts were found in the field blanks, from the average amount in the field blank plus 3 standard deviations (FB + 3 SDs). Recoveries were evaluated using deuterated PAHs and averaged 78%. Relative standard deviations (RSD) in the quantification of average amounts accumulated by replicate CHEM-PUF at each site averaged 13% in both summer and winter. Average ambient air concentrations (C_{AIR}, ng m^{-3}) for all PAHs were determined from the amount accumulated (ng) within the exposure period (field blank corrected), and the volume of air sampled (V_A, m^3). PRCs (or depuration compounds) loaded into CHEM-PUF samplers prior to deployment, were used to determine the volume of air sampled in winter. Sampling rates (R_S, m^3 day^{-1}) and hence V_A for summer deployments, were derived from winter sampling rates, using the known influence of differences in temperature (Fuller et al., 1966) and wind speed (Tuduri et al., 2006) on air side mass transfer coefficients. These derivations, the PRC loading into PUF and the determination of R_S (m^3 day^{-1}) in each season for these deployments have been described in detail previously (Kennedy et al., 2010).

2.3. *umu*C (Genotoxicity)

The *umu*C genotoxicity assay (Oda et al., 1985; Reifferscheid et al., 1991) utilises *Salmonella typhimurium* strain TA1535 with an *umu*C′-lac′Z fusion plasmid pSK1002. The assay protocol was adapted from ISO 13829 (ISO, 2000), with further details for the procedure and all calculations (induction ratios and growth) provided in Supporting Material (SM). In brief TA1535/pSK1002 were incubated with serial dilutions of the sample in triplicate (6 point, 2 fold dilution series in 3% DMSO) both with (+S9 rat Aroclor 1254, Moltox) and without (-S9) metabolic activation. Positive controls (4-nitroquinoline-N-oxide [-S9] and 2-aminoanthracene [+S9]), water controls (negative control), bacterial controls and solvent controls (3% DMSO) were run on each plate for both −S9 and + S9 testing (see SM for further details).

Reference compounds (REF) for both +S9 (benzo[*a*]pyrene (B[*a*]P)) and −S9 (6-nitrochrysene, 6-nCHY), were also tested in triplicate (12 point, 2 fold dilution series in 3% DMSO). B[*a*]P is a prototypical indirect acting genotoxic compound (Nakamura et al., 1987). 6-nCHY is found in both diesel exhaust and ambient air particulate matter (Murahashi et al., 1999) at ultra-trace levels (i.e., pg m^{-3}) and as such, levels in Australia have not been published to our knowledge. This reference compound was identified through individual compound testing of a range of nitroarenes available within our laboratories as a suitable and potent reference compound for this assay (refer SM and Figure S2).

$$B[a]P\ Eq_{BIO}\ (+S9)\ or\ 6-nCHY\ Eq_{BIO}\ (-S9)=\frac{EC_{IR1.5\ REF}}{EqV_{A\ BIO\ IR1.5\ SAMPLE}}(ng\ m^{-3}) \quad (1)$$

B[*a*]P (+S9) and 6-nCHY (-S9) equivalent air concentrations (Equation 1) were determined from the ratios of the effective concentrations for the reference compound ($EC_{IR\ 1.5}$, ng

well^{-1}) and the equivalent volume of air dosed (Eq V_A, m^3 $well^{-1}$) for the BIO-PUF derived samples, which induced an induction ratio of 1.5. An induction ratio of 1.5 was only considered significant while the growth of the bacteria remained above 0.5 and the β-galactosidase activity of the sample was twice that of the solvent control. Effective concentrations (IR = 1.5) were interpolated from best-fit non-linear regression curves (hillslope = 1) using Graph Pad Prism 5 software.

2.4. CAFLUX (AhR activity)

The CAFLUX bioassay utilises a recombinant rat hepatoma cell line (H4G1.1c2) that contains a stably transfected AhR-responsive enhanced green fluorescent protein (EGFP) reporter gene plasmid (pGreen1.1) (Nagy et al., 2002; Zhao and Denison, 2004). Cell growth and seeding is described in more detail in SM and the testing protocol has been described in detail previously (Kennedy et al., 2009). In brief for dosing, the media was replaced with 100 μL of non-selective media containing in triplicate the BIO-PUF sample (5 point, 10-fold dilution series, maximum 1% DMSO), the reference compound (TCDD) dilution series (9 point, 2×10^{-7} M – 1×10^{-12} M), a positive control (β-naphthoflavone) and solvent/negative control (1% DMSO) on each plate. Testing from a maximum of 1% DMSO ensures no solvent induction of EGFP since these concentrations of DMSO do not induce a dose response and are not significantly different in response to medium only controls. Solvent control responses were subtracted from both reference compound, individual compound testing (PAHs) and the BIO-PUF sample induced responses.

