Chapter 15
Salivary Bioscience and Environmental Exposure Assessment

Parinya Panuwet, Priya E. D'Souza, Emily R. Phillips, P. Barry Ryan, and Dana Boyd Barr

Abstract Human exposure to toxic, environmental chemicals can be assessed by measuring levels of parent chemicals or their metabolites in biological matrices such as urine, plasma, and whole blood. This technique is known as biomonitoring. If these measurements are accurate, the internal dose of the target chemicals in humans can be estimated. Biomonitoring has become an important tool for epidemiological studies linking exposures to toxic, environmental chemicals to health outcomes. Selection of the appropriate biological matrices for biomonitoring is crucial and requires a comprehensive understanding of the physiochemical properties of the target chemicals, their toxicokinetic properties, as well as the biological properties of the matrix. Different matrices may provide different information on the magnitude and frequency of dose, toxicity, and biological endpoint. Saliva has been used as a matrix for biomonitoring of toxic, environmental chemicals, but with far less frequency than urine or plasma. The success of using saliva as a matrix for exposure assessment has been proven for cotinine, a principal metabolite of nicotine. However, a number of limitations and challenges remain for other chemicals such as plasticizers and heavy metals. In this chapter, the current knowledge on salivary biomonitoring is provided, including the associated challenges researchers face. More importantly, practical recommendations are provided with the intention of supporting future research using saliva as a matrix to assess exposure to toxic, environmental chemicals.

Keywords Saliva · Biomonitoring · Environmental exposure · Toxic chemicals · Exposure assessment

P. Panuwet (✉) · P. E. D'Souza · E. R. Phillips · P. B. Ryan · D. B. Barr
Laboratory of Exposure Assessment and Development for Environmental Research (LEADER), Department of Environmental Health, Rollins School of Public Health, Emory University, Atlanta, GA, USA
e-mail: ppanuwe@emory.edu

D. A. Granger, M. K. Taylor (eds.), *Salivary Bioscience*,
https://doi.org/10.1007/978-3-030-35784-9_15

15.1 History of Salivary Bioscience in Environmental Exposure

At present, in addition to naturally occurring inorganic and organic chemicals, humans are exposed to a wide variety of anthropogenic chemicals through their environment (e.g., air, water, soil, etc.). This is a result of industrial growth, urbanization, industrial and nonindustrial product consumption and substitution, and other human practices. Product substitution refers to the replacement of a key chemical ingredient that was proven toxic to humans with a newly developed substance for which toxicological evidence in humans is not yet available. For instance, bisphenol A has been phased out and replaced with bisphenol F or bisphenol S in consumable plastic materials (Lehmler, Liu, Gadogbe, & Bao, 2018).

Examples of chemicals to which humans are commonly exposed include heavy metals/metalloids, dioxins, pesticides, plasticizers (phthalates, bisphenols), cosmetic and pharmaceutical preservatives (parabens), flame retardants (polybrominated diphenyl ethers or PBDEs, organophosphate flame-retardants), polycyclic aromatic hydrocarbons, gasoline emissions and combustion products, per- and poly-fluoroalkyl substances, tobacco smoke, environmental phenols, volatile organic compounds, and perchlorate. Some of these chemicals enter the food chain and are ingested by humans, while others become suspended in the air or attached to particulate matter or dust particles and are inhaled into human lungs. Other environmental chemicals dissolve in water and are ingested or absorbed through human skin. Some chemicals remain in the soil and enter the human body via dermal absorption or ingestion. Other chemicals (i.e., key ingredients in cosmetic products) are specifically designed to be applied to human skin and therefore are readily absorbed by the human body.

Some environmental chemicals, such as pesticides, heavy metals/metalloids, and tobacco smoke have well-defined toxicological endpoints that are associated with health outcomes in humans (Damalas & Koutroubas, 2016; IARC, 2012; Kakkar & Jaffery, 2005; Wallace, 2012). However, the toxicological endpoints for many chemicals are ambiguous, particularly when exposures to them are chronic and low level. Epidemiological and risk assessment studies continue to play an important role in quantifying the associated health risks or in investigating the relationships between exposures to these environmental chemicals and health outcomes. It is undeniable that a key factor in these studies is the ability to determine accurate and precise exposure levels in the study population. While there are other tools (i.e., questionnaires and ecological and environmental assessments) that can be used to estimate exposure levels, *biomonitoring* is a key analytic process in defining exposure-disease risk/outcomes, largely due to the biological significance of internal dose measurements (Needham et al., 2005). In short, biomonitoring refers to the measurement of parent compounds, their metabolites, or reaction products, in biological matrices such as urine, blood, and serum as well as other collectible biological matrices (Angerer, Ewers, & Wilhelm, 2007). Biomonitoring and biological measurement of exposure can be used interchangeably.

Upon entering the human body, a chemical undergoes four complex steps in the pharmacokinetic process: absorption, distribution, metabolism, and excretion (ADME). In order to assess human exposure to a given chemical, biological measurements of the chemical can be made after the absorption step or during each of the subsequent steps of ADME. Measurement of the concentration of a chemical involves the selection of appropriate biological matrices. The concentrations in each matrix depend on the amount of the intake and uptake of the absorbed chemical, the pharmacokinetics of the chemical, and the exposure scenario, including the timing of the exposure (Sexton, Callahan, & Bryan, 1995). Biomonitoring data integrate all routes of exposure and are independent of the exposure pathway. Ideally, measurements of the biologically effective dose, the dose at the target site that causes an adverse health effect, are preferred. However, in most circumstances, the target organ is unknown or unavailable for collection. As such, the chemical is measured in a proximal matrix, typically blood or urine, to estimate the internal dose (Barr, Wang, & Needham, 2005; Pirkle, Needham, & Sexton, 1995).

Selection of the most appropriate matrix for biomonitoring depends upon the different classes of environmental chemicals to be monitored. For example, some persistent organic pollutants, such as dioxins and PBDEs, are nonpolar and lipophilic and thus they tend to partition in lipid stores in the body (CDC, 2017). Therefore, biological matrices that are rich in lipid content (e.g., serum, adipose tissue, brain, and breast milk) are likely to be appropriate matrices for biological measurements of these chemicals (Barr et al., 2005). For biological measurements of environmental chemicals, blood or urine is often the matrix of choice. Blood has inherent, toxicological advantages for biomonitoring because the majority of chemicals must be absorbed by the bloodstream and circulated to the tissues to have an effect. Additionally, measurements are automatically normalized because blood volume is constant per given body weight, allowing for the estimation of total toxicant body burden. Urine is the other most commonly used biological matrix. Several environmental chemicals are nonpersistent (i.e., they have short environmental and biological half-lives) and are excreted in the urine in their original form, as metabolites, or both, depending on their pharmacokinetic characteristics. Thus, urine is considered an excellent matrix for measuring those compounds (Needham et al., 2005).

Saliva has been used as a matrix for the biological measurement of exposure to environmental chemicals, mainly those found in tobacco smoke, for more than 38 years (Greenberg, Haley, Etzel, & Loda, 1984; Jarvis et al., 1985; Peyton III, Wilson, & Benowitz, 1981; Wall, Johnson, Jacob, & Benowitz, 1988). Historically, saliva was used, together with serum and/or urine, to quantify nicotine and its major metabolite, cotinine, using gas chromatographic methods (Feyerabend & Russell, 1990; Peyton III et al., 1981). A major limitation of these methods was low selectivity of the analytical instrumentation available at the time, making the elimination of chemical interferences impossible. This also led to difficulties in quantifying low levels of nicotine and cotinine resulting from second- or third-hand smoke exposure (also collectively called environmental tobacco smoke (ETS) exposure). In the mid- to late-1990s, the advance of efficient and effective interfaces between

liquid chromatography and mass spectrometry enabled better separation and detection of cotinine and nicotine in biological samples, allowing for the detection of cotinine in serum and saliva resulting from ETS exposure (Bernert et al., 1997).

Since then, saliva has been widely used as the matrix of choice for biological measurement of cotinine to assess exposure to ETS in adults. Because the collection procedure is minimally invasive and relatively easy in comparison to other biological matrices (e.g., blood), saliva has also been explored as a suitable matrix for the analysis of other environmental chemicals (either in the form of metabolites or the original parent compounds) for exposure assessment purposes. However, concentrations of the environmental chemicals in saliva are usually very low and may not be in equilibrium with concentrations in plasma. This is a major factor that limits the application of saliva as a matrix for exposure assessment of environmental chemicals.

15.2 Current Status of Knowledge in Environmental Exposure

Saliva is considered a nontraditional matrix for biomonitoring of most environmental chemicals. As reviewed in Hold, de Boer, and Zuidema (1999), saliva is produced by a number of specialized glands and then discharged into the oral cavity of mammalian vertebrates. The major salivary glands (i.e., parotid, submandibular, and sublingual) are responsible for producing most of the saliva. A small portion of saliva also comes from the small labial, buccal, and palatal glands that line the mouth. In adults, the total volume of saliva produced each day is 1000–1500 mL (Humphrey & Williamson, 2001).

There are three primary mechanisms by which a given biomolecule or small chemical circulating in plasma can enter or be transported into saliva. These mechanisms include passive diffusion, active transport against a concentration gradient, and ultrafiltration through pores in the membrane. Some biomolecules, such as steroid hormones, can pass through the membrane of the glandular epithelial cells via a passive diffusion process because their molecules contain fatty acids, which permits their transcellular diffusion through the lipophilic layer of the epithelial membrane. An active transport mechanism is responsible for the presence of electrolytes, proteins such as immunoglobulin A, as well as some drugs in saliva. For environmental chemicals, the primary transport mechanism is thought to be ultrafiltration. Due to the hydrophilic properties of these chemicals, they are unlikely to pass through to the saliva compartment via passive transcellular diffusion or active transcellular transport. More detailed information on the transport of biomolecules from blood to saliva can be found in the article by Pfaffe, Cooper-White, Beyerlein, Kostner, and Punyadeera (2011). It is possible that the free form of environmental chemicals that pass into the saliva compartment will later bind to proteins that are present in saliva (Fig. 15.1).

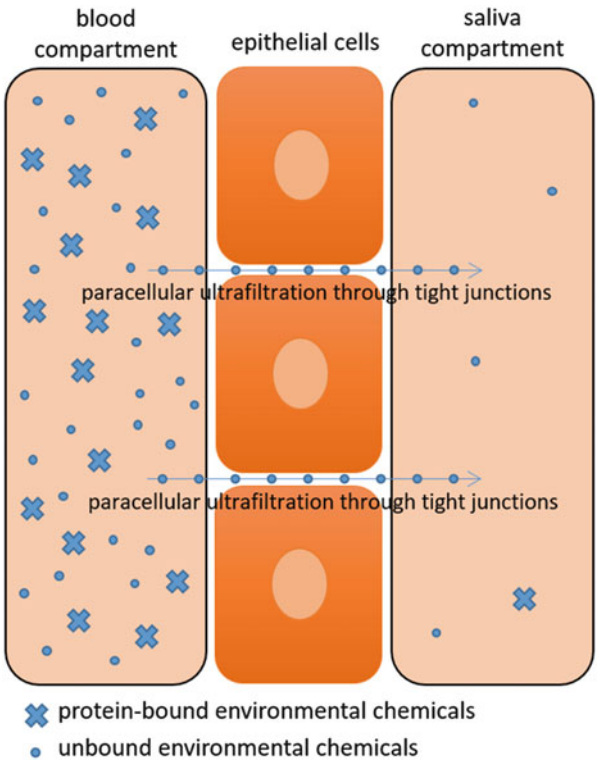


Fig. 15.1 Transport of environmental chemicals from the blood compartment to saliva. Note: Information was extracted from several published articles (Cooper, 2000; Hold et al., 1999; Pfaffe et al., 2011)

In comparison to plasma, saliva contains more water (it is 97–99.5% water) and less protein, with a range of 0.15–0.64 g protein/100 mL saliva (which is less than 1% of the protein found in plasma). The pH of saliva is between 5.3 and 7.8, depending on the level of stimulation. Due to the low protein content, protein-bound molecules are unlikely to be transported into saliva. Therefore, the unbound fraction of any compound is likely to partition into saliva (Hold et al., 1999; Humphrey & Williamson, 2001).

Due to its unique characteristics and the ease by which it is collected, saliva has been explored for its use as a matrix for biomonitoring. So far, saliva is a proven reliable matrix for biomonitoring of exposure to nicotine found in tobacco smoke (Avila-Tang et al., 2013; Demkowska, Polkowska, & Namiesnik, 2011). Other applications of saliva in biomonitoring are for the analysis of some heavy metals, phthalate metabolites, and perchlorate, although these applications still have some limitations.

15.2.1 Analysis of Salivary Cotinine

Nicotine is responsible for tobacco addiction and influences tobacco use patterns (Bruijnzeel, 2012). It is the principal tobacco alkaloid, making up about 1.5% by weight of commercial cigarette tobacco, and comprising about 95% of the total alkaloid content. Nicotine is primarily metabolized by liver enzymes into a number of metabolites (Benowitz, Hukkanen, & Jacob, 2009).

In humans, 70–80% of nicotine is converted to cotinine. This transformation involves the liver enzyme CYP2A6 to produce nicotine-Δ1′ (5′)-iminium ion, which is catalyzed by a cytoplasmic aldehyde oxidase to cotinine. Cotinine is then converted to cotinine glucuronide by uridine 5′-diphospho-glucuronosyltransferase (UGT) enzymes. Other nicotine metabolites detected in smoker urine are *trans*-3′-hydroxycotinine and its glucuronide conjugate. Cotinine is excreted as an unchanged compound in urine to a small degree (10–15% of the nicotine and metabolites in urine). The remainder is converted to metabolites, primarily *trans*-3′-hydroxycotinine (33–40%), cotinine glucuronide (12–17%), and *trans*-3-′-hydroxycotinine glucuronide (7–9%). *Trans*-3′-hydroxycotinine, in its free form, is typically the most detected tobacco-related compound in smokers' urine (Benowitz et al., 2009; Byrd, Chang, Greene, & de Bethizy, 1991). The primary metabolism of nicotine is summarized in Fig. 15.2.

In the urine of smokers, the sum of nicotine, cotinine, *trans*-3′-hydroxycotinine, their respective glucuronide conjugates, and nicotine *N*-oxide accounts for >85% of the consumed nicotine dose. This summation is referred to as total nicotine equivalents, which serves as an excellent biomarker of tobacco exposure (Murphy et al., 2017; Wang, Liang, Mendes, & Sarkar, 2011). A lack of UGT-2B10 enzyme activity (as a result of a high frequency of nonfunctional alleles) responsible for the conversion of cotinine into cotinine glucuronide largely influences the level of cotinine in serum and saliva in an individual. African Americans are more likely to have no active UGT-2B10 enzyme, leading to higher cotinine concentrations in their plasma and lower cotinine glucuronide levels in their urine. This could mediate the toxicological mechanisms and biological outcomes of nicotine exposure. In addition, when comparing cotinine levels in serum or saliva, across different races, genetic polymorphisms should be characterized and taken into account before interpreting the data (Fowler et al., 2015; Murphy et al., 2017).

