SCREENING FOR ARYL HYDROCARBON RECEPTOR ACTIVITY IN AMBIENT AIR USING PASSIVE SAMPLING (SPMDS) AND CAFLUX

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Abstract
Ambient air samples may be screened for aryl hydrocarbon receptor activity using bioassays such as CAFLUX. This screening may provide an estimate of overall potency which is then a function of all components of the complex mixture including uncharacterized components and may reduce the need for costly analysis in all cases. Passive air samplers are particularly suited for use as a screening tool due to their cost-effectiveness and capacity to be deployed over wide spatial scales simultaneously. In this study passive air samplers were deployed at five sites in Queensland, Australia. Co-deployed samplers were analysed for priority pollutant polycyclic aromatic hydrocarbons and the aryl hydrocarbon receptor activity of the samples from each site, were assessed using CAFLUX. TCDD Eq concentrations for each sample were determined. Samples from exposed sites were significantly different in response to the field blank response except for one site where samplers were deployed in a eucalypt forest. This activity could be detected from an equivalent air volume of < 2 m^3 per well. This activity showed an apparent decrease with increasing distance from the central business district, for sites within the same air shed. These sample concentrations were converted to a TCDD equivalent air concentration and ranged from 0.045 – 0.64 $pg.m^{-3}$. These specific sites were a eucalypt forest site in outer Brisbane and an inner urban site respectively. These levels are consistent with though lower than overseas estimates of aryl hydrocarbon receptor activity in ambient air.

Introduction
There are problems associated with targeting individual chemicals for monitoring and regulation and the extent to which this protects public health [1]. An alternative approach is to assess ambient air in terms of overall toxic potency for specific mechanisms of toxicity. Overall potency is a function of all components of the complex mixture. This incorporates both the influence of uncharacterised components of the mixture, and the nature of the interactions between components [2]. Assessing overall toxic potency may be achieved using bio-analytical techniques.

Bio-analytical techniques provide a relatively rapid and cost effective means of screening large numbers of samples for their ability to elicit a biological response through a specific mode of action [3]. Biological responses may occur at exposure levels below the detection limit of standard chemical analysis methods [4]. This response then may be a more sensitive means of assessing potential effect for low concentration exposures as is the case for many semivolatile organic chemicals in air including dioxins. These techniques have been utilised in a range of contexts relevant to the assessment of hazardous air pollutants (HAPs) in ambient air. These include assessments using specific HAPs or HAPs mixtures [5], relevant HAPs emission sources [6], rain water [7], low-high volume air sampling [8] and passive air sampling [9,10].

Passive sampling is particularly suited for use as a screening tool as these samplers are relatively cost effective, may be deployed over wide spatial scales simultaneously are non-intrusive and may be deployed even in remote areas which lack infrastructure. *In vitro* bioassays such as the CALUX and CAFLUX have been developed which may sensitively and rapidly screen the overall potency of a sample for aryl hyrdrocarbon receptor (AhR) mediated or dioxin-like activity. These assays use reporter genes (luciferase and green fluorescent protein respectively) under transcriptional control of dioxin responsive elements to monitor potency. These assays are extremely sensitive with detection limits 0.03-1 pg and 0.03-0.07 pg per assay [11] respectively and may significantly reduce the need for expensive analysis in all cases.

Overall potency may be reported as a maximum TCDD equivalent concentration for a specific equivalent air

volume dosed per well. This equivalent air volume (EqV_A, m^3) may be derived from the sampling rate of the passive sampler (R_S, $m^3.day^{-1}$), the time of deployment (t, days), and corrected for the proportion of final extract volume dosed into the test well accounting for known losses (m) (Equation 1).

$$EqV_A = m \cdot R_S \cdot t \qquad \textbf{Equation 1}$$

The CALUX assay has been used to monitor overall potency of particulate and vapour phase ambient air samples [12]. However the CAFLUX assay's EGFP (enhanced green fluorescent protein) reporter gene activity has the advantage of being able to be measured in living cells without the cost of lysis or reporter gene substrates [11]. This study evaluates the use of the CAFLUX assay in combination with passive air sampling to monitor AhR activity in ambient air.