TCDD equivalent air concentrations (TCDD Eq_{BIO}, pg m^{-3}, Equation 2) for each site were determined from the ratio of the effective concentration of TCDD (pg $well^{-1}$) and the equivalent volume of air (Eq $V_{A\,BIO}$, m^3 $well^{-1}$) dosed for the BIO-PUF derived samples, which induced the 50% TCDD max effect level after 24 hours incubation.

$$TCDD\,Eq_{BIO} = \frac{EC_{50\ TCDD}}{EqV_{A50\ SAMPLE}} (pg\ m^{-3}) \tag{2}$$

Best fit non-linear regressions (hillslope = 1) were used for all samples, with goodness of fit (R^2) averaging 0.99 for both summer and winter testing.

2.5. Relative potency of individual PAH with respect to 2,3,7,8-TCDD on CAFLUX

Relative potencies (REP) for individual PAHs with respect to TCDD (Equation 3) were determined using the ratio of the effective concentrations (M) of TCDD, and individual PAH (Accustandard, 1% DMSO) at the 50% TCDD max effect level after 24, 48 and 72 hour incubation periods.

$$REP = \frac{EC_{50\ TCDD}\,(M)}{EC_{50\%\ PAH}\,(M)} \tag{3}$$

All average REP were determined from a minimum of two independent tests in triplicate using non-linear regression (bottom = 0) with two models (hillslope = 1 vs. variable) compared using the extra sum of squares F test to determine the best-fit.

Individual PAH REP values with respect to TCDD were then used to determine the sum of TCDD equivalent air concentration (ΣTCDD Eq_{CHEM}, Equation 4). These values were

derived for each location by correcting individual PAH levels (C_{AIR}) derived from CHEM-PUF, by the respective REP values.

$$\sum TCDD\,Eq_{CHEM} = \sum REP \cdot C_{AIR}\,(pg\,m^{-3}) \quad (4)$$

Since there are a wide range of parameters used to quantify either exposure or effect in this study, a summary of each is provided in SM Table S2.

3. Results and Discussion

In this study, seasonal PAH levels in Australia were monitored by direct chemical analysis and indirect effect based assessments of exposure were made. The potencies of effect based monitoring potentially relevant to subsequent carcinogenesis for these compounds including genotoxicity (*umu*C assay) and AhR activity (CAFLUX assay), were expressed as equivalent air concentrations for these effects. All equivalent air concentrations are the average of two independent tests (in triplicate) of replicate BIO-PUF samples from each location. The relationships between PAH levels and these equivalent air concentrations were explored and the significance of these PAH levels to the observed AhR activity in particular examined, using relative potencies for individual PAHs determined on the CAFLUX assay.

3.1. Volume of air sampled/dosed

Sampling rates (R_S, $m^3\ day^{-1}$), the total volume of air sampled (V_A, m^3) and the equivalent volume of air available for dosing in bioassays ($V_{A\ BIO}$, m^3 of air per μL of BIO-PUF sample) for the summer and winter sampling periods for each site are provided in SM Table S3.

3.2. Seasonal PAH exposures

Average ambient air concentrations (C_{AIR}, ng m^{-3}) derived from the amounts accumulated in replicate CHEM-PUF samplers for all PAHs in summer and winter at sites across Australia are provided in SM Table S4. Among the PAHs quantified, benzo[*a*]pyrene is classified by the IARC as being a human carcinogen [1], dibenz[*a*,*h*]anthracene as a probable human carcinogen [2A], and benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]-fluoranthene and indeno[*1,2,3-c,d*]pyrene as possible human carcinogens [2B] (IARC, 2008). The ambient concentrations of these PAHs were converted to toxic equivalent benzo[*a*]pyrene air concentrations (B[*a*]P TEQ) using carcinogenic toxic equivalency factors (TEF) (Nisbet and LaGoy, 1992) and summed to derive the sum of benzo[*a*]pyrene toxic equivalent air concentration ΣB[*a*]P TEQ. The average ambient concentration estimates for the PAH exposure markers B[*a*]P and the ΣB[*a*]P TEQ for each site in summer and winter are provided in Table 1. The influence of season on both of these markers was found to be statistically significant with paired t-testing ($p < 0.05$ and 0.03 respectively). The slopes of the relationships (linear regression) between summer and winter data for each marker were 2.1 ± 0.12 and 2.8 ± 0.12 ($p < 0.0001$) respectively. Urban capital cities are key sources for these chemicals and therefore as expected the ambient levels of both indicators of PAH exposure are higher in Perth, Brisbane and Adelaide.