According to a recent research study using a highly sensitive and selective quadrupole-time of flight tandem mass spectrometer, nicotine, cotinine, and *trans*-3′-hydroxycotinine were found in saliva samples (Carrizo, Nerin, Domeno, Alfaro, & Nerín, 2016). In this study, only the free forms of nicotine, cotinine, and *trans*-3′-hydroxycotinine were found in the salivary fluid. The conjugated forms were not detected in the saliva samples. This may suggest that the conjugated forms of the primary nicotine metabolites are unable to cross into salivary fluid, despite the fact that cotinine glucuronide and *trans*-3′-hydroxycotinine glucuronide were detected in human plasma samples from individuals exposed to tobacco smoke (de Leon et al., 2002). On the other hand, the conjugate forms may partition in saliva before

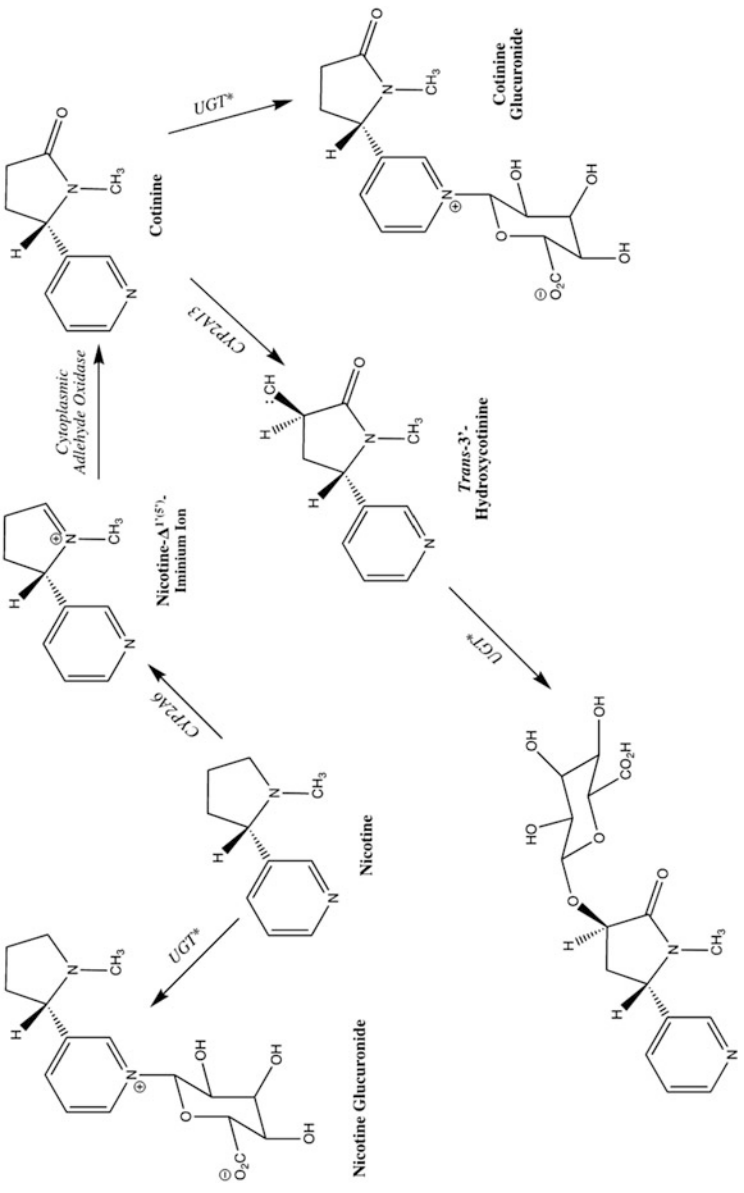


Fig. 15.2 Primary metabolism of nicotine to cotinine, *trans*-3′-hydroxycotinine, and their conjugated metabolites

glucuronidase enzyme breaks the glucuronide bond and liberates the free forms (Chauncey, Lionetti, Winer, &Lisanti, 1954).

Concentrations of cotinine vary across different biological matrices. A comparative study conducted by Wall et al. (1988) found that concentrations of cotinine found in serum, saliva, and urine of active smokers ($\leq$ 10 cigarettes per day) were 78 ng/mL, 66.9 ng/mL, and 673.4 ng/mL, respectively (Wall et al., 1988). Another study, where paired urine–saliva samples from pregnant women who reported not smoking were analyzed for cotinine levels, showed that the geometric mean concentration of cotinine in saliva and urine are similar ($n = 52$, during third trimester, 3.06 ng/mL vs. 3.07 ng/mL, respectively). However, among the smoking pregnant women ($n = 17$, third trimester), the geometric mean concentrations of cotinine in saliva and urine were drastically different (128.45 ng/mL vs. 341.72 ng/mL) (Stragierowicz, Mikołajewska, Zawadzka-Stolarz, Polańska, & Ligocka, 2013).

A study that analyzed paired serum-saliva samples for cotinine levels also indicated that, on average, the concentration of salivary cotinine is approximately 27% higher than the concentration in serum. The difference in concentrations between the two matrices was much more pronounced in ETS-exposed individuals than active smokers. When the blood compartment becomes saturated with cotinine, a portion of the cotinine is transported into the saliva compartment, making the ratio of cotinine in blood versus saliva close to 1:1. However, saliva fluid content is largely dependent on pH and the amount of protein that can bind to cotinine (Bernert Jr, McGuffey, Morrison, & Pirkle, 2000). Overall, these results suggest that salivary cotinine concentrations are closer to those found in serum but much less than those found in urine, particularly for smokers.

A correlation of cotinine concentrations in serum (or plasma), urine, and saliva is of great interest because it suggests that chemical equilibrium can be established across these biological matrices. This equilibrium is important to determine if salivary concentrations can be used as an indicator of exposures to nicotine or to predict exposure magnitudes. According to a study by Bernert Jr et al. (2000), a simple, linear relationship between serum and saliva cotinine concentrations can be seen in an individual exposed to nicotine. This study found that this relationship can be used to reasonably estimate the serum cotinine concentration in an individual given his or her salivary cotinine result (Bernert Jr et al., 2000). Another study concluded that urinary cotinine concentrations (which were not creatinine-adjusted to account for differences in urinary concentration/dilution) were highly correlated with salivary cotinine concentrations (Stragierowicz et al., 2013). The correlation of cotinine concentrations across serum (or plasma), urine, and saliva means that any of these biological matrices can be used to assess nicotine exposures, although the low nicotine metabolite concentrations found in serum or saliva samples from ETS-exposed individuals may limit detection ability, leading to low frequency of detection and low statistical power. A laboratory method that will be used to analyze these samples should have a suitable limit of detection (LOD) or limit of quantification (LOQ) for all matrices.

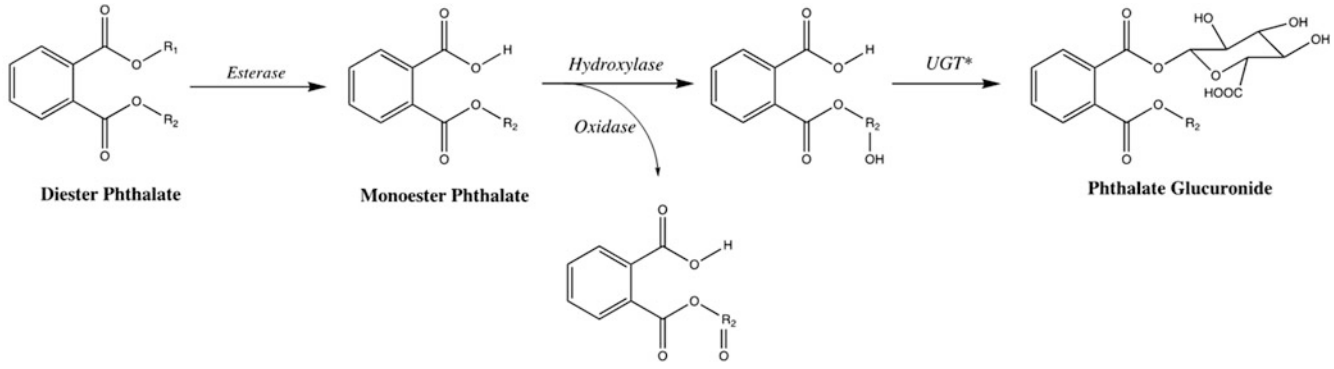
Success in the analysis of salivary cotinine has led to its widespread application in research studies. For instance, salivary cotinine levels were used to evaluate the

association between Spanish smoking legislation and second-hand smoke exposure in a nonsmoking adult cohort in Barcelona, Spain. This study analyzed 397 saliva samples from nonsmokers before and after the implementation of national smoking bans. The results showed a decrease in the frequency of detection of salivary cotinine (from 92.4% to 64.2%) among the study subjects following the implementation of the national smoking bans (LOQ = 0.05 ng/mL). The geometric mean of salivary cotinine concentration significantly decreased from 0.98 ng/mL to 0.12 ng/mL after the implementation of the smoke-free legislation. This decrease was independent of sociodemographic variables (Lidón-Moyano et al., 2017). Another study used salivary cotinine levels to evaluate the impact of ETS exposure during pregnancy on child neurodevelopment within the first 2 years of life. Saliva samples from 461 non-smoking pregnant women were analyzed for cotinine levels. The results indicated that ETS exposures in the first and second trimesters of pregnancy were associated with a decrease in child language function at the age of 1 year and 2 years. A negative association was found for cotinine level in all trimesters of pregnancy and child motor abilities at 2 years of age. This study, using saliva samples, could confirm that ETS exposure during pregnancy could have a negative impact on child psychomotor development within the first 2 years of life (Polanska et al., 2017).

15.2.2 Analysis of Phthalate Metabolites in Saliva

Phthalates, often called plasticizers, are a group of chemicals used to increase the flexibility and durability of plastic or to hold color, shine, or fragrance in various consumer products. They are used in vinyl flooring, adhesives, detergents, lubricating oils, automotive plastics, plastic clothes (raincoats), and personal care products (soaps, shampoos, hair sprays, and nail polishes). Phthalates are key ingredients in plastic packaging film and sheets, household products, children's toys, and medical materials. Once phthalates enter the human body, they are quickly metabolized and excreted in urine (CDC, 2017). Examples of phthalates are di-2-ethylhexyl phthalate (DEHP), dibutyl phthalate (DBP), and diethyl phthalate (DEP). Figure 15.3 summarizes the primary metabolism of phthalates.

Phthalate metabolites have been reportedly detected in saliva samples (Silva et al., 2005). In Silva et al. (2005), salivary concentrations of phthalate metabolites were measured in 39 anonymous adult volunteers using high-performance liquid chromatography–tandem mass spectrometry. Seven out of the 14 measured phthalate metabolites were detected. These include phthalic acid, monomethyl phthalate (MMP), monoethyl phthalate (MEP), mono-n-butyl phthalate (MnBP), monoisobutyl phthalate (MiBP), monobenzyl phthalate (MBzP), and mono-2-ethylhexyl phthalate (MEHP). The detection frequencies ranged from 8% (for MMP) to 85% (for MnBP). The concentrations of these metabolites varied. The highest concentration, 353.6 ng/mL, was observed for MBzP. The frequency of detection and the salivary levels of each phthalate monoester in this study population



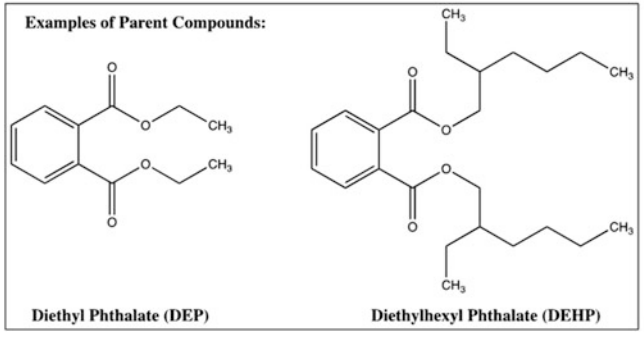


Fig. 15.3 Primary metabolism of phthalates. Note: *UGT∗ = UGT isoenzymes*

were lower than those found in urine for the same monoester in the general US population (Silva et al., 2005).

In the study of Silva et al. (2005), the metabolites detected in saliva samples were non-oxidative metabolites normally formed by hydrolysis enzymes (esterases) in serum, breast milk, and saliva (Calafat, Slakman, Silva, Herbert, & Needham, 2004; Kato et al., 2003; Silva et al., 2005). The levels of these metabolites, for example, MEHP, in these matrices can be a result of DEHP contamination. To prevent this issue, an appropriate post-collection treatment to denature these enzymes is needed (Calafat et al., 2004; Kato et al., 2003).

It is known that the oxidative metabolites are better biomarkers of phthalate exposure, as they are the metabolic products of specific liver enzymes (Choi et al., 2012). Thus, the concentrations of oxidative metabolites are not subject to contamination of the parent compounds (Silva et al., 2006). However, the concentrations of oxidative metabolites, mostly present as glucuronide-bound metabolites, are much lower than the non-oxidative metabolites. In addition, the concentrations of glucuronide-bound, oxidative metabolites in serum are much lower than those found in urine (Kato et al., 2004).

Another study has attempted to investigate the relationships among phthalate metabolites across biological matrices such as breast milk, urine, saliva, and serum collected from 33 US lactating women. Using the gold-standard liquid chromatographic–tandem mass spectrometric method, this study only detected two phthalate metabolites that are oxidative metabolites: mono-(3-carboxypropyl) phthalate (MCPP) (2.2 μg/L) and mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP) (2.3 μg/L). This detection was from a single saliva sample from one woman on one visit. All other phthalate metabolites were not detected (Hines, Calafat, Silva, Mendola, & Fenton, 2009).

The results from both studies (Silva et al., 2005; Hines et al., 2009) are not in agreement in terms of the detection of oxidative metabolites of phthalates. In addition, the detection frequencies for these metabolites vary greatly. No detection of some oxidative metabolites, such as mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), may indicate no transport of these metabolites from blood plasma to saliva fluids (lack of chemical equilibrium), or insufficient sensitivity of the employed method. If chemical equilibrium can only be established for some metabolites between blood plasma and saliva and the likelihood of pre-analytic and analytic contamination is high, saliva will likely be an unsuitable matrix for exposure assessment of phthalates. Besides, for a comprehensive assessment of phthalate exposures, a panel of metabolites must be analyzed altogether (CDC, 2017).

15.2.3 Analysis of Toxic Heavy Metals in Saliva

There is enough scientific evidence to indicate that toxic heavy metals can also be detected in saliva, similarly to electrolytes. A recent study showed that arsenic (As),

chromium (Cr), and lead (Pb) were detected in the saliva of healthy individuals (Dame et al., 2015).

In the study by Bhowmick et al., (2013), 50 saliva samples were collected from male residents of Nadia district in West Bengal, India, where the groundwater is reported to be heavily contaminated with manganese (Mn) and As. In this study, Mn, As, Cr, and Pb were detected in saliva samples with mean concentrations of 5.4 μg/L (range 0.69–22 μg/L), 6.3 μg/L (range 0.70–29 μg/L), 0.78 μg/L (range < LOD-5.9 μg/L), and 0.94 μg/L (range < LOD-4.2 μg/L), respectively. Because the water source was contaminated predominantly by Mn and As, a significant correlation between Mn and As was observed in these saliva samples (Bhowmick et al., 2013).

Wang et al. (2017) reported the concentrations of total As and its species in urine and saliva samples of 70 residents exposed to As from drinking water in Shanxi, China. The result showed that the total As concentration in saliva was relatively lower than in urine samples (mean concentrations, 12.31 μg/L in saliva and 124.93 μg/g in urine). Salivary total As concentrations were positively correlated with total urinary As and drinking water As concentrations. This study also found that in saliva, most As species were not methylated. The major species in saliva was As^{III} and As^{V} (76.18% of total), followed by dimethylarsinate (13.08%) and methylarsonic acid (9.13%). This study suggests that the methylated As species have a limited transfer capability from blood plasma into saliva fluid (Wang et al., 2017).

In an occupational study by Gil et al. (2011), where cadmium (Cd), Cr, Mn, nickel (Ni), and Pb levels in whole blood, urine, and saliva (axillary hair included) from 178 individuals were analyzed, no correlation was found for Mn concentrations across all biological matrices analyzed. Salivary Ni concentrations did not correlate with blood or urinary concentrations. Salivary Cd concentrations did not correlate with blood Cd or urinary Cd concentrations, but significantly correlated with concentrations in hair. Salivary Cr concentrations were only correlated with urinary concentrations, but not with those found in other matrices. Salivary Pb concentrations were inversely, significantly correlated with blood Pb concentrations. No correlation was found between salivary Pb and urinary Pb concentrations. Overall, the results of this study indicate a lack of uniform correlations between salivary metal concentrations and those found in other biological matrices (Gil et al., 2011).

In a study by Nriagu, Burt, Linder, Ismail, and Sohn (2006), a total of 904 pairs of blood and saliva samples were collected and analyzed for Pb levels. The population was composed of low-income adults who lived in Michigan, USA. The average blood Pb and salivary Pb levels were 2.7 μg/dL (or 27 μg/L) and 2.4 μg/L, respectively. The log-transformed Pb concentrations derived from both matrices were statistically correlated, but the regression coefficient was very weak ($r = 0.135$) (Nriagu et al., 2006).

