Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

HHS Public Access

Author manuscript

Environ Res. Author manuscript; available in PMC 2016 October 01.

Published in final edited form as:

Environ Res. 2015 October ; 142: 387–406. doi:10.1016/j.envres.2015.06.032.

Tooth Matrix Analysis for Biomonitoring of Organic Chemical Exposure: Current Status, Challenges, and Opportunities

Syam S. Andra[1,*], **Christine Austin**[1,2], and **Manish Arora**[1,2,*]

[1]Exposure Biology, Lautenberg Environmental Health Sciences Laboratory, Department of Preventive Medicine, Icahn School of Medicine at Mount Sinai, New York, NY, USA

[2]Faculty of Dentistry, University of Sydney, Sydney, NSW, Australia

Abstract

Epidemiological evidence supports associations between prenatal exposure to environmental organic chemicals and childhood health impairments. Unlike the common choice of biological matrices such as urine and blood that can be limited by short half-lives for some chemicals, teeth provide a stable repository for chemicals with half-life in the order of decades. Given the potential of the tooth bio-matrix to study long-term exposures to environmental organic chemicals in human biomonitoring programs, it is important to be aware of possible pitfalls and potential opportunities to improve on the current analytical method for tooth organics analysis. We critically review previous results of studies of this topic. The major drawbacks and challenges in currently practiced concepts and analytical methods in utilizing tooth bio-matrix are (i) no consideration of external (from outer surface) or internal contamination (from micro odontoblast processes), (ii) the misleading assumption that whole ground teeth represent prenatal exposures (latest formed dentine is lipid rich and therefore would absorb and accumulate more organic chemicals), (iii) reverse causality in exposure assessment due to whole ground teeth, and (iv) teeth are a precious bio-matrix and grinding them raises ethical concerns about appropriate use of a very limited resource in exposure biology and epidemiology studies. These can be overcome by addressing the important limitations and possible improvements with the analytical approach associated at each of the following steps (i) tooth sample preparation to retain exposure timing, (ii) organics extraction and pre-concentration to detect ultra-trace levels of analytes, (iii) chromatography separation, (iv) mass spectrometric detection to detect multi-class organics simultaneously, and (v) method validation, especially to exclude chance findings. To highlight the proposed improvements we present findings from a pilot study that utilizes tooth matrix biomarkers to obtain trimester-specific exposure information for a range of organic chemicals.

[*]Correspondence to: Syam S. Andra (Instructor) and Manish Arora (Associate Professor), Icahn School of Medicine at Mount Sinai, Preventive Medicine Department, One Gustave L. Levy Place, Box 1057, New York, NY 10029, USA. syam.andra@mssm.edu and manish.arora@mssm.edu.

Competing financial interests: The authors declare no competing financial interests.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Keywords

Biomarker; Chromatography; Dentine; Mass spectrometry; Organics; Prenatal; Teeth

1. Introduction: Tooth analysis for the assessment of prenatal exposure to environmental chemicals

Perinatal exposures to environmental chemicals have been linked to a multitude of health outcomes, including impaired neurodevelopment (Bellinger, 2013) and metabolic syndrome (Behl et al., 2013; La Merrill and Birnbaum, 2011; Wang et al., 2014) in children. Exposure to environmental chemicals in utero has gained much attention because of the heightened susceptibility of developing organs, particularly the brain (Adams et al., 2000; Grandjean and Landrigan, 2006; Grandjean and Landrigan, 2014; Rice and Barone, 2000). The concept of 'critical windows of susceptibility' supports the rationale that disruption of developmental processes during early life would change the trajectory of long-term health status (Cory-Slechta et al., 2008; Grandjean and Landrigan, 2006; Grandjean and Landrigan, 2014). The fetal organs are highly susceptible to environmental chemical exposures because of (i) greater absorption of chemicals due to immature chemical transport mechanisms, (ii) underdeveloped biological mechanisms to detoxify chemicals, (iii) greater exposure due to greater intake (pound for pound) compared to adults, and (iv) heightened susceptibility to even small amounts of chemicals which alters subsequent growth and development (Grandjean and Landrigan, 2006; Grandjean and Landrigan, 2014; Landrigan et al., 2004).

This review will focus on risk associated with early life exposure to organic toxicants and we direct the reader to several other reviews for information on risks associated with metal exposures (e.g. arsenic, cadmium, and manganese: (Rodriguez-Barranco et al., 2013); lead: (Guilarte et al., 2012); mercury: (Gundacker et al., 2010; Yoshimasu et al., 2014). The risks from prenatal and early childhood exposure are particularly significant for certain organic toxicants including polybrominated diphenyl ethers (PBDEs), environmental tobacco smoke, and organophosphate (OP) and organochlorine (OC) pesticides. PBDEs can be transferred to the developing fetus across the placenta and to children via breast milk and fat-containing food (Lorber, 2008). A prior U.S. study has shown almost identical PBDE concentrations in maternal blood collected at delivery and in cord blood, suggesting that the placental barrier offers limited protection to the fetus (Mazdai et al., 2003). Neurodevelopment outcomes in children from prenatal exposures to environmental organics are a widely studied research topic. Example studies are for (i) PBDE: (Chen et al., 2014; Eskenazi et al., 2013; Gascon et al., 2011; Herbstman JB, 2010); (ii) bisphenol A: (Braun et al., 2009; Harley et al., 2013; Perera et al., 2012; Yolton et al., 2011); and (iii) phthalates: (Kobrosly et al., 2014; Polanska et al., 2014; Tellez-Rojo et al., 2013; Yolton et al., 2011).