Materials and Methods

Semipermeable membrane devices (SPMDs) were deployed at five sites in Queensland (QLD), Australia. The study sites included South Brisbane, Hemmant, Mount Cotton, Mackay, and Gladstone (Table 1). Standard dimension SPMDs [13] were prepared and deployed for 30 days or 36 days (Mackay only) from March to April 2006. A sampling rate of 4 $m^3.day^{-1}$ was assumed based on an average of literature values for a broad range of SOCs [14-17]. Field blanks (n=2) for analysis and bioassay were transported to the sites for both deployment and retrieval.

Table 1: Field Site Descriptions for Passive Sampler Deployments

Site Name	Description
South Brisbane	Brisbane, South East Queensland:Inner urban motorway site affected by vehicular emmissions
Hemmant	Brisbane, South East Queensland: Industrial & port area for Brisbane 10 km from CBD
Gladstone	Central Queensland:regional area adjacent to alumina refinery
Mackay	Central Queensland: regional area mix of beef cattle, sugar cane farm
Mount Cotton	Brisbane, South East Queensland: Eucalypt forest site approximately 40 kms from CBD

Two SPMDs from each site were combined for each sample, with three samples in total per site. Replicate samples were assessed bio-analytically with one retained for effect directed analysis. Samples were extracted in 300 mL of hexane (Licrosolv-Merck) for 24 hours in teflon sealed glass jars on a shaker. Solvent was replaced and samplers extracted for a further 24 hours and these extracts were combined. Each sample was evaporated and subjected to gel permeation/size exclusion chromatography. The fraction collected has previously been calibrated for classes of SOCs including polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), dioxins and furans. Samples were evaporated under nitrogen and solvent exchanged to 120 µL DMSO for bioassay or 100 µL in hexane for analysis using GC-MS (SIM mode).

Each replicate sample (n=2) was tested in triplicate on the CAFLUX[18] assay (n=6) using a rat hepatoma cell line (H4G1.1c2) stably transfected with pGreen 1.1 (EGFP), seeded at 1 x 10^5 cells.$well^{-1}$ and dosed when 90 % confluent. The maximum EqV_A dosed was 1.4 – 1.7 m^3 for 30 – 36 days deployment respectively at 1 % dmso in a final well volume of 100 µL. An eight point TCDD standard curve of original concentration from 0.1 nM – 20 µM was run for each sample. Relative fluorescence units (RFU) were measured (excitation filter 485 nm, emission filter 520 nm, gain 1500) after 48 hours exposure and a TCDD Eq concentration was interpolated with Graph Pad Prism from an RFU vs. [TCDD] standard curve for each sample dilution using the measured RFU in each case.

Results and Discussion

The average maximum CAFLUX derived TCDD Eq concentrations for each site and for the field blank samples are shown in Figure 1. These results indicate that AhR activity is detected from less than 2 m^3 EqV_A per well. ANOVA with Tukey's multiple comparison post testing indicates that all sites are significantly different ($p < 0.05$-0.001) in potency to both the Mount Cotton site and the field blank samples. Mount Cotton however was not significantly different in potency to the field blank. South Brisbane and Mount Cotton were assessed as

having the highest and the lowest average maximum TCDD Eq (M) for the sites respectively. Of the Brisbane sites (South Brisbane, Hemmant and Mount Cotton), the derived potencies reflect distance from the CBD in magnitude with decreasing estimates obtained as the distance from the CBD increases. South Brisbane and Hemmant were not significantly different in potency however. The coefficients of variation in TCDD Eq amounts (pg) per sample derived from these concentrations averaged 23 %. Passive sampler replicates as were pooled in this case for each sample, typically have average CVs < 20 % for quantification.

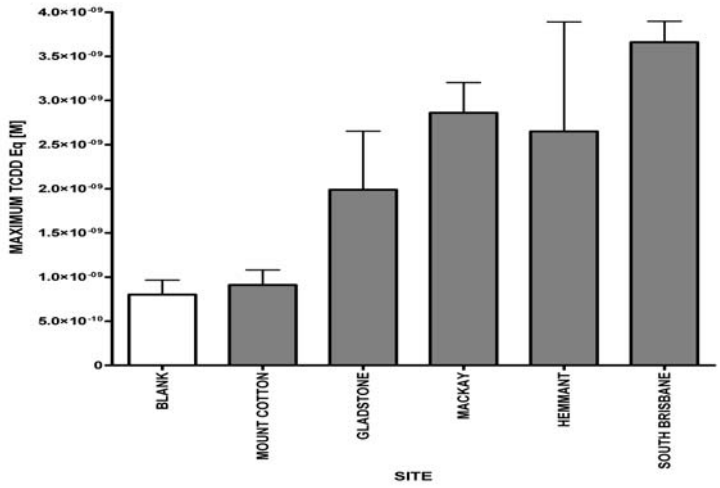

Figure 1: Average Maximum TCDD Equivalent Sample Concentrations [M] for each Site and the Field Blanks. The equivalent air volume per well was higher for the Mackay site at 1.7 m^3 in comparison to the other sites at 1.4 m^3.

The TCDD Eq amounts per sample were converted to a TCDD Equivalent air concentration ($pg.m^{-3}$) for each site using the final volume of extract and the total air volume sampled at each site, 240 & 288 m^3 (Mackay only). The total air volume was corrected (5/7) for proportion of sample fractionated during size exclusion chromatography to 171 & 206 m^3 respectively. The average blank TCDD Eq amount (31 $pg.sample^{-1}$) was deducted in each case. The average CAFLUX derived TCDD Eq air concentration for each site ranged from 0.045 (Mount Cotton) – 0.64 $pg.m^{-3}$(South Brisbane) and are provided in Table 2. These estimates are lower than estimates derived using CALUX (24 hr) for ambient air (vapour plus particulate phase) at two sites in the Czech Republic, which ranged from 69 – 130 $pg.m^{-3}$[19].

Previous air monitoring in Brisbane, Australia has reported average WHO TEQs of 8.89 & 2.47 $fg.m^{-3}$ for dioxins/furans and dioxin like PCBs respectively [20]. The difference in magnitude of these results indicate that analytical assessments may underestimate overall toxic potency as determined bioanalytically for this end-point by only considering specific dioxins/furans and dioxin like PCBs. Previous studies assessing AhR activity in vapour or particulate phase ambient air samples have proposed that observed activity may arise from ambient PAH levels as opposed to dioxin/furan or PCB levels[12,19]. This observation has been supported through the use of sulfuric acid silica gel treatment of samples[19]. Several of the higher molecular weight PAHs including benzo[k]fluoranthene, dibenzo[a,h]anthracene and indeno[1,2,3-cd]pyrene are potent AhR agonists [21,22] and PAHs tend to be found in air at levels several orders of magnitude higher than those found for dioxins.

Table 2: CAFLUX (48 hour) derived estimates of TCDD Equivalent Air Concentration ($pg.m^{-3}$) compared with potency estimates derived through PAH analysis corrected with CALUX relative potency factors

Site	TCDD Eq Air Concentration ($pg.m^{-3}$)[a]	ΣTCDD Eq ($pg.m^{-3}$)	
		6 hr[b]	24 hr[c]
South Brisbane	0.64 ± 0.053	55	0.14
Hemmant	0.42 ± 0.30	20	0.039
Gladstone	0.39 ± 0.12	18	0.052
Mackay	0.39 ± 0.064	0.22	0.0017
Mount Cotton	0.045 ± 0.027	0.19	0.0012

[a] CAFLUX derived estimates of ambient air concentration based on 48 hour TCDD Eq maximum; [b,c] Individual PAHs quantified converted

to TCDD equivalent amounts using IEF_{TCDD} EC_{50} 6 hour and 24 hour respectively derived using CALUX [21].

Individual PAH levels quantified in co-deployed SPMDs have been converted to the sum of the TCDD Equivalent Air Concentrations (ΣTCDD Eq) for each site (Table 2), using CALUX derived relative potency estimates for individual PAHs with respect to TCDD [21]. Spearman rank correlation between CAFLUX derived TCDD Eq Air Concentrations and these overall potency estimates derived from individual PAHs quantified were significant for the ΣTCDD $Eq_{6\ hour}$ ($r = 0.97$; $p < 0.05$) but not significant for the ΣTCDD $Eq_{24\ hour}$ ($r = 0.82$; $p = 0.13$). These potency estimates will vary depending on the specific compounds selected for analysis and whether they are detected in all cases. The cell line used to assess potency and the exposure period will also influence potency estimates in each case.[22]. Further work is required to confirm the influence of PAHs specifically in this case for this exposure period (48 hours) and to obtain CAFLUX derived relative potency estimates for the individual PAHs quantified for this exposure.

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