3.3. Seasonal genotoxicity (*umu*C) and AhR activity (CAFLUX)

The potency of air from each location in both seasons was expressed as average equivalent air concentrations (Table 1) for both effects. Genotoxic potency assessed both with (+S9) and without (-S9) metabolic activation on the umuC assay are expressed as B[*a*]P Eq_{BIO} and 6-nCHY Eq_{BIO} (ng m^{-3}) respectively (Equation 1). AhR activity assessed on the

CAFLUX assay was expressed as TCDD Eq_{BIO} (pg m^{-3}) determined at the 50% effect level after 24 hours incubation (Equation 2). Relative standard deviations in the determination of these average equivalent air concentrations from replicate BIO-PUF sample extracts averaged 17% (+S9 winter only) and 31% (-S9) on the *umu*C genotoxicity assay and 31% on the CAFLUX AhR activity assay.

Direct acting (-S9) genotoxicity ranged from < 0.39 ng m^{-3} (Mutdapilly - rural background) to 1.8 ng m^{-3} (Brisbane - urban capital) in summer and from 1.5 ng m^{-3} (Mutdapilly - rural back-ground) to 5.3 ng m^{-3} (Adelaide - urban capital) in winter. Indirect acting (+S9) genotoxicity ranged from < 3.4 ng m^{-3} (Gladstone -regional centre) to 7.5 ng m^{-3} (Perth - urban capital) in summer and from 3.6 ng m^{-3} (Bunbury - regional centre) to 11 ng m^{-3} (Adelaide -urban capital) in winter. Notably, the rural background site Mutdapilly was either not genotoxic in summer (< 7.0 ng m^{-3}) or not significantly different (t-test, $p = 0.30$) from the site with the lowest indirect acting genotoxicity in winter (Bunbury).

Both direct (-S9) and indirect (+S9) acting genotoxicity were higher in winter by an average factor of 3.3 and 1.2 respectively. Indirect acting genotoxicity (+S9) was mostly at less than detectable levels in summer. For direct acting genotoxicity (-S9) then, the significance of this observed seasonal effect (linear regression of summer vs. winter for 6-nCHY equivalent air concentrations) was assessed for the South Australian sites only, since standard deviations were available in both seasons. The slope for this relationship (3.3 ± 0.26) was found to be statistically significant ($p = 0.0062$). Direct acting genotoxic compounds in air include nitrated and oxygenated PAHs which may be both directly emitted or formed in-situ from parent PAH compounds (Atkinson and Arey, 1994). The levels of these compounds may be higher in winter in association with high parent PAH levels (Wada et al., 2001; Tang et al., 2005; Albinet et al., 2008).

This observation derived from passive sampling, that genotoxicity may be elevated in winter is consistent with previous assessments of genotoxicity using active sampling, which have indicated that activity is typically higher in winter (Binkova et al., 1999; Topinka et al., 2000; Binkovda et al., 2003) and in urban areas (Binkovda et al., 2003; Brits et al., 2004; Skarek et al., 2007b). However, both direct and indirect acting genotoxicity in specific regional areas (i.e. Bunbury, Gawler, and Mt Gambier) in winter can be similar to the activity within urban capitals (i.e. Brisbane) indicating that season may be a more important factor for effect than location (i.e. direct acting genotoxicity, see Figure S3 in SM).

AhR activity assessed as TCDD equivalent air concentrations (TCDD Eq_{BIO}, pg m^{-3}) ranged from 1.5 pg m^{-3} (Mt. Gambier -regional centre) to 46 pg m^{-3} (Brisbane - urban capital) in summer and from 2.2 pg m^{-3} (Mutdapilly - rural background) to 251 pg m^{-3} (Adelaide - urban capital) in winter. These equivalent air concentrations were on average a factor of 3.0 times higher in winter. The slope (2.3 ± 0.10) for this relationship (summer vs. winter linear regression, excluding Bunbury and Adelaide with no replication) was found to be statistically significant ($p < 0.0001$). The importance of urban areas and winter exposure periods for AhR activity determined in this study is illustrated in Figure 1. These findings derived from passive sampling of ambient air are again consistent with previous studies which sampled air using active sampling techniques which have demonstrated: that AhR-inducible cytochrome P450-1A1 activity is greater in winter than in summer in PM_{10} extracts (Brown et al., 2005); that there is significantly higher competitive binding of the AhR in inner city than in rural air total suspended particulate matter (TSP) (Mason, 1994); and that urban air has higher TCDD equivalent air concentrations than background air when testing TSP + vapour-phase extracts (Skarek et al., 2007b). However, these findings may well be location specific with other studies finding no strong association between either summer vs. winter or urban vs. rural AhR activity for TSP and vapour phases of ambient air

(Klein et al., 2006) and no significant differences found between the AhR activity of urban and rural particulate matter (< 1 μm) extracts during an intense winter smog event (Wenger et al., 2009).

3.4. Relationships between PAH exposure markers, genotoxicity and AhR activity

Un-substituted PAHs require metabolic activation (+S9) for reactive binding to DNA and potential subsequent carcinogenesis. Since indirect acting genotoxicity was mostly below the detection limit in summer, a relationship between the PAH markers (B[*a*]P and ΣB[*a*]P TEQ) and indirect acting genotoxic potency (B[*a*]P Eq_{BIO}) was only evaluated in winter. Spearman rank correlation showed that these relationships were statistically significant for both B[*a*]P (r = 0.95, $p = 0.0011$) and ΣB[*a*]P TEQ (r = 0.93, $p = 0.0022$). Similarly, relationships between AhR activity and both B[*a*]P and ΣB[*a*]P TEQ were found to be statistically significant in both summer (r = 0.86, $p = 0.0107$ and r = 0.90, $p = 0.005$) and winter (r = 0.90, $p = 0.0046$, and r = 0.93, $p = 0.0022$), respectively. The relationship between AhR activity and the carcinogenic PAH marker ΣB[*a*]P TEQ in the winter deployment period is illustrated in Figure 2 (log transformed linear regression to illustrate differences between sites $r^2 = 0.85$).

These findings that markers of PAH exposure are related to these effects using PUF passive samplers, are consistent with previous studies which have monitored both genotoxicity (Binkova et al., 1999; Topinka et al., 2000; Brits et al., 2004; De Kok et al., 2005; Skarek et al., 2007a; Skarek et al., 2007b) and AhR activity (Arrieta et al., 2003; Brown et al., 2005; Skarek et al., 2007b; Cavanagh et al., 2009; Wenger et al., 2009) in active samples of ambient air. Where passive samplers such as PUF or semipermeable membrane devices have been used to monitor PAH in either ambient (Cupr et al., 2006) or indoor and outdoor air in an occupational exposure setting (coke plant) (Bonetta et al., 2009), the highest genotoxic effects are associated with locations where the highest PAH levels have been accumulated by these samplers. Similarly, we previously determined that differences in the potency of AhR activity in indoor vs. outdoor air sampled by PUF passive air samplers reflected potential PAH profiles, although many of the carcinogenic PAHs were not detected (Kennedy et al., 2009). Together these results indicate that passive samplers may provide a convenient means of conducting effect based monitoring consistent with PAH exposures and with results derived from more traditional active sampling of ambient air.

3.5. Proportion of AhR activity accounted for by monitored PAH exposures

The relationships established between the markers for PAH exposure and AhR activity indicate that higher PAH levels in urban areas and in winter are indicative of high AhR activity in these contexts. In order to confirm the importance of non-HAH compounds like the PAH to this “total” AhR activity the winter samples were also treated with H_2SO_4/silica gel, which degrades such compounds (Villeneuve et al., 2002). No AhR activity was quantifiable above the assay detection limits (< 0.19 to < 0.40 pg m^{-3}) in samples that were treated with H_2SO_4 silica gel. This indicates that HAHs which would be resistant to this treatment are not contributing significantly to the “total” AhR activity measured for these locations. This is consistent with previous findings in indoor and ambient air using the PUF sampler (Kennedy et al., 2009), and pooled TSP + vapour-phase (PUF) or PM_{10} samples from outdoor sites with both high and moderate traffic intensities (Ciganek et al., 2004). Total AhR activity has also been found to be 45 to 700 fold greater than dioxin-like activity in TSP + vapour phase ambient air samples assessed post multilayer silica gel (KOH/H_2SO_4/$AgNO_3$) and activated carbon column treatment of samples (Anezaki et al., 2009). Dioxin-like AhR activity was quantified in this latter study using samples concentrated to between 8–14 m^3 μL^{-1} which is significantly higher than the 1.2–2.5 m^3 μL^{-1} estimated in winter for this study (SM, Table S3). It is likely that in order to quantify this activity a

combination of both relatively high dosage (equivalent air volumes per well) and more exhaustive sample clean-up is required.

An alternative approach to confirming the significance of compounds such as the PAHs, is to monitor a reduction in potency with respect to the sustained induction of TCDD, as the time of incubation of samples with cells increases (Behnisch et al., 2001). This approach (employing 6 hour vs. 24 hour incubation periods) has been used to confirm the relative contribution of more readily metabolised AhR agonists like PAHs to the AhR activity of TSP extracts (Hamers et al., 2000) with a luciferase reporter gene assay. In order to validate the use of this approach in the CAFLUX assay the TCDD Eq_{BIO} for PUF extracts were also quantified at the 50% effect level after 48 and 72 hours incubation (SM, Table S5). These indicate a reduction in equivalent air concentration with respect to TCDD as the incubation period increases. This is illustrated for an urban (Adelaide), regional centre (Bunbury) and a rural background site (Mutdapilly) during the winter deployment (Figure 3).

It is important however to determine whether PAHs exhibit a similar reduction in potency with respect to TCDD as the incubation period increases on this assay. Therefore, average relative potencies (REP) for individual PAHs with respect to TCDD at the 50% effect level, after 24, 48 and 72 hour incubation periods (Equation 3) were determined (Table 2). REP estimates for both 5 and 20% effect levels are also provided in SM Table S6. These REP values exhibit a reduction in potency with incubation period which is consistent with the reduction in TCDD Eq_{BIO} observed in the exposed samples.

All PAHs classified as IARC group 1, 2A and 2B human carcinogens are the relatively more potent agonists out of the priority pollutant PAHs which demonstrate AhR activity. High correlations between AhR affinity and cancer data have been found previously for PAHs (Sjogren et al., 1996). Several of the five to six ring probable/possible carcinogenic PAHs (dibenz[*a*,*h*]anthracene, indeno[*1*,*2*,*3-c*,*d*]pyrene and benzo[*k*]fluoranthene), are particularly potent agonists. These REP values for PAHs determined for the first time on the CAFLUX bioassay are typically within an order of magnitude of those derived with rat hepatoma H4IIE, dioxin responsive chemical activated luciferase gene expression (DR-CALUX, EC_{50} 24 hour incubation) by others (Machala et al., 2001; Behnisch et al., 2003). PAHs which were less potent on the CAFLUX assay included benzo[*a*]pyrene, benz[*a*]anthracene, benzo[*k*]-fluoranthene and benzo[*b*]fluoranthene while more potent PAHs included dibenz[*a*,*h*]anthracene, indeno[*1*,*2*,*3-c*,*d*]pyrene, chrysene, fluoranthene and pyrene. These differences illustrate the importance of the use of REP specific to the bioassay used to assess AhR activity as significant differences in accounting for observed activity may otherwise arise (Villeneuve et al., 2002).

Assuming that the individual responses of PAHs quantified in this study (SM, Table S4) are additive for AhR activity, these REP were used to estimate the total TCDD equivalent air concentrations which could be accounted for through chemical analysis of PAHs (ΣTCDD Eq_{CHEM}, pg m^{-3}, Equation 4) for all locations in summer and winter (Table 3). The ΣTCDD Eq_{CHEM} ranged from 0.0039 pg m^{-3} (Mutdapilly – rural background) to 0.28 pg m^{-3} (Adelaide – urban capital) in summer, and from 0.021 pg m^{-3} (Mutdapilly – rural background) to 1.1 pg m^{-3} (Adelaide – urban capital) in winter. The dominant contributors to ΣTCDD Eq_{CHEM} in both seasons were chrysene (34%), indeno[*1*,*2*,*3-c*,*d*]pyrene (35%), benzo[*b*+*k*]fluoranthene (15%) and benz[*a*]anthracene (6.6%), while dibenzo[*a*,*h*]anthracene (26%) was a dominant contributor in winter, where detected. The PAHs quantified however only account for an average of 0.55% and 1.4% of the total AhR activity (Table 1) in summer and winter respectively (% TCDD Eq_{BIO}, Table 3).

These findings indicate that other un-quantified non-HAH AhR inducible compounds sampled by the PUF passive sampler are the dominant contributors to observed total AhR activity at these locations. Many other compounds, including un-substituted PAHs and nitrated, methylated and oxygenated PAH derivatives are both present in ambient air and demonstrate AhR activity (Machala et al., 2001; Misaki et al., 2007; Bekki et al., 2009). Interestingly, many of the more polar PAH derivatives are likely to be directly genotoxic, and direct acting genotoxicity was found to be higher in winter together with a concomitant increase in total AhR activity. Further work is required to quantify the contribution of these less frequently quantified PAH derivatives to total AhR activity, given the potential for adverse biological outcomes resulting from these exposures.

4. Conclusion

Direct (chemical analysis) and indirect effect based exposure assessments potentially relevant for subsequent carcinogenesis for PAH exposures in ambient air were significantly related and demonstrate the importance of urban areas and winter exposure periods. Unlike indirect acting genotoxicity, AhR activity was quantifiable at all locations in both seasons. IARC classified carcinogenic PAHs were the most potent inducers of AhR activity on the CAFLUX assay. A significant proportion of the total AhR activity at these locations is attributable to non-HAH AhR ligands like the PAHs, but remains unresolved.

While this study demonstrates the feasibility of combining passive sampling with multiple bioanalytical assessments, it illustrates the need for further chemical analysis to resolve the observed potency in different locations. It is also important to recognise the limitations and uncertainties which remain associated with the application and indeed the combination of passive sampling in particular with these techniques. Estimates of both ambient concentration and toxicity derived from passive samplers will be influenced by differences in vapour vs. particulate-phase sampling rates particularly if the compounds of interest are found predominantly in coarser particle size ranges which are not as efficiently sampled by these samplers (Chaemfa et al., 2009a; Chaemfa et al., 2009b). These differences have been reported to amount to as much as a 90% reduction in particulate-phase sampling rates (Klanova et al., 2008; Chaemfa et al., 2009c). Total concentration and total toxicity assessments in this case may be underestimated when derived using passive samplers compared with more traditional active sampling techniques. Estimates are more likely to be reasonable where toxicity is concentrated in the vapour and finer particle size ranges of ambient air and there is greater agreement between sampling rates for these different phases of ambient air.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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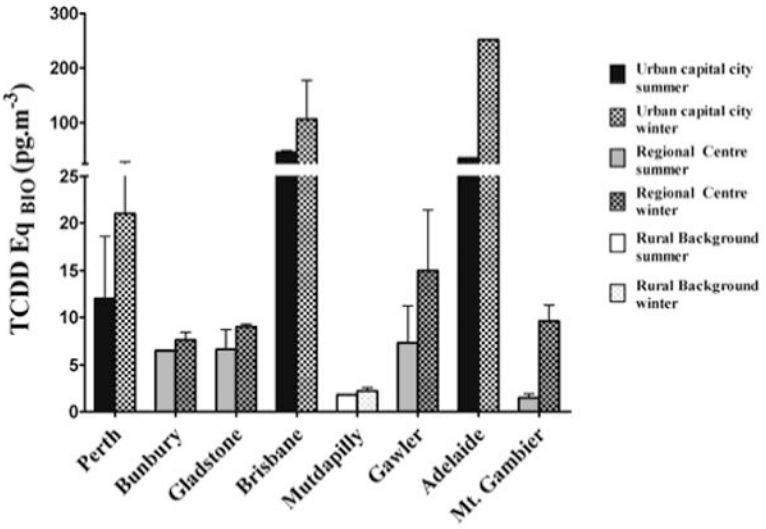

Figure 1. Seasonal AhR activity expressed as TCDD Eq_{BIO} (pg m^{-3}) for the 50% effect level (24 hour incubation) derived from PUF passive air samplers for urban capitals, regional centres and rural background locations

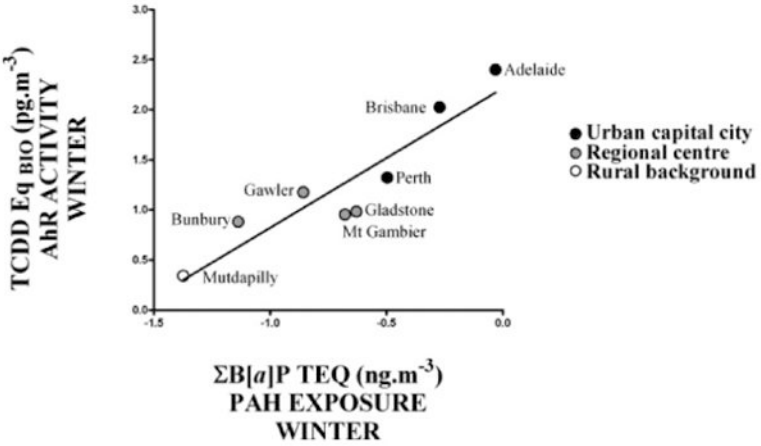

Figure 2. Linear regression between the PAH exposure marker ΣB[a]P TEQ (ng m^{-3}) and AhR activity (TCDD Eq_{BIO}, pg m^{-3}), as determined from passive sampling at urban capitals, regional centres and a rural background location in winter

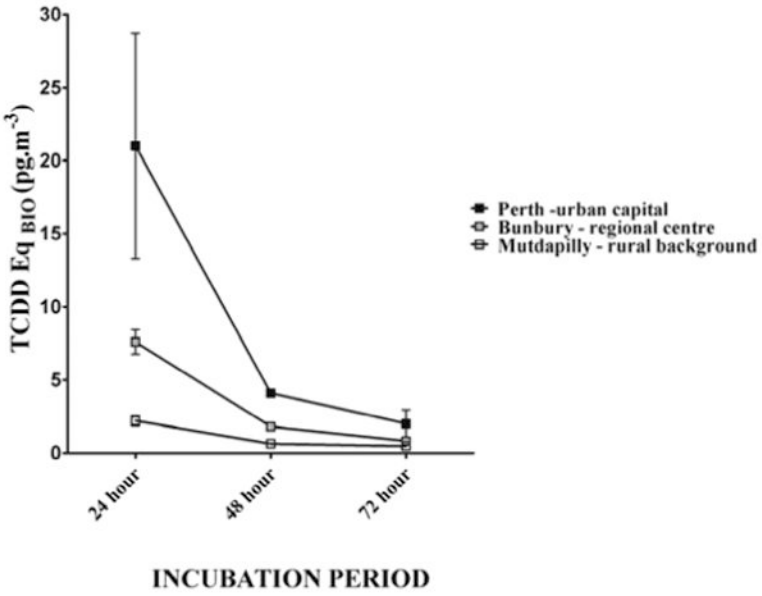

Figure 3. The decline in the CAFLUX derived TCDD equivalent air concentrations (pg m^{-3}) at the 50% effect level with an increase in the incubation period which is consistent with relatively more labile compounds being the major inducers of AhR activity at these locations

Table 1

Markers of PAH exposure B[a]P and ΣB[a]P TEQ (ng m^{-3}), umuC derived genotoxic potencies expressed as 6-nitrochrysene (-S9) and benzo[a]pyrene (+S9) equivalent air concentrations (ng m^{-3}) and CAFLUX derived AhR potencies expressed as TCDD equivalent air concentrations (pg m^{-3}) using PUF passive air samplers to sample exposure in both summer and winter

	Ambient PAH Exposure Markers				*umu*C Genotoxicity				CAFLUX Ahr Activity	
	B[*a*]P (ng m^{-3})		Σ B[*a*]P TEQ (ng m^{-3})		Direct Acting (-S9) 6-nCHY Eq_{BIO}[a] (ng m^{-3})		Indirect Acting (+S9) B[*a*]P Eq_{BIO}[b] (ng m^{-3})		TCDD Eq_{BIO}[c] (ng m^{-3})	
Sampling Locations	**Summer**	**Winter**	**Summer**	**Winter**	**Summer**	**Winter**	**Summer**	**Winter**	**Summer**	**Winter**
Perth	0.037±0.0062	0.084±0.0055	0.13±0.017	0.32±0.0082	1.5	5.1±1.6	7.5	8.5±0.035	12±6.6	21±7.7
Bunbury	0.0051±0.00028	0.013±0.0010	0.048±0.0014	0.073±0.0032	1.0	2.6±0.17	<7.0	3.6±1.6	6.5	7.6±0.83
Gladstone	0.025±0.0096	0.060±0.023	0.066±0.018	0.21±0.080	0.34	2.0±1.1	<3.4	7.9±1.1	6.6±2.1	9.0±0.28
Brisbane	0.088±0.0021	0.16±0.014	0.18±0.0063	0.53±0.019	1.8	2.6±1.2	6.3	8.4±1.5	46±3.3	106±72
Mutdapilly	<0.0055	0.0071±0.00047	0.036±0.00012	0.042±0.00090	<0.39	1.5±0.21	<5.1	5.2±0.10	1.8	2.2±0.35
Gawler	0.0092±0.000029	0.037±0.000054	0.070±0.000030	0.14±0.0044	0.91±0.12	2.4±0.60	<10	7.3±2.0	7.3±3.9	15±6.4
Adelaide	0.15±0.0051	0.33±0.068	0.33±0.010	0.93±0.12	1.5±0.7	5.3±0.62	<8.3	11±0.0052	35	251
Mt Gambier	0.0056±0.0011	0.054±0.0032	0.060±0.00069	0.23±0.0053	0.79±0.14	2.8±2.0	<6.4	7.6±2.2	1.5±0.4	9.6±1.7

[a] Direct acting (-S9) genotoxic potency (Equation 1 where reference compound is 6-nitrochrysene)

[b] Indirect acting (+S9) genotoxic potency (Equation 1 where reference compound is benzo[*a*]pyrene)

a and b The first replicate summer sample for Perth, Bunbury, Gladstone, Brisbane and Mutdapilly was rejected in summer based on positive control response

[c] Quantified at the 50% effect level after 24 hours incubation (Equation 2)

Table 2

CAFLUX derived average relative potency estimates for individual polycyclic aromatic hydrocarbons (REP) with respect to 2,3,7,8-TCDD with different incubation periods (24, 48 and 72 hour)

Polycyclic Aromatic Hydrocarbons			50% Effect level REP[a] [M ratio] Incubation Period		
Sixteen Priority PAHs		No. of Rings	24 hour	48 hour	72 hour
1	Naphthalene	2	-	-	-
2	Acenaphthene	3	-	-	-
3	Acenaphthylene	3	-	-	-
4	Fluorene	3	-	-	-
5	Phenanthrene	3	-	-	-
6	Anthracene	3	-	-	-
7	Fluoranthene	4	1.1×10^{-7}	2.9×10^{-8}	-
8	Pyrene	4	1.1×10^{-7}	5.7×10^{-8}	5.0×10^{-8}
9	Chrysene	4	1.2×10^{-4}	1.0×10^{-5}	3.1×10^{-6}
10	Benzo[*a*]anthracene	4	4.2×10^{-5}	5.1×10^{-6}	3.0×10^{-6}
11	Benzo[*a*]pyrene	5	3.4×10^{-5}	7.5×10^{-6}	4.2×10^{-6}
12	Benzo[*b*]fluoranthene	5	2.2×10^{-5}	4.4×10^{-6}	2.1×10^{-6}
13	Benzo[*k*]fluoranthene	5	1.7×10^{-4}	2.1×10^{-5}	1.2×10^{-5}
14	Indeno[*1,2,3-c,d*]pyrene	6	9.3×10^{-4}	9.9×10^{-5}	4.8×10^{-5}
15	Benzo[*g,h,i*]perylene	6	-	-	-
16	Dibenz[*a,h*]anthracene	5	3.6×10^{-3}	3.5×10^{-5}	1.2×10^{-3}
Other					
17	Benzo[*e*]pyrene	5	-	-	-
18	Perylene	5	4.2×10^{-6}	1.9×10^{-6}	1.5×10^{-6}
19	2-methylphenanthrene	3	-	-	-
20	1-nitropyrene	4	3.4×10^{-5}	7.5×10^{-6}	4.2×10^{-6}

[a] All REP values are the average of two independent tests in triplicate; '-' indicates PAHs which did not induce 50% TCDD max effect level

Table 3

TCDD equivalent air concentrations for each site (ΣTCDD Eq_{CHEM}, pg m^{-3}) derived using average relative potency estimates for individual PAHs (REP) with respect to TCDD and average ambient concentration estimates, indicating the proportion of the total AhR potency (TCDD Eq_{BIO}) for each site accounted for by these known PAHs

	ΣTCDD Eq_{CHEM} [a] (pg m^{-3})		% TCDD Eq_{BIO} [b] (%)	
Sampling Locations	**Summer**	**Winter**	**Summer**	**Winter**
Perth	0.12	0.42	0.99	1.9
Bunbury	0.015	0.067	0.24	0.89
Gladstone	0.059	0.23	0.90	2.6
Brisbane	0.14	0.55	0.31	0.52
Mutdapilly	0.0039	0.021	0.22	0.97
Gawler	0.010	0.16	0.14	1.1
Adelaide	0.28	1.1	0.82	0.43
Mt Gambier	0.011	0.29	0.76	3.0

[a] $\Sigma TCDD\ Eq_{CHEM}$ (Equation 4) using REP for the 50% effect level with 24 hour incubation (Table 2) and average C_{AIR} for individual PAH (SM Table S4) where C_{AIR} for benzo(*b*+*k*)fluoranthene treated as 50% for both REP for benzo(*b*)fluoranthene and benzo(*k*)fluoranthene)

[b] $\%\ TCDD\ Eq_{BIO}$ is the proportion of this total AhR activity (Table 1) accounted for by the known AhR activity of individual PAH levels at each site ($\Sigma\ TCDD\ Eq_{CHEM}$)