Similar to PBDEs, OC pesticides such as dichlorodiphenyl trichloroethane (DDT) and its metabolites are higher in fetal circulation than maternal matrices. For example, a study of 90 mother/infant pairs from Mexico with no known occupational exposure found that in most cases the concentrations of the pesticide residues in cord blood were higher than maternal blood. Of the OCs detected, p,p′-dichlorodiphenyl dichloroethylene was the most

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

concentrated (lipid-adjusted means, 4.4 ppm maternal; 4.7 ppm cord blood), followed by p,p′-DDT (1.8 ppm maternal, 2.8 ppm cord blood), and o,p′-DDT (0.30 ppm maternal, 0.35 ppm cord blood) (Waliszewski et al., 2001). Studies have shown that OP pesticides readily cross the placental barrier to reach the fetus (Rauh et al., 2006; Whyatt et al., 2009) and dialkyl phosphate metabolites have been detected in amniotic fluid (Bradman et al., 2003). Emerging evidence suggests that prenatal, but not postnatal OP exposure is associated with Attention Deficit Hyperactivity Disorder (Eskenazi et al., 2007; Marks et al., 2010) and impaired cognitive development (Bouchard et al., 2011; Harari et al., 2010; Rauh et al., 2006). Impacts on neurodevelopment in children from prenatal exposures to pesticides are widely recognized and reviewed elsewhere (Ding and Bao, 2014; Muñoz-Quezada MT, 2013; Polanska et al., 2013). However, direct fetal measurements of the intensity and timing of intrauterine exposure have not been undertaken for OP and OC pesticides, primarily due to the absence of a direct fetal biomarker of environmental chemical exposure.

Tobacco smoke contains many toxicants and one recent study found higher levels of tobacco smoke markers (cotinine and nicotine) in the first urine of newborns of actively smoking mothers than offspring of non-smokers (Florek et al., 2011). Potential health impacts in infants and children from their mothers smoking during pregnancy are multifarious (a critical review by (Bruin et al., 2010)) and examples are (i) low birth weight (Ricketts et al., 2005) or childhood overweight (Moller et al., 2014), (ii) stillbirth risk and congenital malformation (meta-analysis report by (Leonardi-Bee et al., 2011)), (iii) wheezing and asthma incidence (meta-analysis study by (Burke et al., 2012)) and (iv) neurodevelopment impairment (reviewed by (Herrmann et al., 2008)). Prenatal exposure assessment is one of the greatest challenges in developmental epidemiologic studies. This is limited by (i) the lack of biomarkers that directly measure fetal (vs maternal) exposure at specific intrauterine developmental periods; (ii) the inability to objectively reconstruct past exposure at specific life stages outside the use of questionnaires; and (iii) the expense and time needed to conduct prospective studies of prenatal exposure and health. No contemporary biomarker such as chemicals measured in cord blood, placenta, and maternal markers during pregnancy, or infant samples can provide a direct measure of exposure timing at different times of intrauterine development in large epidemiologic studies. To address these issues studies have attempted to use primary teeth to provide a direct measure of the timing and intensity of exposure to metals from approximately the 14th gestational week to birth and into early childhood (e.g. lead (Arora et al., 2014; Gulson et al., 1997; Needleman et al., 1974; Needleman HL, 1974; Rabinowitz et al., 1976; Uryu et al., 2003), manganese (Arora et al., 2012; Arora et al., 2011; Ericson et al., 2001; Gunier et al., 2013), and barium and strontium (Austin et al., 2013; Humphrey et al., 2008).

Teeth are uniquely positioned to capture childhood chemical exposures in continuum starting from early (second trimester in utero) to progressive life stages (until deciduous teeth are shed around 6–12 year’s age). Unlike the common choice of bio-matrices such as urine and blood that can be limited by short half-lives for some chemicals, exposure signals in teeth remain stable enabling a longer detection window (Gulson et al., 1997). Although hair also provides longer detection windows, it is limited to a monthly time scale (Gareri and Koren, 2010; Hinners et al., 2012). The human tooth as a bio-matrix of past exposures to metals was detailed in a recent review, which also discussed tooth components and tooth

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

development process (Arora and Austin, 2013). In brief, enamel and dentine are the primary mineralized components of a tooth crown that differ significantly in their mineralization pattern. This characteristic determines their usefulness in capturing and measuring chemical exposures.

Previous applications of tooth-chemical concentrations in epidemiologic studies did not provide detailed temporal (i.e. weekly, monthly or trimester-specific) information on prenatal and early childhood exposure to metals. In early studies, whole teeth were digested and metal toxicants concentrations reported on a whole-tooth basis [reviewed by (Arora and Austin, 2013)]. Although teeth have been used for exposure assessment to inorganic chemicals since the 1960s (Altshuller et al., 1962; Arora and Austin, 2013; Needleman et al., 1974; Needleman and Shapiro, 1974), the use of teeth for assessing exposures to organic chemicals is in the early 2000s (Table 1). Moreover, the use of tooth chemical biomarkers for detailed assessment of exposure timing is a relatively recent development, unlike the use of other conventional biomarkers in blood or urine that have a long tradition. These studies cannot give exposure information on a finely resolved temporal scale for organic chemicals, which prevent the study of critical windows of vulnerability. Grinding whole teeth or utilizing large fragments of teeth ignores the complex developmental physiology and microstructure of teeth and can lead to gross exposure misclassification, as has been shown in an important study by Rabinowitz and colleagues (Rabinowitz et al., 1993), which will be discussed in Section 3. The goal of this review is to present a comprehensive background on the current state-of-the-art of tooth organics analysis, its limitations, challenges and future opportunities. We discuss the different parameters affecting each of the following steps: (i) tooth collection, (ii) decontamination and pre-treatment, (iii) analytes extraction, clean-up and pre-concentration, and (iv) separation, detection and quantification using chromatography and mass spectrometry tools. We summarize the novel application of tooth bio-matrix analysis for reconstructing prenatal exposures to environmental chemicals and the practical applications. The review concludes with emphasizing the need to understand which chemical classes may be affecting specific critical windows of development related to childhood health disorders so that the source of exposure may be identified and thereby provide a means of intervention to reduce exposure and the consequent health effects.

2. Literature review: Methodological aspects of organic chemical analysis in teeth

Peer-reviewed publications on organic chemicals analysis in teeth were identified from a comprehensive search in Scopus (1960 onwards). Keywords for the search were ((teeth OR tooth) AND (chromato*) OR (“mass spec*”)). Resulting articles were assessed for inclusion by reading either the abstract or full text or both. Further, the cited bibliography in each of these articles of interest was screened to obtain relevant back referenced citations for inclusion. From the detailed search, we found 14 articles that measured organic chemicals in tooth bio-matrix. In Table 1, we summarize these studies, providing key details of exposure source, detection rate, and concentrations of the organic chemicals detected in teeth (Camann et al., 2013; Cattaneo et al., 2003; Fos P, 2011; Garcia-Algar et al., 2003; Jan et al., 2006; Jan et al., 2013; Jan and Vrbic, 2000; Jan et al., 2001; Kanjanawattana et al., 2001;

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Marchei et al., 2008; Pascual et al., 2003; Pellegrini et al., 2006; Schussl et al., 2014; Zeren et al., 2013). These studies ranged from (i) intentional drug abuse (Cattaneo et al., 2003; Pellegrini et al., 2006), (ii) environmental exposures (Garcia-Algar et al., 2003; Pascual et al., 2003), (iii) clinical administration (Fos P, 2011; Schussl et al., 2014) and (iv) personal habits of alcohol consumption (Zeren et al., 2013). These studies have used teeth from human children (Camann et al., 2013; Garcia-Algar et al., 2003; Jan and Vrbic, 2000; Marchei et al., 2008; Pascual et al., 2003), adults (Fos P, 2011; Schussl et al., 2014; Zeren et al., 2013) and deceased people (Cattaneo et al., 2003), as well as from animals (Jan et al., 2006; Jan et al., 2013; Jan et al., 2001; Kanjanawattana et al., 2001) (Table 1). The tooth bio-matrix has been successfully analyzed for the presence of numerous organics classes e.g. organochlorines (Jan et al., 2006; Jan et al., 2013; Jan and Vrbic, 2000; Jan et al., 2001), anesthetics (Kanjanawattana et al., 2001), illegal drugs (Kanjanawattana et al., 2001; Pellegrini et al., 2006), tobacco (Marchei et al., 2008; Pascual et al., 2003; Pichini et al., 1997), analgesics, pesticides and plastics additives (Camann et al., 2013), alcohol (Zeren et al., 2013), and antibiotics (Fos P, 2011; Schussl et al., 2014) (Table 1). Reported methodologies for extraction and analysis of organic chemicals in teeth are complex. In the following sub-sections, we review the analytical approaches used at each stage of sample analysis as reported in previous studies.

2.1. Tooth collection

For deciduous/baby teeth, collection typically occurred upon natural shedding (Camann et al., 2013; Garcia-Algar et al., 2003; Marchei et al., 2008; Pascual et al., 2003), while in other studies teeth were extracted because of dental disease (Jan and Vrbic, 2000; Pellegrini et al., 2006; Schussl et al., 2014) or for the purpose of the study in animals (Fos P, 2011; Jan et al., 2006; Jan et al., 2013; Jan et al., 2001; Kanjanawattana et al., 2001; Zeren et al., 2013), and in certain cases after death (Cattaneo et al., 2003) (Table 2). Benefits of collecting naturally shed teeth include non-invasive bio-matrix collection and these teeth cover the prenatal period whereas only the first molar in permanent teeth start forming before or near birth. The amount of sample available from teeth extracted in response to dental health issues is reduced due to the need to restrict sampling to sound areas of the tooth. Moreover, teeth can be stored at room temperature for long periods of time, unlike blood and urine. Although the methods for storage of collected teeth lack clarity in the literature, it appears that they were stored under dry conditions with no additional precautions. Organics were detected in teeth stored for long periods of time. For example, morphine and codeine were detected in teeth from cadavers with different post-mortem intervals and different conditions of preservation, with the longest postmortem interval being 2 years (Cattaneo et al., 2003). Similarly, acetaminophen was quantified in shed molars stored for greater than 17 years (Camann et al., 2013). However, no systematic studies have been conducted on the storage stability of multiple classes of organic chemicals in either intact or spiked tooth matrix.

2.2. Tooth pre-analysis preparation: decontamination and pulverization

Prior to analysis teeth samples were usually cleaned of residual blood with cleaning solutions (e.g. Pellegrini et al (Pellegrini et al., 2006)) and saliva with sterile swabs (e.g. Schussl et al (Schussl et al., 2014)). Tooth surface decontamination consisted of either a single step of cleaning with dichloromethane (Camann et al., 2013; Garcia-Algar et al.,

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

2003; Marchei et al., 2008; Pascual et al., 2003) or a sequence with hypochlorite, saline and distilled water solutions (Pellegrini et al., 2006). Adherent tissue was also removed with scalpels and bone curettes by Schussl et al. (Schussl et al., 2014). Wash solutions obtained from tooth decontamination procedures were typically free of organic analytes (Camann et al., 2013) or negligible (Pascual et al., 2003), indicating an absence of external surface contamination. However, (i) not all the studies performed a decontamination step and analyzed the obtained wash fraction and (ii) no comparative analysis was performed between decontamination with protic solvents such as methanol or phosphate buffer versus non-protic solvents such as acetone or dichloromethane. Protic solvents might extract or leach the incorporated organics in tooth during washing. Further research is required to assess the use of wash solutions. Moreover, internal contamination from odonobalstic processes within a tooth matrix was never addressed before.

Teeth are usually cut and pulverized to powder, which is probably the most widely used pre-analysis step (all studies in literature shown in Table 2). Pulverization was carried out with a high energy mini bead-beater (Zeren et al., 2013) or a ceramic mill (Cattaneo et al., 2003), ball mill (Garcia-Algar et al., 2003; Marchei et al., 2008; Pascual et al., 2003; Pellegrini et al., 2006), bone mill (Schussl et al., 2014), or pestle (Jan et al., 2006; Jan et al., 2013; Jan and Vrbic, 2000; Jan et al., 2001). Powdered tooth samples were dried over phosphorus pentoxide by a particular research group (Jan et al., 2006; Jan et al., 2013; Jan et al., 2001). While the common practice was to store the pulverized sample at room temperature, teeth were also reported to be frozen with liquid nitrogen and stored at −80°C either prior to sample preparation (Fos P, 2011; Kanjanawattana et al., 2001) or after pulverization (Schussl et al., 2014). Pulverized tooth sample mass obtained for organics analysis reported in literature was in the range from 15 mg (Kanjanawattana et al., 2001) to 2–4 g (Jan et al., 2006; Jan et al., 2013; Jan and Vrbic, 2000; Jan et al., 2001) (sample weights from the other studies are presented in Table 2). The typical mass ranges for human deciduous teeth were about 50 mg.

Extraction of organic chemicals has been exclusively performed on pulverized whole teeth. Several types of equipment were used for tooth homogenization. However, it appears that no study has looked into the loss of organics analytes sorbed onto the surfaces of material and media used for tooth pulverization. It is also unclear whether the current studies monitored degradation or modifications of organics due to heat generated from tooth pulverization. It is suggested to pulverize teeth samples in liquid nitrogen or cryogenic grinding mills instead of mortar and pestle under ambient conditions to reduce the risk of degradation of the organics.

2.3. Tooth organics extraction, clean-up and pre-concentration

Considering the complex structure and composition of the tooth matrix, tooth analysis generally requires an incubation step followed by sample extraction, and evaporation and reconstitution of the extract for analysis. Extraction of organics from teeth often require time and labor intensive protocols that involve incubation of samples in specific solutions for varying periods followed by clean-up procedures involving solid-phase extraction, liquid-liquid extraction or both. Studies reported the length of sample extraction taking from few minutes to half a day. Of the few studies that used an internal standard, structural analogs

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

(Garcia-Algar et al., 2003; Kanjanawattana et al., 2001; Marchei et al., 2008; Pascual et al., 2003; Pellegrini et al., 2006) or deuterated forms (Camann et al., 2013; Cattaneo et al., 2003; Zeren et al., 2013) of the study chemicals were usually spiked at a known concentration prior to tooth sample preparation to calculate percent extraction recovery (Table 2). Depending on the organic chemical of interest, researchers incubated either in acidic or basic solutions and either at ambient or specific temperature with or without ultra-sonication. Acidification of powdered tooth for incubation was achieved by using sulfuric acid and hexane (Jan et al., 2006; Jan et al., 2013; Jan and Vrbic, 2000; Jan et al., 2001) or hydrochloric acid (Cattaneo et al., 2003; Pellegrini et al., 2006). Incubation of tooth powder in an alkaline media was by using sodium hydroxide (Garcia-Algar et al., 2003; Kanjanawattana et al., 2001; Marchei et al., 2008; Pascual et al., 2003). A combination of solutions consisted of acetonitrile with glacial acetic acid and ammonium hydroxide were used in a sequence for incubating powdered whole tooth to extract multiple classes of organics that required acidic, neutral and alkaline pH for extraction (Camann et al., 2013). Almost all the studies reported an extraction protocol unique for a particular class of organic analytes in their respective study. More details on the composition and proportion of incubation mixtures, and temperature and duration of incubation are presented in Table 2. However, because each group studied a different set of organics, there is a lack of method optimization for (i) extract combination and concentration, and (ii) extraction duration. Studies have not explored whether extraction equilibrium was reached between the tooth powder sample and the incubation media. It is unclear whether a particular organic analyte was extracted to the maximum possible extent from the tooth powder. The stability of the organics was also not determined during the incubation period. Hence, it is essential to study incubation time and temperature versus analyte recovery for each organic analyte of interest.

Deposition of organics in dental tissues is driven by (i) their lipophilic properties which favors accumulation in high lipid tissues depending on their Octanol-Water Partition Coefficient (K_{ow}) (Bertelsen et al., 1998), (ii) sorption onto organic biomolecules with diol and amino groups such as sugars and aminoacids, depending on Carbon-Water Partitioning Coefficient (K_{oc}) (Müller, 1994), and (iii) the diffusion coefficient into hard tissues (Hackenberg R, 2003). Jan et al (2013) evaluated for the first time how physicochemical properties of certain organochlorines and tissue specific characteristics of pulp, dentine and enamel determine their accumulation in lamb's primary dental tissue. Hence, the simultaneous or sequential combination of multiple solvents is essential to extract organic analytes with different solubility and situated in different tooth components. The most popular extraction method for pre-concentration of analytes in the incubated powdered tooth solution is liquid-liquid extraction (LLE) (Camann et al., 2013; Cattaneo et al., 2003; Garcia-Algar et al., 2003; Kanjanawattana et al., 2001; Pascual et al., 2003; Pellegrini et al., 2006; Zeren et al., 2013) followed by solid-phase extraction (SPE) (Marchei et al., 2008; Schussl et al., 2014) or a method similar to SPE that uses Florisil material to retain organics (Camann et al., 2013; Jan et al., 2006; Jan et al., 2013; Jan and Vrbic, 2000; Jan et al., 2001). The choice of tooth sample clean-up for analyte pre-concentration steps was associated with the choice of separation and detection instrumentation used in each study (reviewed in the section below).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

LLE was performed with (i) tert-butyl methyl ether for lidocaine (Kanjanawattana et al., 2001), (ii) a mixture of chloroform, isopropanol and n-heptane for morphine and codeine (Cattaneo et al., 2003), (iii) a mixture of chloroform and isopropanol for opiates and cocaine (Pellegrini et al., 2006), (iv) a mixture of acetonitrile and water for ethyl glucuronide (Zeren et al., 2013), (v) a two-stage process consisting of (a) extraction with dichloromethane and treating the resulted organic fraction with hydrochloric acid and (b) collecting the aqueous fraction from the previous step, mixing with sodium hydroxide and extracting with dichloromethane for nicotine and cotinine (Garcia-Algar et al., 2003; Pascual et al., 2003), and (vi) a three-stage process using acetonitrile, glacial acetic acid and ammonium hydroxide for extracting acetaminophen, 4-aminophenol, anandamide, ibuprofen, trichloro-2-pyridinol, isopropyl-6-methyl-4-pyrimidinol, organophosphate, pyrethroid and cyfluthrin insecticide metabolites, and oxidative-metabolites of mono-2-ethylhexyl phthalate (Camann et al., 2013). SPE columns used for pre-concentrating tooth organics were (i) isolute HCX, a mixed-mode sorbent for nicotine and cotinine (Marchei et al., 2008) and (ii) Oasis HLB, a reversed-phase sorbent for amoxicillin and clindamycin (Schussl et al., 2014). Similar column-based tooth clean-up consisting of silica and Florisil sorbents was used for extracting polychlorinated biphenyls (Jan and Vrbic, 2000) and hexachlorobenzene and dichlorodiphenyl dichloroethylene (Jan et al., 2006; Jan et al., 2013; Jan et al., 2001), and organochlorine, organophosphate and pyrethroid pesticides (Camann et al., 2013). Clarithromycin extraction details are unavailable from a study by Fos et al (Fos P, 2011). Further information on the materials, solvents and solutions used for powdered tooth clean-up, and evaporation and reconstitution of the extracts are detailed in Table 2.

Metabolic stability (K_{metab}) of organic chemicals also determines their levels in dental tissues (Jan et al., 2013). For example, metabolically stable organochlorines such as PCB-169 and hexachlorobenzene tend to enrich in dental tissues (Jan et al., 2013) because of their ability to resist metabolic breakdown in blood (Nanci, 2008). This is not true for PCB-155 that degrades rapidly in blood circulation and hence lower levels were detected in dental pulp, compared to enamel where metabolic degradation is slow (Jan et al., 2013). Pulp is rich in blood vessels and was included in homogenization of the tooth sample in certain studies. This presents a major problem as chemicals and contaminants levels in the pulp will be much higher than those archived in the mineralizing tissue. Whole teeth that include the pulp may therefore only represent exposure at the time of collection, not cumulative exposure. The odontoblast processes that run from the pulp all through the dentin, transfer nutrients and chemicals and is also pulverized in the tooth sample used for organics analysis (Camann et al., 2013). This obscures whether the organics signal originated from the hard tissues or the intertwined cell processes. Hence, the challenge is to differentiate the chemical signatures repositories that are specific to timing of chemical exposure versus time of sample collection.

2.4. Tooth organics separation

Most of the published works on tooth analysis focused on one or a few closely-related analytes from a single chemical class; except for Camann et al (2013). Preference of either gas or liquid chromatography as the preferred separation technique for tooth organics analysis appears to be driven by target analytes being screened and preference of analyst.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Gas chromatography (GC) was the preferred separation technique for polychlorinated biphenyls and similar persistent organic chemicals (Jan et al., 2006; Jan et al., 2013; Jan and Vrbic, 2000; Jan et al., 2001), illegal drugs (Cattaneo et al., 2003; Pellegrini et al., 2006), tobacco exposure markers (Garcia-Algar et al., 2003; Pascual et al., 2003), and pesticides (Camann et al., 2013). For GC amenable analysis, certain studies followed a priori chemical modification of analytes, commonly referred to as derivatization (Cattaneo et al., 2003; Pellegrini et al., 2006). Derivatization is commonly required for gas chromatography analysis of organics, in particular for those with a free amino, hydroxyl or carboxyl functional groups. Hence, it is beneficial to consider multiple derivatization agents to separate multiple organics analytes with different functional groups. A possible alternative to increase sensitivity in gas chromatography based methodologies is to use the chemical ionization mode, which unfortunately are specific to particular sets of organics analytes. The derivatization step was dependent on the nature of the analyte and separation technique (Table 2). Underivatized and thermo-labile organics are best separated using liquid chromatography based methodologies. Liquid chromatography (LC) was an appropriate separation technique used for the analysis of an anesthetic (Kanjanawattana et al., 2001), tobacco metabolites (Marchei et al., 2008), analgesics, and metabolites of pesticides and phthalates (Camann et al., 2013), clarithromycin (Fos P, 2011), alcohol metabolite (Zeren et al., 2013), and antibiotics (Schussl et al., 2014).

The nature of GC columns used in literature for tooth organics analysis varied widely, including (i) a SPB-5 capillary non-polar column with fused silica material and boned phase of poly (5% diphenyl/95% dimethyl siloxane) and poly(50% n-octyl/50% methyl siloxane) for persistent organic chemicals (Jan et al., 2006; Jan et al., 2013; Jan and Vrbic, 2000; Jan et al., 2001), (ii) a HP-MS5 with (5%-phenyl)-methylpolysiloxane phase for illegal drugs (Cattaneo et al., 2003; Pellegrini et al., 2006), and (iii) a Zerbon 5% dimethyl poly-siloxane phase for tobacco metabolites (Garcia-Algar et al., 2003; Pascual et al., 2003). LC columns used for tooth organics analysis included (i) C8 phase for anesthetics (Kanjanawattana et al., 2001)), tobacco metabolites (Marchei et al., 2008), antibiotics (Fos P, 2011), or (ii) a pentaflourophenyl reversed phase for antibiotics (Schussl et al., 2014), or (iii) a hydrophilic interaction on non-bonded silica phase for alcohol metabolite (Zeren et al., 2013). Information on the GC and LC columns used for multi-class organics separation from whole tooth extract by Camann et al (Camann et al., 2013) is unavailable (Table 3).

Extract volume injected onto to the GC or LC columns for separation of analytes was between 1 μL (Pellegrini et al., 2006) to 30 μL (Marchei et al., 2008; Schussl et al., 2014) (information for the rest of studies is presented in Table 2). Individual study details on the GC conditions including (i) injector mode and temperature, (ii) carrier gas and flow rate, and (iii) oven temperature, gradient and duration is available in Table 3. Similarly, LC conditions that include (i) binary solvent composition and pH, (ii) mobile phase gradient, flow duration and rate, and (iii) column temperature are presented in Table 3. Due to differences in polarities, molecular weights and isomeric forms, it is suggested to use more than one stationary phase on either gas or liquid chromatography column and/or more than one mobile phase on the liquid chromatography method.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

2.5. Tooth organics detection

Mass spectrometry is the preferred detection technique for organic chemicals extracted from teeth (Table 3). Exceptions are (i) electron capture detector for persistent organic chemicals (Jan et al., 2006; Jan et al., 2013; Jan and Vrbic, 2000; Jan et al., 2001), and (ii) UV detector for antibiotics (Kanjanawattana et al., 2001). Detection with a low resolution single quad mass spectrometer (mass selective detector, MSD) or a high resolution tandem mass spectrometer (MS/MS) was of equal choice in the tooth organics studies. MSD was commonly used in conjunction with GC-based separation for analyzing illegal drugs (Cattaneo et al., 2003; Pellegrini et al., 2006) and tobacco markers (Garcia-Algar et al., 2003; Pascual et al., 2003). MS/MS was the preferred detection method applied with LC-based separation applied for quantifying tobacco metabolites (Marchei et al., 2008), metabolites of pesticides, phthalates (Camann et al., 2013) and alcohol consumption (Zeren et al., 2013), and antibiotics (Schussl et al., 2014). Electron impact was the most commonly used ionization method for GC-based separation methods (Garcia-Algar et al., 2003; Pascual et al., 2003; Pellegrini et al., 2006) and electrospray ionization for LC-based separation methods (Camann et al., 2013; Fos P, 2011; Marchei et al., 2008; Zeren et al., 2013). Mass spectrometry platforms coupled with liquid chromatography has a greater advantage in separating organics that occur at lower levels and with varying hydrophilic properties in dental tissues compared to gas chromatography methodology (Marchei et al., 2008).

Individual study details on the mass spectrometry conditions such as (i) ion source, quadrupole and interface temperatures, (ii) electron impact ionization voltage, (iii) collision energy, (iv) capillary and cone voltage, (v) desolvation (sheath) and nebulizer (cone) gas nature, pressure and flow rate, and (vi) m/z transitions used for selected ion monitoring or multiple reaction monitoring are presented in Table 3. The most notable limitation of a multi-class organics analysis will be decreased analytical sensitivity due to differences in polar functional groups and physico-chemical properties of the analytes from different classes. However, given the importance of understanding exposures to mixtures rather than a particular set of chemicals it is necessary to develop analytical methodologies for multiple classes without a compromise on sensitivity. One such effort will be combining solid phase micro-extraction with a GC-MS/MS as was achieved for simultaneous analysis of different classes of pesticides in hair (Salquebre et al., 2012). Irrespective of the chromatography and mass spectrometry platform selection, it is essential to report data on percent recovery and variability, and accuracy and precision for each organic analyte considered in a given study.

2.6. Tooth organics analytical performance and quantitation

Extraction recovery of organics in powdered tooth sample ranged from as low as 19–36% for iso-propyl-6-methyl-4-pyrimidinol to as high as 81–126% for mono-6-ethylhexyl phthalate in the case of the multi-class chemical extraction protocol followed by Camann et al (Camann et al., 2013). In general, studies that looked at monitoring one (Kanjanawattana et al., 2001) or few analytes (Pellegrini et al., 2006) reported satisfactory average recoveries in the range of 80–90%. Matrix spike recoveries for individual analytes, where available, are presented in Table 2. Limits of detection (LOD) and quantification (LOQ) varied widely, which was primarily determined by the separation and detection instrumentations used in the respective studies (Table 3). Most sensitive LOD and LOQ were reported for (i) codeine

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

analysis with GC-MS at a 2.0 ng g^{-1} and 6.0 ng g^{-1} sensitivity, respectively (Pellegrini et al., 2006) and (ii) ethyl glucuronide analysis with LC-MS/MS at a 0.48 ng g^{-1} and 1.61 ng g^{-1} sensitivity, respectively (Zeren et al., 2013). Inter- and intra-day variability of the analyses, where available, is presented in Table 3.

The analytical methods developed to quantify organics in whole tooth, were used to differentiate, for example, (i) total polychlorinated biphenyl levels in permanent teeth of children between cases (38 ng g^{-1}) and controls (7 ng g^{-1}) (Jan and Vrbic, 2000), (ii) lidocaine levels in upper (0.21 mg g^{-1}) versus lower canine (0.17 mg g^{-1}) in dogs (Kanjanawattana et al., 2001), (iii) morphine in deceased adults molar (8 – 83 ng g^{-1}), premolar (7 – 35 ng g^{-1}) versus incisor (77 ng g^{-1}) (Cattaneo et al., 2003), (iv) nicotine in shed teeth of children who were exposed to environmental tobacco (27 ng g^{-1}) versus controls (14 ng g^{-1}) (Marchei et al., 2008), (v) hexachlorobenzene levels in pulp (0.6 pmol g^{-1}), dentine (0.7 pmol g^{-1}) and enamel (0.2 pmol g^{-1}) in sheep (Jan et al., 2013), (vi) clarithromycin levels in inflamed pulp (0.2 – 4.4 ng mg^{-1}) versus lower canine (0.2 – 1.3 ng mg^{-1}) in extracted teeth from adult patients (Kanjanawattana et al., 2001), (vii) ethyl glucuronide levels in whole teeth of adult men who were alcohol abusers (22 pg mg^{-1}), light users (6.2 pg mg^{-1}) versus controls (<0.5 pg mg^{-1}) (Zeren et al., 2013), and (viii) amoxicillin levels in crown (0.2 μg g^{-1}) versus root (0.5 μg g^{-1}) in adult patients whole tooth (Schussl et al., 2014). Similar information for other analytes in the literature is detailed in Table 1.

Percent recovery of organics extraction from tooth matrix was calculated based on the recovery of spiked analytes. Given the difficulties in finding teeth that are free from environmental organics, it is essential to quantify the background concentrations before use for spiked quality-controls. In addition, spiking does not necessarily result in a homogenous distribution of the organic chemical in the pulverized tooth powder. Hence, it is not a true representation of the real recovery of accumulated organics in the deeper tissues of a tooth. Also, not all the available studies used an internal standard to assess the organics extraction procedure efficiency. Addition of an internal standard will help minimize the between-run variability and thereby improves the method precision and accuracy. In some of the studies where an internal standard was used, it was not clear at which point it was added during the extraction process. It is suggested to spike the internal standard in the very beginning of the extraction protocol for calculating absolute recovery. Approaches behind deriving the limit of detection and limit of quantification is not clear in certain studies. It is not clear in most of the studies whether calibration standards were prepared in tooth extracts or deionized water. If spiked in a tooth extract, it is essential to test and report for matrix interferences and sorption of spiked analyte to the tooth material in solution. The lack of standard reference material for organics in tooth matrix is another potential limitation for analytical method validation. Calibration standards were usually prepared daily. No information is available on stability of the spiked standards in tooth matrix extracts. Need for participation in programs to identify inter- and intra-laboratory variability in organics analysis is essential.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

3. Environmental organics analysis in tooth bio-matrix: Current challenges and future opportunities

3.1. Conceptual drawbacks and challenges

Given the potential of the teeth to reconstruct exposures to environmental organic chemicals, it is important to be aware about possible pitfalls and potential opportunities to improve on the current analytical method for tooth organics analysis. The major challenges in currently used methodologies in utilizing teeth are (i) no consideration of external (from outer surface) and internal contamination (from micro odontoblast processes), (ii) the latest formed dentine is the most lipid rich and therefore would absorb and accumulate most organic chemicals and compounds. Therefore, the assumption that whole ground teeth represent prenatal exposures is misleading, (iii) reverse causality in exposure assessment due to whole ground teeth, and (iv) teeth are a precious bio-matrix. Hence, grinding them raises ethical concerns about appropriate use of a very limited resource in exposure biology and epidemiology studies.

Permanent teeth were used in majority of the reviewed studies (for example, (Cattaneo et al., 2003; Fos P, 2011; Jan et al., 2013; Jan and Vrbic, 2000; Jan et al., 2001; Kanjanawattana et al., 2001; Pellegrini et al., 2006; Schussl et al., 2014; Zeren et al., 2013), compared to deciduous teeth (for example, (Camann et al., 2013; Garcia-Algar et al., 2003; Marchei et al., 2008; Pascual et al., 2003). Analysis of deciduous teeth offers several advantages such as the relative ease of collection of naturally shed teeth at 6 – 12 years and the inclusion of dentine and enamel formed prenatally. In comparison, permanent teeth can be more difficult to collect and the earliest records of exposure information start around the time of birth for the first molar. However, permanent teeth enable the study of exposures over a much longer timeline.

3.2. Methodological limitations and research directions

A limited number of procedures were reported in the literature for measuring organic chemicals in teeth. The measurement of chemical concentrations in pulverized whole teeth cannot reconstruct the timing of exposure. The use of whole teeth is purported to represent chemical concentration accumulated over the period of tooth formation but more likely only represents what was in circulation at the time of tooth collection due to the major influence of the pulp. Therefore, grinding whole teeth for chemical analysis may lead to exposure misclassification. The resorption that precedes exfoliation of deciduous teeth has a major effect on exposure signals when analyzed using large fragments of dentine that include the pulp area (Rabinowitz et al., 1993). In a study on lead, it was proposed that resorption may affect sites as much as 2 – 3 mm away from the final edge of resorption and effectively ‘resets’ the dentine lead level in this area to the then current blood levels (Rabinowitz et al., 1993). Therefore, large fragments of dentine, or whole teeth, are more a representation of the blood levels during the resorption, rather than lifetime cumulative exposure. Using large fragments or whole teeth for such determinations would therefore have a high risk of misclassification if exposure varied significantly before resorption.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

The major limitation of the whole tooth analysis approach lies in not knowing the extent of organics signal contribution from pulp and what has been deposited in dentine or enamel. One way of overcoming this shortcoming is by narrowing sample collection to dentine formed at specific time periods, such as trimester-specific formed tooth layers, to determine the timing of exposure (detailed in a later section with example from a case study). This approach requires specialized skills and instrumentation to identify and dissect trimester-specific tooth layers. The amount of tooth material that can be harvested from the micro-meter scale trimester-specific tooth layers is expectedly smaller in volume than whole teeth. The analysis of such samples, therefore, requires a thorough analyte pre-concentration for detection and quantification. No specific guidelines are available for tooth sample preparation and extraction for organics. Priority needs to be given to developing new analytical methods that are suitable for limited sample mass.

Moreover, the limitations in using teeth as a valid bio-matrix for measuring exposure biomarkers to environmental organic chemicals are (i) lack of validation of concentrations in teeth against conventional biological matrices such as cord blood, placenta, pregnant mother and infant urine, etc., (ii) lack of standardized analytical protocols, (iii) lack of standard reference materials, and (iv) lack of quality control/quality assurance procedures for both intra- and inter-laboratory comparisons. Additional limitations that are relevant to existing analytical methodologies are (i) lack of information on stability of the analytes in either intact or spiked tooth matrix stored at room temperature, (ii) lack of consideration of analytes contamination from the internal odonobalstic processes within a tooth matrix, (iii) lack of knowledge on the loss of organic analytes sorbed onto the surfaces of material and media used for tooth preparation and pulverization, (iv) lack of exploration on whether extraction equilibrium was reached between the tooth powder sample and incubation media that influence percent recovery of analytes, and (v) lack of a single analytical methodology for simultaneous extraction, chromatographic separation, and mass spectrometric detection of a wide range of analytes belonging to multiple chemical classes with varying polar functional groups and physico-chemical properties.

4. Future perspectives: Tooth as a novel bio-matrix to determine in utero exposure timing to environmental organics and later-life health outcomes in children

Presently, it is not feasible to routinely sample fetal blood in epidemiologic studies, and while maternal blood, urine and other biological matrices may be collected during pregnancy, chemicals in maternal samples may not accurately reflect fetal exposure for all toxicants (Needham et al., 2011; Rudge et al., 2009). For example, there are differences in maternal-fetal partitioning of organic compounds (reviewed by (Aylward et al., 2014)). The utility of blood measurements is further limited by the short half-lives of some chemical pollutants (Barbosa et al., 2005). Many organophosphate pesticides are cleared rapidly from blood; for example, chlorpyrifos in blood and urine following controlled oral exposures has a half-life of ~1 hour (Eaton et al., 2008). Therefore, a single maternal blood measurement taken during pregnancy does not accurately reflect the magnitude of exposure to the fetus throughout intrauterine development. Cord blood is often of value in measuring fetal

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

exposure but cannot provide temporal information on chemical exposure beyond late third trimester. Concentrations of chemicals in plasma, urine, hair and other media, while useful in monitoring adults, are also similarly limited in studies addressing fetal chemical exposure.

To address these issues, an analytical methodology for organics that utilizes human deciduous teeth to provide a direct measure of the timing and intensity of chemical exposure from approximately the 14th gestational week to birth and into early childhood is essential. This methodology should exploit the normal growth pattern of teeth, which is analogous to rings in a tree, and utilizes micro-spatial chemical measurements of specific growth rings that correspond to specific critical developmental windows. Between the 14th to 19th weeks of intrauterine development, the tooth germ enters the advanced bell stage characterized by the appearance of enamel and dentine at the future dentine-enamel junction on the cusp tip (Berkovitz, 2002). Subsequently, enamel and dentine deposition occurs in a rhythmic manner, forming incremental lines in both enamel and dentine. At birth, an accentuated incremental line, the neonatal line, is formed due to stunting of the ameloblasts and odontoblasts that deposit enamel and dentine matrix respectively (Sabel et al., 2008). This line forms a clear histological landmark that demarcates pre- and postnatal formed tooth layers (Sabel et al., 2008).

Dentine is composed of a lipid-containing protein matrix and a mineral component of substituted hydroxyapatite. Deposition of dentine occurs in two phases; first, the protein matrix is deposited followed by mineralization with influx of calcium, phosphorus and also any chemicals that the fetus is exposed to concurrently (Hamada, 1989; Smith and Lesot, 2001; Smith, 1998). Dentine is mineralized to its final state of ~70% immediately after the incremental layer-by-layer deposition of the protein matrix (Berkovitz BKB., 2009). Therefore, measurements of chemicals at any single location in dentine can be readily linked to timing of exposure unless a pathological process (e.g. dental caries) causes dissolution of the dentine. Dentine has a tubular structure that has blood vessels and cellular processes penetrating it. Only the mineralized fraction retains the original exposure information from the period of tooth development, whereas the soft tissue components essentially add noise to that important temporal exposure information.

Selective and sensitive analytical methods development is required because of the limited sample mass available from trimester-specific tooth layers. Detailed method development and validation procedure, and its application towards tooth analysis from a children cohort study are underway. So far, we have developed an analytical method that consists of (i) micro-sectioning of teeth to achieve trimester-specific tooth layers, and (ii) extraction of acidic, basic and neutral analytes and concentrating these on polymeric sorbent using SPE, and (iii) reversed phase chromatographic separation and analyte detection and quantification using polarity switching and multiple reaction monitoring on a triple quadruple mass spectrometer. The method was applied to quantify 15 phthalates metabolites and 3 tobacco metabolites in teeth from two children for which we obtained second and third trimester-specific tooth layers (Figure 1A). An example plot for the time of exposure versus concentration graph for mono-benzyl phthalate (MBzP) levels is shown in Figure 1B. Looking at the cumulative prenatal exposure using a pooled sample (similar to whole tooth analysis situation or single blood measurement), one cannot differentiate the exposure

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

between two children (Figure 1B). However, individuals' concentrations differ significantly when trimester specific fractions were analyzed. Child B (triangle) was exposed to much higher levels during the second trimester. This is a typical scenario leading to exposure misclassification and thereby misleading the inferences about causality.

Though we focused on identifying and quantifying metabolites of phthalates and tobacco for the exploratory analysis, this approach can be extended to quantifying other environmental chemical classes of interest such as phenols, pesticides, brominated flame retardants and persistent organic pollutantsin the near future. Our preliminary data shows that organic analytes in epidemiological studies can be retrospectively detected in tooth layers formed during the second and third trimesters. Work is in progress to fully exploit the potential of trimester-specific tooth layers analysis to pin-point the timing and intensity of a chemical or mixture exposure. Further method development and new instrumentation is required for this approach to reach its full potential.

5. Conclusion and Outlook

Perinatal exposures to environmental chemicals in children are associated with major public health outcomes including impaired neurodevelopment (Bellinger, 2013; