Université de Montréal

Évaluation du 4,5-dihydrodiol-benzo[a]pyrène et du 7,8-dihydrodiol-benzo[a]pyrène en tant que biomarqueurs spécifiques alternatifs d'exposition au benzo[a]pyrène

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Résumé

Reconnu par le Centre international de recherche sur le cancer (CIRC) comme cancérigène chez l'être humain, le benzo[a]pyrène (BaP) est un des hydrocarbures aromatiques polycycliques (HAP) les plus étudiés. Souvent rencontrés dans de nombreux milieux de travail, les HAP sont un groupe de polluants omniprésents dans l'environnement, formé par des processus de combustion incomplets.

Bien que le BaP présente un risque élevé pour la santé des travailleurs, il n'existe aucun biomarqueur spécifique au composé permettant le suivi et la surveillance d'exposition au BaP dans un lieu de travail. Le 3-hydroxybenzo[a]pyrène (3-OHBaP) est le métabolite du BaP le plus développé comme biomarqueur. Ce métabolite est principalement excrété dans les fèces, ainsi qu'une quantité infime dans l'urine, ce qui le rend difficile à mesurer. De plus, le 3-OHBaP montre une certaine rétention rénale, un facteur qui rend plus compliqué son utilisation en tant que biomarqueur car il oblige de prendre plusieurs critères en compte dans l'analyse de sa cinétique temporelle. Par ailleurs, le 1-hydroxypyrène (1-OHP) est un métabolite urinaire du pyrène souvent utilisé comme biomarqueur d'exposition aux HAP. Neanmoins, il s'agit du métabolite d'un HAP non-cancérigène et par conséquent sa capacité de démontrer le risque de cancer associé à une exposition donnée est faible.

Ce mémoire visait à détecter et à évaluer l'exposition au BaP en suivant ses métabolites urinaires: le 4,5-dihydrodiol-benzo[a]pyrène et le 7,8-dihydrodiol-benzo[a]pyrène (le 4,5-diolBaP et le 7,8-diolBaP; les diolBaP).

L'évaluation du 4,5-diolBaP et du 7,8-diolBaP s'est déroulée dans deux études:

La première étude, surnommée « l'expérience du shampooing », portait sur un volontaire qui s'est exposé aux HAP dans un environnement contrôlé en utilisant un shampooing à base de goudron de houille. Cette étude a été conçue afin d'évaluer l'évolution temporelle du 7,8-diolBaP chez l'homme et de vérifier son potentiel en tant que biomarqueur d'exposition par comparaison avec le 1-OHP dans le même cadre expérimental. Elle a été réalisée avec deux expériences. La première portait sur une seule exposition et la seconde sur une exposition multiple.

La deuxième étude, surnommée « l'étude des travailleurs », reposait sur une analyse comparative du 4,5-diolBaP, du 7,8-diolBaP, du 1-OHP et du 3-OHBaP dans un milieu de travail. Cette étude avait pour objectif d'évaluer les compétences des diolBaP dans un contexte réel à côté des biomarqueurs établis d'exposition au BaP et aux HAP. Cinq travailleurs d'une usine de production d'anodes en carbone ont accepté de participer à cette étude.

Dans le cadre de ces deux études, les échantillons d'urine étaient analysés par la chromatographie en phase liquide à ultra-haute performance (UHPLC) couplée à la fluorescence.

« L'expérience du shampooing » : L'expérience de l'exposition unique et celle de l'exposition multiple ont révélé une élimination de façon mono-exponentielle du 7,8-diolBaP, identique à celle du 1-OHP, avec des concentrations dans le même ordre de grandeur. Nous avons également confirmé un taux d'élimination plus rapide pour le 1-OHP en regardant ses pics. Le 7,8-diolBaP augmente en valeur maximale après chaque exposition, et cette découverte a mis en évidence une accumulation tout au long de la semaine, alors que pour le 1-OHP, le deuxième pic est plus grand, mais le troisième est plus petit, montrant ainsi moins d'accumulation pendant la même période temporelle.

« L'étude des travailleurs »: Selon les résultats, la méthode analytique utilisée était incapable de discerner correctement le 4,5-diolBaP des autres contaminants urinaires éluant pendant le même temps de rétention. Le 7,8-diolBaP, quant à lui, élue à des concentrations urinaires d'un ordre de grandeur similaire au 1-OHP tel que vu chez tous les travailleurs évalués. Chez certains travailleurs, la concentration urinaire du 7,8-diolBaP était toujours plus élevée avant le début d'un quart de travail avec l'élimination qui avait lieu pendant le quart de travail pour fournir une valeur de concentration inférieure à la fin du quart de travail. Cependant, la concentration du 1-OHP a eu une hausse immédiate avec l'exposition, culminant à la fin de chaque quart de travail. Pour les autres travailleurs, les concentrations du 7,8-diolBaP et du 1-OHP étaient systématiquement plus élevées à la fin du quart de travail. Il est probable que ces variations indiquent les différentes voies d'exposition.

Le présent mémoire a montré le potentiel du 7,8-diolBaP en tant que biomarqueur d'exposition spécifique au BaP et par conséquent, il fournit un point de départ pour explorer la

quantification du lien entre l'exposition au BaP et ses effets néfastes sur la santé de l'être humain. L'utilisation de la spectrométrie de masse est nécessaire à confirmer l'identité des diolBaP avant d'aller de l'avant.

Mots-clés : Benzo[a]pyrène, métabolisme, biomarqueurs, toxicocinétique, 4,5-dihydrodiol-benzo[a]pyrène, 7,8-dihydrodiol-benzo[a]pyrène, 1-hydroxypyrène

Abstract

Benzo[a]pyrene (BaP) is one of the more commonly studied polycyclic aromatic hydrocarbons (PAHs), a group of omnipresent pollutants in the environment formed through incomplete combustion processes. It is listed as a confirmed carcinogen to human beings by the International Agency for Research on Cancer (IARC) and is highly present in many workplaces.

Although presenting a significant health risk to workers, there are currently no convenient compound-specific biomarkers that enable the tracking and monitoring of occupational exposure to BaP. 3-Hydroxybenzo[a]pyrene (3-OHBaP) is the most developed, as a biomarker, amongst the metabolites of BaP. It's mostly excreted with the faeces, presenting in trace amounts in urine, which makes it difficult to measure; it is also demonstrates renal retention, which adds a layer of complexity in its use as a biomarker because there are many factors to take into consideration when looking at its kinetic time course. 1-Hydroxypyrene (1-OHP), in the other hand, is a urinary metabolite of pyrene that serves as a good representative of PAH presence. It is, however, the metabolite of a non-carcinogenic PAH, and is not fully capable of representing the cancer risk posed in a given scenario.

This thesis sought to detect and assess BaP exposure through tracking its urinary metabolites: 4,5-dihydrodiol-benzo[a]pyrene and 7,8-dihydrodiol-benzo[a]pyrene (4,5-diolBaP and 7,8-diolBaP; diolBaPs).

4,5-DiolBaP and 7,8-diolBaP were evaluated through two studies:

The first study, the "shampoo experiment", featured a volunteer who self-exposed to PAHs in a controlled setting by using a coal-tar-based shampoo. The study consisted of two experiments. The first focused on a single exposure and the second on multiple exposures. This study was set to evaluate the time course of 7,8-diolBaP in humans and verifying its potential as a biomarker of exposure through a comparison with 1-OHP in the same experimental framework.

The second study consisted of a comparative analysis of 4,5-diolBaP, 7,8-diolBaP, 1-OHP and 3-OHBaP in an occupational setting, evaluating the competency of the diolBaPs in a

real-world setting alongside established biomarkers of BaP and PAH exposure. Five workers at a carbon anode production plant volunteered to participate in this study.

For both of these studies, the urine samples were analysed by ultra-high-pressure liquid chromatography (UHPLC) coupled with fluorescence.

"Shampoo Experiment": The single and multiple exposures revealed a monoexponential elimination on the part of 7,8-diolBaP, identical to 1-OHP, with similar magnitudes of concentration. 1-OHP was also confirmed to undergo a more rapid elimination from the system, where after each exposure for 7,8-diolBaP, the ensuing peak value is higher. This finding demonstrated evidence of accumulation of 7,8-diolBaP throughout the week, whereas with 1-OHP, the second peak is larger, then the third one is smaller, thus showing less accumulation over the same time frame.

"Worker Study": The results showed that the analytical method used was unable to properly discern 4,5-diolBaP from other urinary contaminants eluting during the same retention time. 7,8-DiolBaP, on the other hand, eluted at urinary concentrations that were a similar order of magnitude to 1-OHP, as can be seen in all of the workers evaluated. For some workers, the urinary concentration of 7,8-diolBaP was consistently at its peak prior to the start of a shift and elimination took place during the shift, to provide a lower concentration value at the end of the shift. With 1-OHP, the rise was immediate with exposure, peaking at the end of every shift. For other workers, both 7,8-diolBaP and 1-OHP are consistently higher at the end of the shift. These variations are likely to indicate different routes of exposure.

This thesis showed the potential use of 7,8-diolBaP as a compound-specific biomarker of exposure for BaP and thus provides a starting point in exploring the quantification of BaP exposure and negative health effects in humans. Confirmation of the compound's identity is needed through the use of mass spectrometry.

Keywords: Benzo[a]pyrene, metabolism, biomarkers, toxicokinetics, 4,5-dihydrodiol-benzo[a]pyrene, 7,8-dihydrodiol-benzo[a]pyrene, 1-hydroxypyrene

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BaP: Benzo[a]pyrene

BaPDE: (+)-Benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide

diolBaPs: 4,5-Dihydrodiol-benzo[a]pyrene and 7,8-dihydrodiol-benzo[a]pyrene

DMBA: 7,12-Dimethylbenzoanthracene

DNA: Deoxyribonucleic acid

GC/MS: Gas chromatography coupled to mass spectrometry

HPLC: High-Performance Liquid Chromatography

IARC: International Agency for Research on Cancer

PAH: Polycyclic Aromatic Hydrocarbon

UHPLC: Ultra-High-Performance Liquid Chromatography

UV: Ultraviolet

1-OHP: 1-Hydroxypyrene

3-OHBaP: 3-Hydroxybenzo[a]pyrene

4,5-diolBaP: 4,5-Dihydrodiol-benzo[a]pyrene

7-OHBaP: 7-Hydroxybenzo[a]pyrene

7,8-diolBaP: 7,8-Dihydrodiol-benzo[a]pyrene

9-OHBaP: 9-Hydroxybenzo[a]pyrene

This thesis is dedicated to my two incredible sisters. Your existence makes my existence that much more significant

J'aimerais dédier ce mémoire à mes deux sœurs incroyables. Vos existences me donnent le bonheur infini et font que la mienne soit beaucoup plus signifiante

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1. General Overview

Benzo[a]pyrene (BaP) is one of the better studied polycyclic aromatic hydrocarbons (PAHs), a group of organic chemicals formed through incomplete combustion processes that are typically identified through a shared particularity - multiple fused aromatic rings (Bjorseth 1983). It is classified as a known carcinogen to human beings (Group 1) according to the IARC Working Group on the Evaluation of Carcinogenic Risks to Humans (2012) and presents both epigenetic and genotoxic characteristics based on *in vivo* experiments. It creates mutations through direct reactions with deoxyribonucleic acid (DNA) and enhances the growth of existing tumours by increasing gene expression (Moreau 2013).

Although BaP is naturally present in trace amounts in the environment, having been detected in the air, water and earth (Douben 2003), most notable human exposure occurs in the workplace, especially during the processing of natural resources (Rengarajan et al. 2015). Industries showing a high level of employee exposure to BaP include coal, aluminium production, carbon-electrode manufacture and power plants (Field & Withers 2012). The exposure is generally through the air or via the cutaneous route, but given the persistent nature of the compound and the way in which it is formed, it can often be ingested as well (Gündel et al. 2000). Workplace exposure to BaP is most commonly associated with lung cancer (Field & Withers 2012), though the potential for other forms of cancer such as stomach and gastrointestinal cancer remain prolific (Lippman & Hawk 2009).

Lung cancer is the most prominent killer in Canada amongst the cancers, representing 26% percent of cancer-related deaths (Société canadienne du cancer 2015). The high death toll may be attributed to how hard it is to suspect lung cancer, with early symptoms manifesting as simple coughs or respiratory infections. As a result, testing for, and thus acknowledging a risk of cancer, tend to be done late in the tumour development process, ergo making treatment difficult. It is estimated that in 2017 alone, 28600 Canadians would develop lung cancer, and 21100 would die from it in the same year (Société canadienne du cancer 2015).

In order to counteract this and other workplace risks, measures need to be put into place to understand and lower harmful interactions between employees and xenobiotics. In this case,

the risk associated with BaP exposure needs to be reduced and one of the necessary components for this to happen is a reliable method of detection that tracks said exposure. Originally, air sampling was used, but it was found to be insufficient as it did not take into consideration any other pathways of exposure, and it was not specific to how much had been absorbed into the body (Gündel et al. 2000). Biological indicators were the next logical step whereby compounds whose presence in the system could be traced to exposure to BaP were used to quantify said exposure.

Currently, 1-hydroxypyrene (1-OHP) is the best biomarker of exposure to PAHs; however, given that it comes from the breakdown of the pyrene, it indicates contact with PAHs, but not BaP specifically (Jongeneelen 2001). With the known carcinogenicity of BaP versus the suspected, unknown or unlikely cancer-causing abilities of many other PAHs, the importance of finding a compound-specific reliable biomarker is paramount to properly assess, quantify and thus regulate BaP exposure (Moreau & Bouchard 2015), all in the overall aim of reducing the incidence of occupational cancer.

3-Hydroxybenzo[a]pyrene (3-OHBaP) has been explored and found to be inadequate in both concentration eluted in human urine and kinetic behaviour such that alternative biomarkers are warranted (Leroyer et al. 2010). It presents challenges in fully gathering data surrounding exposure to BaP, and PAHs in general, since, when compared to other metabolites of PAHs, it not only is hard to detect at trace amounts, but is eliminated in two phases from the system and can take up to fifty hours to hit its half life (Lutier et al. 2016).

This thesis aims to detect and assess BaP exposure through tracking its urinary metabolites: 4,5-dihydrodiol-benzo[a]pyrene and 7,8-dihydrodiol-benzo[a]pyrene (4,5-diolBaP and 7,8-diolBaP; diolBaPs). Their use as compound-specific biomarkers of occupational BaP exposure is gauged by comparing results obtained to 1-OHP in identical exposure circumstances, and occasionally 3-OHBaP. The results of this study have the potential to positively influence workplace health and safety regulations and standards, thus reducing the risk of cancer for certain groups of workers.

1.1 Polycyclic Aromatic Hydrocarbons

1.1.1 Definition

Polycyclic Aromatic Hydrocarbons (PAHs), also known as Polyaromatic Hydrocarbons, are a large group of chemicals that are classified based upon their shared structural trait of multiple fused organic rings with delocalized electrons (Choi et al. 2010). They are ubiquitous pollutants in the environment, formed from both anthropogenic and natural sources, with numerous health consequences (Baklanov et al. 2006).

There are currently no unanimously agreed upon boundaries as to what constitutes a PAH (Lee 2000). Although all definitions contain the trademark aromatic rings in the plurality, there is discussion as to whether compounds containing elements other than carbon and hydrogen can be included or if compounds with only two fused aromatic rings can be considered polycyclic (Lee 2000). For the purposes of this thesis, the term PAH is used to refer to the many definitions and names of these compounds containing three fused carbon rings or more, being inclusive in the broadest sense possible.

According to this chosen definition, phenanthrene and anthracene are the smallest true PAHs, consisting of three aromatic rings with a variance in stereochemistry (Richter et al. 2001). Naphthalene and benzene, with two and one aromatic ring respectfully, are not considered to be true PAHs in this instance (Comandini & Brezinsky 2011), although they are both very much aromatic hydrocarbons. PAHs containing more than six rings are considered large whereas those of six or less are labelled as small (Abdel-Shafya & Mansour 2016).

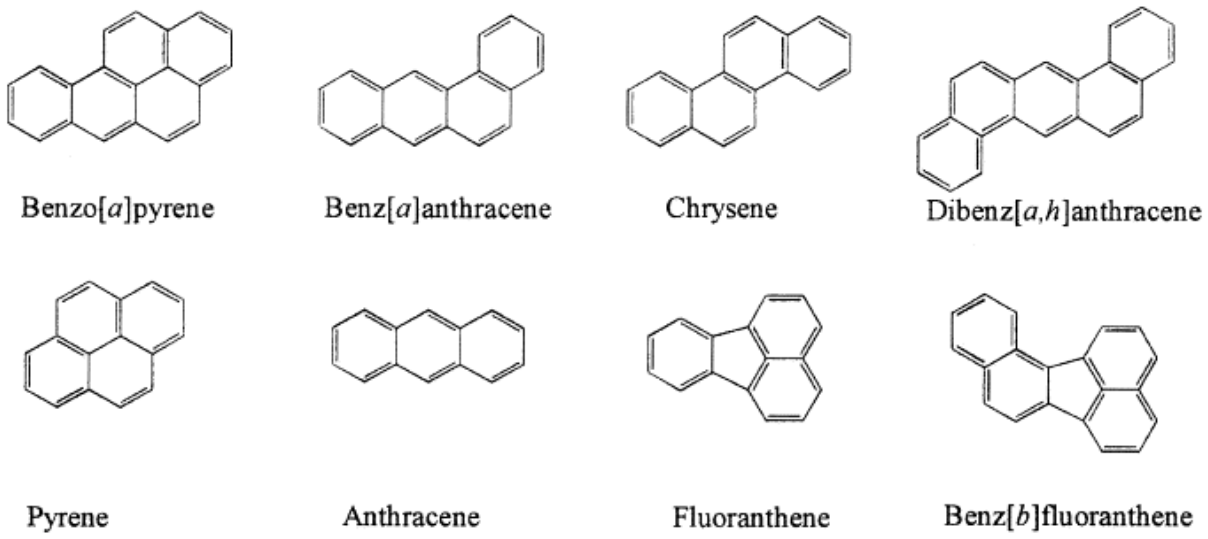

Figure 1: Some common polycyclic aromatic hydrocarbons (Phillips 1999)

1.1.2 Physico-chemical Properties

PAHs are formed through incomplete combustion processes involving organic compounds and components (Arey & Atkinson 2003). Incomplete combustion takes place when not enough oxygen is readily available for the reaction process to produce the desired compounds. As a result of this, PAHs can be formed through both natural means, such as forest fires, and anthropogenic actions such as melting down metal (Arey & Atkinson 2003).

This group of compounds typically exhibit higher molecular weights which result in higher boiling and melting points. They tend to be colourless, white, or pale-yellow solids at room temperature (Abdel-Shafya & Mansour 2016); however, those featuring two-three rings occur predominantly in vapour phase (Choi et al. 2010). The larger the hydrocarbon, the more stable it tends to be. Leading from the electron displacement that arises from the benzene ring, the compounds are nonpolar and lipophilic, with the larger compounds being a lot more insoluble than the smaller PAHs (Thorsen et al. 2004). Their lipophilicity means that they can be found stored in almost every organ, but they do not have a penchant for biomagnification (United Nations Environment Programme et al. 1998; Abdel-Shafya & Mansour 2016). Regardless, they are still commonly found in water.

When in the environment, PAHs attach to particulate matter. The larger PAHs (five or more rings) can be found in the atmosphere bound to particles and forming aerosols, others may

be found adsorbed onto soil, even when in water as they have extremely low aqueous solubility (Choi et al. 2010).

The aromatic ring ensures stability meaning that PAHs do not easily biodegrade in the environment and can persist a long time after formation; however, when subjected to the biodegradation process, each PAH reacts and behaves differently (Nadarajah et al. 2002). These differences are also apparent when considering photooxidation, soil adsorption and bioaccumulation. As a general trend, the linear and clustered PAHs degrade faster under the light since the angular PAHs are considered more structurally stable (Korfmacher et al. 1981). The opposite holds true for enzyme or bacteria-based biodegradation where the increased surface area makes angular PAHs more vulnerable (Abdel-Shafya & Mansour 2016).

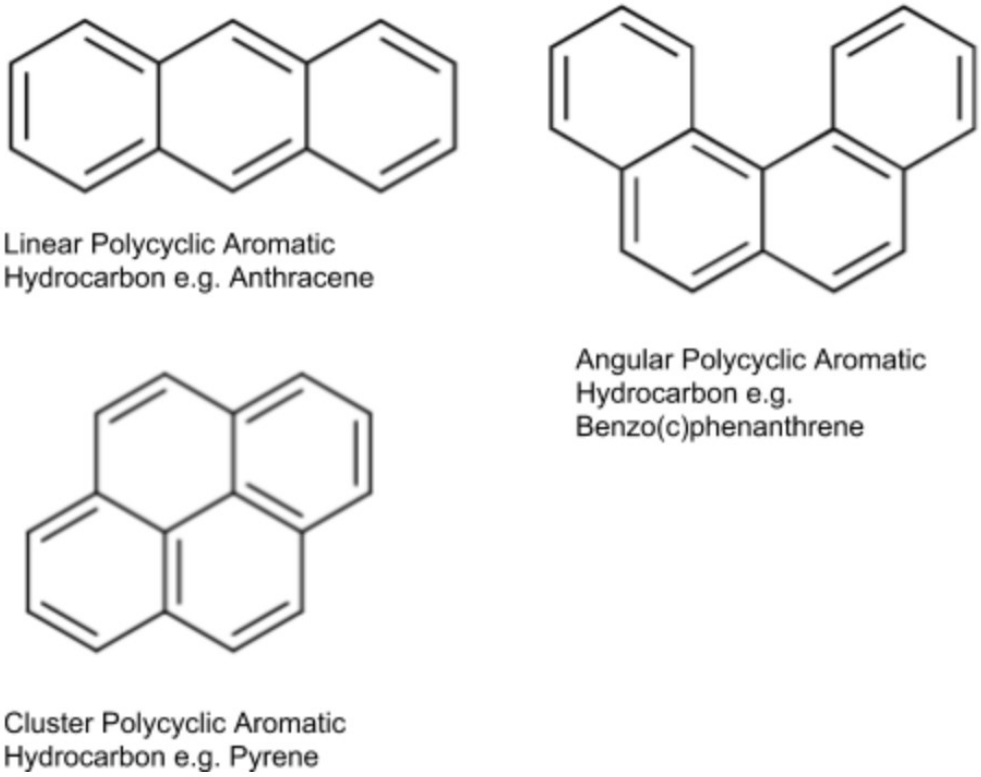

Figure 2: Molecular arrangement of the polycyclic aromatic hydrocarbons adapted from (Arey & Atkinson 2003)

Each ring projects differently on the ultraviolet (UV) spectrum, which gives PAHs a unique presence when tested through a spectrometer (Abdel-Shafya & Mansour 2016). This means that each compound is easily identifiable by its light signature. This also confirms attributes such as their fluorescence, relative sensitivity to light, and ability to resist and conduct heat (Abdel-Shafya & Mansour 2016).

1.1.3 Sources

The majority of PAH emissions come from anthropic sources as is evidenced by the higher concentrations in or near urban centres (Van Metre et al. 2000; Peng et al. 2011). Combustion processes are widespread in modern society, with personal habits such as smoking, the burning of logs for heating or cooking, and, hydrocarbon-based fuels providing the energy for vehicles (OECD 2012; Nacoulma et al. 2016; Lim et al. 2007). Typically, PAHs are formed at temperatures ranging from 350°C to more than 1200°C, though they can form slowly at 100°C over decades as is the case in crude oils (Abdel-Shafya & Mansour 2016). Higher temperatures are linked to shorter alkyl chains which translate to smaller PAHs versus lower temperatures and the longer reaction time which produces larger PAHs.

Industrial actions release significant concentrations of PAHs - especially those that burn fuel like coal, gas, oil, or process raw materials such as aluminium (Lee et al. 1977). Some of the more notorious workplaces include power plants, coke plants, steel plants, foundries, and carbon electrodes production facilities (Rengarajan et al. 2015). In these cases, PAHs enter the environment through waste release such as smoke stacks in the oil and gas industry, accidents like petrochemical spills, and through human transfer where someone regularly exposed to PAHs carries them on their clothes or body (Abdel-Shafya & Mansour 2016). Additional sources of PAHs include the manufacture of cement, bitumen and asphalt, wood preservation, waste incineration, and car tire production/reuse (Abdel-Shafya & Mansour 2016).

PAHs can also originate from non-human sources or actions. Some of these natural processes include the erosion of rocks containing petroleum hydrocarbons, vegetation decay, forest fires and volcano eruptions (Abdel-Shafya & Mansour 2016). These reactions involve exothermic energy whereby there may be insufficient oxygen present as combustion takes place, thus creating PAHs. Stemming from this, PAHs can be found in terrestrial and aquatic plants,

soils and water (National Research Council (US) Committee on Pyrene and Selected Analogues 1983). Forest fires are particularly large contributors to atmospheric PAH content, although studies do show that the kind of forest fire, including wind and meteorological conditions, have a significant impact on air concentrations of PAHs produced (McMahon & Tsoukalas 1978).

There is also a natural synthesis of PAHs in plants that is not fully understood, such as 1-methyl-7-isopropylphenanthrene in pine tar (National Research Council (US) Committee on Pyrene and Selected Analogues 1983). This provides uncertainties as to the true extent to which PAHs are pollutants.

It is as a result of atmospheric dispersion that PAHs are omnipresent in the environment. In the atmosphere, PAHs are found as either aerosols (small solid particles in the air) or as a liquid in vaporous form (Choi et al. 2010). Compounds with lower vapour pressure were more likely to be in particle form versus those with high vapour pressure (R. P. Schwarzenbach 2017). Some of these PAHs are then deposited, polluting the soil and water. Other PAHs found in the soil and water may come about more directly, through natural PAH synthesis or an oil spill for example. Those that land on the earth normally bind to soil particles, which then defines their movement (Riccardi et al. 2008). They go where the soil goes.

1.1.4 Health Concerns

PAHs are a complex and diverse group of compounds that have differing effects on human health, not all of which are fully known or understood (Choi et al. 2010). Concerns have been raised at a global scale regarding the carcinogenic potential of some of the more structurally complex PAHs as well as the overall risks associated with exposure to PAH mixtures (Lima et al. 2005). To ensure the safety of the general population despite the limited knowledge, precautionary regulations have been made at national and international levels (EU Science Hub 2015; Food Standards Agency 2018; CCCF 2009).

The average citizen encounters PAHs through the gastrointestinal route (Fiala et al. 2001). PAHs are especially common in grilled food (notably meat and fish), though they can be found when food is cooked in other ways as well (Kim et al. 2013). Occasionally, the contamination does not originate from the manner in which the food is prepared, but from PAHs that have contaminated the soil and water, thus being absorbed into growing food and/or

bioaccumulating across the food chain (Wickström et al. 1986). It is because of this that the majority of PAH food-based exposure is through cereals and vegetables (Phillips 1999).

Studies show, however, that the principal area of significant exposure is in the workplace (Unwin et al. 2006). Occupational exposure to PAHs can occur in a variety of industrial activities and tasks, such as aluminum and coke production, iron foundries, steel plants, tar distillation, the extraction of oil, wood impregnation, carbon electrode production, roofing and road paving to name a few (Boffetta et al. 1997). In these instances, exposure tends to either be respiratory or cutaneous. Research surrounding PAH exposure has narrowed down the lungs as the primary target organ of concern, with the skin and the bladder being secondary (Armstrong et al. 2004).

Toxicological experiments on animals have confirmed a number of PAHs that can lead to cancer in animals, and some epidemiological studies are making a strong case of PAH carcinogenicity in human beings (Armstrong et al. 2004). The IARC has recognized well studied PAHs like 7,12-dimethylbenzoanthracene (DMBA) and BaP as cancer-causing agents in humans, with many other PAHs being listed as definitely, probably or possibly carcinogenic to humans (Rengarajan et al. 2015; Boström et al. 2002). Given their omnipresence in the environment, it is impossible to avoid contact with them, which makes risk assessment a necessary component in the study of PAHs.

PAHs are non-polar lipophilic substances, which allows them to be quickly absorbed and stored in the body, regardless of route of entry (Kim et al. 2013). Some compounds have been recognized as epigenetic, increasing tumour growth, or genotoxic, reacting directly with cellular DNA. In order to achieve this level of toxicity, the stable PAHs must undergo metabolic transformation to be thus rendered unstable and highly reactive (Choi et al. 2010). Their metabolism generally occurs with the help of cytochrome P450, usually with oxidation or hydroxylation as a first step. This leads to epoxides, quinones and other highly-electrophilic derivatives that target the nucleus (Choi et al. 2010). On top of frameshift mutations, PAH metabolites are capable of base-pair substitutions, strand breaks and altering chromosomes (Jung et al. 2013).

The intricacy in understanding the health effects of PAHs is their diversity as a chemical group (Masih et al. 2012). Exposure to PAHs is rarely to one PAH at a time, but more so a mixture. This adds a layer of complexity in understanding the related health risks because the different compounds will behave differently in the body (Jang et al. 2018). Their ability to cause cancer has been proven in numerous cases, or at a minimum implied, however, the relationship between exposure and negative health response has yet to be fully quantified (Boffetta et al. 1997). This information is normally considered paramount in the establishment of occupational health and safety regulations (Schrenk 2002). DMBA has been linked to breast cancer whereas BaP is more associated with lung cancer (Lai & Singh 2006; Denissenko et al. 1996). Observations have been made theorising a connection between exposure to coal tar, containing a mixture of over five hundred compounds, and bladder cancer (Tremblay et al. 1995). The route of entry is equally as important where PAHs that are eaten will more likely cause stomach cancer versus those that are inhaled causing lung cancer (Kim et al. 2013).

Acute health effects are not well documented or understood in regards to PAHs. Workers in PAH-heavy industries often claim eye-irritation, nausea, vomiting, diarrhoea, skin irritation and inflammation; however, these were mixtures of pollutants that contained not just multiple PAHs, but other compounds as well (Cocker et al. 2003; United Nations Environment Programme et al. 1998). Non-cancerogenic long term effects may include cataracts, kidney and liver damage, asthma and other respiratory problems (Bach et al. 2003). As a mechanism for toxicity, PAHs are strong immunosuppressants. They tend to interfere with cellular membrane function through the inhibition of enzymes via the same mechanisms by which they cause cancer (Armstrong et al. 2004).

The lipophilicity of PAHs is to the point where they are able to cross the placental barrier and have been detected in foetal tissues and umbilical cord blood (United Nations Environment Programme et al. 1998; Sexton et al. 2011). Experiments conducted on rats have brought about worries of teratogenicity whereby birth defects are seen on the pups of exposed rats. Observed deficiencies include low birth weight, premature delivery and heart problems in rats (Perera et al. 2006). It is unknown which teratogenic effects are identical for human beings, but behavioural problems have been observed in human children where the parent was exposed to high levels of PAHs during pregnancy (Edwards et al. 2010).

It should be noted that PAHs are more toxic in the presence of ultraviolet light, where photooxidation works to destabilise the compound and thus make it more reactive (Abdel-Shafya & Mansour 2016).

1.1.5 Monitoring Exposure

Given the potential negative health effects that could arise upon exposure to PAHs, it is important to establish reliable tools that can monitor exposure. This serves to both prevent undesirable health outcomes by minimising risk and establish the relationship between dose and effect (Harper 2004). When considering environmental pollutants that are ubiquitous, the first step is to survey the amount that one is exposed to (World Health Organization 1996).

When considering PAH content in food, various regulatory bodies, such as Health Canada and the Canadian Food Inspection Agency, take the lead in setting out the maximum content of PAHs on an individual basis (Zelinkova & Wenzl 2015). That is to say, regulations are established per individual chemical, rather than with PAHs as a group. Quality assurance tools have been developed to quantify PAH content in food, typically through analytical methods (Zelinkova & Wenzl 2015). In Canada specifically, only benzo[a]pyrene content in olive-pomace oil (at 3 μg/kg maximum) and water (at 0.1 μg/L maximum) are regulated, with a general provision prohibiting poisonous or harmful substances regardless of whether a maximum has been set for it (Santé Canada 2017).

In an occupational setting, it is a little more complicated. With food and drink, the PAH content ingested is the PAH content that one is exposed to (Evenson et al. 1988). In the workplace, given that PAHs there are rarely consumed, but more so inhaled or touched, the amount one is exposed to is not necessary the amount that enters the body (World Health Organization 1996). This can be for a variety of personal factors ranging from age, gender, genetics, height and weight, as well as protective measures put in place such as personal protective wear and ventilation (Llobet et al. 2003; Jagt et al. 2004).

An estimated 350,000 Canadians, making up two percent of the working population, are exposed on a regular basis through work to PAHs: 120,000 live in Ontario, 84,000 in Québec and 41,000 in Alberta (CAREX Canada 2016). These workers mainly come from the restaurant industry, petroleum industries, automotive repair, firefighters, coal tar production and distillation, coal gasification, aluminum production, carbon electrode manufacture, mining,

metalworking, calcium carbide production, and municipal trash incinerators (CAREX Canada 2016).

Previously, air monitoring was used to estimate PAH exposure. The air would be sampled and, through a filtration system, analyzed to gain an estimate of the concentrations of PAHs in a given area (Unwin et al. 2006). Using the results obtained, occupational health and safety regulations were developed. The American Conference of Governmental Industrial Hygienists, for example, had set up a maximum exposure limit of 0.2 mg/m^3 over an eight-hour period for coal workers (American Conference of Governmental Industrial Hygienists 1998). This system, while providing important information, did not take into consideration the multiple pathways of entry needed to be fully cognisant of PAH exposure (Choi et al. 2010). Furthermore, it lacked the ability to transition the concentration of PAH in an area versus the quantity that successfully enters the body by any given pathway (Zhao et al. 1990). As a result of these weaknesses, there was a natural transition to biosurveillance as the preferred method of monitoring exposure (Hayes et al. 2007).

A biological marker, shortened to biomarker, refers to a broad range of compounds that can be observed in accessible biological matrices, often blood, that give accurate reproducible results regarding happenings inside the body (Strimbu & Tavel 2010). That is to say, these are substances that indicate xenobiotic infiltration using normal biological processes and responses as a base. A chemical compound or its metabolites in blood or a biochemical change are examples of biomarkers. The key challenge, when developing a biomarker for a given substance or scenario is always determining the relationship between the biomarker and said substance/scenario (Strimbu & Tavel 2010).

In this instance, the internal dose of PAH is found by evaluating biomarkers in human urine and estimating exposure based off of the results (Lutier et al. 2016). Urine is the preferred matrix because it requires a less invasive sampling procedure when compared to blood, and unlike faeces, urine is excreted multiple times a day, which enables continuous monitoring (Vaidya & Bonventre 2010). These biomarkers may even be used to follow and determine the kinetics of PAHs (the timing surrounding when they enter, metabolise and leave the body) (Lutier et al. 2016).

Extensive research has been done by many laboratories around the world and currently the preferred biomarker for PAH exposure is 1-OHP for cutaneous, oral and respiratory entries

(Srogi 2007; Viau 1999; Klöslová et al. 2016). It is the main metabolite of pyrene, a non-cancerogenic PAH, representing an estimated ninety percent of all of pyrene's metabolites (Ifegwu et al. 2012). The high levels of pyrene present in most PAH mixtures means that this biomarker truly functions as a good reference for the internal dosage of PAHs as a whole. Given the half-life of urinary 1-OHP, it represents the last twenty-four hours of PAH exposure (Ifegwu et al. 2012).

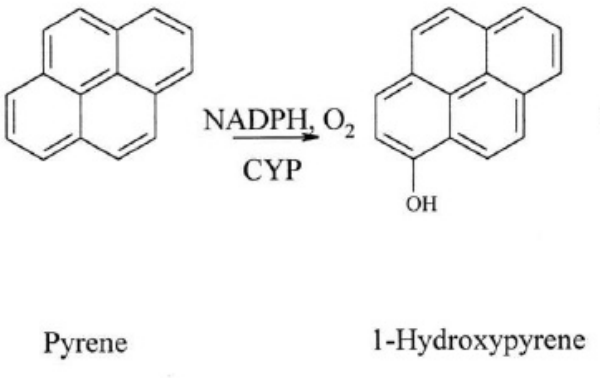

Figure 3: The relationship between pyrene and 1-hydroxypyrene (Jongeneelen 2001).

The limitation with this is that each PAH is a different compound providing different dangers. Some PAHs are more toxic than others. In grouping them all together, 1-OHP gives an insight as to whether someone has been in contact with PAHs, but is not specific as to which ones and thus which countermeasures need to be taken to ensure safety (Kang et al. 2005). Moreover, regulating contact with unknown and ever-changing mixtures is not likely to be practical. When looking at PAH food regulations, they are compound specific, and in establishing similar guidelines for the workplace, it is important to target the PAHs known to be dangerous, thus having a biomarker unique to that PAH. This enables scenarios whereby one can assess the risk of, for example cancer, given a worker's exposure by looking at biomarkers for the carcinogenic PAHs in that person's urine.

1.2 Benzo[a]pyrene

1.2.1 Chemical Properties

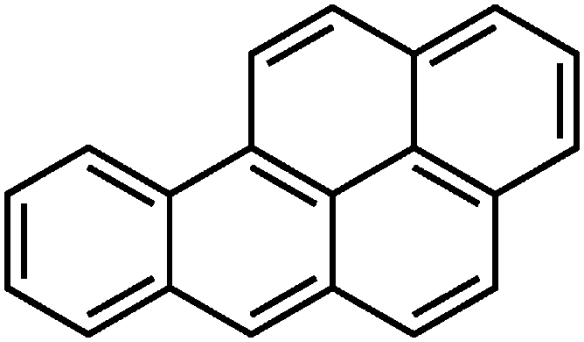

Figure 4 : The chemical structure of benzo[a]pyrene (IARC Working Group on the Evaluation of Carcinogenic Risk to Humans 2012).

The chemical formula of benzo[a]pyrene (BaP) is $C_{20}H_{12}$ with a molecular mass of 252.31 g/mol. Alternatively named 3,4-Benzopyrene, it takes the form of a pale-yellow solid at room temperature with a boiling point ranging from 310°C to 496°C and a melting point of 178-179°C (IARC Working Group on the Evaluation of Carcinogenic Risk to Humans 2012a; Choi et al. 2010; Comité fédéral-provincial-territorial sur l'eau potable 2015). Chemically, BaP is formed through the highly exothermic fusion of benzene with pyrene between 300°C and 600°C (R. P. Schwarzenbach 2017). Alternative outcomes include benzo[e]pyrene which is not as well understood or as studied as BaP (Sharma et al. 2008).

BaP exhibits similar traits to most of the PAHs; it is non-polar, highly lipophilic and, with a solubility of less than 0.1 mg/L, does not easily dissolve in water (IARC Working Group on the Evaluation of Carcinogenic Risk to Humans 2012). As a result of the stability provided by the five fused rings, the compound does not easily biodegrade and remains in the environment for a long time. It is found in car exhausts, cigarette smoke, coal tar, grilled food and asphalt, but easily penetrates the soil, groundwater and waterways (Armstrong et al. 2004). When in the atmosphere, BaP has a vapour density of 8.7 (with air as 1) and tends to form aerosols. It has

one of the lower vapour pressures in comparison with other large PAHs (Abdel-Shafya & Mansour 2016).

1.2.2 Sources

Similar to the other PAHs, BaP is omnipresent in the environment. Although most PAH mixtures tend to be randomised, there are certain instances where BaP content is known to be higher.

In total, an estimated 19,000 to 22,000 kg of BaP are emitted every year in Canada from anthropogenic sources, including industrial processes and fossil fuel combustion (National Research Council of Canada 1983). One of the more prominent sources of exposure that is more unique to BaP is tobacco smoke. Sidestream smoke has been reported to have between 52 to 95 ng of BaP per cigarette, which is more than three times higher than the BaP concentration found in mainstream smoke (IARC Working Group on the Evaluation of Carcinogenic Risk to Humans 2012).

BaP content in water has been measured in the Great Lakes as well, with an estimated 9.1 to 9.8 ng of BaP per cm^2 of surface area (National Research Council of Canada 1983). The major source of this BaP is believed to be direct atmospheric input. BaP is, however, sparse in drinking water as is evidenced by a survey of seven water treatment plants in the Niagara area of Ontario, Canada (International Joint Commission 1983).

As one of the larger PAHs, BaP is not regularly found in gaseous or vapour state, but in solid form. In the atmosphere, as with most other mediums, BaP adsorbs onto the nearby particles in the air, forming aerosols, and on land, collating to soil and sediment particles which are then transported by the various earth cycle processes (e.g. the rock cycle, the water cycle) or the weather (Mackay & Paterson 1991). It is estimated that 82% of BaP found in the environment is in the soils, 17% in sediment, 1% in water and less than 1% in the air (Hattemer-Frey & Travis 1991).

1.2.3 Health Concerns

1.2.3.1 Non-Cancerous Effects

The physicochemical properties of BaP enable quick uptake and circulation within the body. Experiments in toxicology demonstrate the harm that BaP can cause to organisms in development. Studies in animals and humans confirm the presence of BaP or its metabolites in foetal tissues, thus confirming that it is capable of crossing the placental barrier as a result of its lipophilicity (Madhavan & Naidu 1995; Withey et al. 1993); furthermore, breast milk can be contaminated by BaP, especially in the case of smokers (Yu et al. 2011).

A single injection of BaP in young rats reduced their rate of growth (Greim 2007); and this was further seen in human beings through a cohort study that followed exposed mothers and confirmed stunted growth in their children (Tang et al. 2006). There is also an overall decrease in foetal survival according to studies on animals, with the potential for causing infertility, though those on humans were inconclusive (Wu et al. 2010; MacKenzie & Angevine 1981). Reduced fertility was confirmed for both the rats exposed to BaP (male and female) as well as their offspring (MacKenzie & Angevine 1981).

BaP exposure in young children can alter learning capabilities, memory, motor function, and electrophysiological pathways in the brain (Broekman et al. 2009; F. Perera et al. 2012; Chen et al. 2012). There have been links made to increased anxiety, hyperactivity and depression (F. P. Perera et al. 2012).

BaP also functions as an immunosuppressant, like many other PAHs. The thymus gland, where T cells that are essential for organism immunity mature, was smaller in rats that had been orally exposed to BaP in comparison with the controls (Kroese 2001). Further studies showed a reduction in the number of B cells, another component in organism defense, around their production area - the bone marrow and the spleen (De Jong 1999). In human beings, the evidence shows similar results. Apoptosis, cell death and destruction, have also been observed amongst lymphocytes and monocytes, both important immunoprotection cells, in occupationally exposed coke oven workers (Zhang et al. 2012).

In terms of immediate short-term negative health effects, dermal application can lead to verrucae, a series of warts that are treatable (United Nations Environment Programme et al. 1998). Other effects have not been conclusive. Beyond this, the only other non-cancer effects of BaP that are noteworthy include the reduction of size of most major organs in the body including the liver and the kidneys in treated animals (De Jong 1999).

1.2.3.2 Cancer

Although BaP itself may have negative health consequences, it is most dangerous when metabolised. In this form, it is capable of reacting with DNA and causing mutations, which may lead to tumours and the eventuality of cancer (Stowers et al. 1985). Given the biochemical disruptions that BaP has been linked to, these developmental malformations and mutations may also lead to cancer.

BaP has produced tumours in mice, rats, hamsters, guinea-pigs, rabbits, ducks, newts and monkeys via the various routes of exposure: oral, dermal, inhalation, subcutaneous, intraperitoneal and intravenous (IARC Working Group on the Evaluation of Carcinogenic Risk to Humans 2012). Animals developed lung cancer when exposure to BaP was by respiration, stomach cancer from eating food laced with BaP, and skin cancer from cutaneous entry (Heinrich et al. 1994; Rigdon & Neal 1969; Levin et al. 1977).

In humans, occupational exposure to BaP has been linked to a variety of cancers: BaP exposure in coke production, coal gasification, paving and roofing, and aluminum smelting are associated with lung cancer; coal gasification and aluminum smelting have been linked to bladder cancer; and, coal tar distillation provides evidence of skin cancer (IARC Working Group on the Evaluation of Carcinogenic Risk to Humans 2012). Mastrangelo et al. (1996), for example, compiled studies that showed that workers in a tin mine who were exposed to 1.6 to 108 $\mu g/m^3$ of BaP per year were twice as likely to get lung cancer as the general population, and those exposed to 108 to 250 $\mu g/m^3$ per year were at 2.7 times the risk. Amongst aluminium factory workers, exposure to 10 to 99 $\mu g/m^3$ of BaP per year augmented the risk of bladder cancer by two, 100 to 199.9 $\mu g/m^3$ per year revealed a risk of 6.2 for bladder cancer, and 200 to299.9 $\mu g/m^3$ per year held a risk of 6.7 (Mastrangelo et al. 1996).

When in the body, BaP is quickly absorbed because of its lipophilic properties and stored in fatty tissues (Moreau 2013). When reaching the liver, cytochrome P450, a metabolic enzyme, transforms the aromatic hydrocarbon into an electrophile, whether it be a diol-epoxide, quinone or another electrophile that is highly unstable; one of the more well-known ones being (+)-benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide (BaPDE). Metabolism usually makes the compound more soluble in water, and thus easier to excrete, but it has the added side effect of making BaP that much more bioactive (Shimada & Fujii-Kuriyama 2004).

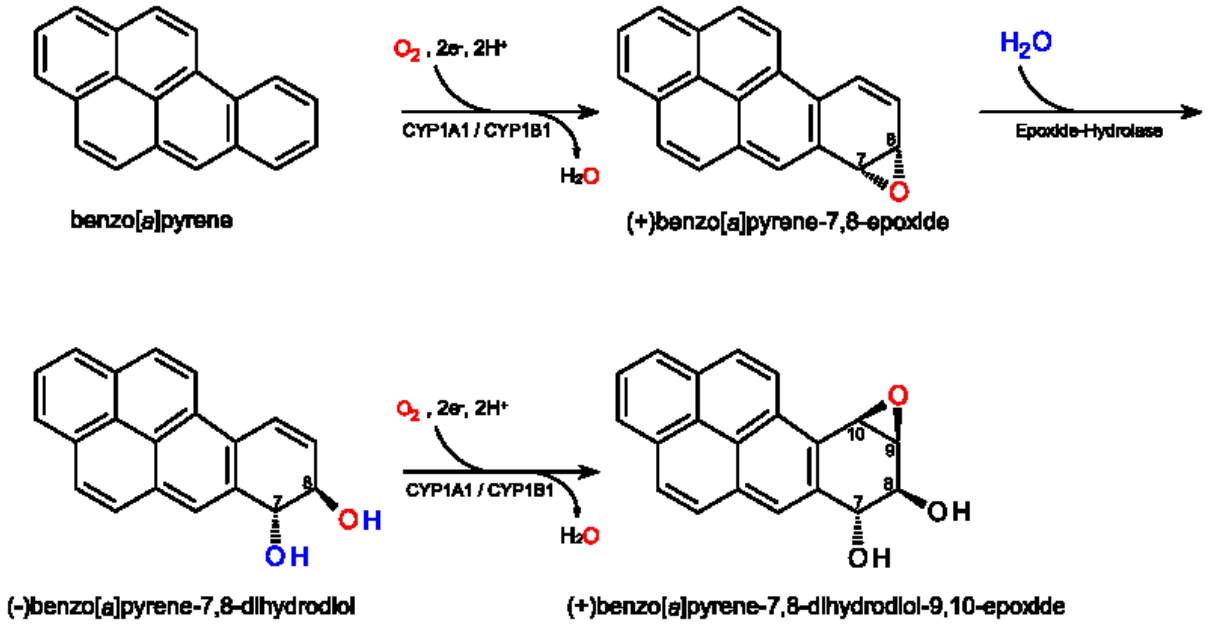

Figure 5: The synthesis of (+)-Benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide (BaPDE) (Kleiböhmer 2001). Note that CYP1A1 and CYP1B1 refer to the specific subtype of cytochrome P450 that deals with BaP.

This electrophilic compound is able to bind to nucleic acids as a result of the bay region located between the carbon 10 and 11 on the BaPDE molecule (Koreeda et al. 1978). This reaction can cause a change in the DNA codons that define what proteins are to be produced, thus a point mutation, which in turn can lead to tumours and irregular behaviour in the cells that could function as a starting point of carcinogenesis (Jiang et al. 2007).

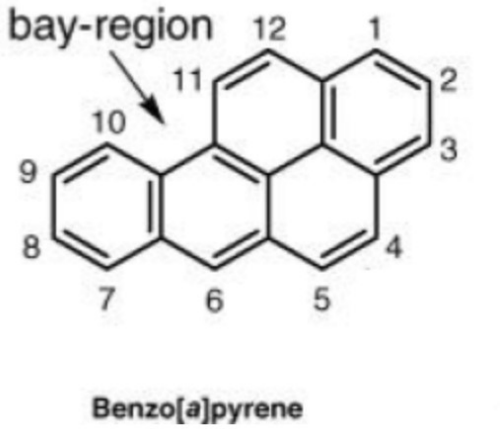

Figure 6: The bay-region of Benzo[a]pyrene (BaP) adapted from (Platt et al. 2008).

Other metabolites of BaP are also implicated in the carcinogenesis of BaP. A radical can be formed, once again with the help of cytochrome P450, which is highly reactive (Stack et al. 1995). The radical cation tends to target guanine or adenine amongst the codons and thus creates apurinic sites where the codon lacks its pair on the opposing DNA strand.

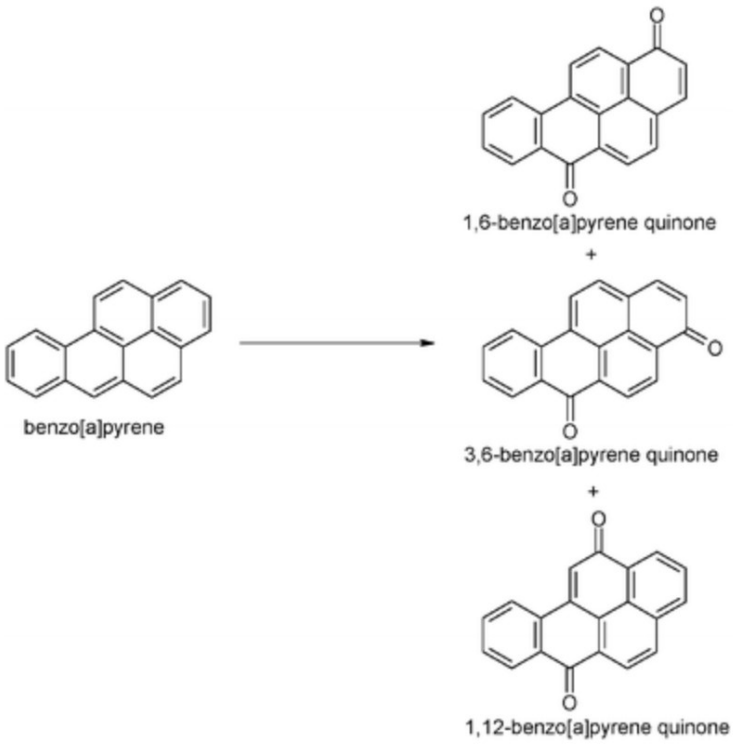

Figure 7: An example of some quinones of benzo[a]pyrene (Hartmann & Jung 2010).

The third possibility that creates cancerous circumstances is through the transformation of BaP, with the aid of cytochrome P450, to a quinone. The BaP quinones can either react directly with DNA, or through forming extremely reactive oxygenated compounds, such as a radical anion of superoxide ($O_2^{\cdot-}$) or hydrogen peroxide (H_2O_2), which in turn form hydroxide anions (OH^-) which are extremely reactive with DNA (Xue & Warshawsky 2005). The BaP quinones may also be biotransformed into BaP diones which increase cytochrome P450 activity, which in turn leads to the formation of more BaPDE, which increases the damage to DNA (Burczynski et al. 2000).

1.2.4 Monitoring Exposure

Monitoring exposure to BaP in an occupational setting is presently a complex affair.

Environmental monitoring is relatively straightforward. Analytical instruments are used to measure the quantity of BaP in a given matrix (water, air, soil). The results can then be used to verify that any regulations surrounding BaP content and exposure are being met. A prominent example is in drinking water. After the water has been sampled, liquid-liquid extraction or liquid-solid extraction may be used to concentrate the contaminants before they are analysed by gas chromatography coupled to mass spectrometry (GC/MS) or high-performance liquid chromatography (HPLC) (Comité fédéral-provincial-territorial sur l'eau potable 2015).

Environmental monitoring can reveal the quantity of BaP available for exposure, that is to say the amount that one may come into contact with in the line of their work duties. It does not, however, give a true representation of the quantity that one is actually exposed to (Srogi 2007). Due to a variety of factors, whether it be intrinsic (such as weight, body-fat content, age, height, pregnancy status, health status) or external (for example, the clothes worn, personal protective equipment used etc.), no two people are exposed to the same amount when looking at routes of entry alternative to ingestion and injection, or a combination thereof (Wagner 2006).

Biosurveillance is used to avoid these inconsistencies and track exposure in occupational settings. At present, the most developed biomarker for BaP is 3-OHBaP, a metabolite that can be found in the urine and the faeces. It is produced through the metabolism of BaP in the liver, where the rate of clearance from the body is governed by the rate of transfer of the bile to the gastrointestinal tract (Ortiz 2014).

Marie et al. (2010) modelled the kinetics of 3-OHBaP in rats. The plasma profile of this metabolite showed a biexponential elimination after an intravenous injection, while the excretion profile showed a build up in urine followed by a progressive elimination, compatible with a renal retention. The experiment also presented the need for a sensitive analytical tool to measure excretion since 3-OHBaP eliminated represents only 0.21% of the BaP dose injected in rats, the majority of which is excreted in the faeces (Marie et al. 2010).

Urine is the preferred matrix for biomarker measurement in the workplace as it is non-invasive to sample, and relatively easy to obtain in comparison to faecal samples. Methods involving 3-OHBaP, as adapted for humans, noticed a delay in 3-OHBaP hitting peak value. Lutier et al. (2016) showed through a study comparing 1-OHP and 3-OHBaP that post-shift, 1-

OHP concentration increased in urinary quickly whereas 3-OHBaP levels remained steady, or decreased, before finally increasing.

The toxicokinetics of 3-OHBaP are not very conducive for the biomonitoring of exposure. It adds a layer of complexity to the relationship between exposure and effect that is key in biomonitoring. Lutier et al. (2016) recommend urinary collection of employees a day after exposure when using 3-OHBaP to accommodate for this. Therefore, 3-OHBaP can be used to confirm BaP exposure, but it has not been particularly appropriate for monitoring exposure, assessing the timing or the dosage involved (Moreau & Bouchard 2015; Barbeau et al. 2014).

1.3 4,5-dihydrodiol-benzo[a]pyrene and 7,8-dihydrodiol-benzo[a]pyrene

1.3.1 Chemical Properties

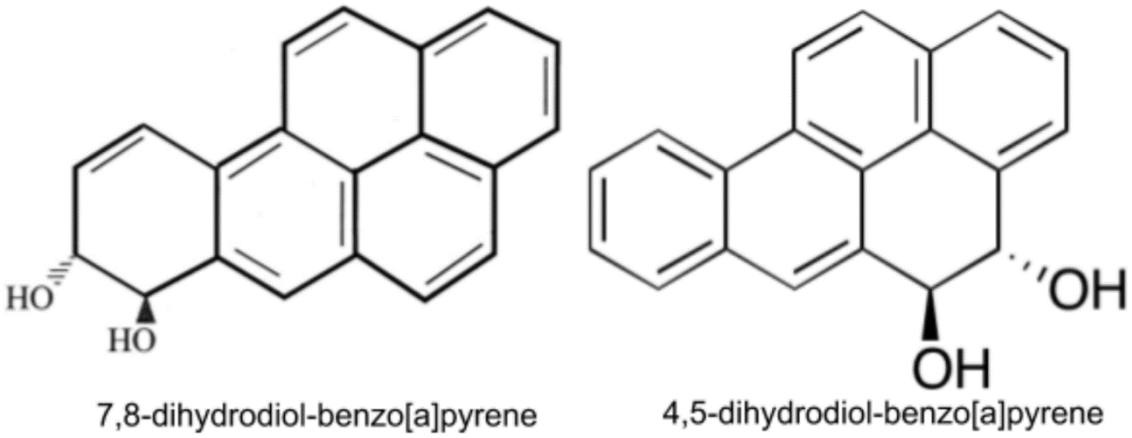

Figure 8 : The chemical structure of 4,5-dihydrodiol-benzo[a]pyrene and 7,8-dihydrodiol-benzo[a]pyrene (Pessah et al. 2001; Toronto Research Chemicals n.d.)

4,5-DiolBaP and 7,8-diolBaP both have the chemical formula $C_{20}H_{14}O_2$ with a molecular weight of 286.33 g/mol (Pubchem n.d.). Not much is known about these compounds in terms of their vapour density or other characteristics. 7,8-DiolBaP has been however documented as being more carcinogenic than BaP in newborn mice (Kapitulnik et al. 1977); when metabolically

activated by liver microsomes, the ensuing compound binds more to DNA than BaP when activated.

1.3.2 Potential as Biomarkers

In looking for an ideal biomarker for BaP exposure, and thus carcinogenic PAHs in total, researchers have been comparing the temporal profiles of several BaP metabolites via various routes of entry in animals: 3-OHBaP, 7-hydroxybenzo[a]pyrene (7-OHBaP), 9-hydroxybenzo[a]pyrene (9-OHBaP), trans-9,10-dihydrodiobenzo[a]pyrene, 4,5-diolBaP and 7,8-diolBaP (Marie et al. 2010; Moir 1996; Aramandla Ramesh Michael Greenwood et al. 2001; Ramesh et al. 2001; Likhachev et al. 1992; Weyand & Bevan 1986; Moody et al. 1995).

Moreau & Bouchard (2015), in order to better compare these biomarkers, evaluated them together in the same experimental framework in rats, looking to create direct comparisons. They found that during the 72 hours after exposure, the relative amounts of the biomarkers were as follows in the rat urine (in order of descending relative concentration): after intratracheal and intravenous exposure: 4,5-diolBaP, 3-OHBaP, 7-OHBaP, 7,8-diolBaP; after oral dosing: 3-OHBaP, 4,5-diolBaP and 7-OHBaP, 7,8-diolBaP; after cutaneous application: 3-OHBaP and 7-OHBaP, 4,5-diolBaP, 7,8-diolBaP. They further noticed that the urinary time course curves demonstrated a faster elimination of the diolBaPs (Moreau & Bouchard 2015).

This brought attention to the diolBaPs as potential alternative biomarkers of BaP exposure. They were produced in similar quantities to 3-OHBaP, which was currently in use, they were compound-specific to BaP, and they hinted at more favourable kinetics through their faster elimination rates (Moreau 2013; Ortiz 2014). The diolBaPs undergo monoexponential elimination, unlike 3-OHBaP and 7-OHBaP, which follow two phases (see Introduction Chapter 1.2.4 Monitoring Exposure). Their rapid elimination can be explained by their polar nature, whereas 3-OHBaP's atypical profile can potentially be attributed to kidney retention or the reabsorption of the free form, which the diolBaPs seem to not be impacted by (Heredia-Ortiz & Bouchard 2013; Schänzer et al. 1991).

Due to the promising results from Moreau & Bouchard (2015), the next logical step was to assess diolBaPs in a human context, which is explored in this thesis.

An assessment of the use of these two diolBaPs has never been done in human beings. Previous studies took place in animals, but the transition between species is often not the easiest to predict. Laboratory rats are raised and exposed to only one compound through the intravenous, intraperitoneal or subcutaneous route, which is rarely the case in human exposure. There is also the fact that humans are very rarely exposed to one compound at a time, but experience contact with mixtures.

2. Research Hypothesis and Objectives

2.1 Hypothesis

This project is based on the hypothesis that 4,5-diolBaP and 7,8-diolBaP, two urinary metabolites of BaP, are useful compound-specific biological markers that can be used to detect, assess and quantify exposure to BaP. This comes from the reasoning that existing biomarkers used in the evaluation of BaP exposure are either not optimal based on toxicokinetics, such as 3-OHBaP, or do not represent BaP exposure specifically, but rather PAHs as a whole, i.e. 1-OHP.

2.2 Objectives and Aims

This study has two central objectives, all culminating in providing key components for the establishment of workplace health and safety standards surrounding BaP exposure. They are as follows:

- **Objective 1**: Assessing, in a case-study, the potential use of 7,8-diolBaP as a biomarker of BaP exposure by comparing its kinetic time course to that of the validated biomarker, 1-OHP, in a volunteer following two different exposure scenarios to coal-tar shampoo containing PAHs.

- **Objective 2**: Exploring the use of 4,5-diolBaP and 7,8-diolBaP as biomarkers of BaP exposure in an occupational setting versus 1-OHP and 3-OHBaP by studying the kinetic time courses of these metabolites in a group of occupationally-exposed workers.

Moreau & Bouchard (2016) found low excretion concentrations of 7,8-diolBaP in experimentally exposed rat urine, and as such they recommended developing a more sensitive method to further exposure 7,8-diolBaP as a potential biomarker of BaP exposure. Objective 1 serves to verify that 7,8-diolBaP is indeed detectable in measurable concentrations in human urine in a controlled setting before testing its concentration level in an occupational setting (Objective 2). This was not seen as necessary for 4,5-diolBaP given its prominent excretion concentrations observed in rat urine.

3. Methodology

3.1 General Overview

There were two major studies conducted in the course of this thesis.

The first was a case-study comparing the urinary time course of 7,8-diolBaP and 1-OHP metabolites following the application of a coal-tar based medicated shampoo often used for psoriasis treatment. Two sets of experiments took place where a volunteer used the shampoo and collected all micturition over the two-day period preceding exposure and a two-to-four-day period post-exposure. By controlling the exact moment of exposure and, as a direct result of having all the pre-and post-exposure urine of the volunteer for an extended period of time, this study allowed a preliminary understanding of the kinetics of 7,8-diolBaP in a human context.

The second study followed the time courses of 4,5-diolBaP and 7,8-diolBaP excretion in the urine of occupationally exposed workers during a typical work week. Five workers from an anode production plant in Grenoble, Auvergne-Rhône-Alpes, France, volunteered. These workers were exposed on a regular basis to PAHs, including BaP, in their line of work. Samples were collected at the beginning and at the end of their shift, and at random points throughout the weekend where minimal non-occupational exposure was expected. The goal here was to have pre-exposure and post-exposure samples from the workplace, verifying whether a pattern was emergent and confirming the ability to track BaP exposure through measurements of the metabolites in a series of urine samples.

These two overarching components of the study have been aptly named the “shampoo experiment” and the “worker study”. They each used a similar method of analysis and identical chemicals, with the only differences being in the volunteer population and sample collection.

3.2 Sampled Subjects

3.2.1 "Shampoo Experiment"

A case-study volunteer participated in the “shampoo experiment” (Person X). The volunteer was a male subject in good health, aged 47, who took part in two sampling sessions; the first being a single exposure event, the second assessing multiple consecutive exposures spread out over three days. Person X did not have any health issues that would require him to use the medicated coal-based shampoo, and the first collection session also marks the first time he has used this kind of shampoo. He reported being a non-smoker and without regular contact with PAHs in the workplace.

3.2.2 "Worker Study"

Six industrial workers regularly exposed to BaP on the job at an anode producing plant in Grenoble, Auvergne-Rhône-Alpes, France, volunteered to allow the collection of their urine at the start and at the end of their work shift, with some samples collected over the weekend when they were not working as well. The detailed description of the sampled population is provided in Barbeau et al. (2015).

3.3 Urine Samples

3.3.1 "Shampoo Experiment"

The methodology used was adapted from a study by Viau & Vyskocil (1995). Prior to exposure, the volunteer was instructed to collect all his urine for twenty-four hours in order to provide a baseline standard as to what his urine normally consists of without shampoo use. On the day of exposure, he was asked to take one final pre-exposure micturition and immediately enter the shower with the express purpose of using the shampoo. The reasoning behind this was to reduce the dilution of the first post-exposure sample with urine formed pre-exposure. Post-exposure collection continued for three days thereupon. Every time the volunteer urinated during the experimental process, he noted the time and volume prior to bottling the sample.

3.3.1.1 Single Exposure

The volunteer used an estimated 6 mL of the shampoo and followed the instructions on the back of the bottle to the letter, using a timer and a measuring cylinder to keep a modicum of precision. He applied the shampoo to his hair after it was wet, massaging it into his scalp for two minutes, rinsed immediately, reapplied and left the shampoo on his scalp for five minutes. During the rinsing, the volunteer was careful to avoid letting the runoff touch anywhere else in his body such that cutaneous absorption from the scalp or the hands were the sole routes of entry.

3.3.1.2 Multiple Exposure

This second sample collection took place four weeks after the first, thus giving adequate time for the BaP and other PAHs from the shampoo application to leave his system. In the multiple exposure scenario, he followed the same steps as in the single exposure scenario with two exceptions. He used 10 mL of the shampoo each time, and he self-exposed three times at roughly twenty-four-hour intervals.

3.3.2 "Worker Study"

Urine samples were collected from exposed workers at an anode producing plant in France. They were taken at the beginning and the end of each work shift over a week, and at additional points in time over the weekend. Dr. Anne Maître's team from the Université Joseph-Fourier de Grenoble (Grenoble, Auvergne-Rhône-Alpes, France) were responsible for collecting the samples from the plant workers and sending them to the team in Montréal, Quebec, Canada. Samples were collected in polypropylene bottles and kept frozen at -20°C until analysis.

3.4 Chemicals

Chemicals were sourced with the express purpose of creating standards, processing the samples, and running the Ultra-High-Performance Liquid Chromatography (UHPLC) system.

Reference standards of 1-hydroxypyrene, 4,5-dihydrodiol-benzo[a]pyrene and 7,8-dihydrodiol-benzo[a]pyrene were obtained from the National Cancer Institute Chemical Carcinogen Reference Standards distributed by Midwest Research Institute (Kansas City, Missouri, USA). β-Glucuronidase/arylsulfatase (100 000 Fishman U ml–1 and 800 000 Roy U ml–1 from Helix pomatia) was procured from Roche Diagnostics (Laval, Quebec, Canada).

High-performance liquid chromatography (HPLC)-grade methanol (MeOH), ethyl acetate, ascorbic and citric acids were derived from Fisher Scientific Company (Ottawa, Ontario, Canada).

The shampoo chosen was a Canadian brand labelled as medicated "for the control of itching, redness and scaling associated with stubborn seborrhea or psoriasis of the scalp" produced in Montréal, Quebec, Canada. The active ingredient targeted is listed as 10% coal tar topical solution by weight.

3.5 Urine Analysis

Moreau & Bouchard (2015) successfully established a method to detect diolBaPs in the urine of rats exposed to high doses of BaP, using a UHPLC-fluorescence system. The method was adapted in the current work to attempt to quantify dioBaP at the much lower concentration levels found in human urine.

The urine collected was stirred and had to be concentrated ten-fold. 10 mL of each urine sample was taken and mixed with an equal amount of sodium acetate buffer (0.1 M, pH 5), then hydrolysed with 20 µL of β-glucuronidase/arylsulfatase at 37°C in a shaking bath for an hour to obtain the sum of free and conjugated metabolites.

The next step was solid phase extraction to isolate the metabolites from polar (water-soluble) components found in urine (using Sep-Pak C18 cartridges; Waters Corporation, Milford, Massachusetts, USA). The cartridges were conditioned with 4 mL of methanol and 8 mL of water. The urine samples were then passed through the cartridges. For diolBaP analysis, the cartridges were then washed with 8 mL of water and 8 mL of 15% methanol:water solution in an effort to reduce polar contamination. For 1-OHP analysis, cartridges were washed with a 50% methanol:water solution instead for the "worker study" only since the compound is more

difficult to elute, and thus a larger quantity of impurities could be removed without losing the desired compound. Subsequently elution took place for all samples and analytes using 8 mL of pure methanol. Nitrogen gas was used to aid throughout this process.

Afterwards, evaporation of the methanol at 40°C took place, and the resulting residue was dissolved in 200 µL of pure methanol. Samples were then centrifuged for 30 sec. The liquid layer was then transferred to vials for UHPLC-fluorescence analysis.

The analysis was carried out using an Agilent 1290 Infinity Ultra-HPLC system (Mississauga, Ontario, Canada). It is comprised of five modules: a binary pump, an autosampler, a thermostatted column compartment, a diode array detector and a fluorescence detector. There were two columns used in the process: a Poroshell 120 SB-C18 (2.1 X 50 mm; 2.7 µm) from Agilent #689775-902, and a Zorbax Eclipse plus C18 RRHD (2.1 X 50 mm; 1.8 µm) from Agilent # 959759-902. The first column was used to "load" the sample and clean it whilst preventing elution and the second served as an analytical column that passed the liquid to the detectors. The temperature of the analytical column was set at 40°C. Switching from the loading column to the analytical column was set at 11.5 min.

Passing of samples through the columns was performed using a methanol:water gradient mobile phase also containing 5 mM of citric acid and 10 mg/L of ascorbic acid. Ascorbic acid was added to the mobile phase in accordance with a previous study conducted by Bouchard et al. (1994). The flow rate into the analytical system was 0.2 mL/min, the injection volume was 10 µL. Samples were kept at 13°C on the injection tray, and the PMT gain was kept at 11. The run time was set at 30 minutes per sample with three minutes postrun. The gradient elution conditions during each run were as follows: at 5.5 minutes, the water:methanol ratio was 90:10; 35:65 at 9.2 minutes; 0:100 at 21.5 minutes and, 35:65 at 29 minutes. The excitation and emission wavelengths for the fluorescence analysis of 4,5-diolBaP were 274 nm and 390 nm respectively, and 264 nm and 415 nm for 7,8-diolBaP.

Both positive and negative controls were used for quality control. Quantification was performed using an external calibration curve of each metabolites prepared in blank volunteer urine before enzymatic hydrolysis and analysed as previously described for the other volunteer samples.

3.6 Data Analysis

After UHPLC-fluorescence analysis, the peaks associated with 4,5-diolBaP, 7,8-diolBaP and 1-OHP were evaluated using their estimated retention time to obtain the surface area for each compound in each sample. The values were put into the formula below along with information obtained during the urine collection process (such as urine volume):

$$\left(\frac{\left(\frac{Peak\ Surface\ Area - \ intercept}{Slope \times urinary\ dilution}\right) \times Redissolution\ Volume\ (MeOH)}{Volume\ of\ urine\ analysed}\right)$$

$$= pmol\ per\ mL\ of\ urine$$

Afterwards, the preliminary value of each biomarker candidate was adjusted for urinary creatinine content. Since creatinine is a side product of the energy consumption by muscle tissue eliminated by the kidneys by glomerular filtration, it is excreted in urine at a relatively constant rate with time (Wagner et al. 2010). As this is typically a reliable process, adjusting the urinary content of each biomarker for creatinine corrects for the impact of urine dilution of measured concentrations (Wagner et al. 2010).

$$(pmol\ per\ mL\ of\ urine) \div \left(\frac{Creatinine\ Content}{Molecular\ weight\ of\ creatinine}\right)$$

$$= Quantity\ per\ mol\ of\ creatinine$$

4. Results

4.1 General Overview

The results of this study have been broken into two papers: the first outlining what was learnt from the case study in a controlled setting featuring the self-exposed volunteer, and the second focusing on the workers and how effective the diolBaPs were in a real-life context.

4.2 “Shampoo Experiment”

4.2.1 Author Contributions

Chúk Odenigbo

- Worked with co-author and research director Dr. Michèle Bouchard to create the volunteer self-exposure methodology
- Worked with the laboratory technicians Ross Thuot and Marc Mantha to collect and analyse the volunteer urine samples
- Integrated sample spectrum results from the UHPLC and compiled data into spreadsheets
- Wrote all of the sections of this research article and integrated comments from co-author and research director Dr. Michèle Bouchard, as well as research professional Jonathan Côté

Michèle Bouchard

- Successfully applied for and received funding for this project to take place
- Created the sample analysis methodology used to analyse the urine samples
- Recruited the volunteer featured in this study
- Provided the spreadsheet with the necessary calculations to compile concentrations, rates, cumulative excretions and revised the spreadsheets with raw results inserted by Chuk Odenigbo
- Worked on creating graphic interpretations of the results with research professional Jonathan Côté
- Wrote the research report for the funding agency, associated with the projet
- Revised the manuscript and provided comments and suggestions

4.2.2 Mapping the kinetic profile of 7,8-dihydrodiol-benzo[a]pyrene as a biomarker of cutaneous exposure to polycyclic aromatic hydrocarbons in human beings.

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Declarations of interest: none

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4.2.2.1 Abstract

Benzo[a]pyrene (BaP) is one of the better studied polycyclic aromatic hydrocarbons (PAHs) and a known carcinogen according to the International Agency for Research on Cancer (IARC). Produced through incomplete combustion processes, it features heavily in occupational settings, yet there is no trustworthy economic method for monitoring exposure in workers.

7,8-Dihydrodiol-benzo[a]pyrene (7,8-diolBaP) is produced when BaP undergoes monooxygenation, catalysed by Cytochrome P450 1A1 or 1B1, followed by hydrogenation with epoxide hydrolase. It has previously demonstrated promising results as a biomarker of PAH exposure in rats and as such, this study sought to evaluate its capabilities in a human context.

Through organising a series of experiments where exposure took place in a controlled setting, a volunteer was engaged to self-expose cutaneously to a coal-tar-based shampoo, an off-the-counter brand geared towards those with excessive dandruff or psoriasis of the scalp. The first experimental session focused on a single exposure event, followed by three days of being monitored, whereas the second was oriented around three exposure events roughly twenty-four hours apart, with two additional days of monitoring. Samples were run by Ultra-High-Performance Liquid Chromatography coupled to fluorescence detection, from which the spectra were integrated and analysed. The results obtained for 7,8-diolBaP were compared to 1-hydroxypyrene (1-OHP), the current preferred biomarker for PAH exposure and the benchmark as to what forms a good biomarker in this instance.

7,8-DiolBaP was excreted at a comparable rate to 1-OHP and held a similar order of magnitude. There was a 2-6 hour difference between when 1-OHP and 7,8-diolBaP would attain peak value, but the kinetic profiles revealed a clear rise and fall in both compounds that corresponded with the moment of exposure. 1-OHP was found to be eliminated faster from the system and hence accumulated at a slower pace, eventually being excreted at lower levels than 7,8-diolBaP as the week progressed in both of the experimental sessions.

The results successfully outline 7,8-diolBaP as a good candidate for further study through the lens of occupational health and safety. It is commensurate kinetically, and in terms of quantity excreted, to 1-OHP. Next steps include confirming the compounds' identities through mass spectrometry and adapting the methodology to trial in the workplace.

Key words: 7,8-dihydrodiol-benzo[a]pyrene, benzo[a]pyrene, polycyclic aromatic hydrocarbon, biological monitoring, biomarker, toxicokinetics

4.2.2.2 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous pollutants widespread throughout the environment (Choi et al. 2010). They typically form as a cumulation of incomplete combustion processes whereby organic compounds undergo exothermic reactions without sufficient oxygen (Nikolaou et al. 1984). They can be characterised by their multiple fused carbon rings and share common chemical properties that render them very stable, thus, persistent in the atmosphere (Bjorseth 1983). Although similar in many respects, each of the group members demonstrate different qualities, including effects on human health (Choi et al. 2010).

Benzo[a]pyrene (BaP) is one of the better known and more dangerous PAHs (Ohura et al. 2004). It is ranked as a group 1 carcinogen by the IARC Working Group on the Evaluation of Carcinogenic Risks to Humans (2012), meaning that it is a confirmed cancer-causing agent to human beings, and a number of animal and human-based studies have demonstrated negative long term impacts on organ growth and the immune system (Weyand & Bevan 1986; van Grevenynghe et al. 2005; Mackenzie & Murray Angevine 1981). It is often a significant component of randomised PAH mixtures and features prominently in occupational settings such as iron foundries, anode production plants and diesel refineries (Lindstedt & Sollenberg 1982; Rengarajan et al. 2015).

Even with all these identified dangers, regulations and workplace policies regarding BaP are few and far between because it is hard to monitor exposure. BaP is a genotoxin that interacts directly with deoxyribonucleic acid (DNA) to cause mutations that may lead to cancer (Rengarajan et al. 2015), and as a result, it is difficult to understand what level of exposure leads to negative outcomes in the average person, thus preventing the creation of occupational exposure limits (Brzeźnicki et al. 2009).

At first, air monitoring was used, whereby the level of contaminant in the air was quantified and regulated, but this was found to be inaccurate due to the context of exposure (Zhu et al. 2011). Physiological differences in people mean that two people can be exposed to the same quantity of a substance but if it is in the air, the amount that each one absorb, and thus are truly exposed to, varies greatly (Forster et al. 2008). The focus soon shifted to biological

monitoring, quantifying exposure through substances or reactions in the body that are present solely due to said exposure (Choi et al. 2010).

In monitoring PAHs, 1-hydroxypyrene (1-OHP), a metabolite of pyrene, is quite established. Pyrene is present in large quantities in most PAH mixtures and 1-OHP is produced in easily detectable quantities in human beings (Viau 1999). It presents a simple kinetic profile where concentration increases steadily immediately after exposure, hitting its peak value within eight hours, and diminishing quickly after that. It is, for all intensive purposes, an ideal biomarker for PAHs (Jongeneelen 2001). Pyrene, however, is not carcinogenic nor considered especially dangerous (Rengarajan et al. 2015). This effectively means that although it is used to monitor PAH exposure, the risk of cancer or other negative consequences of exposure is implied through assuming that BaP and other harmful PAHs are present (Choi et al. 2010).

In order to better target the risk, compound-specific biomarkers for BaP are being looked into. The current forerunner is 3-hydroxybenzo[a]pyrene (3-OHBaP), the main monohydroxylated BaP metabolite (Forster et al. 2008). This compound presents numerous challenges, the first being that although in rat urine it is produced in sufficient quantities for detection, in human beings it is mainly excreted through the faeces (Marie et al. 2010). Extremely low amounts are released in human urine and given that urine is one of the less invasive culturally-appropriate ways to do biological monitoring, this makes it very difficult to detect (Rossella et al. 2009; Moreau 2013). Furthermore, the kinetic profile of 3-OHBaP is not as intuitive, having a half-life of up to 49.5 hours after exposure in the body (Lutier et al. 2016).

As a result, 1-OHP remains the preferred biomarker for PAH exposure, even in cases where carcinogenic PAHs are being targeted (Choi et al. 2010; Rengarajan et al. 2015; Lutier et al. 2016). An alternative compound-specific biomarker is needed by industry and government to more accurately monitor and control exposure to BaP and other carcinogenic PAH compounds.

Moreau & Bouchard (2015) found promising results in rats for 4,5-dihydrodiol-benzo[a]pyrene and 7,8-dihydrodiol-benzo[a]pyrene (4,5-diolBaP and 7,8-diolBaP; diolBaPs) when comparing their analytical results to those of 1-OHP and 3-OHBaP in the same experimental framework. This paper seeks to further evaluate 7,8-diolBaP as a potential alternative compound-specific biomarker of BaP in humans through evaluating its viability in a controlled PAH-exposure setting.

The long-term objective of this research is to establish a compound-specific biomarker for BaP that can be used to monitor exposure in the workplace and design workplace health and safety regulations to mitigate and abate the incidence of occupational cancer.

4.2.2.3 Materials and Methods

This paper seeks to evaluate the proficiency of 7,8-diolBaP as a compound-specific biomarker of BaP in a controlled PAH-exposure setting. Pursuant to this objective, one volunteer was recruited and brought into contact with PAHs using a coal-tar based shampoo, sold for the treatment of psoriasis and serious cases of dandruff. Using the already established and preferred PAH biomarker, 1-OHP, as a point of comparison, the two metabolites were analysed through an ultra-high-performance liquid chromatography (UHPLC) setup coupled to fluorescence.

4.2.2.3.1 Study Population

Aptly named Person X, the 47 year old genetically male volunteer took part in two sampling sessions; the first being a single exposure event, the second assessing multiple consecutive exposures spread out over three days. Person X did not have any health issues that would require him to use the medicated coal-based shampoo, and the first collection session also marks the first time he has used this kind of shampoo. He is a non-smoker and is not regularly exposed to elevated quantities of PAHs in the workplace.

4.2.2.3.2 Chemicals

Reference standards for 1-OHP and 7,8-diolBaP were obtained from the National Cancer Institute Chemical Carcinogen Reference Standards distributed by Midwest Research Institute (Kansas City, Missouri, USA). β-Glucuronidase/arylsulfatase (100 000 Fishman U ml^{-1} and 800 000 Roy U ml^{-1} from Helix pomatia) was solicited from Roche Diagnostics (Laval, Quebec, Canada).

High-performance liquid chromatography (HPLC)-grade methanol (MeOH), ethyl acetate, ascorbic and citric acids were acquired from Fisher Scientific Company (Ottawa, Ontario, Canada).

The shampoo chosen was a Canadian brand labelled as medicated “for the control of itching, redness and scaling associated with stubborn seborrhea or psoriasis of the scalp” produced in Montréal, Quebec, Canada. The active ingredient targeted is listed as 10% coal tar topical solution by weight.

4.2.2.3.3 Urine Samples

The methodology used was adapted from a study by Viau & Vyskocil (1995). In advance of each exposure session, the Person X was instructed to collect his urine for forty-eight hours to provide a baseline standard as to what his urine normally consists of without the coal-tar shampoo use. Preceding each exposure, he was asked to take one final pre-exposure micturition, following which immediately enter the shower and use the shampoo. The reasoning behind this was to reduce the dilution of the first post-exposure micturition with urine formed prior to exposure.