A recent study from Staff et al. (2014) demonstrated the limited use of saliva as an alternative to whole blood for the biomonitoring of inorganic Pb. In their study, 105 pairs of blood and saliva samples were obtained from occupationally exposed workers. For the paired samples, the median whole blood Pb level was 6.00 μg/dL

(or 60 μg/L) and the median salivary Pb level was 17.1 μg/L. Although a significant correlation between blood and salivary Pb levels was observed, it was weak (Pearson's correlation coefficient = 0.457; 95% C.I. 0.291–0.596). The authors concluded that saliva could only be effective as a surrogate for whole blood in highly exposed populations (Staff et al., 2014). This conclusion is in agreement with the article published previously by Koh et al. (2003) that examined the relationship between salivary Pb and whole blood Pb levels in 82 exposed adults. In this article, the authors concluded that the use of salivary Pb for biomonitoring in individuals with whole blood Pb levels ranging from 10 to 50 μg/dL is not recommended.

Moreover, in a study by Barbosa et al. (2006), salivary Pb levels were compared to blood Pb and plasma Pb levels. A total of 88 subjects participated in this study and provided all samples analyzed. Significant, albeit weak, correlations were found between salivary Pb concentrations, blood Pb concentrations, and plasma Pb concentrations, respectively. However, the salivary Pb to plasma Pb ratio was highly variable (from 0.20 to 18.0), suggesting a high degree of variation in Pb concentrations in both matrices, thus suggesting a lack of chemical equilibrium. Hence, it was concluded that salivary Pb may not be used as a biomarker to indicate Pb exposure nor can it be used to predict plasma Pb levels in a low to moderately exposed population (Barbosa et al., 2006).

The study by de Souza Guerra et al. (2015) investigated whole blood, serum, and saliva Pb levels from the same populations (children and adolescents) at two time points (12 months apart) to assess the effectiveness of the mitigation measures undertaken by the Brazilian authorities to reduce Pb exposures. They found no significant correlations between the salivary Pb and whole blood Pb levels as well as the salivary Pb and serum Pb levels in both time points. Note that the numbers of pairs varied from $n = 50$ to $n = 57$ (de Souza Guerra et al., 2015).

The above information, including the results from Costa de Almeida et al. (2010) and Wilhelm et al. (2002), which were not mentioned in detail here, shows that heavy metal concentrations in saliva and other biological matrices are not always correlated. This suggests that, in some cases, equilibrium may not be established across human compartments for some metals. This may complicate the use of saliva as a matrix to assess exposures to those metals.

15.2.4 Analysis of Perchlorate in Saliva

Perchlorate ion is a negatively charged group of atoms consisting of a central chlorine atom bonded to four oxygen atoms. The molecular formula of perchlorate is ClO_4^-. Perchlorates can form naturally in the atmosphere, leading to trace levels of perchlorate in precipitation. Perchlorates are also manufactured in large amounts for its use in rocket fuels, explosives, temporary adhesives, electrolysis baths, batteries, airbags, drying agents, etching agents, cleaning agents and bleach, and oxygen generating systems. Perchlorate has gained public health concern due to its ability to inhibit partially the thyroid's uptake of iodine. It is anticipated that people

exposed to excessive amounts of perchlorate for a long period of time may develop a diminished capacity to produce thyroid hormones (ATSDR, 2008). A developing fetus or infant, whose normal neurodevelopment depends on adequate iodine intake for the production of thyroid hormones, may be affected upon exposure to perchlorate (Leung, Pearce, & Braverman, 2010).

An exploratory study analyzed a subset of 13 paired saliva–serum samples for perchlorate using a liquid chromatographic–tandem mass spectrometric method. The concentrations of perchlorate in serum correlated well with the concentrations of perchlorate in saliva. Although the sample size was low, the mean saliva–serum concentration ratio of perchlorate was 14:1, suggesting that perchlorate concentrations in saliva are generally higher than those in serum. Although salivary perchlorate concentrations are generally lower than those in urine; this research may lead to the use of salivary perchlorate in epidemiological investigations aiming to assess the associations between environmental exposure to perchlorate and health outcomes (Oldi & Kannan, 2009).

15.3 Methodology Issues, Challenges, and Considerations

Several environmental chemicals, to which humans are commonly exposed, can be measured in saliva in order to provide a snapshot of the internal dose at the time of collection. However, for other chemicals, salivary concentrations are too low to be reliably quantified by current analytical instruments, leading to the limited interpretation of the results. This factor prevents the widespread application of saliva in human biomonitoring of environmental chemicals.

Nonetheless, for the successful utilization of saliva in human biomonitoring of environmental chemicals, several analytical issues must be considered.

15.3.1 Sample Collection

Saliva is easier to collect than blood. Like urine, it can be collected multiple times in a given time period, offering an excellent opportunity for longitudinal assessment of exposure levels.

Saliva can be collected via a self-spitting technique or a variety of commercial devices. The spitting technique provides oral fluid that is relatively viscous and non-uniform. The commercial devices provide advantages such as decreased collection time and ease of use. These devices may be used together with stimulators such as citric acid and chewing gum to increase the volume of saliva (Drummer, 2008). However, the use of such collection devices may not be suitable for use with children because of potential choking hazards.

When the flow rate of saliva increases, the chemical composition changes. The pH of saliva also changes which affects the ability of acidic, e.g., citric acid, and

basic chemicals to partition into the oral fluid. Thus, the use of stimulators can control the salivary pH and concentrations of some chemicals in the oral fluid. Devices that contain absorbent pads must be centrifuged to release the fluid from the pad. The collection process is an important element that can affect the concentrations of target chemicals (Drummer, 2008).

Because the buccal cavity can be contaminated by components derived from previous ingestions, it is recommended that saliva is collected a few hours after drinking or eating (Bessonneau, Pawliszyn, & Rappaport, 2017). In addition, the salivary flow rate and concentrations vary according to the circadian clock (Dawes, 1972). As such, collection time is an important factor in determining the concentrations of target chemicals or metabolites in saliva (Dallmann, Viola, Tarokh, Cajochen, & Brown, 2012). It is important that saliva samples are collected at the same time for repeat measurements (Bessonneau et al., 2017).

15.3.2 Extraction Method and Analysis Technique

Usually, exposure to a given environmental chemical results in a low, circulating concentration in plasma. Only a portion of this chemical (whether it is the parent form or metabolite) is transferred into saliva, while the rest is excreted via urine or feces. Another portion may partition in adipose tissues or bind to macromolecules, depending on its lipophilicity (Barr et al., 2005). Even in the case of exposure to nicotine, the concentrations of cotinine found in saliva can be quite low, especially for those of ETS-exposed individuals. Thus, salivary cotinine cannot be used to estimate more precisely the level of exposure to tobacco smoke in nonsmokers (Stragierowicz et al., 2013).

Environmental chemical concentrations in saliva are relatively low, therefore it is crucial that the appropriate extraction technique and analytical instruments are selected to enable the quantification of these chemicals or their metabolites. The majority of chemicals that partition in saliva are highly polar and may not bind to proteins (Hold et al., 1999). To extract these chemicals, solid-phase extraction or liquid–liquid extraction techniques are normally used, with or without prior protein precipitation. Extraction recovery may vary across different chemicals, but the extractants are clean, as they contain fewer chemicals than in urine or blood plasma (Bessonneau et al., 2017).

The analysis of target environmental chemicals in saliva requires the use of highly sensitive and selective techniques such as mass spectrometry (Barr et al., 2005). Mass spectrometry offers unprecedented capabilities over other techniques such as colorimetric analyses or spectrophotometry; it is able to separate and analyze chemicals based on their masses (i.e., mass-to-charge ratio). When mass spectrometry is operated in tandem, by combining at least two mass spectrometric analyzers into one instrument, it is even more sensitive and is able to analyze chemicals at very low concentrations. However, mass spectrometry requires that the target chemicals

be initially separated with liquid or gas chromatography to achieve optimal sensitivity and selectivity during the analysis.

Mass spectrometric methods are subject to certain limitations as well. Matrix effects, especially ion suppression, can lead to low sensitivity of mass analyzers when used with liquid chromatography to analyze polar chemicals. Ion suppression is caused by co-eluting chemicals that reduce the ionization efficiency of target analytes, therefore allowing a lower volume of the target analytes to enter the mass analyzer (Panuwet et al., 2016). As such, the samples that contain fewer chemical components will likely be free from ion suppression. This is true for saliva samples, as they were found to lack interference from matrix effects during the analysis of environmental pollutants (Russo, Barbato, Mita, & Grumetto, 2019). This may facilitate wider use of saliva as a matrix for chemical analyses in the future.

15.3.3 Lack of Reference Materials

It is important to determine the accuracy of any given analytical method used for human biomonitoring, in order to allow for direct comparisons of the results with other studies. This can be achieved via the analysis of standard reference materials of known concentrations such as Standard Reference Materials produced by the National Institutes of Standards and Technology (NIST). Currently, there is no standard reference material available for the determination of environmental chemicals in saliva.

15.4 Future Directions and Opportunities

With the continued advancement of analytical capabilities, biomonitoring will play a critical role in understanding chemical exposures that have personal and public health significance (Dennis et al., 2017). With the emergence of a new concept, the human exposome, the exposure assessment paradigm has shifted. Saliva may play a significant role in this development.

The concept of the human exposome was proposed in 2005 by molecular epidemiologist Dr. Christopher P. Wild, to draw attention to the need to improve environmental exposure assessment to complement the progress made on the human genome characterization effort. In his view, there was a desperate need to develop methods with the same precision for characterizing an individual's environmental exposures, as there was for an individual's genome. Initially, the concept was defined as, "*. . .life-course environmental exposures (including lifestyle factors), from the prenatal period, onwards*" (Wild, 2005). The original definition was strictly centered on exposure assessment. However, a new definition proposed in 2014 defines the exposome as, "*The cumulative measure of environmental influences and associated biological responses throughout the lifespan, including exposures*

from the environment, diet, behavior, and endogenous processes." This definition allows the inclusion of three new concepts: cumulative biological responses, human behaviors, and endogenous processes. All of these components have a profound role in understanding the etiology of disease development (Miller & Jones, 2014). The human exposome concept emphasizes the fact that the sources of toxic chemicals are both exogenous (such as air, water, diet, drugs, and radiation) and endogenous (from inflammation, lipid peroxidation, oxidative stress, existing diseases, infections, and gut flora) (Rappaport, 2011).

Thus, in the era of the human exposome, exposure assessment of environmental chemicals should not only aim to quantify the concentrations of chemicals but also to understand the resulting biological responses. Some of the biological responses may result in a series of changes in endogenous metabolites. By looking at these changes, subtle alterations in biological pathways that underlie various physiological conditions and aberrant processes, including diseases, can be revealed (Johnson, Ivanisevic, & Siuzdak, 2016). The changes in metabolites can be characterized using metabolomics. Metabolomics was developed to enable the comprehensive measurement of all metabolites (both endogenous and exogenous) and low-molecular weight molecules in a biological specimen (Scalbert et al., 2014). Endogenous metabolites are small molecules synthesized by enzymes or our microflora while exogenous metabolites have their sources from the environment, food, drugs, or other consumable products (Wishart et al., 2013). In a comprehensive metabolomics study, advanced analytical platforms are required to uncover a large set of metabolites. These usually include liquid chromatography–mass spectrometry, nuclear magnetic resonance spectroscopy, gas chromatography–mass spectrometry, and inductively coupled plasma mass spectrometry. The sum of metabolites in a given biological matrix or from a particular origin is called the metabolome (Scalbert et al., 2014). Note that the extraction procedure and analytical method of choice largely influence the types of metabolites detected in each measurement (Johnson et al., 2016).

The saliva metabolome has been characterized to provide a solid foundation for future studies that seek to understand the etiology of the disease (Dame et al., 2015; Takeda et al., 2009). The recent characterization of the saliva metabolome was reported by Dame et al. (2015), in which a large set of metabolites was determined using multiple analytical platforms. In their report, there were at least 853 detectable endogenous and exogenous metabolites (corresponding to 1237 probable chemical species) in human saliva. Approximately 300 of them have been quantified (Dame et al., 2015). Although the saliva metabolome has considerable chemical diversity, saliva contains fewer chemicals than urine, which has been confirmed to contain more than 3000 chemicals and serum, which has more than 4000 chemicals (Bouatra et al., 2013; Psychogios et al., 2011; Wishart et al., 2013). In addition, the saliva metabolome may differ according to gender, sample collection strategy (stimulated versus unstimulated), as well as smoking status (Takeda et al., 2009).

Nevertheless, because saliva is likely in equilibrium with whole blood, and both whole blood and saliva temporally represent diverse groups of small molecules in the body, there is great potential for the use of saliva as a biological matrix in studies

of the human exposome. In a recent study, the saliva metabolome was evaluated for its use in exposome-wide association studies, or E-WAS, to link human metabolic pathways to the development of diseases. This study found that the saliva exposome represents at least 14 metabolic pathways, including amino acid metabolism, the citrate cycle, gluconeogenesis, glutathione metabolism, and butanoate metabolism. These metabolic pathways are connected to human metabolic diseases, central nervous system diseases, and neoplasms (Bessonneau et al., 2017). Therefore, saliva may be useful to discover exposure-risk factors for several chronic diseases. This study also emphasized that saliva specimens offer a more practical way to investigate longitudinal individual exposomes due to the simplicity and noninvasiveness of specimen collection and the ability to collect multiple samples from many individuals at any given time.

With the continued improvement of analytical technologies and instrumentation, the ability to detect and quantify low-level metabolites will be achieved. More metabolites or environmental chemicals in saliva will be uncovered in the future. Standardization of biomonitoring methods will permit a direct comparison between studies, allowing exposure levels to be compared across different populations. It is anticipated that saliva will continue to offer a unique advantage in human biomonitoring studies aiming at linking exposures to environmental chemicals to the development of chronic diseases in humans.

References

Angerer, J., Ewers, U., & Wilhelm, M. (2007). Human biomonitoring: State of the art. *International Journal of Hygiene and Environmental Health, 210*(3–4), 201–228.

ATSDR. (2008). *Toxicological profile for perchlorates*. Retrieved Dec 12, 2018, from https://www.atsdr.cdc.gov/phs/phs.asp?id=892&tid=181#top

Avila-Tang, E., Al-Delaimy, W. K., Ashley, D. L., Benowitz, N., Bernert, J. T., Kim, S., . . . Hecht, S. S. (2013). Assessing secondhand smoke using biological markers. *Tobacco Control, 22*, 164–171.

Barbosa, F., Corrêa Rodrigues, M. H., Buzalaf, M. R., Krug, F. J., Gerlach, R. F., & Tanus-Santos, J. E. (2006). Evaluation of the use of salivary lead levels as a surrogate of blood lead or plasma lead levels in lead exposed subjects. *Archives of Toxicology, 80*(10), 633–637.

Barr, D. B., Wang, R. Y., & Needham, L. L. (2005). Biologic monitoring of exposure to environmental chemicals throughout the life stages: Requirements and issues for consideration for the National Children's Study. *Environmental Health Perspectives, 113*(8), 1083–1091.

Benowitz, N. L., Hukkanen, J., & Jacob, P. (2009). Nicotine chemistry, metabolism, kinetics and biomarkers. *Handbook of Experimental Pharmacology, 192*, 29–60.

Bernert, J. T., Jr., McGuffey, J. E., Morrison, M. A., & Pirkle, J. L. (2000). Comparison of serum and salivary cotinine measurements by a sensitive high-performance liquid chromatography-tandem mass spectrometry method as an indicator of exposure to tobacco smoke among smokers and nonsmokers. *Journal of Analytical Toxicology, 24*(5), 333–339.

Bernert, J. T., Turner, W. E., Pirkle, J. L., Sosnoff, C. S., Akins, J. R., Waldrep, M. K., . . . Sampson, E. J. (1997). Development and validation of sensitive method for determination of serum cotinine in smokers and nonsmokers by liquid chromatography/atmospheric pressure ionization tandem mass spectrometry. *Clinical Chemistry, 43*(12), 2281–2291.

Bessonneau, V., Pawliszyn, J., & Rappaport, S. M. (2017). The saliva exposome for monitoring of individuals' health trajectories. *Environmental Health Perspectives, 125*, 1–10.

Bhowmick, S., Halder, D., Kundu, A. K., Saha, D., Iglesias, M., Nriagu, J., … Chatterjee, D. (2013). Is saliva a potential biomarker of arsenic exposure? A case-control study in West Bengal, India. *Environmental Science and Technology, 47*, 3326–3332.

Bouatra, S., Aziat, F., Mandal, R., Guo, A. C., Wilson, M. R., Knox, C., … Wishart, D. S. (2013). The human urine metabolome. *PLoS One, 8*(9), e73076.

Bruijnzeel, A. W. (2012). Tobacco addiction and the dysregulation of brain stress systems. *Neuroscience and Biobehavioral Reviews, 36*, 1418–1441.

Byrd, G. D., Chang, K. M., Greene, J. M., & de Bethizy, J. D. (1991). Evidence for urinary excretion of glucuronide conjugates of nicotine, cotinine, and trans-3′-hydroxycotinine in smokers. *Drug Metabolism and Disposition, 20*(2), 192–197.

Calafat, A. M., Slakman, A. R., Silva, M. J., Herbert, A. R., & Needham, L. L. (2004). Automated solid phase extraction and quantitative analysis of human milk for 13 phthalate metabolites. *Journal of Chromatography B, 805*(1), 49–56.

Carrizo, D., Nerin, I., Domeno, C., Alfaro, P., & Nerín, C. (2016). Direct screening of tobacco indicators in urine and saliva by atmospheric pressure solid analysis probe coupled to quadrupole-time of flight mass spectrometry (ASAP-MS-Q-TOF-). *Journal of Pharmaceutical and Biomedical Analysis, 124*, 149–156.

Chauncey, H. H., Lionetti, F., Winer, R. A., & Lisanti, V. F. (1954). Enzymes of human saliva: I. the determination, distribution, and origin of whole saliva enzymes. *Journal of Dental Research, 33*(3), 321–334.

Choi, K., Joo, H., Campbell, J. L., Clewell, R. A., Andersen, M. E., & Clewell, H. J., 3rd. (2012). In vitro metabolism of di(2-ethylhexyl) phthalate (DEHP) by various tissues and cytochrome P450s of human and rat. *Toxicology In Vitro, 26*(2), 315–322.

Cooper, G. M. (2000). *Transport of small molecules. The cell: A molecular approach* (2nd ed.). Sunderland, MA: Sinauer Associates. www.ncbi.nlm.nih.gov/books/NBK9847/

Costa de Almeida, G. R., de Freitas Tavares, C. F., de Souza, A. M., Sampaio de Sousa, T., Rodrigues Funayama, C. A., Barbosa, F., Jr., … Gerlach, R. F. (2010). Whole blood, serum, and saliva lead concentrations in 6- to 8-year-old children. *The Science of the Total Environment, 408*(1), 1551–1556.

Dallmann, R., Viola, A. U., Tarokh, L., Cajochen, C., & Brown, S. A. (2012). The human circadian metabolome. *Proceedings of the National Academy of Sciences, 109*, 2625–2629.

Damalas, C., & Koutroubas, S. (2016). Farmers' exposure to pesticides: Toxicity types and ways of prevention. *Toxics, 4*(1), 1.

Dame, Z. T., Aziat, F., Mandal, R., Krishnamurthy, R., Bouatra, S., Borzouie, S., … Wishart, D. S. (2015). The human saliva metabolome. *Metabolomics, 11*, 1864–1883.

Dawes, C. (1972). Circadian rhythms in human salivary flow rate and composition. *The Journal of Physiology, 220*, 529–545.

de Leon, J., Diaz, F. J., Rogers, T., Browne, D., Dinsmore, L., Ghosheh, O. H., … Crooks, P. A. (2002). Total cotinine in plasma: A stable biomarker for exposure to tobacco smoke. *Journal of Clinical Psychopharmacology, 22*(5), 496–501.

de Souza Guerra, C., Magna, G. A. M., Machado, S. L., De Capitani, E. M., Ramos, J., Peixoto, I. T., … de Fátima Carvalho, M. (2015). Lead concentrations in whole blood, serum, saliva and house dust in samples collected at two time points (12 months apart) in Santo Amaro, BA, Brazil. *Environmental Research, 142*(1), 337–344.

Demkowska, I., Polkowska, Z., & Namiesnik, J. (2011). Non-invasive biological fluid matrices analysed to assess exposure to environmental tobacco smoke. *Journal of Exposure Science & Environmental Epidemiology, 21*(6), 656–661.

Dennis, K. K., Marder, E., Balshaw, D. M., Cui, Y., Lynes, M. A., Patti, G. J., … Barr, D. B. (2017). Biomonitoring in the era of the exposome. *Environmental Health Perspectives, 125*(4), 502–510.

Drummer, O. H. (2008). Introduction and review of collection techniques and applications of drug testing of oral fluid. *Therapeutic Drug Monitoring, 30*(2), 203–206.

Feyerabend, C., & Russell, M. A. (1990). A rapid gas-liquid chromatographic method for the determination of cotinine and nicotine in biological fluids. *The Journal of Pharmacy and Pharmacology, 42*(6), 450–452.

Fowler, S., Kletzl, H., Finel, M., Manevski, N., Schmid, P., Tuerck, D., . . . Iglesias, V. A. (2015). A UGT2B10 splicing polymorphism common in African populations may greatly increase drug exposure. *The Journal of Pharmacology and Experimental Therapeutics, 352*, 358–367.

Gil, F., Hernández, A. F., Márquez, C., Femia, P., Olmedo, P., López-Guarnido, O., & Pla, A. (2011). Biomonitorization of cadmium, chromium, manganese, nickel and lead in whole blood, urine, axillary hair and saliva in an occupationally exposed population. *The Science of the Total Environment, 409*, 1172–1180.

Greenberg, R. A., Haley, N. J., Etzel, R. A., & Loda, F. A. (1984). Measuring the exposure of infants to tobacco smoke. Nicotine and cotinine in urine and saliva. *The New England Journal of Medicine, 310*(17), 1075–1078.

Hines, E. P., Calafat, A. M., Silva, M. J., Mendola, P., & Fenton, S. E. (2009). Concentrations of phthalate metabolites in milk, urine, saliva, and serum of lactating North Carolina women. *Environmental Health Perspectives, 117*(1), 86–92.

Hold, K., de Boer, D., & Zuidema, J. (1999). Saliva as an analytical tool in toxicology. *International Journal of Drug Testing, 1*, 1–36.

Humphrey, S. P., & Williamson, R. T. (2001). A review of saliva: Normal composition, flow, and function. *The Journal of Prosthetic Dentistry, 85*, 162–169.

International Agency for Research on Cancer. (2012). *Personal habits and indoor combustions*. WHO (100E):541.

Jarvis, M. J., Russell, M. A., Feyerabend, C., Eiser, J. R., Morgan, M., Gammage, P., & Gray, E. M. (1985). Passive exposure to tobacco smoke: Saliva cotinine concentrations in a representative population sample of non-smoking school children. *BMJ, 291*(6500), 927–929.

Johnson, C. H., Ivanisevic, J., & Siuzdak, G. (2016). Metabolomics: Beyond biomarkers and towards mechanisms. *Nature Reviews. Molecular Cell Biology, 17*, 451–459.

Kakkar, P., & Jaffery, F. N. (2005). Biological markers for metal toxicity. *Environmental Toxicology and Pharmacology, 19*(2), 335–349.

Kato, K., Silva, M. J., Brock, J. W., Reidy, J. A., Malek, N. A., Hodge, C. C., . . . Barr, D. B. (2003). Quantitative detection of nine phthalate metabolites in human serum using reversed-phase high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry. *Journal of Analytical Toxicology, 27*(5), 284–289.

Kato, K., Silva, M. J., Reidy, J. A., Hurtz, D., 3rd, Malek, N. A., Needham, L. L., . . . Calafat, A. M. (2004). Mono(2-ethyl-5-hydroxyhexyl) phthalate and mono-(2-ethyl-5-oxohexyl) phthalate as biomarkers for human exposure assessment to di-(2-ethylhexyl) phthalate. *Environmental Health Perspectives, 112*(3), 327–330.

Koh, D., Ng, V., Chua, L. H., Yang, Y., Ong, H., & Chia, S. (2003). Can salivary lead be used for biological monitoring of lead exposed individuals? *Occupational and Environmental Medicine, 60*, 696–698.

Lehmler, H.-J., Liu, B., Gadogbe, M., & Bao, W. (2018). Exposure to bisphenol A, bisphenol F, and bisphenol S in U.S. adults and children: The National Health and Nutrition Examination Survey 2013–2014. *ACS Omega, 3*, 6523–6532.

Leung, A. M., Pearce, E. N., & Braverman, L. E. (2010). Perchlorate, iodine and the thyroid. *Best Practice & Research. Clinical Endocrinology & Metabolism, 24*(1), 133–141.

Lidón-Moyano, C., Fu, M., Perez-Ortuño, R., Ballbè, M., Sampedro-Vida, M., Martín-Sánchez, J. C., . . . Martínez-Sánchez, J. M. (2017). Assessment of salivary cotinine concentration among general non-smokers population: Before and after Spanish smoking legislations. *Cancer Epidemiology, 51*, 87–91.

Miller, G. W., & Jones, D. P. (2014). The nature of nurture: Refining the definition of the exposome. *Toxicological Sciences, 137*(1), 1–2.

Murphy, S. E., Sipe, C. J., & Choi, K., Raddatz, L. M., Koopmeiners, J. S., Donny, E. C. , & Hatsukami, D. K. (2017). Low cotinine glucuronidation results in higher serum and saliva cotinine in African American compared to white smokers. *Cancer Epidemiology, Biomarkers & Prevention, 26*, 1093–1099.

Needham, L. L., Özkaynak, H., Whyatt, R. M., Barr, D. B., Wang, R. Y., Naeher, L., . . . Zartarian, V. (2005). Exposure assessment in the National Children's Study: Introduction. *Environmental Health Perspectives, 113*(8), 1076–1082.

Nriagu, J., Burt, B., Linder, A., Ismail, A., & Sohn, W. (2006). Lead levels in blood and saliva in a low-income population of Detroit, Michigan. *International Journal of Hygiene and Environmental Health, 209*(1), 109–121.

Oldi, J. F., & Kannan, K. (2009). Perchlorate in human blood serum and plasma: Relationship to concentrations in saliva. *Chemosphere, 77*(1), 43–47.

Panuwet, P., Hunter, R. E., D'Souza, P. E., Chen, X., Radford, S. A., Cohen, J. R., . . . Barr, D. B. (2016). Biological matrix effects in quantitative tandem mass spectrometry-based analytical methods: Advancing biomonitoring. *Critical Reviews in Analytical Chemistry, 46*, 93–105.

Peyton, J., III, Wilson, M., & Benowitz, N. L. (1981). Improved gas chromatographic method for the determination of nicotine and cotinine in biologic fluids. *Journal of Chromatography. B, Biomedical Sciences and Applications, 222*(1), 61–70.

Pfaffe, T., Cooper-White, J., Beyerlein, P., Kostner, K., & Punyadeera, C. (2011). Diagnostic potential of saliva: Current state and future applications. *Clinical Chemistry, 57*, 675–687.

Pirkle, J. L., Needham, L. L., & Sexton, K. (1995). Improving exposure assessment by monitoring human tissues for toxic chemicals. *Journal of Exposure Analysis and Environmental Epidemiology, 5*(3), 405–424.

Polanska, K., Krol, A., Merecz-Kot, D., Ligocka, D., Mikolajewska, K., Mirabella, F., . . . Hanke, W. (2017). Environmental tobacco smoke exposure during pregnancy and child neurodevelopment. *International Journal of Environmental Research and Public Health, 14* (7), 796.

Psychogios, N., Hau, D. D., Peng, J., Guo, A. C., Mandal, R., Bouatra, S., . . . Wishart, D. S. (2011). The human serum metabolome. *PLoS One, 6*(2), e16957.

Rappaport, S. M. (2011). Implications of the exposome for exposure science. *Journal of Exposure Science & Environmental Epidemiology, 21*(1), 5–9.

Russo, G., Barbato, F., Mita, D. G., & Grumetto, L. (2019). Simultaneous determination of fifteen multiclass organic pollutants in human saliva and serum by liquid chromatography-tandem ultraviolet/fluorescence detection: A validated method. *Biomedical Chromatography, 33*, e4427.

Scalbert, A., Brennan, L., Manach, C., Andres-Lacueva, C., Dragsted, L. O., Draper, J., . . . Wishart, D. S. (2014). The food metabolome: A window over dietary exposure. *The American Journal of Clinical Nutrition, 99*, 1286–1308.

Sexton, K., Callahan, M. A., & Bryan, E. F. (1995). Estimating exposure and dose to characterize health risks: The role of human issue monitoring in exposure assessment. *Environmental Health Perspectives, 103*, 17.

Silva, M. J., Reidy, J. A., Preau, J. L., Samandar, E., Needham, L. L., & Calafat, A. M. (2006). Measurement of eight urinary metabolites of di(2-ethylhexyl) phthalate as biomarkers for human exposure assessment. *Biomarkers, 11*(1), 1–13.

Silva, M. J., Reidy, J. A., Samandar, E., Herbert, A. R., Needham, L. L., & Calafat, A. M. (2005). Detection of phthalate metabolites in human saliva. *Archives of Toxicology, 79*, 647–652.

Staff, J. F., Harding, A.-H., Morton, J., Jones, K., Guice, E. A., & McCormick, T. (2014). Investigation of saliva as an alternative matrix to blood for the biological monitoring of inorganic lead. *Toxicology Letters, 231*(1), 270–276.

Stragierowicz, J., Mikołajewska, K., Zawadzka-Stolarz, M., Polańska, K., & Ligocka, D. (2013). Estimation of cutoff values of cotinine in urine and saliva for pregnant women in Poland. *BioMed Research International, 1*, 1–11.

Takeda, I., Stretch, C., Barnaby, P., Bhatnager, K., Rankin, K., Fu, H., ... Slupsky, C. (2009). Understanding the human salivary metabolome. *NMR in Biomedicine, 22*, 577–584.

U.S. Centers for Disease Control and Prevention. (2017). *Environmental chemicals*. Retrieved Dec 12, 2018 from https://www.cdc.gov/biomonitoring/environmental_chemicals.html

Wall, M. A., Johnson, J., Jacob, P., & Benowitz, N. L. (1988). Cotinine in the serum, saliva, and urine of nonsmokers, passive smokers, and active smokers. *American Journal of Public Health, 78*(6), 699–701.

Wallace, A. D. (2012). Toxic endpoints in the study of human exposure to environmental chemicals. *Progress in Molecular Biology and Translational Science*, 89–115.

Wang, J., Liang, Q., Mendes, P., & Sarkar, M. (2011). Is 24h nicotine equivalents a surrogate for smoke exposure based on its relationship with other biomarkers of exposure? *Biomarkers, 16* (2), 144–154.

Wang, D., Shimoda, Y., Wang, S., Wang, Z., Liu, J., Liu, X., ... An, Y. (2017). Total arsenic and speciation analysis of saliva and urine samples from individuals living in a chronic arsenicosis area in China. *Environmental Health and Preventive Medicine, 22*(45), 1–9.

Wild, C. P. (2005). Complementing the genome with an "Exposome": The outstanding challenge of environmental exposure measurement in molecular epidemiology. *Cancer Epidemiology, Biomarkers & Prevention, 14*(8), 1847–1850.

Wilhelm, M., Pesch, A., Rostek, U., Begerow, J., Schmitz, N., Idel, H., & Ranft, U. (2002). Concentrations of lead in blood, hair and saliva of German children living in three different areas of traffic density. *The Science of the Total Environment, 297*, 109–118.

Wishart, D. S., Jewison, T., Guo, A. C., Wilson, M., Knox, C., Liu, Y., ... Scalbert, A. (2013). HMDB 3.0—The human metabolome database in 2013. *Nucleic Acids Research, 41*, D801–D807.