Each time Person X urinated during the experimental period, he was expected to note the time and volume, after which he was to bottle up his urine for further analysis. Samples were then collected for three days after exposure to create an acute kinetic profile of 7,8-diolBaP and 1-OHP.

4.2.2.3.3.1 Single Exposure

When the volunteer took his shower, he used an estimated 6 mL of the shampoo and followed the instructions on the back of the bottle to the letter, using a timer and a measuring cylinder to keep a modicum of precision. He applied the shampoo to his hair after it was wet, massaging it into his scalp for two minutes, rinsed immediately, reapplied and left the shampoo on his scalp for five minutes. During the rinsing, the volunteer was careful to avoid letting the runoff touch anywhere else in his body such that cutaneous absorption from the scalp or the hands were the sole route of entry.

4.2.2.3.3.2 Multiple Exposure

This second sample collection took place four weeks after the first, thus giving adequate time for any PAH and metabolites in his system to return to average levels based on his lifestyle. In the multiple exposure scenario, he followed the same steps as in the single exposure scenario with two exceptions. He used 10 mL of the shampoo each time, and he self-exposed three times in rough twenty-four hour intervals.

4.2.2.3.4 Urine Analysis

Moreau and Bouchard (2015) successfully established a system to detect diolBaPs in rat urine using an ultra-high-performance liquid chromatography (UHPLC) setup coupled to fluorescence. With their method as a starting point, the urine collected was homogenised and had to be concentrated ten-fold. 10 mL of each urine sample was taken and mixed with an equal amount of sodium acetate buffer (0.1 M, pH 5), then hydrolysed with 20 µL of β-glucuronidase/arylsulfatase at 37°C in a shaking bath for an hour to obtain the sum of free and conjugated metabolites.

The next step was solid phase extraction (using Sep-Pak C18 cartridges; Waters Corporation, Milford, Massachusetts, USA). The cartridges were conditioned with 4 mL of methanol and 8 mL of water. The standards and samples were then passed through, and prior to collection, were washed with 8 mL of water and 8 mL of 15% methanol:water solution in an effort to reduce polar contamination. Elution then took place using 8 mL of pure methanol. Adjusted nitrogen gas was used to aid throughout this process.

Afterwards, evaporation at 40°C took place, and the resulting residue was dissolved in 200 µL of pure methanol and centrifuged. The liquid layers were then transferred to vials for UHPLC analysis.

The analysis was carried out using an Agilent 1290 Infinity Ultra-HPLC system (Mississauga, Ontario, Canada). It comprises of five modules: a binary pump, an autosampler, a thermostatted column compartment, a diode array detector and a fluorescence detector. There were two columns used in the process: a Poroshell 120 SB-C18 (2.1 X 50 mm; 2.7 µm) from Agilent #689775-902, and a Zorbax Eclipse plus C18 RRHD (2.1 X 50 mm; 1.8 µm) from Agilent # 959759-902. The first column was used to “load” the sample and clean it whilst preventing elution and the second served as an analytical column that passed the liquid to the detectors. The temperature of the analytical column was set at 40 °C. Switching from the loading column to the analytical column was set at 11.5 min.

Urine analysis was performed using a methanol:water gradient mobile phase also containing 5 mM of citric acid and 10 mg/L of ascorbic acid. Ascorbic acid was added to the mobile phase in accordance with a previous study conducted by Bouchard et al. (1994).

The flow rate was 0.3 mL/min, the injection volume was 10 µL, samples were kept at 13°C on the injection tray, and the PMT gain was kept at 11. The run time was a set 30 minutes per sample with three minutes postrun. The gradient elution conditions during each run were as follows: at 5.5 minutes, the water:methanol ratio was 90:10; 35:65 at 9.2 minutes; 0:100 at 21.5 minutes and, 35:65 at 29 minutes. The excitation and emission wavelengths for the fluorescence analysis were 264 nm and 415 nm for 7,8-diolBaP respectively.

The urine standards analysed obtained consistent coefficients of 1, which indicates a straight calibration curve and a functioning method. In order to ensure quality assurance, positive and negative controls were used. External calibration curves of 7,8-diolBaP and 1-OHP were prepared in blank human urine before undergoing the same analysis as that of the volunteer samples. The limit of quantification for the 10 µL injection into the UHPLC-fluorescence system is 2 fmol/mL and the variation coefficient is below 15%.

4.2.2.3.5 Data Analysis

Using the retention time observed by Moreau & Bouchard (2015) in UHPLC-fluorescence as a guide, the peaks for 7,8-diolBaP and 1-OHP were identified and confirmed through the use of methanol and urine standards. The surface areas provided were then integrated and converted to picomole per millilitre of urine using the formula below:

$$\left(\frac{\left(\frac{Peak\ Surface\ Area\ in\ Volunteer\ Urinary\ Extract\ -\ intercept}{Slope \times urinary\ dilution}\right) \times Redissolution\ Volume\ (MeOH)}{Volume\ of\ urine\ analyzed}\right)$$

$$= pmol\ per\ mL\ of\ urine$$

The units were adjusted from this point depending on the information we wished to display.

To better assess the kinetic profile, the values were adjusted for urinary creatinine content. Creatinine is a side product of the energy consumption by muscle tissue, and through glomerular filtration, it comes out in the urine in regular amounts (Wagner et al. 2010). Since this is a stable process in every individual, adjusting the urinary content of each biomarker for

creatinine mitigates the uncertainties as to the body's processing of the metabolite (Wagner et al. 2010).

$$(pmol\ per\ mL\ of\ urine) \div \left(\frac{Creatinine\ Content}{Molecular\ weight\ of\ creatinine}\right) = Quantity\ per\ mol\ of\ creatinine$$

4.2.2.4 Results

In order to properly assess and understand the relationship between 7,8-diolBaP and PAH contact, experiments focusing on controlled cutaneous exposure took place. Although the exact quantity of BaP in the coal-tar shampoo used is unknown, as it is a part of the group of PAHs found in coal-tar, the exact moment of exposure is known. Furthermore, all the micturition over each experimental period were collected and measured, thus allowing us to fully follow 7,8-diolBaP throughout the excretion process.

For the two sets of experiments, the single and multiple exposures, the results obtained were compiled into three types of figures. The first is a graph demonstrating the concentration of 7,8-diolBaP in the urine throughout the experimental period (in nanomoles per mole of creatinine); the second being the rate at which 7,8-diolBaP was excreted (in picomoles per hour); and, the third being the total cumulative excretion (in picomoles). In each instance, the results obtained for 7,8-diolBaP were compared to those of 1-OHP coming from the same experimental circumstances. The graphs use a broken line with square points to represent 7,8-diolBaP and a solid line with circular points for 1-OHP.

4.2.2.4.1 Single Exposure

The first experiment featured a single exposure session. Figure 9 shows the urinary excretion concentration for both 7,8-diolBaP and 1-OHP in Person X adjusted for creatinine content using the first micturition after exposure as a starting point.

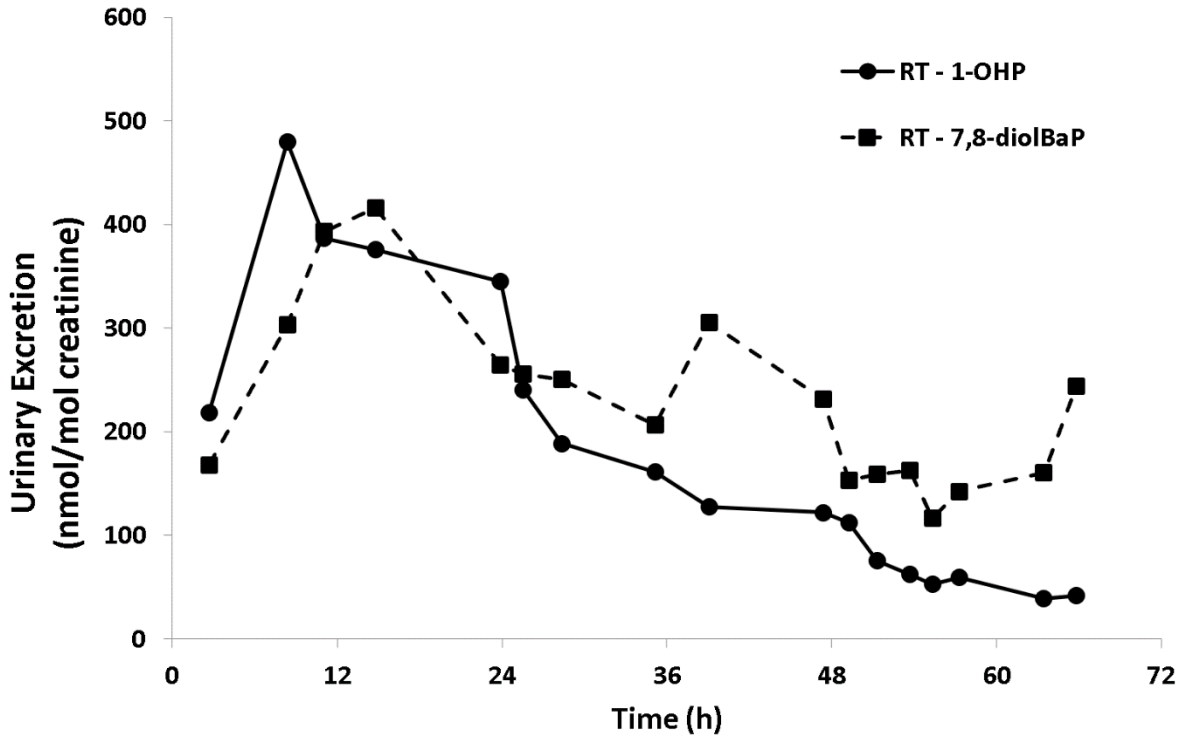

Figure 9: Urinary excretion of 7,8-dihydrodiol-benzo[a]pyrene and 1-hydroxypyrene adjusted for creatinine content after single cutaneous exposure to PAHs

There is a clear peak in both 1-OHP and 7,8-diolBaP where the peak value is reached prior to the concentration waning and returning to pre-exposure levels. 1-OHP peaks around 8-9 hours after exposure, consistent with other literature (Lutier et al. 2016). 7,8-diolBaP reaches similar levels of magnitude, taking a little bit longer to do so at 14-15 hours following exposure. This is then followed by gradual declines on the part of the two metabolites, with 1-OHP achieving lower concentrations faster than 7,8-diolBaP, even though at its peak, it hit 63.15 nmol per mol of creatinine higher than 7,8-diolBaP. There are smaller peaks observed in the excretion of 7,8-diolBaP which may allude to exposure in to smaller quantities of BaP in a context where pyrene is not equally as present, such as second-hand cigarette smoke or through eating grilled food (Vu et al. 2015).

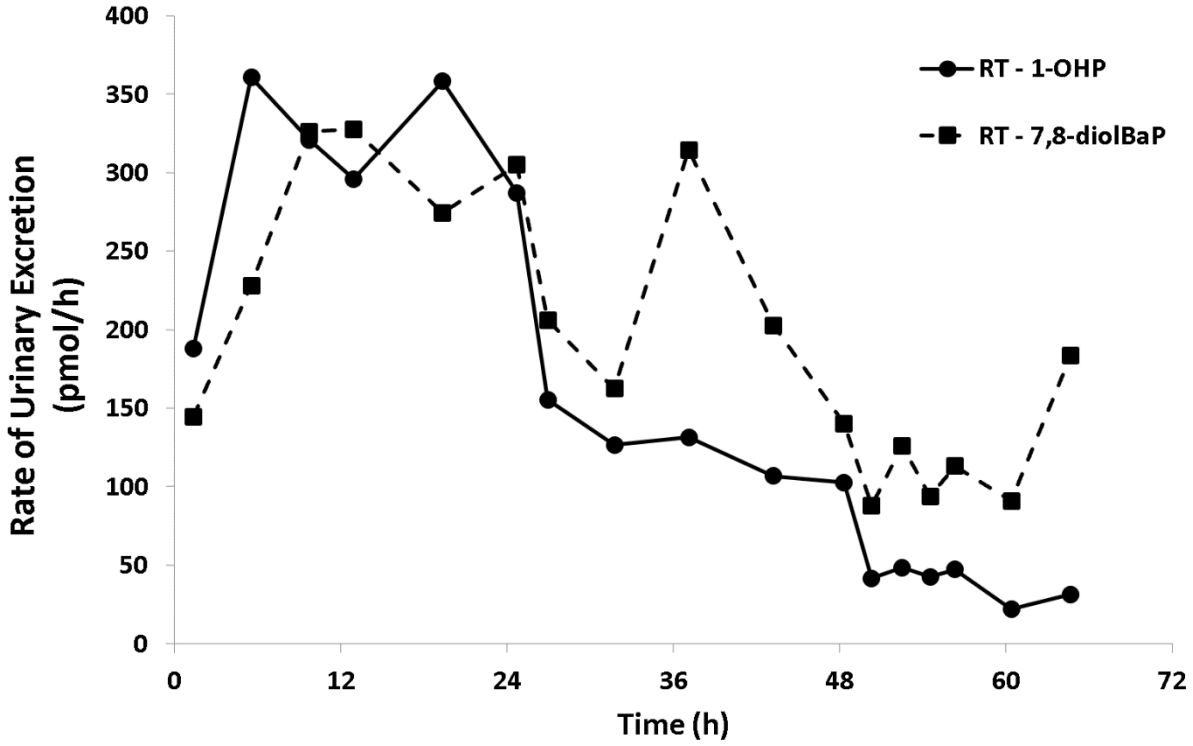

Figure 10: Rate of urinary excretion for 7,8-dihydrodiol-benzo[a]pyrene and 1-hydroxypyrene after single cutaneous exposure to PAHs

According to Figure 10, 7,8-diolBaP is excreted at a slower rate when compared to 1-OHP, which may be the reason for the lag between the peak values observed in Figure 9. The speed at which 1-OHP is excreted reaches 360 pmol/h after five hours from the point of exposure, while 7,8-diolBaP reaches 327 pmol/h after almost thirteen hours. The kinetic profile, however, is quite similar. There are two peaks observed in both 1-OHP and 7,8-diolBaP, where they hit a certain excretion rate, lower and then re-augment before slowing as the last amounts leave the body. The third "speed-peak" in 7,8-diolBaP's profile corresponds with the second excretion peak noted in Figure 9 where the volunteer may have been exposed to BaP due to lifestyle circumstances.

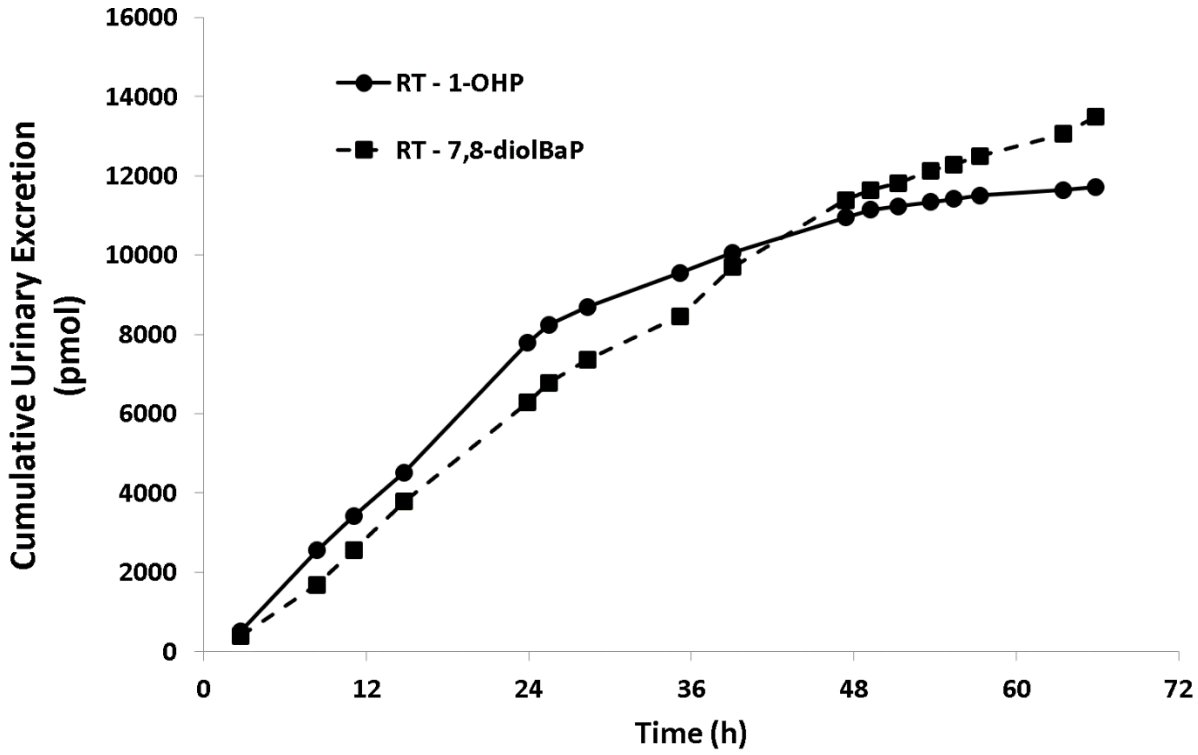

Figure 11: Cumulative urinary excretion of 7,8-dihydrodiol-benzo[a]pyrene and 1-hydroxypyrene after single cutaneous exposure to PAHs

Figure 11: **Cumulative urinary excretion of 7,8-dihydrodiol-benzo[a]pyrene and 1-hydroxypyrene after single cutaneous exposure to PAHs**shows 1-OHP starting off slightly higher in terms of the total amount excreted and as the week progresses, 7,8-diolBaP surpasses 1-OHP at around 50 hours post-exposure. A clear synergy in the kinetics of 7,8-diolBaP and 1-OHP is demonstrated with the two being excreted at similar amounts throughout the observed experimental period and rising and plateauing almost in sync as the observational period comes to a close.

4.2.2.4.2 Multiple Exposure

The second experiment featured a set of multiple expositions, simulating the daily exposure one would experience in the workplace. This takes place four weeks after the first experiment, thus enabling the body to recalibrate. In this instance, Person X self-exposes a total of three times in the morning, with a rough twenty-four hours separating each exposure.

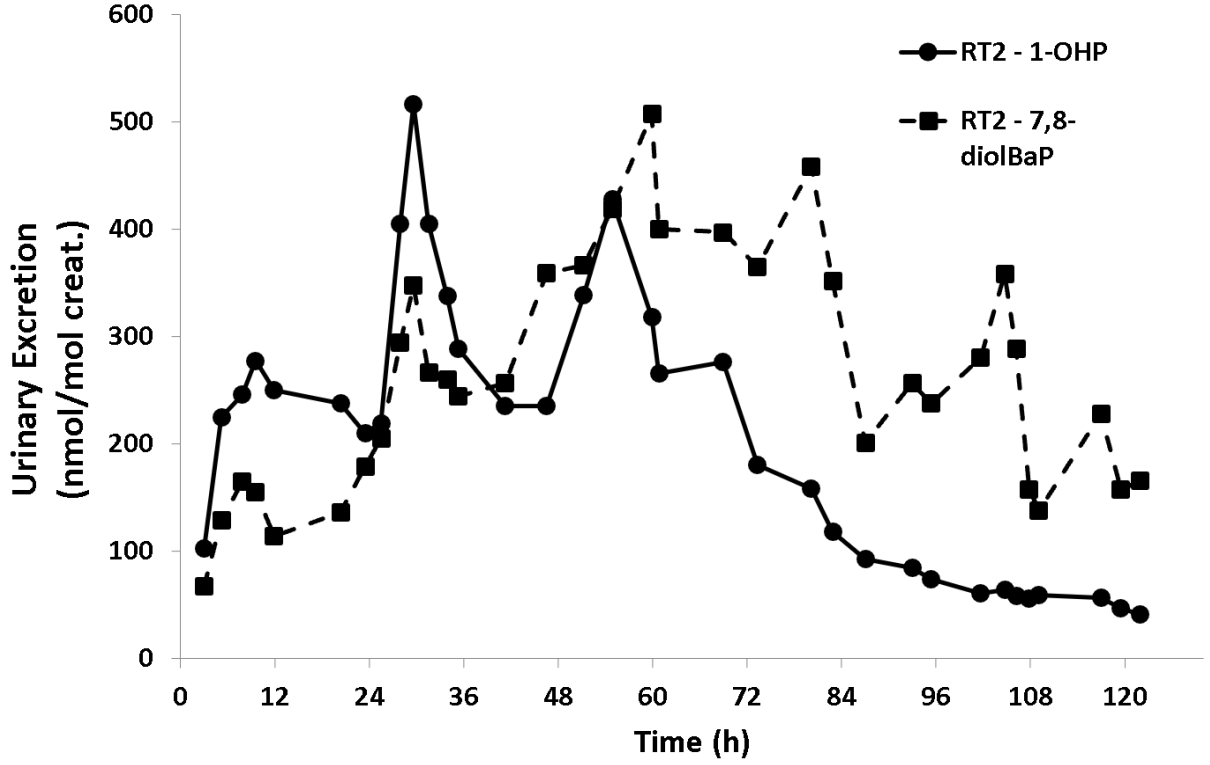

Figure 12: Urinary excretion of 7,8-dihydrodiol-benzo[a]pyrene and 1-hydroxypyrene adjusted for creatinine content after multiple cutaneous exposures to PAHs

There are three distinct peaks in the excretion profile of 1-OHP and six for that of 7,8-diolBaP. In looking at Figure 12, there is a small first peak 9-10 hours following the first exposure for 1-OHP, another larger peak at 30-31 hours post-first exposure, and a third at 54-55 hours post-first exposure. Given that exposure takes place at t = 0, 16 and 38 hours, all 1-OHP the peaks correspond to exposure times, with the second and third peak larger than the first showing accumulation in the body and demonstrating that 1-OHP does not entirely leave the body in the rough twenty-four hour gaps between exposure. The size of the third peak shows that the 1-OHP metabolised as a result of the first exposure had left the body by the time the third exposure took place, hence leading to the second peak being the highest of the three.

7,8-diolBaP, in comparison, emits three peaks 7-8 hours, 29-30 hours and 59-60 hours following the first exposure. These align quite well with 1-OHP, the peak values being attained

at almost the same moment. The second peak is larger than the first, showing cumulation of 7,8-diolBaP as a result of a second exposure before the first set of metabolites have been completely produced and excreted from the body. The third peak is larger, showing a continuation in accumulation and a slower excretion than 1-OHP (Figure 12).

The fourth large peak in 7,8-diolBaP lines up with a smaller peak in 1-OHP, which, although they do not correlate to any exposition as a result of this experiment, could signify exposure to PAHs as a result of lifestyle. The size of the peak for 7,8-diolBaP speaks to the length of time it takes for the metabolite to entirely leave the body, and thus can be attributed to accumulation resulting from previous exposures. The peak, although much smaller in 1-OHP, is clearly influenced by a previous exposition as it is higher than the first peak. The last two peaks in 7,8-diolBaP are not seen in 1-OHP, and this may be as a result of exposure to a source of BaP that does not contain pyrene as well (Figure 12).

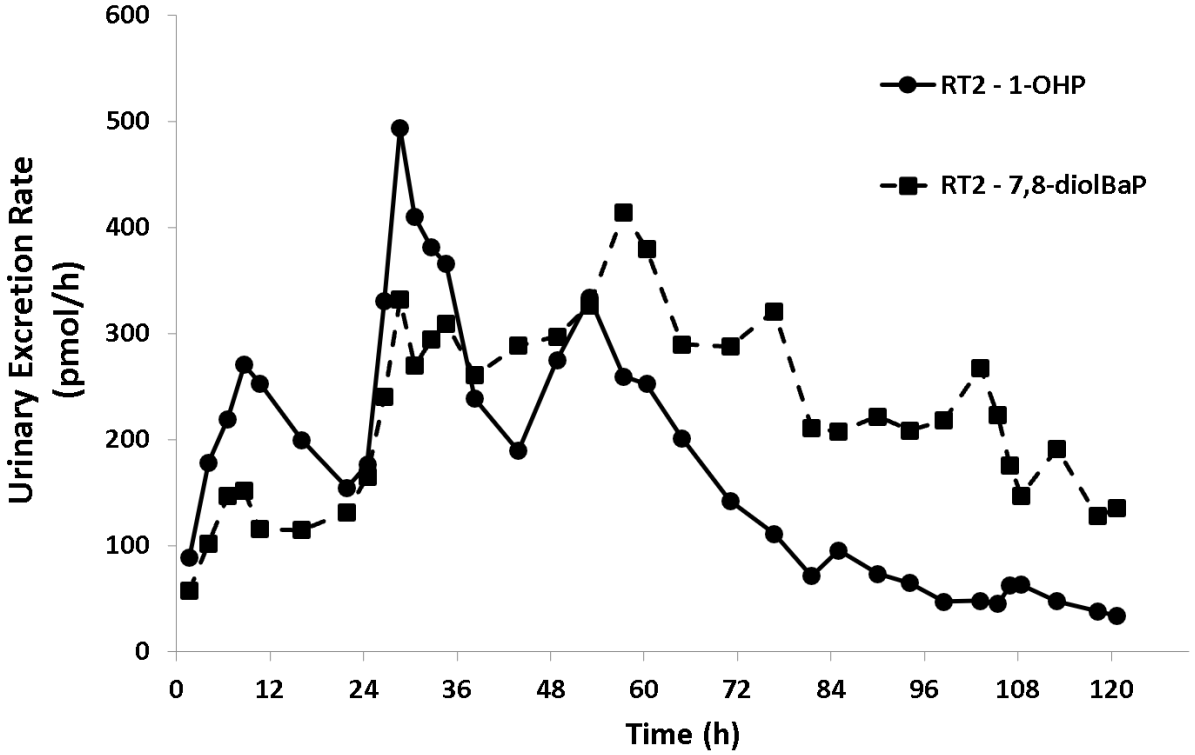

Figure 13: Rate of urinary excretion for 7,8-dihydrodiol-benzo[a]pyrene and 1-hydroxypyrene after multiple cutaneous exposures to PAHs

As seen in the single exposure experiment, the excretion rate for 1-OHP is faster than 7,8-diolBaP, but as the week progresses, 7,8-diolBaP starts being released in higher amounts per hour than 1-OHP. Increases in excretion rate are observed to correlate with moments of exposure and the peaks seen in Figure 12.

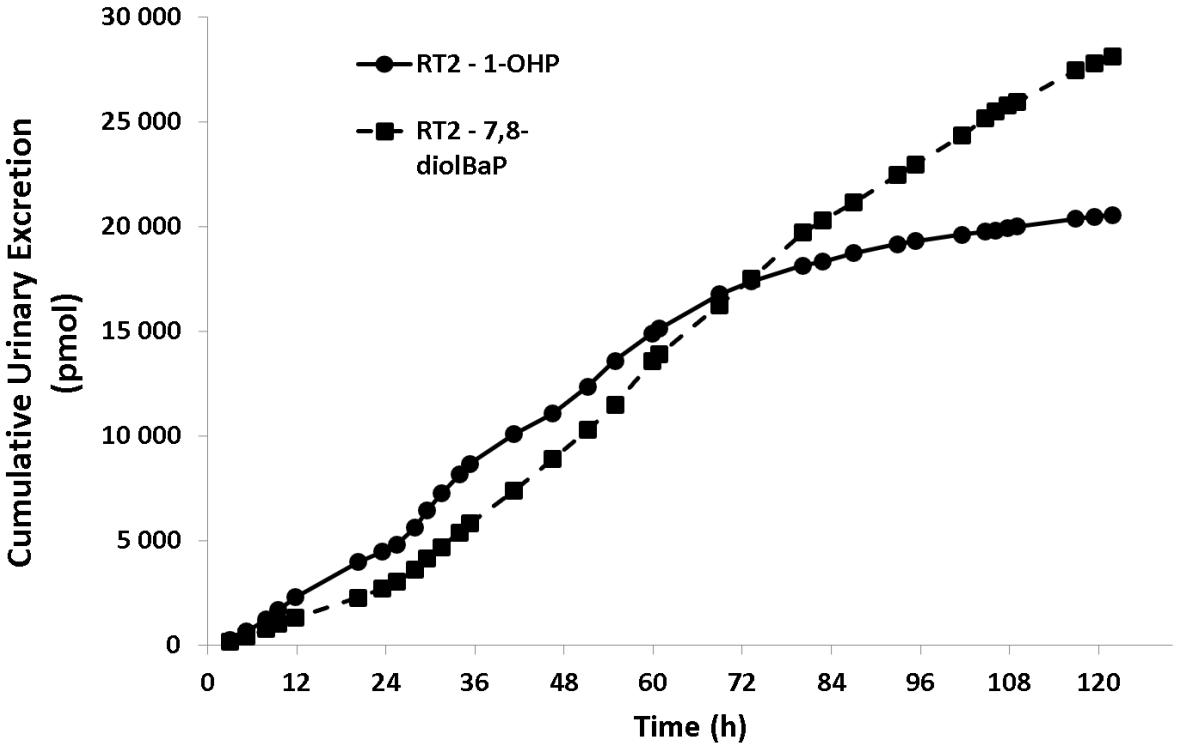

Figure 14: Cumulative urinary excretion of 7,8-dihydrodiol-benzo[a]pyrene and 1-hydroxypyrene after multiple cutaneous exposures to PAHs

Identical to the results in Figure 11, in looking at Figure 14, there is a synergy in both the quantity and the timing of the release of 1-OHP and 7,8-diolBaP. Towards the end of the experimental period, as cumulative urinary excretion of 1-OHP reached a plateau, 7,8-diolBaP continues to increase at the same pace seen throughout the week, reflecting the last two exposure peaks witnessed in Figure 12 for 7,8-diolBaP that are not seen for 1-OHP. As was witnessed in the single exposure experiment, 1-OHP, throughout the three exposures, was excreted at slightly higher overall quantities than 7,8-diolBaP, however, they remained within the same order of magnitude.

4.2.2.5 Discussion

As an environmental pollutant, BaP is quite prominent in urban environments. It forms through incomplete combustion processes which take place everyday in general life, whether it be as a result of cooking or being exposed to car exhaust fumes (Rengarajan et al. 2015). Many studies have demonstrated the negative consequences of living in areas with high PAH content in the air, and for many workers, this is an occupational hazard that cannot be countered (Choi et al. 2010). In order to improve the safety of these workers, maximum exposure amounts need to be derived such that exposure can be carefully monitored and health risks minimised and mitigated.

As part of the efforts to create a dependable, accurate and economic method to monitor exposure to carcinogenic PAHs, our team derived a series of experiments made to establish the kinetic profile of a promising biomarker for BaP, which has been put forth as a good candidate to represent carcinogenic PAHs as a whole (Paal'me et al. 1983). Through *in vivo* studies on rats by Moreau & Bouchard (2015), 7,8-diolBaP was recognised as having potential. Although it was excreted in lower quantities when compared to 1-OHP and 3-OHBaP in rats, it was consistently found in the kidney and intestines, thus presenting the probability that it is mainly excreted through the urine. In instances where it was detected in the rat urine, it was found to be rapidly cleared from the body within eight-hours of dosing, which hinted at more favourable kinetics in comparison to 3-OHBaP (Moreau 2013).

7,8-DiolBaP is produced when BaP is first monooxygenated through a reaction catalysed by microsomal nicotinamide adenine dinucleotide phosphate (NADPH)-dependent cytochrome P450 isoforms 1A1 and 1B1, and then conjugated with water by epoxide hydrolase (Jiang et al. 2007). In comparing this compound with the preferred PAH biomarker, 1-OHP, we were hoping to better ascertain its true utility and reliability. This biomarker candidate needed to avoid the pitfalls of 3-OHBaP while maintaining the advantages of 1-OHP.

The results clearly demonstrate similar kinetics, showing a mono-exponential elimination identical to that of 1-OHP. The first experiment revealed a time lag between when the 1-OHP peak was seen and when that of 7,8-diolBaP attained its summit; however, the second experiment showed values that were more or less in-sync, with the peaks appearing within 2-3 hours of one another. Furthermore, the peaks observed corresponded with the time of exposure.

Within hours of being exposed, there is a steady rise in both compounds, peak concentration is reached, then there is a steady decline. In the multiple exposure scenario, there are a series of increases and decreases, demonstrating both the immediateness at which the two compounds begin being formed as PAH metabolites, and the speed at which they are cleared from the system such that any further exposures would be clear through a kinetic profile.

The multiple exposure scenario shows that 1-OHP is cleared from the system at a faster rate when compared to 7,8-diolBaP, which is more conducive towards spot-testing in an occupational setting. However, given that the trade off is a compound-specific marker to BaP and thus carcinogenic PAHs, this is an acceptable shortcoming to take on.

This study also revealed the importance of non-workplace exposure to BaP. The experiments both revealed other instances of exposure to BaP by Person X throughout the week that were not a part of the experiment. This brings to light the importance of continuous monitoring for employees given that their lifestyle will strongly influence the level of BaP already in their system. As the slower formation rate of 7,8-diolBaP indicates, it takes a little longer for the body to metabolise BaP, ergo enabling it to accumulate faster which increases the risk of negative health consequences.

4.2.2.6 Conclusion

The objective of this study was to evaluate the potential of 7,8-diolBaP as an alternative compound-specific biomarker for exposure to BaP. The study has been successful in meeting this goal. Through establishing a series of controlled experiments where exposure was monitored and timed, following which all micturition during the observational period were collected and analysed, we were able to gain a preliminary understanding of the kinetic tendencies of 7,8-diolBaP and how it compared to existing biomarkers in current use.

7,8-DiolBaP was found to both produce a comparable kinetic profile and be excreted at comparable quantities to 1-OHP, thus making it a viable biomarker for BaP exposure and carcinogenic PAHs as a whole. The next steps include verifying the identity of the compounds assessed through mass spectrometry and refining the methodology for use in an occupational setting.

4.2.2.7 Conflict of Interest

The authors have no conflicts of interest to declare.

4.2.2.8 References

Bjorseth, A., 1983. *Handbook of Polycyclic Aromatic Hydrocarbons Polycyclic Aromatic Hydrocarbons*,

Bouchard, M., Dodd, C. & Viau, C., 1994. Improved Procedure for the High-Performance Liquid Chromatographic Determination of Monohydroxylated PAH Metabolites in Urine. *Journal of analytical toxicology*, 18(5), pp.261–264.

Brzeźnicki, S., Bonczarowska, M. & Gromiec, J.P., 2009. [Occupational exposure limits for polycyclic aromatic hydrocarbons. Current legal status and proposed changes]. *Medycyna pracy*, 60(3), pp.179–185.

Choi, H. et al., 2010. 6. Polycyclic aromatic hydrocarbons. In World Health Organization, ed. *WHO Guidelines for Indoor Air Quality: Selected Pollutants*. NCBI Bookshelf. Geneva: World Health Organization.

Forster, K. et al., 2008. 3-Hydroxybenzo[a]pyrene in the urine of workers with occupational exposure to polycyclic aromatic hydrocarbons in different industries. *Occupational and environmental medicine*, 65(4), pp.224–229.

IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2012. Chemical agents and related occupations. *IARC monographs on the evaluation of carcinogenic risks to humans / World Health Organization, International Agency for Research on Cancer*, 100(Pt F), pp.9–562.

Jiang, H. et al., 2007. Metabolism of benzo[a]pyrene in human bronchoalveolar H358 cells using liquid chromatography-mass spectrometry. *Chemical research in toxicology*, 20(9), pp.1331–1341.

Jongeneelen, F.J., 2001. Benchmark guideline for urinary 1-hydroxypyrene as biomarker of occupational exposure to polycyclic aromatic hydrocarbons. *The Annals of occupational hygiene*, 45(1), pp.3–13.

Lindstedt, G. & Sollenberg, J., 1982. Polycyclic aromatic hydrocarbons in the occupational environment: with special reference to benzo[a]pyrene measurements in Swedish industry. *Scandinavian journal of work, environment & health*, 8(1), pp.1–19.

Lutier, S. et al., 2016. Urinary elimination kinetics of 3-hydroxybenzo(a)pyrene and 1-hydroxypyrene of workers in a prebake aluminum electrode production plant: Evaluation of diuresis correction methods for routine biological monitoring. *Environmental research*, 147, pp.469–479.

Mackenzie, K.M. & Murray Angevine, D., 1981. Infertility in Mice Exposed in utero to Benzo(a)pyrene1. *Biology of reproduction*, 24(1), pp.183–191.

Marie, C. et al., 2010. A toxicokinetic study to elucidate 3-hydroxybenzo(a)pyrene atypical urinary excretion profile following intravenous injection of benzo(a)pyrene in rats. *Journal of applied toxicology: JAT*, 30(5), pp.402–410.

Moreau, M., 2013. *Étude du continuum exposition-effet cancérogène du benzo[a]pyrène*. PhD. Université de Montréal.

Moreau, M. & Bouchard, M., 2015. Comparison of the kinetics of various biomarkers of benzo[a]pyrene exposure following different routes of entry in rats. *Journal of applied toxicology: JAT*, 35(7), pp.781–790.

Nikolaou, K., Masclet, P. & Mouvier, G., 1984. Sources and chemical reactivity of polynuclear aromatic hydrocarbons in the atmosphere — A critical review. *The Science of the total environment*, 32(2), pp.103–132.

Ohura, T. et al., 2004. Polycyclic aromatic hydrocarbons in indoor and outdoor environments and factors affecting their concentrations. *Environmental science & technology*, 38(1), pp.77–83.

Paal'me, L.P., Kirso, U.E. & Urbas, E.R., 1983. [Is benzo(a)pyrene an indicator of carcinogenic pollution?]. *Voprosy onkologii*, 29(7), pp.74–80.

Rengarajan, T. et al., 2015. Exposure to polycyclic aromatic hydrocarbons with special focus on cancer. *Asian Pacific journal of tropical biomedicine*, 5(3), pp.182–189.

Rossella, F. et al., 2009. Urinary polycyclic aromatic hydrocarbons and monohydroxy metabolites as biomarkers of exposure in coke oven workers. *Occupational and environmental medicine*, 66(8), pp.509–516.

van Grevenynghe, J. et al., 2005. Human CD34-positive hematopoietic stem cells constitute targets for carcinogenic polycyclic aromatic hydrocarbons. *The Journal of pharmacology and experimental therapeutics*, 314(2), pp.693–702.

Viau, C. & Vyskocil, A., 1995. Patterns of 1-hydroxypyrene excretion in volunteers exposed to pyrene by the dermal route. *The Science of the total environment*, 163(1-3), pp.187–190.

Viau, M.B., 1999. Urinary 1-hydroxypyrene as a biomarker of exposure to polycyclic aromatic hydrocarbons: biological monitoring strategies and methodology for determining biological exposure indices for various work environments. *Biomarkers: biochemical indicators of exposure, response, and susceptibility to chemicals*, 4(3), pp.159–187.

Vu, A.T. et al., 2015. Polycyclic Aromatic Hydrocarbons in the Mainstream Smoke of Popular U.S. Cigarettes. *Chemical research in toxicology*, 28(8), pp.1616–1626.

Wagner, B.D., Accurso, F.J. & Laguna, T.A., 2010. The applicability of urinary creatinine as a method of specimen normalization in the cystic fibrosis population. *Journal of cystic fibrosis: official journal of the European Cystic Fibrosis Society*, 9(3), pp.212–216.

Weyand, E.H. & Bevan, D.R., 1986. Benzo(a)pyrene disposition and metabolism in rats following intratracheal instillation. *Cancer research*, 46(11), pp.5655–5661.

Zhu, X. et al., 2011. Ambient concentrations and personal exposure to polycyclic aromatic hydrocarbons (PAH) in an urban community with mixed sources of air pollution. *Journal of exposure science & environmental epidemiology*, 21(5), pp.437–449.

4.3 “Worker Study”

4.3.1 Author Contributions

Chúk Odenigbo

- Worked with the laboratory technicians Ross Thuot and Marc Mantha to prepare and analyse the volunteer urine samples
- Integrated sample spectrum results from the UHPLC and compiled data into spreadsheets
- Wrote all of the sections of this research article and integrated comments from co-author and research director Dr. Michèle Bouchard, as well as research professional Jonathan Côté

Michèle Bouchard

- Conceptualised this project and created the sample analysis methodology used to analyse the worker urine
- Successfully applied for and received funding for this project to take place
- Provided the spreadsheet with the necessary calculations to compile concentrations, rates, cumulative excretions and revised the spreadsheets with raw results inserted by Chuk Odenigbo
- Worked on creating graphic interpretations of the results with research professional Jonathan Côté
- Revised the manuscript and provided comments and suggestions
- Maintained correspondance with Dr. Anne Maître's team in France to coordinate the volunteer sampling and receive the urine collected
- Wrote the report to the funding agency associated with the project

4.3.2 An initial assessment of 4,5-dihydrodiol-benzo[a]pyrene and 7,8-dihydrodiol-benzo[a]pyrene as potential biomarkers of exposure to benzo[a]pyrene in an occupational setting

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4.3.2.1 Abstract

Polycyclic aromatic hydrocarbons (PAHs) are a family of compounds produced through incomplete combustion processes. They are ubiquitous in certain workplaces, whereby exposure occurs mainly through inhalation. 1-hydroxypyrene (1-OHP) is a urinary metabolite of pyrene, a non-carcinogenic PAH used for the biosurveillance of PAH exposure. Although useful, pyrene is non-representative of the risks posed by its carcinogenic counterparts and therefore an alternative biomarker is necessary to properly evaluate the cancer-oriented perils of PAHs. Benzo[a]pyrene (BaP) is one of the better studied PAHs and a recognized carcinogen by the International Agency for Research on Cancer. This paper thus seeks to evaluate 4,5-dihydrodiol-benzo[a]pyrene and 7,8-dihydrodiol-benzo[a]pyrene (4,5-diolBaP and 7,8-diolBaP) as biomarkers of exposure specific to BaP and wherefore carcinogenic PAHs in an occupational setting. Five employees at a carbon anode production plant were recruited as volunteers; their urine was collected before and after each work shift for one week, as well as at irregular intervals during their days off. A method proven successful in detecting 4,5-diolBaP and 7,8-diolBaP in rat urine was modified and refined to obtain dependable results in human urine, consequently traversing the species barrier. Samples were run by Ultra-High-Performance Liquid Chromatography coupled to fluorescence detection, from which the spectra were integrated and analyzed. The 7,8-diolBaP and 4,5-diolBaP results obtained were compared with those of 1-OHP in identical contexts. The lack of consistency observed in results coming from 4,5-diolBaP demonstrated that the methodology was not sufficiently sensitive enough to distinguish it from the other urinary contaminants. 7,8-DiolBaP, on the other hand, presented a similar urinary profile to that of 1-OHP whereby the kinetics and amplitude were noted as being very comparable. Given the noticeable sensitivity and consistency of the compound in cases where low doses were expected, it is hence very probable that 7,8-diolBaP would be an excellent compound-specific biomarker for BaP exposure. The next steps include analysis by mass spectrometry to confirm that the compounds identified in fluorescence correspond accordingly and refining the method to increase its accuracy.

Key words: 4,5-dihydrodiol-benzo[a]pyrene, 7,8-dihydrodiol-benzo[a]pyrene, benzo[a]pyrene, biomarkers, polycyclic aromatic hydrocarbons, 1-hydroxypyrene, toxicokinetics

4.3.2.2 Introduction

Benzo[a]pyrene (BaP) is a known cancer-causing agent to human beings, classified as such by the IARC Working Group on the Evaluation of Carcinogenic Risk to Humans (IARC 2010). The compound is widespread, being found in the air, water and soil (Twardowska and Kolodziejczyk 1998), and is produced through incomplete combustion processes. Societal exposure is typically through food and smoking, with high ambient concentrations in certain workplaces (iron foundries, aluminium production sites and oil processing centres to name a few) (Karle et al. 2004; Lutier et al. 2016).

Given the prevalence of BaP and the risk of repeated contact in humans (Buchet et al. 1992; Qiu et al. 2012; Petry et al. 1996), it is important to be able to measure exposure and analyze the potential health impacts. As a genotoxic compound that reacts with DNA directly (Mourón et al. 2006), the mechanisms of action for BaP exposure are well known, especially in animals (Yeo et al. 2017); however, assessing and regulating the risks it presents in an inferred quantity has yet to be achieved in humans (Barbeau et al. 2015). Biological markers, often shortened to biomarkers, are one of the principal methods of evaluating exposure to xenobiotics and quantifying the organism's response to said compound (McCarthy 2018).

The preferred biological marker for BaP exposure is 1-hydroxypyrene (1-OHP), a metabolite of pyrene. Owing to the large proportion of pyrene present in randomized polycyclic aromatic hydrocarbon (PAH) compound mixtures, 1-OHP serves as a universal biomarker, indicating exposure to PAHs in general rather than being specific to BaP (Bouchard et al. 1998; World Health Organization 2006). Seeing the variation within the family of what constitutes a PAH, the properties and thus health effects differ depending on factors such as size, number of aromatic rings, or whether the compound is linear. This effectively means that not all PAHs are equally dangerous, and in kind, exposure to PAHs does not have a set outcome.

BaP exposure is normally within the context of a PAH mixture, nonetheless, studies have verified the carcinogenicity of the compound, and along these lines, the threat to human health that can be associated with it. Pursuant to this is the need to identify a compound-specific biomarker for BaP such that absorbed doses related to workplace exposure may be quantified rather than estimated. That is to say, the biomarker used to assess the risks of cancer related to

PAH exposure in the workplace should be definitive to a proven carcinogenic PAH, in this case, BaP (IARC Working Group on the Evaluation of Carcinogenic Risk to Humans 2010).

Toxicological experiments conducted on rats by Moreau and Bouchard (2015) were able to confirm 4,5-dihydrodiol-benzo(a)pyrene and 7,8-dihydrodiol-benzo(a)pyrene (4,5-diolBaP and 7,8-diolBaP; diolBaPs) metabolites in urine in quantities relative to BaP exposure. When compared to 3-hydroxybenzo(a)pyrene (3-OHBaP), a monohydroxy metabolite of BaP that has also been proposed as a biomarker of human exposure to carcinogenic PAHs, their kinetic profile was found to be more favourable. There was a clear time-exposure correlation versus the atypical profile put forth by 3-OHBaP (Barbeau et al. 2011; Moreau et al. 2015).

4,5-DiolBaP and 7,8-diolBaP result from the interaction between BaP and cytochrome P450 enzymes, followed by hydrolysis by epoxide hydrolase to trans-dihydrodiols. This, coupled with the fact that they are easily detectable in rat urine, made them ideal candidates for further study in human beings (Barbeau et al. 2011; Moreau et al. 2015), which has never been done before.

Existing analytical methods used for determining the concentration of BaP metabolites in urine are based on chromatographic technology: gas chromatography (GC) and high-performance liquid chromatography (HPLC) (Lee et al. 2003). The measurement of BaP metabolites, such as 3-OHBaP, requires highly sensitive methods given that urinary content levels are assessed in nanogram/litre. Most BaP metabolites are, therefore, orders of magnitude lower than better understood PAH biomarkers of exposure such as 1-OHP (Barbeau et al. 2014; Pruneda-Álvarez et al. 2016). Building on from past experiments and taking into consideration the relatively low quantities of BaP metabolites found in urine, evidence suggests that the ultra-high-performance liquid chromatography (UHPLC) setup may provide the best mobilisation in augmenting the sensitivity of the analytical instruments and thereupon the precision of the results (Moreau and Bouchard 2015).

The aim of this paper was to establish a sensitive analytical method, using the UHPLC-fluorescence, to determine diolBaP quantities in the urine of workers that have been occupationally exposed to BaP; and, to assess the possibility of using either or both of the diolBaPs as reliable biomarkers of exposure through comparing them with 1-OHP in the same context. In light of the uncertainty surrounding exposure to genotoxic compounds, the results of

this study could lead to enhanced institutional precautionary measures implemented to reduce the health risks from BaP and potentially carcinogenic PAHs in general.

4.3.2.3 Materials and Methods

4.3.2.3.1 Study Population

With the aim of providing information that can eventually be used in the creation of occupational health and safety standards regarding contact with BaP, this project targets industrial workers who are regularly exposed on the job. This study is possible thanks to an agreement with an anode producing plant in Grenoble, Auvergne-Rhône-Alpes, France. Five workers volunteered to allow the collection of their urine at the start and at the end of their work day, for a week, and four accepted having some samples collected over the weekend when they were not working as well. Each worker was also confirmed to be a non-smoker to avoid the potential confounding variable.

Dr. Anne Maître's team from the Université Joseph-Fourier de Grenoble (Grenoble, Auvergne-Rhône-Alpes, France) were responsible for collecting the samples from the plant workers and sending them to our team in Montréal, Quebec, Canada. Samples were collected in polypropylene bottles and kept frozen at -20°C until analysis.

4.3.2.3.2 Data Analysis

Retention time of standards in methanol and in spiked blank urine was used to identify the compounds in workers' urinary extracts. From the fluorescence spectrum obtained, the profiles of the diolBaPs in workers' urinary extracts were integrated and, using standard curves in urine, the resulting surface areas were then converted to picomole per milliliter of urine for further analysis. After which, the raw value of each biomarker candidate was adjusted for urinary creatinine content to accommodate for the uncertainties as to the body's processing of the metabolite and impact of urine dilution on concentrations of metabolites (Wagner et al. 2010).

The following equation was used in the conversion:

$$\left(\frac{\left(\frac{Peak\ Surface\ Area\ in\ worker\ urinary\ extract\ -\ intercept}{Slope\ of\ standard\ curve\ in\ urine \times urinary\ dilution}\right) \times Redissolution\ Volume\ (MeOH)}{Volume\ of\ urine\ analyzed}\right)$$

$$\div \left(\frac{Creatinine\ Content}{Molecular\ weight\ of\ creatinine}\right) = Quantity\ per\ mol\ of\ creatinine$$

4.3.2.3.3 Chemicals

1-OHP and BaP metabolite reference standards (4,5-diolBaP and 7,8-diolBaP) were sourced from the National Cancer Institute Chemical Carcinogen Reference Standards distributed by Midwest Research Institute (Kansas City, Missouri, USA). β-Glucuronidase/arylsulfatase (100 000 Fishman U ml^{-1} and 800 000 Roy U ml^{-1} from Helix pomatia) was procured from Roche Diagnostics (Laval, Quebec, Canada).

High-performance liquid chromatography (HPLC)-grade methanol (MeOH), ethyl acetate, ascorbic and citric acids were obtained from Fisher Scientific Company (Ottawa, Ontario, Canada).

4.3.2.3.4 Urine Analysis

Moreau and Bouchard (2015) were successful in using the UHPLC-fluorescence in detecting 4,5-diolBaP and 7,8-diolBaP in rat urine, blood and feces. Their method of urinary analysis was ergo used as a starting point. It was modified and refined through a combination of trial and error as well as the usage of relevant literature in order to obtain dependable and sensitive results in human urine, thus traversing the species barrier.

The worker urine collected was thawed, homogenized and had to be concentrated ten-fold prior to being passed through the UHPLC system for diolBaP detection. 10 mL of each urine sample was mixed with an equal amount of sodium acetate buffer (0.1 M, pH 5), then hydrolyzed with 20 µL of β-glucuronidase/arylsulfatase at 37°C in a shaking bath for an hour to obtain the sum of free and conjugated metabolites.

The samples were then put through solid phase extraction (SPE) (using Sep-Pak C18 cartridges; Waters Corporation, Milford, Massachusetts, USA). Prior to running the samples, the cartridges were conditioned with 4 mL of methanol and 8 mL of water. In an effort to reduce polar contaminants, samples were washed with 8 mL of water and 8 mL of 15% methanol:water solution after SPE. 1-hydroxypyrene standards were washed with a 50% methanol:water solution instead since a larger quantity of impurities could be removed without losing the desired compound. What remained on the cartridges was then eluted into test tubes using 8 mL of pure methanol. Adjusted nitrogen gas was used to aid throughout this process.

The ensuing mixture was evaporated at 45°C, following which the resulting residue was dissolved in 200 µL of pure methanol and centrifuged prior to being placed in vials for UHPLC-fluorescence analysis.

The analysis was carried out using an Agilent 1290 Infinity Ultra-HPLC system (Mississauga, Ontario, Canada). It is comprised of five modules: a binary pump, an autosampler, a thermostatted column compartment, a diode array detector and a fluorescence detector. There were two columns used in the process: a Poroshell 120 SB-C18 (2.1 X 50 mm; 2.7 µm) from Agilent #689775-902, and a Zorbax Eclipse plus C18 RRHD (2.1 X 50 mm; 1.8 µm) from Agilent # 959759-902. The first column was used to "load" the sample and clean it whilst preventing elution and the second served as an analytical column that passed the liquid to the detectors. The temperature of the analytical column was set at 40 °C. Switching from the loading column to the analytical column was set at 11.5 min.

Urine analysis was performed using a methanol:water gradient mobile phase also containing 5 mM of citric acid and 10 mg/L of ascorbic acid. Ascorbic acid was added to the mobile phase in accordance with a previous study conducted by Bouchard et al. (1994). The flow rate was 0.2 mL/min, the injection volume was 10 µL, samples were kept at 13°C on the injection tray, and the PMT gain was kept at 11. The run time was a set 30 minutes per sample with three minutes post run. The gradient elution conditions during each run were as follows: at 5.5 minutes, the water:methanol ratio was 90:10; 35:65 at 9.2 minutes; 0:100 at 21.5 minutes; 35:65 at 29 minutes. The excitation and emission wavelengths for the fluorescence analysis of 4,5-diolBaP were 274 nm and 390 nm respectively, and 264 nm and 415 nm for 7,8-diolBaP.

Both positive and negative controls were used for quality control. Their quantification was performed using an external calibration curve of each of the metabolites prepared in blank human urine before enzymatic hydrolysis, and analyzed as described for the worker samples. Their results presented as being within reasonable variation to that of the urine standards used. The negative controls (spiked with 0 pmol/mL of 7,8-diolBaP) held an average surface area of 4.8 versus 4.3 for the urine standards. The positive controls (spiked with 12.5 pmol/mL and 50 pmol/mL of 7,8-diolBaP) had an average surface area of 14.4 and 35.7 respectively in comparison to 12.8 and 34.5 for the urine standards.

The limit of quantification for 7,8-diolBaP was 5 fmol/mL for the 10 µL injection into the UHPLC-fluorescence system with a variation coefficient below 15%. The calibration curve

held a coefficient of 1, demonstrating linearity. The 4,5-diolBaP signal, on the other hand, had significant amounts of interference from other urinary impurities and thus was unable to be quantified or accurately detected.

4.3.2.4 Results

The retention time for 4,5-diolBaP corresponded with that of many other impurities. This lead to difficulty in fully integrating the peak produced, providing an erratic temporal profile that did not correlate well to what was observed in the animal studies conducted by Moreau and Bouchard (2015). 7,8-DiolBaP, on the other hand, produced clear peaks in the workers' urinary extracts at a similar elution time to that of the 7,8-diolBaP reference standard that created theoretically sound serial urine sample profiles.

To carry out a comprehensive assessment of the viability of 7,8-diolBaP as a biological marker of BaP, it was collated to 1-OHP due to its current status as the preferred biomarker for PAH exposure. To further validate the findings and conclusions, verified 3-OHBaP concentrations, as obtained and analyzed by Dr. Anne Maître's team in France, were juxtaposed with the 7,8-diolBaP results to compare and contrast.

The graphs used combine the results on two vertical axes, the one on the right being for 7,8-diolBaP and the one on the left for either 1-OHP or 3-OHBaP. The dark grey columns represent a work shift. 7,8-diolBaP is plotted using a dotted line and square points whereas the 1-OHP or 3-OHBaP are represented with solid lines and circular points.

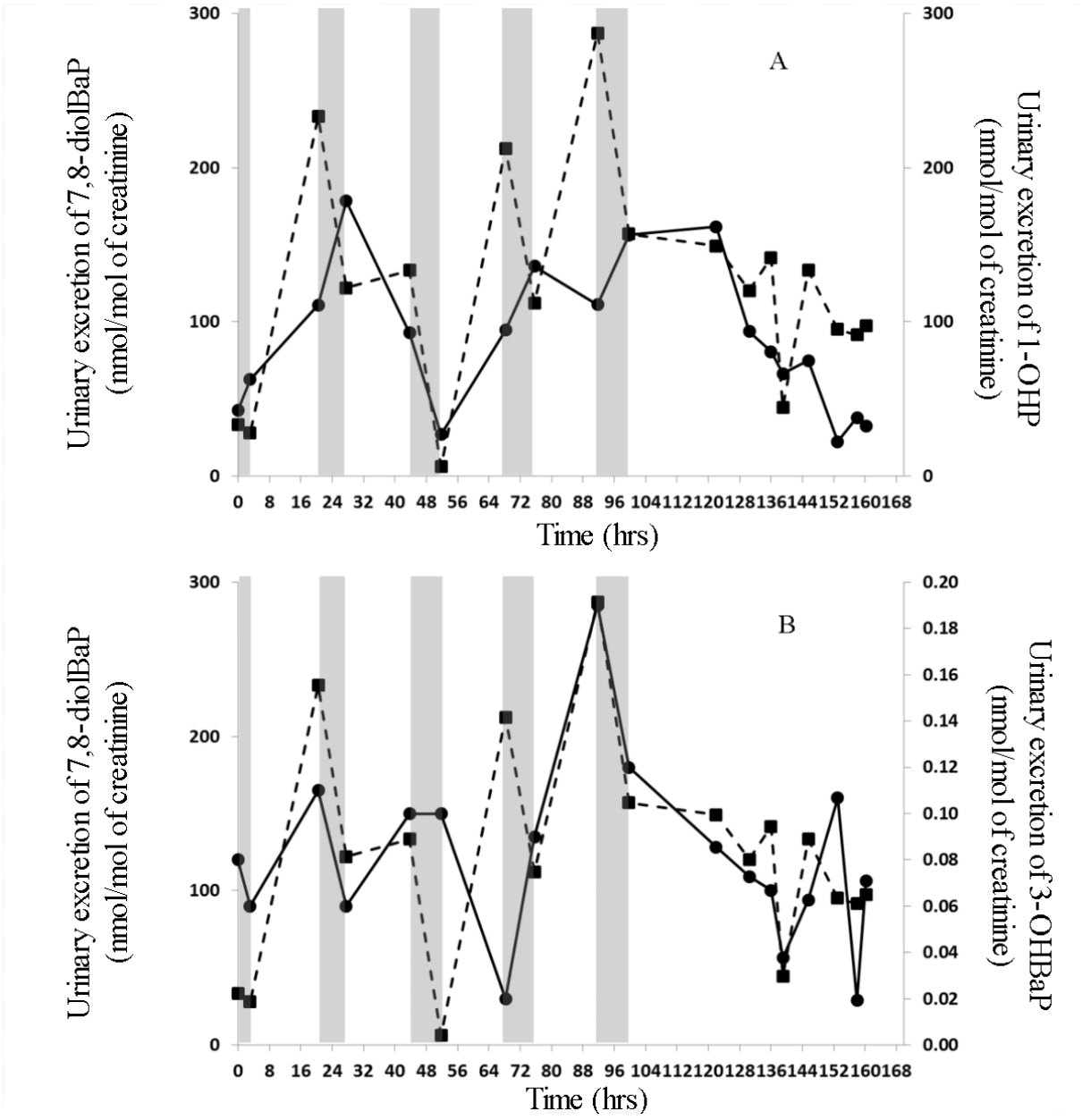

Figure 15: Time profile for the urinary concentrations of 7,8-diolBaP (dotted line and squares) for Worker A, juxtaposed against a time profile of 1-OHP (solid line and circles) (A) and juxtaposed against a time profile of 3-OHBaP (solid line and circles) (B), as sampled by the team at Grenoble.

Figure 15 shows that for worker A, the results of 1-OHP and 7,8-diolBaP are directly comparable in that they elute in similar quantities and maintain a consistent zig-zag pattern whereby their quantity is constantly rising and falling as a function of the work shifts and hence exposure to PAHs. However, there is a difference in kinetics that appears to be consistent between the two compounds. For this worker, 7,8-diolBaP is higher at the start of the work shift and lower afterwards, whereas 1-OHP is generally lower before the start of the work shift and higher at the end on four of the five sampled workdays. This demonstrates that 1-OHP content in urine increases during the course of a workday at a faster rate than that of 7,8-diolBaP, which appears to take a longer time to form and be excreted in urine.

In regard to the comparison with 3-OHBaP, as analyzed by the team in Grenoble, there is a visible similarity, especially the spike in concentrations between 88 and 96 h, that upon closer inspection reveal integral differences. The first being that 3-OHBaP elutes in much smaller quantities. The moles of 3-OHBaP per mole of creatinine are orders of magnitude smaller. There is also no consistency as to whether 3-OHBaP will be higher at the start of the shift, as can be seen around 20 h, or at the end of the shift, as can be seen around 75 hours (Figure 15).

For worker B, Figure 16 shows once again a clear synergy in the kinetics between 1-OHP and 7,8-diolBaP. The standout difference is that 1-OHP seems to be produced and released in larger quantities towards the beginning of the work week whereas 7,8-diolBaP slowly increases throughout the week as it accumulates in the body. This demonstrates that as the week progresses, it takes 7,8-diolBaP longer to leave the body, and it thus accumulates in the body faster than 1-OHP. 7,8-DiolBaP increases significantly during the off-hour periods, reflecting the same kinetic profile witnessed in worker A. In the last shift, given that both 1-OHP and 7,8-diolBaP reduce significantly, it can be deduced that B did not come into contact with PAHs during that time.

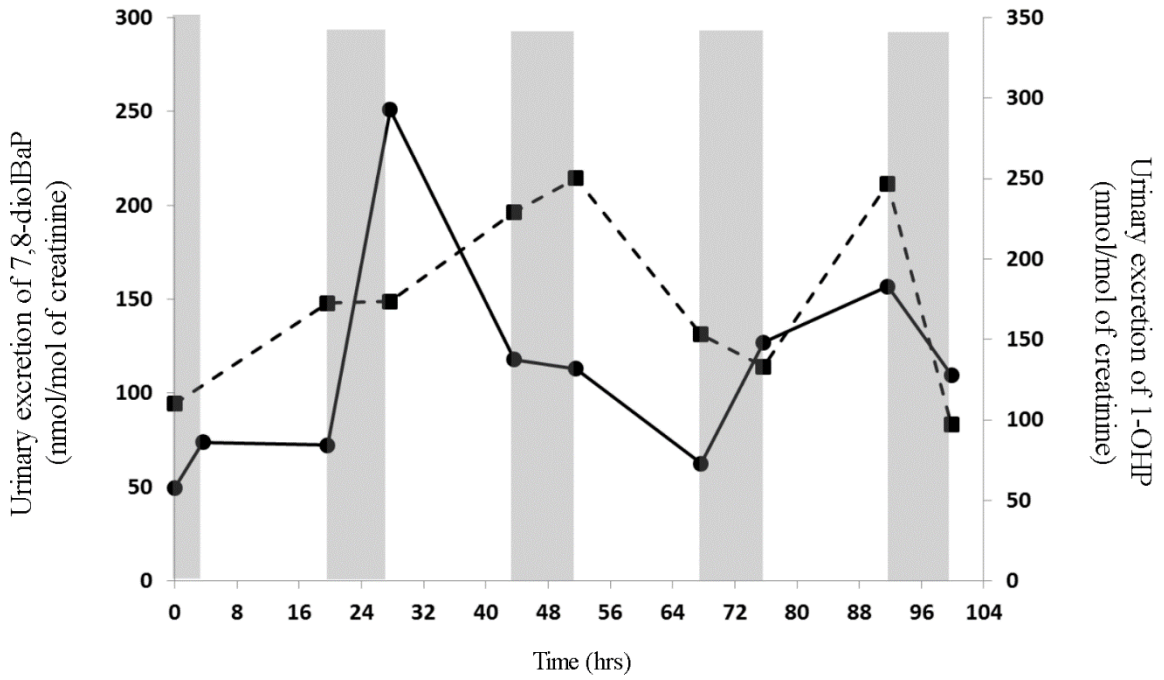

Figure 16: Time profile for the urinary concentrations of 7,8-diolBaP (dotted line and squares) for Worker B, juxtaposed against a time profile of 1-OHP (solid line and circles).

For worker D, Figure 17 depicts that observations made with worker A and B's results appear paralleled in this case. During the weekend when worker D is not at work, the time-course curve tapers off for 7,8-diolBaP and 1-OHP, clearly demonstrating a correlation between BaP exposure and the peaks seen. The increase in 7,8-diolBaP relative to 1-OHP as the week progresses reiterates the kinetic profiles previously observed whereby 7,8-diolBaP accumulates over the week, taking longer to achieve its peak and reduce than 1-OHP.

The last peak, however, is seen four hours after the last shift preceding the weekend for both 1-OHP and 7,8-diolBaP, showing a similar time delay. The peaks look identical as well, with differences in magnitude correlating to the excretion rates throughout the week, thus the route of exposure is clearly different in this instance since the behaviour of 7,8-diolBaP changes, as does its relationship to 1-OHP.

3-OHBaP is once again observed in very low quantities and the kinetics are not consistent between shifts; however, it does taper off at the end in a similar fashion to the other two biomarkers as the source of exposure is no longer present (Figure 17).

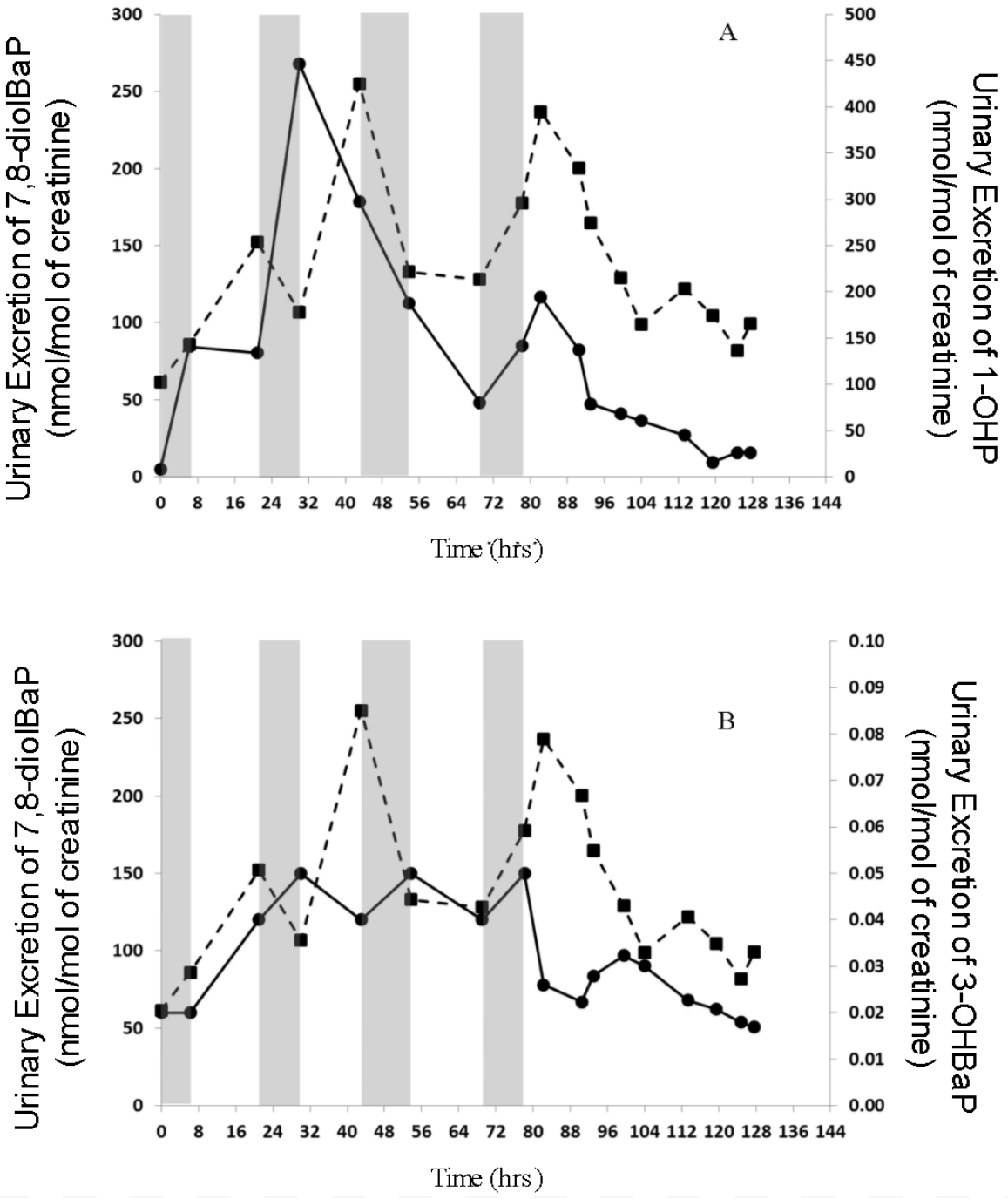

Figure 17: Time profile for the urinary concentrations of 7,8-diolBaP (dotted line and squares) for Worker D, juxtaposed against a time profile of 1-OHP (solid line and circles) (A) and juxtaposed against a time profile of 3-OHBaP (solid line and circles) (B), as sampled by the team at Grenoble.

For worker E, Figure 18 shows that during the workweek, the curves of both 7,8-diolBaP and 1-OHP are very similar, denoting PAH exposure. There is a significant spike observed in 7,8-diolBaP that is not seen in 1-OHP, otherwise, there is a near-perfect juxtaposition. Given the different correlation between 1-OHP and 7,8-diolBaP witnessed in this case, where 7,8-diolBaP increases during the work shift and reduces in the off-peak hours as 1-OHP does, a difference in occupational exposure is evident. They also retain similar magnitudes throughout the week, further re-emphasizing an enhanced similarity in kinetics that is not as prominently observed in the other workers.

3-OHBaP appears in very low quantities, almost appearing unchanging throughout the workweek.

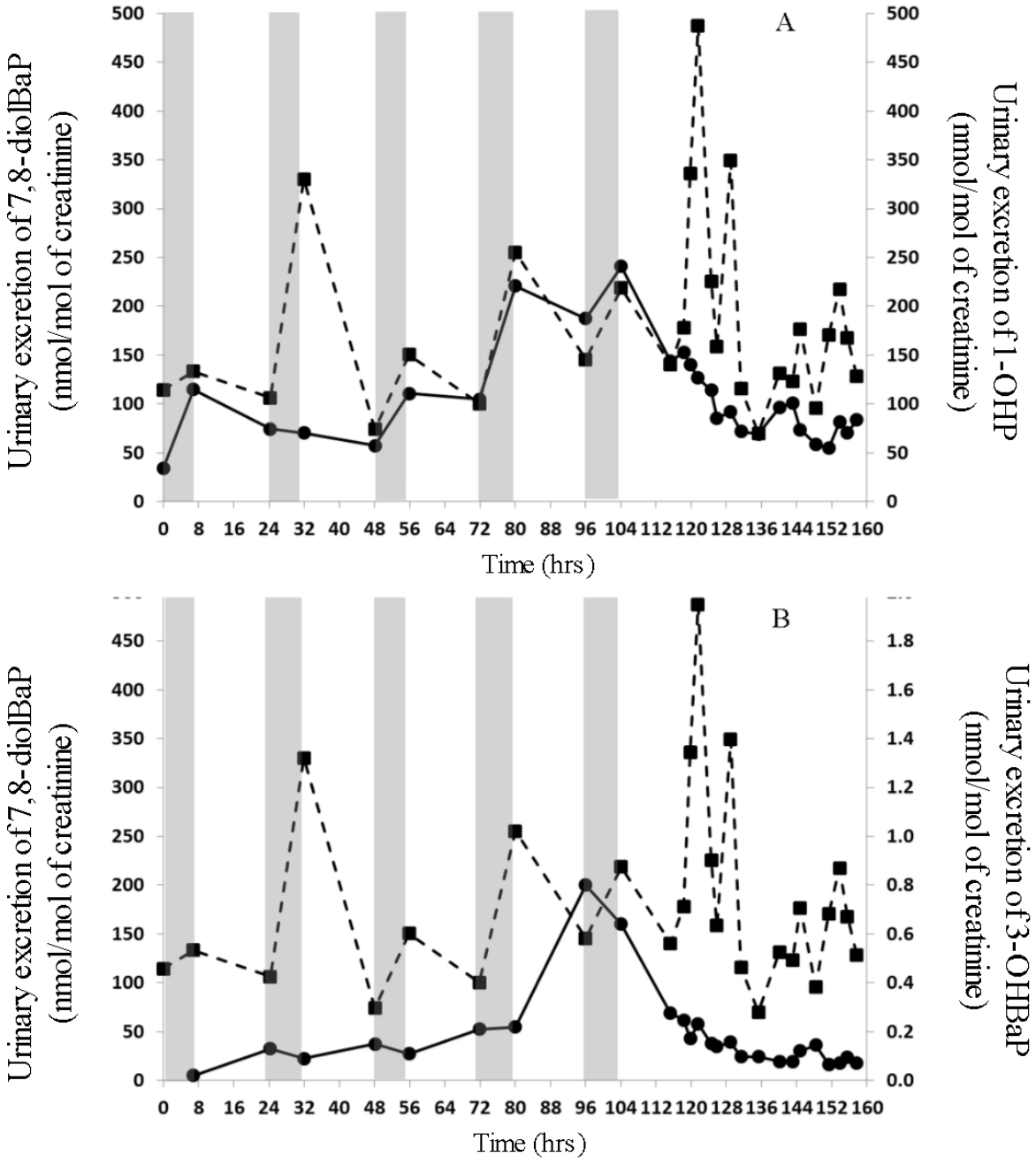

Figure 18: Time profile for the urinary concentrations of 7,8-diolBaP (dotted line and squares) for Worker E, juxtaposed against a time profile of 1-OHP (solid line and circles) (A) and juxtaposed against a time profile of 3-OHBaP (solid line and circles) (B), as sampled by the team at Grenoble.

The weekend, on the other hand, denotes a change where peaks in 7,8-diolBaP are seen while both 1-OHP and 3-OHBaP diminish. Although not confirmed, it is possible that E got in contact with a significant a source of PAHs that contains very little, if any, pyrene during this time period (Figure 18).

For worker G, Figure 19 shows an interesting dichotomy. The first half of the week demonstrates a kinetic pattern similar to that seen in worker A, B and D, whereas from the fourth shift onwards, the kinetic pattern is more similar to worker E. Furthermore, as is seen with worker E, there are peaks throughout the course of the weekend indicating exposure to PAHs during G's time away from work. The reason, however, for the increases throughout the weekend is not clear and may be related to lifestyle habits and behaviours of the volunteer.

The results as seen with 3-OHBaP are showing very little difference throughout the week, which may be attributed to the smaller elution quantities and accordingly, the difficulty in detection.

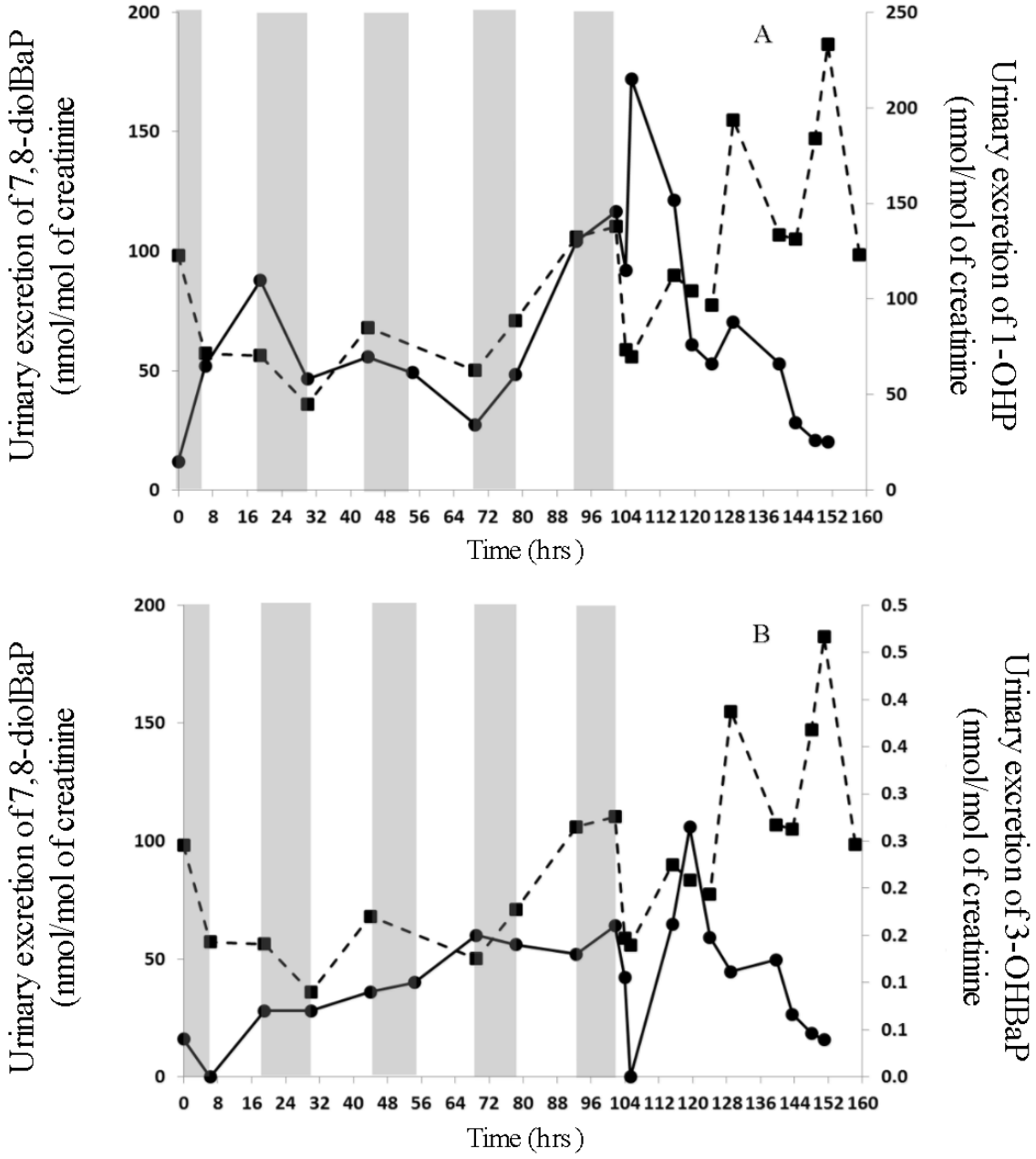

Figure 19: Time profile for the urinary concentrations of 7,8-diolBaP (dotted line and squares) for Worker G, juxtaposed against a time profile of 1-OHP (solid line and circles) (A) and juxtaposed against a time profile of 3-OHBaP (solid line and circles) (B), as sampled by the team at Grenoble.

4.3.2.5 Discussion

The excretion of organic compounds varies wildly between species, which makes it difficult to extrapolate results obtained through *in vivo* studies in animals to human beings when looking at biomarkers. The current study was conducted with five volunteers who performed various tasks at an anode production plant in Grenoble, Auvergne-Rhône-Alpes, France, aptly named A, B, D, E and G. Samples were taken before the beginning of a work shift, at the end of that same shift, then repeated for the remainder of that worker's shifts for the week. This means that there is an unequal number of samples per worker as they all worked different amounts within the week when sampling took place. Furthermore, some workers consented to providing samples over the weekend when they did not have any shifts whereas others did not.

In addition to these differences, a regular smoker, C, who did not work at the anode plant, or in an environment known to contain an above average amount of PAHs, was sampled at random intervals over the period of a day. C self-reported smoking an average of seventeen cigarettes a day and their results functioned to compare the BaP exposure that comes from smoking with that which comes from the workplace.

Based on the kinetics observed in *in vivo* studies conducted in rats, the expectation was that prior to BaP exposure, there would be trace diolBaPs found in the workers' urine, with a sharp increase noted at the end of the workday and a continual reduction to baseline over the weekend. Furthermore, the peaks at the end of the shift were expected to increase progressively throughout the week, assuming that there was a steady accumulation in the body.

The results obtained in this series of experiments are directly comparable to previous animal studies that took place. Bouchard and Viau (1996) compared the kinetics between 1-OHP, 3-OHBaP and 4,5-diolBaP in rats based upon BaP and pyrene exposure using very direct routes of entry (intravenous and intraperitoneal). 3-OHBaP, regardless of route of entry, took considerably longer than 4,5-diolBaP and 1-OHP to reach its excretion peak, and eluted in smaller quantities than both 4,5-diolBaP and 1-OHP. The maximum excretion rate of 1-OHP occurred immediately following injection whereas 3-OHBaP peak values were reached at least 10 hours postdosing. This is seen in all the workers as well where 1-OHP peaks quickly after the workday starts, yet 3-OHBaP takes a while to hit its peak. 4,5-DiolBaP, in rats, was found to emit a kinetic profile similar to that of 1-OHP.

Although it exhibited promising results in rats, 4,5-diolBaP did not demonstrate the same prominence in human beings. 4,5-DiolBaP, based on the same calculations used to convert the peak area to moles per mole of creatinine, were present in larger quantities than the other biomarkers, but the fluorescence analysis of the compound demonstrated many instances of interference, rendering the numbers obtained as mere speculation and as such, they were not included in this report.

7,8-DiolBaP, on the other hand, eluted in clear distinguished peaks at on average 15.7 min. Through a thorough analysis and comparison with the existing biomarkers, 1-OHP and 3-OHBaP, the time-courses of 7,8-diolBaP in workers were more compatible with those of 1-OHP than those of 3-OHBaP. A literature review gave an overview as to the conventional behaviour of 3-OHBaP and 1-OHP. While 1-OHP peaks shortly after exposure and is completely eliminated from the body within 24 hours, 3-OHBaP has a time lag whereby it peaks circa 16 hours after the end of an exposure episode in humans (Jongeneelen et al. 1988; Barbeau et al. 2015; Gendre et al. 2002, 2004). The consequence of the kinetic complexity of 3-OHBaP makes it a second choice when selecting biomarkers for BaP, even though it is compound-specific. While 1-OHP and 3-OHBaP are relatively well understood due to the surrounding literature, 7,8-diolBaP is comparatively new to the scene and demonstrates promise.

Barbeau et al. (2015) conducted a study with volunteers who were occupationally exposed to PAHs during their shifts in an effort to compare the kinetic profiles of 1-OHP and 3-OHBaP in a variety of work-based circumstances. They found that the kinetic profiles differed with worker duties, the aerosol content in the air and the mode of exposure. This meant that not only did profiles differ from volunteer to volunteer, but that the correlation between the profiles of 1-OHP and 3-OHBaP differed greatly as well.

This is also observed in our study between 1-OHP and 7,8-diolBaP. In worker A, 7,8-diolBaP measured levels were consistently lower at the end of the work shift, and higher at the beginning. As soon as the peak value was achieved, the 7,8-diolBaP concentrations were reduced dramatically in the seven-to-eight-hour window of the worker's shift. This same timeline was observed most clearly in D and G. With B and E, it is more difficult to ascertain that eight-hour time lag since spot sampling was used and the reduction occurred off-hours when worker urine was not collected. 1-OHP, on the other hand, increased from the start of the shift, peaking at the end, and reducing during the hours away from exposure.

Certain shifts, most notably in E and G, also demonstrate a pattern whereby 7,8-diolBaP is lower at the beginning of the shift, accumulates and increases throughout the shift as exposure takes place, and finishes higher, as is seen with 1-OHP. This may be attributed to the aerosol content in the air favouring the absorption of more BaP and less pyrene or vice versa at certain moments, or a change in the worker duty and hence the route of exposure. In essence, the changing correlation and kinetics are evidence of a difference in the occupational environment.

In a previous study conducted by Odenigbo et al. (pending), a volunteer was cutaneously exposed to very low levels of PAHs in a controlled setting. The first experiment was a single exposure where the first samples of urine taken within three hours after exposure demonstrated elevated levels of both 1-OHP and 7,8-diolBaP; 1-OHP reached its peak amount eight hours after exposure, whereas 7,8-diolBaP took fourteen hours. In an identical multiple exposure experiment, similar results were seen where 7,8-diolBaP accumulated faster with each exposure (roughly spaced 24 hours apart) while 1-OHP was eliminated faster and accumulated at a slower pace, eventually demonstrating smaller peaks than 7,8-diolBaP towards the end of the week.

The trend remains consistent with our results in that with workers A, B and D, there is a quick rise in 1-OHP within the eight-hour shift, and a drop soon after the source of exposure is no longer present, versus 7,8-diolBaP where a much slower rise is seen during the hours after the shift. As the week progresses, the level of 7,8-diolBaP starts to exceed that of 1-OHP, which may indicate that these workers were primarily exposed through cutaneous absorption. The differing kinetic profiles noticed in certain shifts of E and G may allude to exposure through inhalation.

Another possibility, especially when looking at the third shift of the week for Worker G, is that their tasks changed such that exposure was either reduced to negligible levels or non-existent. This specific instance in worker G shows a continual decline throughout the third shift as if there was no exposure at all, and this is mimicked by 1-OHP in the same instance. Since 1-OHP has a faster decline when there is no longer a source of exposure present, the reduction observed may be indicative of a change of environment where PAHs are not as prominent in the surroundings.

Subject C (results not shown) provides contextualisation in this study. Although smokers are exposed to more PAHs than the average person, the amounts noted through biosurveillance using 1-OHP indicate trace levels. This can be attributed to the fact that when analyzing

individual PAH components in cigarettes, pyrene is noted as being present in very small quantities (Wynder and Hoffmann 1959). BaP is considered a significant component of cigarette smoke (Wynder and Hoffmann 1959). When using 3-OHBaP as a biomarker, the compound eluted in extremely small quantities since it is not primarily excreted through the urine as noted by Leroyer et al. (2010). Looking at 7,8-diolBaP on the other hand, higher levels were noted, exceeding 150 nmol/mol of creatinine. C also serves to add an additional layer of certainty to the identification by fluorescence spectroscopy in that the compound we are looking at is most likely 7,8-diolBaP.

The implication of these results is that there is now a method to biologically survey PAH exposure for carcinogenic concerns. When using 1-OHP to detect and evaluate exposure to PAHs, the fact that it is a pyrene metabolite both plays to and against its utility. Pyrene is often featured in most PAHs mixtures and takes on a large percentage of the overall melee, which means that large quantities of 1-OHP are produced through the metabolic pathways in the body and can be measured in the urine. The negative side of this is that pyrene is considered one of the least dangerous PAH compounds, and is not carcinogenic. This effectively signifies that confirming 1-OHP in one's urine is not emblematic of a potential health risk, nor a potential risk of cancer, it is instead insinuated that PAH mixtures will most certainly contain some of the more carcinogenic compounds.

7,8-DiolBaP opens the doors to greater clarity in this manner. As a compound-specific biomarker, it is produced through the monooxygenation of BaP catalyzed by microsomal nicotinamide adenine dinucleotide phosphate (NADPH)-dependent cytochrome P450 isoforms 1A1 and 1B1; this produces arene oxides, which are then conjugated with water by epoxide hydrolase to create 7,8-diolBaP (Reed and Marnett 1982; Jiang et al. 2007).

BaP too is a constant feature in most PAH mixes. By using 7,8-diolBaP to monitor BaP exposure, finding it in one's urine does confirm contact, not just with PAHs, but with carcinogenic PAHs that could have negative health effects. In a workplace setting where exposure takes place every shift, this kind of information is invaluable to both employers and employees alike because it enables to planning and subsequent creation of workplace health and safety policies that enhance the corporal integrity of their workers. It ergo alleviates any uncertainty as to whether there is increased risk and allows the focus to be on mitigating it.

4.3.2.6 Conclusion

The present study was unable to accurately measure 4,5-diolBaP in human urine as a result of the elution time corresponding with that of many other impurities. It has been, however, successful in both confirming that it is possible to reach the analytical sensitivity needed to identify what is believed to be 7,8-diolBaP in the urine and in demonstrating its viability as a compound-specific biomarker for assessing individual exposure to BaP, and thus carcinogenic PAHs in general, in occupational settings. There are similarities with the kinetic patterns of 1-OHP, an established biological marker in this instance, hence creating a certain level of reliability. 7,8-diolBaP can hereafter be used either in conjunction with, or as an alternative to, 1-OHP when monitoring workplace PAH exposure and evaluating the danger and risk involved for the staff involved.

The next steps include confirming that the eluted compound is indeed 7,8-diolBaP through mass spectroscopy analysis, then create a temporal profile of 7,8-diolBaP excretion through collecting all urinary micturition over a determined period and assessing both the rate of excretion and the cumulative excretion with workplace exposure. Further studies need to be conducted to fine-tune the methodology used such that it is better able to characterise 4,5-diolBaP as well; create a dose-response assessment that links negative health effects to the amount of 7,8 diolBaP content in one's urine to help establish workplace health and safety regulations; and, explore other facets for mitigation and expulsion of BaP from the body using 7,8-diolBaP as an indicator tool.

4.3.2.7 Acknowledgements

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4.3.2.8 References

Barbeau, D. et al., 2014. Relevance of urinary 3-hydroxybenzo(a)pyrene and 1-hydroxypyrene to assess exposure to carcinogenic polycyclic aromatic hydrocarbon mixtures in metallurgy workers. *The Annals of occupational hygiene*, 58(5), pp.579–590.

Barbeau, D., Maître, A. and Marques, M., 2011. Highly sensitive routine method for urinary 3-hydroxybenzo[a]pyrene quantitation using liquid chromatography-fluorescence detection and automated off-line solid phase extraction. *The Analyst*, 136(6), pp.1183–1191.

Barbeau, D. et al., 2015. Occupational exposure to polycyclic aromatic hydrocarbons: relations between atmospheric mixtures, urinary metabolites and sampling times. *International archives of occupational and environmental health*, 88(8), pp.1119–1129.

Bouchard, M., Dodd, C. and Viau, C., 1994. Improved Procedure for the High-Performance Liquid Chromatographic Determination of Monohydroxylated PAH Metabolites in Urine. *Journal of analytical toxicology*, 18(5), pp.261–264.

Bouchard, M. & Viau, C., 1996. Urinary Excretion Kinetics of Pyrene and Benzo(a)pyrene Metabolites Following Intravenous Administration of the Parent Compounds or the Metabolites. *Toxicology and applied pharmacology*, 139(2), pp.301–309.

Bouchard, M., Krishnan, K. and Viau, C., 1998. Kinetics of Tissue Distribution and Elimination of Pyrene and 1-Hydroxypyrene Following Intravenous Administration of [14C]Pyrene in Rats. *Toxicological sciences: an official journal of the Society of Toxicology*, 46(1), pp.11–20.

Buchet, J.P. et al., 1992. Evaluation of exposure to polycyclic aromatic hydrocarbons in a coke production and a graphite electrode manufacturing plant: assessment of urinary excretion of 1-hydroxypyrene as a biological indicator of exposure. *Occupational and environmental medicine*, 49(11), pp.761–768.

Gendre C., Lafontaine M., Morele Y., Payan J.P., Simon P., 2002. Relationship between urinary levels of 1-hydroxypyrene and 3-hydroxybenzo[a]pyrene for workers exposed to polycyclic aromatic hydrocarbons. *Polycyclic Aromatic Compounds*, 22, pp.761–769

Gendre, C., Lafontaine, M., Delsaut, P., Simon, P., 2004. Exposure to polycyclic aromatic hydrocarbons and excretion of urinary 3-hydroxybenzo(a)pyrene: assessment of an appropriate sampling time. *Polycyclic Aromatic Compounds,* 24, pp.433–439.

IARC Working Group on the Evaluation of Carcinogenic Risk to Humans, 2010. Chemical Agents and Related Occupations. In *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*. Lyon: International Agency for Research on Cancer.

Jiang, H. et al., 2007. Metabolism of benzo[a]pyrene in human bronchoalveolar H358 cells using liquid chromatography-mass spectrometry. *Chemical research in toxicology*, 20(9), pp.1331–1341.

Jongeneelen, F.J. et al., 1988. Airborne concentrations, skin contamination, and urinary metabolite excretion of polycyclic aromatic hydrocarbons among paving workers exposed to coal tar derived road tars. *American Industrial Hygiene Association journal*, 49(12), pp.600–607.

Karle, I.L. et al., 2004. Crystal and molecular structure of a benzo[a]pyrene 7,8-diol 9,10-epoxide N2-deoxyguanosine adduct: absolute configuration and conformation. *Proceedings of the National Academy of Sciences of the United States of America*, 101(6), pp.1433–1438.

Lee, W. et al., 2003. Studies on the Analysis of Benzo(a)pyrene and Its Metabolites in Biological Samples by Using High Performance Liquid Chromatography/Fluorescence Detection and Gas Chromatography/Mass Spectrometry. *Bulletin of the Korean Chemical Society*, 24(5), pp.559–565.

Leroyer, A. et al., 2010. 1-Hydroxypyrene and 3-hydroxybenzo[a]pyrene as biomarkers of exposure to PAH in various environmental exposure situations. *The Science of the total environment*, 408(5), pp.1166–1173.

Lutier, S. et al., 2016. Urinary elimination kinetics of 3-hydroxybenzo(a)pyrene and 1-hydroxypyrene of workers in a prebake aluminum electrode production plant: Evaluation of diuresis correction methods for routine biological monitoring. *Environmental research*, 147, pp.469–479.

McCarthy, 0., 2018. *Biomarkers of Environmental Contamination*, CRC Press.

Moreau, M., 2013. *Étude du continuum exposition-effet cancérogène du benzo[a]pyrène*. PhD. Université de Montréal.

Moreau, M., Ayotte, P. and Bouchard, M., 2015. Kinetics of Diol and Hydroxybenzo[a]pyrene Metabolites in Relation to DNA Adduct Formation and Gene Expression in Rats. *Journal of toxicology and environmental health. Part A*, 78(12), pp.725–746.

Moreau, M. and Bouchard, M., 2015. Comparison of the kinetics of various biomarkers of benzo[a]pyrene exposure following different routes of entry in rats. *Journal of applied toxicology: JAT*, 35(7), pp.781–790.

Mourón, S.A. et al., 2006. Genotoxic Effects of Benzo[a]pyrene and Dibenzo[a,l]pyrene in a Human Lung Cell Line. *International journal of toxicology*, 25(1), pp.49–55.

Odenigbo, C. and Bouchard, M., pending. Mapping the kinetic profile of 7,8-dihydrodiol-benzo[a]pyrene as a biomarker of cutaneous exposure to polycyclic aromatic hydrocarbons in human beings. *Science of the Total Environment*, pending.

Petry, T., Schmid, P. and Schlatter, C., 1996. The use of toxic equivalency factors in assessing occupational and environmental health risk associated with exposure to airborne mixtures of polycyclic aromatic hydrocarbons (PAHs). *Chemosphere*, 32(4), pp.639–648.

Pruneda-Álvarez, L.G. et al., 2016. Urinary 1-hydroxypyrene concentration as an exposure biomarker to polycyclic aromatic hydrocarbons (PAHs) in Mexican women from different hot spot scenarios and health risk assessment. *Environmental science and pollution research international*, 23(7), pp.6816–6825.

Qiu, C. et al., 2012. The effect of occupational exposure to benzo[a]pyrene on neurobehavioral function in coke oven workers. *American journal of industrial medicine*, 56(3), pp.347–355.

Reed, G.A. and Marnett, L.J., 1982. Metabolism and activation of 7,8-dihydrobenzo[a]pyrene during prostaglandin biosynthesis. Intermediacy of a bay-region epoxide. *The Journal of biological chemistry*, 257(19), pp.11368–11376.

Twardowska, I. and Kolodziejczyk, A.M., 1998. Benzo[a]pyrene in soils and ground water: Occurrence, sources, distribution, interrelation. *Toxicological and environmental chemistry*, 66(1-4), pp.127–144.

Wagner, B.D., Accurso, F.J. and Laguna, T.A., 2010. The applicability of urinary creatinine as a method of specimen normalization in the cystic fibrosis population. *Journal of cystic fibrosis: official journal of the European Cystic Fibrosis Society*, 9(3), pp.212–216.

World Health Organization. Regional Office for Europe and World Health Organization, 2006. *Air Quality Guidelines: Global Update 2005 : Particulate Matter, Ozone, Nitrogen Dioxide, and Sulfur Dioxide*, World Health Organization.

Wynder, E.L. and Hoffmann, D., 1959. A study of tobacco carcinogenesis. VII. The role of

higher polycyclic hydrocarbons. *Cancer*, 12, pp.1079–1086.

Yeo, C.D. et al., 2017. Roflumilast treatment inhibits lung carcinogenesis in benzo(a)pyrene-induced murine lung cancer model. *European journal of pharmacology*. Available at: http://dx.doi.org/10.1016/j.ejphar.2017.07.004.

5. Discussion

5.1 General Discussion

The goal of this thesis was to evaluate the use of 4,5-diolBaP and 7,8-diolBaP as biomarkers of exposure for BaP. In order to do this, two studies were carried out: one being a case-study in a controlled exposure setting focusing on obtaining a time course for 7,8-diolBaP and comparing it to the current preferred biomarker of PAH exposure, 1-OHP (the "shampoo experiment"); and, the other being a comparative analysis of 4,5-diolBaP, 7,8-diolBaP, 3-OHBaP and 1-OHP in an occupational setting to verify utility in a real-life context (the "worker study").

This goal falls in the larger context of reducing and regulating workplace exposure to carcinogenic PAHs. These steps serve to authenticate a new compound-specific biomarker. From here, one can actively monitor BaP exposure in the workplace and through a combination of epidemiological techniques and toxicological studies, set out industry regulations which will then influence mitigation measures and thus reduce the incidence of occupational cancer.

While the experiments conducted in this thesis were not designed to specifically study the universality of the toxicokinetics of the diolBaPs, useful information was nonetheless gleaned with respect to the excretion trend of 7,8-diolBaP in comparison with 3-OHBaP and 1-OHP in human beings. A dose-dependent relationship is clear between 7,8-diolBaP and BaP, whereby exposure caused an increase in concentrations observed in the urine. This confirms the general trend seen in rats where higher levels of the diolBaPs were measured in the gastrointestinal tract and the urine soon after exposure (Moreau 2013). It also increases the level of certainty that the compound being assessed is the urinary metabolite of a PAH since its presence increases noticeably with exposure.

In addition to the impact of different tasks, aerosol content and route of exposure, many chemical and physiological factors play a role in the differences noticed in the kinetics between the various experiments and compounds. When looking at cutaneous exposure, the skin reservoir effect combined with exposure conditions could explain the high variability of 1-OHP and 7,8-diolBaP in the single and multiple scenarios in the "shampoo experiment" and in various workers in the "worker study". The reservoir effect can prolong the duration that a substance

may remain active in the skin layers, with the effect possible in the stratum corneum, in the viable avascular tissue and in the dermis (Roberts et al. 2004). Pyrene is a smaller compound that traverses the skin's layers faster than BaP, which enables it to get metabolised faster, and may explain the time lag between 1-OHP and 7,8-diolBaP. Moreover, the scalp has the thickest skin in the body, which means that the rate of production of the metabolites is expected to be slower than if absorption is through another part of the body, such as one's hands (Zoltan & Vasconez 1985).

When exposed through inhalation, pyrene is typically present in vapour phase in the air of workplaces while BaP is known to be adsorbed onto the surface of particles (Choi et al. 2010). Absorption following inhalation of pyrene vapour is thus expected to be rapid. On the other hand, while adsorbed to particles, BaP entering the respiratory tract may be retained, which has a direct impact on the absorption rate through the lung epithelium barrier (slower absorption from the respiratory tract) (Bosquillon et al. 2017).

Although the methodology was not capable of elucidating a clear distinction of what appears to be 4,5-diolBaP from the impurities in the urine, it demonstrated success in evaluating and analysing what is presumed to be 7,8-diolBaP. From the observations made, it is possible to extrapolate certain conclusions that need further testing to be confirmed.

Volunteer C, a regular smoker who did not work in an environment known to have significant levels of PAHs, was sampled at random intervals over a twenty-four-hour period. Although his/her results are not used in this study, C provides contextualisation because this is a case where exposure is to PAHs at relatively low levels that have a heavy percentage of BaP and a weak pyrene presence. Studies show that while BaP is very much present in cigarettes, pyrene barely registered when assessing it as a PAH component in cigarette smoke (Wynder & Hoffmann 1959). In demonstrating high concentrations of 7,8-diolBaP and trace concentrations of 1-OHP, we receive confirmation that the biomarker we are looking is linked to BaP, or a carcinogenic PAH.

However, there are uncertainties with this assessment. The first being that although chromatography is capable of aiding in the constant analysis of compounds in mixtures, it does not identify the compound to a perfect level of certainty. Previous experiments by Moreau &

Bouchard (2015) indicate that 7,8-diolBaP tends to elute in similar amounts to 3-OHBaP, whereas our findings are showing a greater similarity to 1-OHP, although the retention time is in essence identical to reference standards (Moreau & Bouchard 2015). According to Klaassen & Watkins (1984), there are differences in metabolism between species, as well as difference in the excretion rates. This effectively makes the extrapolation of results between species extremely difficult and this may be at play in this instance.

Mass spectrometry is a needed component to confirm that the compound measure is truly 7,8-diolBaP and not another compound coeluting at the same retention time and fluorescing in the same conditions. In the event that it is not 7,8-diolBaP, the clear dose-response relationship indicates another PAH metabolite, and because it peaks at higher levels (when compared to 1-OHP) when looking at the Control C, who is a smoker, it most likely references a carcinogenic PAH.

The postulation that follows is less on whether what we believe to be 7,8-diolBaP is viable as a biomarker of BaP, and PAH mixtures, but more so how to improve the accuracy in measuring it in urine to further increase confidence in the results. From there, in having a biomarker for BaP, it then becomes possible to conduct epidemiological experiments on a large scale with workers from various PAH-rich industries that link the levels of this compound found in urine to negative health effects.

6. Conclusion

6.1 Conclusion

Although there is more work to be done, this study has been successful in finding an alternative biomarker for occupational PAH exposure in human beings. A compound eluting in the same time frame as 7,8-diolBaP, as seen in a similar set of studies conducted on rats (Moreau & Bouchard 2015), was identified as having the potential to provide the same baseline information in the context of PAH exposure as 1-OHP, a pyrene derivative and the principal biomarker of exposure for PAHs.

The methodology proved inefficient with regards to 4,5-diolBaP. The retention-time by which the compound was recognised coincided with multiple impurities and the signal was not able to successfully distinguish itself from the background "noise", which lead to difficulties in accurately assessing the peak area, and thus calculating quantity in urine. This means that the results obtained in the context of this experiment were not useable for a proper analysis but given the preliminary findings in rats by Moreau et al. (2015), it is worth exploring further.

Each step, however, was successful in that the method developed seems fully capable of identifying 7,8-diolBaP, and to a lesser extent, 4,5-diolBaP, thus confirming BaP exposure as these compounds are metabolic derivatives to BaP. When analysing the results of the volunteer present in the case study and those who were spot-sampled in the workplace, the toxicokinetics of 7,8-diolBaP proved to be comparable in timing and pattern to 1-OHP, thus inferring its potential as a biomarker of BaP, and an alternative biomarker of PAHs in general. 7,8-diolBaP also proved to be more consistent and easier to both follow, read and understand in human beings when compared to 3-OHBaP in an occupational setting.

If mass spectrometry or another identification method confirms that different compounds had been captured throughout this report, then this thesis remains successful. In this context, it has been able to find a new alternative biomarker of carcinogenic PAHs with the understanding, that the select compound has expressed a definitive dose-response relationship with carcinogenic PAH exposure.

6.2 Future Work

The immediate action needed is a proper unequivocal identification of the biomarkers followed throughout this project by mass spectrometry. Assuming that the compounds found were indeed 4,5-diolBaP and 7,8-diolBaP, the results of this study allow for three trains of consecutive research continuation, each following through with the overall project aim of mitigating or reducing the risk of cancer associated with PAH exposure in the workplace:

- A calibrative study aimed at reducing the interference observed when measuring 4,5-diolBaP in order to properly assess its potential as a biological marker of BaP exposure
- A study geared towards the characterisation of 7,8-diolBaP in its capacity as a biological marker of BaP through which reliable predictable results can be obtained consistently such that workplace health and safety surveillance may be standardised and improved
- Discovering effective mitigation and reduction strategies through using the biomarkers of BaP and other PAHs such that workplace health and safety may be updated appropriately

Given the positive results found in studies on rats, the potential for 4,5-diolBaP as a key biomarker in assessing both BaP, and by extension, carcinogenic PAH exposure is there. Although the retention time in human beings coincides with many impurities and obstructions, improving the method by which the sample is cleaned and further extending the length of the analytical column in the UHPLC may be excellent starting places in establishing a 4,5-diolBaP biosurveillance methodology. The results can then be compared to those of 7,8-diolBaP to evaluate ease of process, accuracy and reliability of results as well as whether they provide the same information in an exposure case.

While this project has yielded results, it is imperfect in that although a general trend can be observed, it lacks specifics. A full toxicokinetic study needs to be launched to better understand the kinetic patterns of 7,8-diolBaP and allow its characterisation such that one can immediately comprehend the level of exposure an employee has been subjected to. This should then be paired with a dose-response study in individuals who are exposed in the workplace, in order to pair the level of 7,8-diolBaP found in the urine, to the negative health consequences of

exposure. Although BaP is genotoxic, and thus is not bound by a set limit in promoting cancer, it is still important to get estimates so that exposure thresholds can be determined and followed to protect the majority of workers.

7. References

Abdel-Shafya, H.I. & Mansour, M.S.M., 2016. A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation. *Egyptian Journal of Petroleum*, 25(1), pp.107–123.

American Conference of Governmental Industrial Hygienists, 1998. *1997 TLVs and BEIs: Threshold Limit Values for Chemical Substances and Physical Agents, Biological Exposure Indices*,

Aramandla Ramesh Michael Greenwood, Ramesh, A. & Greenwood, M., 2001. TOXICOKINETICS OF INHALED BENZO[a]PYRENE: Plasma and Lung Bioavailability. *Inhalation toxicology*, 13(6), pp.533–553.

Arey, J. & Atkinson, R., 2003. Photochemical Reactions of PAHs in the Atmosphere. In P. E. T. Douben, ed. *Ecological and Environmental Toxicology Series*. West Sussex: John Wiley & Sons, Ltd, pp. 47–63.

Armstrong, B. et al., 2004. Lung cancer risk after exposure to polycyclic aromatic hydrocarbons: a review and meta-analysis. *Environmental health perspectives*, 112(9), pp.970–978.

Bach, P.B. et al., 2003. Screening for Lung Cancer*. *Chest*, 123(1), p.72S–82S.

Baklanov, A. et al., 2006. Integrated systems for forecasting urban meteorology, air pollution and population exposure. *Atmospheric Chemistry and Physics Discussions*, 6(2), pp.1867–1913.

Barbeau, D. et al., 2015. Occupational exposure to polycyclic aromatic hydrocarbons: relations between atmospheric mixtures, urinary metabolites and sampling times. *International archives of occupational and environmental health*, 88(8), pp.1119–1129.

Barbeau, D. et al., 2014. Relevance of urinary 3-hydroxybenzo(a)pyrene and 1-hydroxypyrene to assess exposure to carcinogenic polycyclic aromatic hydrocarbon mixtures in metallurgy workers. *The Annals of occupational hygiene*, 58(5), pp.579–590.

Bjorseth, A., 1983. *Handbook of Polycyclic Aromatic Hydrocarbons Polycyclic Aromatic Hydrocarbons* M. Dekker, ed.,

Boffetta, P., Jourenkova, N. & Gustavsson, P., 1997. Cancer risk from occupational and environmental exposure to polycyclic aromatic hydrocarbons. *Cancer causes & control: CCC*, 8(3), pp.444–472.

Bosquillon, C. et al., 2017. A Comparison of Drug Transport in Pulmonary Absorption Models: Isolated Perfused rat Lungs, Respiratory Epithelial Cell Lines and Primary Cell Culture. *Pharmaceutical research*, 34(12), pp.2532–2540.

Boström, C.-E. et al., 2002. Cancer risk assessment, indicators, and guidelines for polycyclic aromatic hydrocarbons in the ambient air. *Environmental health perspectives*, 110 Suppl 3, pp.451–488.

Bouchard, M. & Viau, C., 1996. Urinary Excretion Kinetics of Pyrene and Benzo(a)pyrene Metabolites Following Intravenous Administration of the Parent Compounds or the Metabolites. *Toxicology and applied pharmacology*, 139(2), pp.301–309.

Broekman, B.F.P. et al., 2009. The influence of birth size on intelligence in healthy children. *Pediatrics*, 123(6), pp.e1011–6.

Burczynski, M.E. et al., 2000. The Reactive Oxygen Species- and Michael Acceptor-inducible Human Aldo-Keto Reductase AKR1C1 Reduces the α,β-Unsaturated Aldehyde 4-Hydroxy-2-nonenal to 1,4-Dihydroxy-2-nonene. *The Journal of biological chemistry*, 276(4), pp.2890–2897.

CAREX Canada, 2016. CAREX Canada. *PAHs*. Available at: https://www.carexcanada.ca/en/polycyclic_aromatic_hydrocarbons/ [Accessed January 18, 2018].

CCCF, 2009. *Code of Practice for the Reduction of Contamination of Food with Polycyclic Aromatic Hydrocarbons (PAH) from Smoking and Direct Drying Processes*, Codex Alimentarius. Available at: http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCAC%2BRCP%2B68-2009%252FCXP_068f.pdf.

Chen, C. et al., 2012. Early postnatal benzo(a)pyrene exposure in Sprague-Dawley rats causes persistent neurobehavioral impairments that emerge postnatally and continue into adolescence and adulthood. *Toxicological sciences: an official journal of the Society of Toxicology*, 125(1), pp.248–261.

Choi, H. et al., 2010. 6. Polycyclic aromatic hydrocarbons. In World Health Organization, ed. *WHO Guidelines for Indoor Air Quality: Selected Pollutants*. NCBI Bookshelf. Geneva: World Health Organization.

Cocker, J. et al., 2003. 173. A Control-Based Biological Monitoring Guidance Value for PAHs. In *AIHce 2003*. Available at: http://dx.doi.org/10.3320/1.2757845.

Comandini, A. & Brezinsky, K., 2011. Theoretical study of the formation of naphthalene from the radical/π-bond addition between single-ring aromatic hydrocarbons. *The journal of physical chemistry. A*, 115(22), pp.5547–5559.

Comité fédéral-provincial-territorial sur l'eau potable, 2015. Le benzo[a]pyrène dans l'eau potable. *Consultations concernant la santé de l'environnement et du milieu de travail*. Available at: https://www.canada.ca/fr/sante-canada/services/sante-environnement-milieu-travail/consultations/benzo-pyrene-eau-potable-sante-canada-2015/document-consultation.html [Accessed August 11, 2018].

De Jong, W., 1999. Detection of immunotoxicity of benzo[a]pyrene in a subacute toxicity study after oral exposure in rats. *Toxicological sciences: an official journal of the Society of Toxicology*, 50(2), pp.214–220.

Denissenko, M.F. et al., 1996. Preferential formation of benzo[a]pyrene adducts at lung cancer mutational hotspots in P53. *Science*, 274(5286), pp.430–432.

Douben, P.E.T., 2003. *PAHs: An Ecotoxicological Perspective*, John Wiley & Sons.

Edwards, S.C. et al., 2010. Prenatal Exposure to Airborne Polycyclic Aromatic Hydrocarbons and Children's Intelligence at 5 Years of Age in a Prospective Cohort Study in Poland. *Environmental health perspectives*, 118(9), pp.1326–1331.

EU Science Hub, 2015. Legislation on Polycyclic Aromatic Hydrocarbons (PAHs) - EU Science Hub - European Commission. *EU Science Hub*. Available at: https://ec.europa.eu/jrc/en/eurl/pahs/legislation [Accessed August 10, 2018].

Evenson, M.L. et al., 1988. Estimation of human dose of staphylococcal enterotoxin A from a large outbreak of staphylococcal food poisoning involving chocolate milk. *International journal of food microbiology*, 7(4), pp.311–316.

Fiala, Z. et al., 2001. Environmental exposure of small children to polycyclic aromatic hydrocarbons. *International archives of occupational and environmental health*, 74(6), pp.411–420.

Field, R.W. & Withers, B.L., 2012. Occupational and environmental causes of lung cancer. *Clinics in chest medicine*, 33(4), pp.681–703.

Food Standards Agency, 2018. Polycyclic aromatic hydrocarbons. *Food Standards Agency*. Available at: https://www.food.gov.uk/business-guidance/polycyclic-aromatic-hydrocarbons [Accessed August 10, 2018].

Greim, H., 2007. *The MAK-Collection for Occupational Health and Safety: Part I: MAK Value Documentations*, Wiley-VCH.

Gündel, J., Schaller, K.H. & Angerer, J., 2000. Occupational exposure to polycyclic aromatic hydrocarbons in a fireproof stone producing plant: biological monitoring of 1-hydroxypyrene, 1-, 2-, 3- and 4-hydroxyphenanthrene, 3-hydroxybenz(a)anthracene and 3-hydroxybenzo(a)pyrene. *International archives of occupational and environmental health*, 73(4), pp.270–274.

Harper, M., 2004. Assessing workplace chemical exposures: the role of exposure monitoring. *Journal of environmental monitoring: JEM*, 6(5), pp.404–412.

Hartmann, M. & Jung, D., 2010. Biocatalysis with enzymes immobilized on mesoporous hosts: the status quo and future trends. *Journal of materials chemistry*, 20(5), pp.844–857.

Hattemer-Frey, H.A. & Travis, C.C., 1991. Benzo-a-pyrene: environmental partitioning and human exposure. *Toxicology and industrial health*, 7(3), pp.141–157.

Hayes, A., Bakand, S. & Winder, C., 2007. Novel In-Vitro Exposure Techniques for Toxicity Testing and Biomonitoring of Airborne Contaminants. In U. Marx & V. Sandig, eds. *Drug Testing In Vitro: Breakthroughs and Trends in Cell Culture Technology*. Berlin: WILEY-VCH, pp. 103–120.

Heinrich, U., Roller, M. & Pott, F., 1994. Estimation of a lifetime unit lung cancer risk for benzo(a)pyrene based on tumour rates in rats exposed to coal tar/pitch condensation aerosol. *Toxicology letters*, 72(1-3), pp.155–161.

Heredia-Ortiz, R. & Bouchard, M., 2013. Understanding the linked kinetics of benzo(a)pyrene and 3-hydroxybenzo(a)pyrene biomarker of exposure using physiologically-based pharmacokinetic modelling in rats. *Journal of pharmacokinetics and pharmacodynamics*, 40(6), pp.669–682.

IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2012. Chemical agents and related occupations. *IARC monographs on the evaluation of carcinogenic risks to humans / World Health Organization, International Agency for Research on Cancer*, 100(Pt F), pp.9–562.

IARC Working Group on the Evaluation of Carcinogenic Risk to Humans, 2012a. Benzo[a]pyrene. In *Chemical Agents and Related Occupations.* IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Lyon: International Agency for Research on Cancer.

Ifegwu, C. et al., 2012. Urinary 1-Hydroxypyrene as a Biomarker to Carcinogenic Polycyclic Aromatic Hydrocarbon Exposure. *Biomarkers in cancer*, 4, pp.7–17.

International Joint Commission, 1983. *Report on the Great Lakes water quality*, International Joint Commission.

Jagt, K. van der et al., 2004. Effectiveness of Personal Protective Equipment: Relevance of Dermal and Inhalation Exposure to Chlorpyrifos Among Pest Control Operators. *Journal of occupational and environmental hygiene*, 1(6), pp.355–362.

Jang, T.-W. et al., 2018. The standards for recognition of occupational cancers related with polycyclic aromatic hydrocarbons (PAHs) in Korea. *Annals of Occupational and Environmental Medicine*, 30(1). Available at: http://dx.doi.org/10.1186/s40557-018-0224-1.

Jiang, H. et al., 2007. Metabolism of benzo[a]pyrene in human bronchoalveolar H358 cells using liquid chromatography-mass spectrometry. *Chemical research in toxicology*, 20(9), pp.1331–1341.

Jongeneelen, F.J., 2001. Benchmark guideline for urinary 1-hydroxypyrene as biomarker of occupational exposure to polycyclic aromatic hydrocarbons. *The Annals of occupational hygiene*, 45(1), pp.3–13.

Jung, K.H. et al., 2013. Characteristic molecular signature for the early detection and prediction of polycyclic aromatic hydrocarbons in rat liver. *Toxicology letters*, 216(1), pp.1–8.

Kang, D. et al., 2005. Design issues in cross-sectional biomarkers studies: urinary biomarkers of PAH exposure and oxidative stress. *Mutation research*, 592(1-2), pp.138–146.

Kapitulnik, J. et al., 1977. Benzo[a]pyrene 7,8-dihydrodiol is more carcinogenic than benzo[a]pyrene in newborn mice. *Nature*, 266(5600), pp.378–380.

Kim, K.-H. et al., 2013. A review of airborne polycyclic aromatic hydrocarbons (PAHs) and their human health effects. *Environment international*, 60, pp.71–80.

Klaassen, C.D. & Watkins, J.B., 3rd, 1984. Mechanisms of bile formation, hepatic uptake, and biliary excretion. *Pharmacological reviews*, 36(1), pp.1–67.

Kleiböhmer, W., 2001. *Environmental Analysis*, Elsevier.

Klöslová, Z. et al., 2016. The Relations between Polycyclic Aromatic Hydrocarbons Exposure and 1-OHP Levels as a Biomarker of the Exposure. *Central European journal of public health*, 24(4), pp.302–307.

Koreeda, M. et al., 1978. Binding of benzo[a]pyrene 7,8-diol-9,10-epoxides to DNA, RNA, and protein of mouse skin occurs with high stereoselectivity. *Science*, 199(4330), pp.778–781.

Korfmacher, W.A. et al., 1981. Nonphotochemical decomposition of fluorene vapor-adsorbed on coal fly ash. *Environmental science & technology*, 15(11), pp.1370–1375.

Kroese, E.D., 2001. *Tumorigenic Effects in Wistar Rats Orally Administered Benzo[a]pyrene for Two Years (gavage Studies): Implications for Human Cancer Risks Associated with Oral Exposure to Polycyclic Aromatic Hydrocarbons* National Institute for Public Health and the Environment, ed.,

Lai, H. & Singh, N.P., 2006. Oral artemisinin prevents and delays the development of 7,12-dimethylbenz[a]anthracene (DMBA)-induced breast cancer in the rat. *Cancer letters*, 231(1), pp.43–48.

Lee, H.K., 2000. POLYCYCLIC AROMATIC HYDROCARBONS | Gas Chromatography. In *Encyclopedia of Separation Science*. pp. 3860–3866.

Lee, M.L. et al., 1977. Source identification of urban airborne polycyclic aromatic hydrocarbons by gas chromatographic mass spectrometry and high resolution mass spectrometry. *Biomedical mass spectrometry*, 4(3), pp.182–185.

Leroyer, A. et al., 2010. 1-Hydroxypyrene and 3-hydroxybenzo[a]pyrene as biomarkers of exposure to PAH in various environmental exposure situations. *The Science of the total environment*, 408(5), pp.1166–1173.

Levin, W. et al., 1977. Carcinogenicity of benzo-ring derivatives of benzo(a)pyrene on mouse skin. *Cancer research*, 37(9), pp.3356–3361.

Likhachev, A.J. et al., 1992. Biomarkers for individual susceptibility to carcinogenic agents: excretion and carcinogenic risk of benzo[a]pyrene metabolites. *Environmental health perspectives*, 98, pp.211–214.

Lima, A.L.C., Farrington, J.W. & Reddy, C.M., 2005. Combustion-Derived Polycyclic Aromatic Hydrocarbons in the Environment—A Review. *Environmental Forensics*, 6(2), pp.109–131.

Lim, M.C.H. et al., 2007. Influence of fuel composition on polycyclic aromatic hydrocarbon emissions from a fleet of in-service passenger cars. *Atmospheric environment*, 41(1), pp.150–160.

Lippman, S.M. & Hawk, E.T., 2009. Cancer prevention: from 1727 to milestones of the past 100 years. *Cancer research*, 69(13), pp.5269–5284.

Llobet, J.M. et al., 2003. Concentrations of arsenic, cadmium, mercury, and lead in common foods and estimated daily intake by children, adolescents, adults, and seniors of Catalonia, Spain. *Journal of agricultural and food chemistry*, 51(3), pp.838–842.

Lutier, S. et al., 2016. Urinary elimination kinetics of 3-hydroxybenzo(a)pyrene and 1-hydroxypyrene of workers in a prebake aluminum electrode production plant: Evaluation of diuresis correction methods for routine biological monitoring. *Environmental research*, 147, pp.469–479.

Mackay, D. & Paterson, S., 1991. Evaluating the multimedia fate of organic chemicals: a level III fugacity model. *Environmental science & technology*, 25(3), pp.427–436.

MacKenzie, K.M. & Angevine, D.M., 1981. Infertility in mice exposed in utero to benzo(a)pyrene. *Biology of reproduction*, 24(1), pp.183–191.

Madhavan, N.D. & Naidu, K.A., 1995. Polycyclic aromatic hydrocarbons in placenta, maternal blood, umbilical cord blood and milk of Indian women. *Human & experimental toxicology*, 14(6), pp.503–506.

Marie, C. et al., 2010. A toxicokinetic study to elucidate 3-hydroxybenzo(a)pyrene atypical urinary excretion profile following intravenous injection of benzo(a)pyrene in rats. *Journal of applied toxicology: JAT*. Available at: http://dx.doi.org/10.1002/jat.1511.

Masih, J. et al., 2012. Seasonal Variation and Sources of Polycyclic Aromatic Hydrocarbons (PAHs) in Indoor and Outdoor Air in a Semi Arid Tract of Northern India. *Aerosol and Air Quality Research*, 12(4), pp.515–525.

Mastrangelo, G., Fadda, E. & Marzia, V., 1996. Polycyclic aromatic hydrocarbons and cancer in man. *Environmental health perspectives*, 104(11), pp.1166–1170.

McMahon, C.K. & Tsoukalas, S.N., 1978. Polynuclear aromatic hydrocarbons in forest fire smoke. *Carcinogenesis, Vol. 3: Polynuclear Aromatic Hydrocarbons*, 3, pp.61–73.

Moir, D., 1996. The pharmacokinetics of benzo(a)pyrene in the rat. *Toxicology letters*, 88(1), p.88.

Moody, R.P., Nadeau, B. & Chu, I., 1995. In vivo and in vitro dermal absorption of benzo[a]pyrene in rat, guinea pig, human and tissue-cultured skin. *Journal of dermatological science*, 9(1), pp.48–58.

Moreau, M., 2013. *Étude du continuum exposition-effet cancérogène du benzo[a]pyrène*. PhD. Université de Montréal.

Moreau, M. & Bouchard, M., 2015. Comparison of the kinetics of various biomarkers of benzo[a]pyrene exposure following different routes of entry in rats. *Journal of applied toxicology: JAT*, 35(7), pp.781–790.

Nacoulma, B.M.I. et al., 2016. Impact of bark and foliage harvesting on fruit production of the multipurpose tree Afzelia africana in Burkina Faso (West Africa). *Agroforestry Systems*, 91(3), pp.565–576.

Nadarajah, N. et al., 2002. Enhanced transformation of polycyclic aromatic hydrocarbons using a combined Fenton's reagent, microbial treatment and surfactants. *Applied microbiology and biotechnology*, 59(4-5), pp.540–544.

National Research Council of Canada, 1983. *Polycyclic aromatic hydrocarbons in the aquatic environment: formation, sources, fate and effects on the aquatic environment*, Associate Committee on Scientific Criteria for Environmental Quality.

National Research Council (US) Committee on Pyrene and Selected Analogues, 1983. POLYCYCLIC AROMATIC HYDROCARBONS FROM NATURAL AND STATIONARY ANTHROPOGENIC SOURCES AND THEIR ATMOSPHERIC CONCENTRATIONS. In

Polycyclic Aromatic Hydrocarbons: Evaluation of Sources and Effects. Washington DC: National Academies Press (US).

OECD, 2012. Adult population smoking daily, 2010 and change in smoking rates, 2000-10 (or nearest year). Available at: http://dx.doi.org/10.1787/9789264183896-graph60-en.

Ortiz, R.H., 2014. *Modélisation toxicocinétique du benzo(a)pyrène et 3- hydroxybenzo(a)pyrène pour l'interprétation des données de surveillance biologique de l'exposition chez les travailleurs*. PhD. Université de Montréal.

Peng, C. et al., 2011. Polycyclic aromatic hydrocarbons in urban soils of Beijing: Status, sources, distribution and potential risk. *Environmental pollution* , 159(3), pp.802–808.

Perera, F. et al., 2012. Effects of prenatal polycyclic aromatic hydrocarbon exposure and environmental tobacco smoke on child IQ in a Chinese cohort. *Environmental research*, 114, pp.40–46.

Perera, F. et al., 2006. In Utero and Childhood Environmental Exposures and Multiple Health Outcomes: NIEHS/EPA Children???s Center Cohort Studies. *Epidemiology* , 17(Suppl), pp.S411–S412.

Perera, F.P. et al., 2012. Prenatal polycyclic aromatic hydrocarbon (PAH) exposure and child behavior at age 6-7 years. *Environmental health perspectives*, 120(6), pp.921–926.

Pessah, I.N. et al., 2001. A bioactive metabolite of benzo[a]pyrene, benzo[a]pyrene-7,8-dione, selectively alters microsomal Ca2+ transport and ryanodine receptor function. *Molecular pharmacology*, 59(3), pp.506–513.

Phillips, D.H., 1999. Polycyclic aromatic hydrocarbons in the diet. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 443(1-2), pp.139–147.

Platt, K.L., Grupe, S. & Fickler, M., 2008. The 3,4-oxide is responsible for the DNA binding of benzo[ghi]perylene, a polycyclic aromatic hydrocarbon without a "classic" bay-region. *Chemico-biological interactions*, 176(2-3), pp.179–187.

Pubchem, trans-Benzo(a)pyrene-4,5-dihydrodiol. Available at: https://pubchem.ncbi.nlm.nih.gov/compound/37785 [Accessed December 30, 2017].

Ramesh, A. et al., 2001. Metabolism, bioavailability, and toxicokinetics of Benzo(α)pyrenein F-344 rats following oral administration. *Experimental and toxicologic pathology: official journal of the Gesellschaft fur Toxikologische Pathologie*, 53(4), pp.275–290.

Rengarajan, T. et al., 2015. Exposure to polycyclic aromatic hydrocarbons with special focus on cancer. *Asian Pacific journal of tropical biomedicine*, 5(3), pp.182–189.

Riccardi, C. et al., 2008. Characterization and distribution of petroleum hydrocarbons and heavy metals in groundwater from three Italian tank farms. *The Science of the total environment*, 393(1), pp.50–63.

Richter, H. et al., 2001. Detailed Kinetic Study of the Growth of Small Polycyclic Aromatic Hydrocarbons. 1. 1-Naphthyl Ethyne†. *The journal of physical chemistry. A*, 105(9), pp.1561–1573.

Rigdon, R.H. & Neal, J., 1969. Relationship of Leukemia to Lung and Stomach Tumors in Mice Fed Benzo(a) Pyrene. *Experimental biology and medicine*, 130(1), pp.146–148.

Roberts, M.S., Cross, S.E. & Anissimov, Y.G., 2004. Factors affecting the formation of a skin reservoir for topically applied solutes. *Skin pharmacology and physiology*, 17(1), pp.3–16.

R. P. Schwarzenbach, P.M.G.A.D.M.I., 2017. Environmental organic chemistry, 3rd edition. *International journal of environmental analytical chemistry*, 97(4), pp.398–399.

Santé Canada, 2017. Concentrations maximales établies par Santé Canada à l'égard de contaminants chimiques dans les aliments. *Contaminants chimiques*. Available at: https://www.canada.ca/fr/sante-canada/services/aliments-nutrition/salubrite-aliments/contaminants-chimiques/concentrations-maximales-etablies-egard-contaminants-chimiques-aliments.html [Accessed December 27, 2017].

Schänzer, W., Geyer, H. & Donike, M., 1991. Metabolism of metandienone in man: identification and synthesis of conjugated excreted urinary metabolites, determination of excretion rates and

gas chromatographic-mass spectrometric identification of bis-hydroxylated metabolites. *The Journal of steroid biochemistry and molecular biology*, 38(4), pp.441–464.

Schrenk, D., 2002. Regulatory toxicology: objectives and tasks defined by the working group of the German society of experimental and clinical pharmacology and toxicology. *Toxicology letters*, 126(3), p.167.

Sexton, K. et al., 2011. Polycyclic aromatic hydrocarbons in maternal and umbilical cord blood from pregnant Hispanic women living in Brownsville, Texas. *International journal of environmental research and public health*, 8(8), pp.3365–3379.

Sharma, A. et al., 2008. Effects of Benzo(e)Pyrene, a Toxic Component of Cigarette Smoke, on Human Retinal Pigment Epithelial Cells In Vitro. *Investigative Opthalmology & Visual Science*, 49(11), p.5111.

Shimada, T. & Fujii-Kuriyama, Y., 2004. Metabolic activation of polycyclic aromatic hydrocarbons to carcinogens by cytochromes P450 1A1 and 1B1. *Cancer science*, 95(1), pp.1–6.

Société canadienne du cancer, 2015. Société canadienne du cancer. *www.cancer.ca*. Available at: http://www.cancer.ca/fr-ca/region-selector-page/?region=on [Accessed February 1, 2018].

Srogi, K., 2007. Monitoring of environmental exposure to polycyclic aromatic hydrocarbons: a review. *Environmental chemistry letters*, 5(4), pp.169–195.

Stowers, S.J., Jill Stowers, S. & Anderson, M.W., 1985. Formation and Persistence of Benzo(a)pyrene Metabolite-DNA Adducts. *Environmental health perspectives*, 62, p.31.

Strimbu, K. & Tavel, J.A., 2010a. What are biomarkers? *Current opinion in HIV and AIDS*, 5(6), pp.463–466.

Strimbu, K. & Tavel, J.A., 2010b. What are biomarkers? *Current opinion in HIV and AIDS*, 5(6), pp.463–466.

Tang, D. et al., 2006. PAH-DNA Adducts in Cord Blood and Fetal and Child Development in a Chinese Cohort. *Epidemiology* , 17(Suppl), p.S416.

Thorsen, W.A., Gregory Cope, W. & Shea, D., 2004. Bioavailability of PAHs: Effects of Soot Carbon and PAH Source. *Environmental science & technology*, 38(7), pp.2029–2037.

Toronto Research Chemicals, TRC | Details of CAS = 37571-88-3, ChemicalName = trans-Benzo[a]pyrene-4,5-dihydrodiol, synonym = (4R,5R)-rel-4,5-Dihydrobenzo[a]pyrene-4,5-diol; trans-4,5-Dihydrobenzo[a]pyrene-4,5-diol; (±)-Benzo[a]pyrene-trans-4,5-dihydrodiol; 4,5-Dihydrobenzo[a]pyrene-4,5-trans-(e,e)-diol; Benzo[a]pyrene-trans-4,5-dihydrodiol; trans-4,5-Dihydro-4,5-dihydroxybenzo[a]pyrene; trans-4,5-Dihydrodiolbenzo[a]pyrene; trans-4,5-Dihydroxy-4,5-dihydrobenzo[a]pyrene;, MolFormula = $C_{20}H_{14}O_2$. *B205830*. Available at: https://www.trc-canada.com/product-detail/?B205830 [Accessed December 29, 2017].

Tremblay, C. et al., 1995. Estimation of risk of developing bladder cancer among workers exposed to coal tar pitch volatiles in the primary aluminum industry. *American journal of industrial medicine*, 27(3), pp.335–348.

United Nations Environment Programme, International Labour Organisation & World Health Organization, 1998. SELECTED NON-HETEROCYCLIC POLICYCLIC AROMATIC HYDROCARBONS. In *INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY*. ENVIRONMENTAL HEALTH CRITERIA 202. Geneva: World Health Organization.

Unwin, J. et al., 2006. An Assessment of Occupational Exposure to Polycyclic Aromatic Hydrocarbons in the UK. *The Annals of occupational hygiene*, 50(4), pp.395–403.

Vaidya, V.S. & Bonventre, J.V., 2010. *Biomarkers: In Medicine, Drug Discovery, and Environmental Health*, John Wiley & Sons.

Van Metre, P.C., Mahler, B.J. & Furlong, E.T., 2000. Urban Sprawl Leaves Its PAH Signature. *Environmental science & technology*, 34(19), pp.4064–4070.

Viau, C. & Vyskocil, A., 1995. Patterns of 1-hydroxypyrene excretion in volunteers exposed to pyrene by the dermal route. *The Science of the total environment*, 163(1-3), pp.187–190.

Viau, M.B., 1999. Urinary 1-hydroxypyrene as a biomarker of exposure to polycyclic aromatic hydrocarbons: biological monitoring strategies and methodology for determining biological

exposure indices for various work environments. *Biomarkers: biochemical indicators of exposure, response, and susceptibility to chemicals*, 4(3), pp.159–187.

Wagner, B.D., Accurso, F.J. & Laguna, T.A., 2010. The applicability of urinary creatinine as a method of specimen normalization in the cystic fibrosis population. *Journal of cystic fibrosis: official journal of the European Cystic Fibrosis Society*, 9(3), pp.212–216.

Wagner, M.M., 2006. Introduction. In M. M. Wagner, A. W. Moore, & R. M. Aryel, eds. *Handbook of Biosurveillance*. Burlington: Elsevier Academic Press, pp. 3–12.

Weyand, E.H. & Bevan, D.R., 1986. Benzo(a)pyrene disposition and metabolism in rats following intratracheal instillation. *Cancer research*, 46(11), pp.5655–5661.

Wickström, K. et al., 1986. Polycyclic aromatic compounds (PAC) in leaf lettuce. *Zeitschrift fur Lebensmittel-Untersuchung und -Forschung*, 183(3), pp.182–185.

Withey, J.R. et al., 1993. Distribution of benzo[a]pyrene in pregnant rats following inhalation exposure and a comparison with similar data obtained with pyrene. *Journal of applied toxicology: JAT*, 13(3), pp.193–202.

World Health Organization, 1996. *Biological Monitoring of Chemical Exposure in the Workplace Guidelines*, Geneva: World Health Organization.

Wu, J. et al., 2010. Exposure to polycyclic aromatic hydrocarbons and missed abortion in early pregnancy in a Chinese population. *The Science of the total environment*, 408(11), pp.2312–2318.

Wynder, E.L. & Hoffmann, D., 1959. A study of tobacco carcinogenesis. VII. The role of higher polycyclic hydrocarbons. *Cancer*, 12, pp.1079–1086.

Xue, W. & Warshawsky, D., 2005. Metabolic activation of polycyclic and heterocyclic aromatic hydrocarbons and DNA damage: a review. *Toxicology and applied pharmacology*, 206(1), pp.73–93.

Yu, Y. et al., 2011. Polycyclic aromatic hydrocarbon residues in human milk, placenta, and umbilical cord blood in Beijing, China. *Environmental science & technology*, 45(23), pp.10235–10242.

Zelinkova, Z. & Wenzl, T., 2015. The Occurrence of 16 EPA PAHs in Food – A Review. *Polycyclic aromatic compounds*, 35(2-4), pp.248–284.

Zhang, H.M. et al., 2012. Characteristic analysis of peripheral blood mononuclear cell apoptosis in coke oven workers. *Journal of occupational health*, 54(1), pp.44–50.

Zhao, Z.-H., Quan, W.-Y. & Tian, D.-H., 1990. Urinary 1-hydroxypyrene as an indicator of human exposure to ambient polycyclic aromatic hydrocarbons in a coal-burning environment. *The Science of the total environment*, 92, pp.145–154.

Zoltan, J. & Vasconez, L.O., 1985. Atlas of Skin Repair. *Plastic and reconstructive surgery*, 76(2), p.325.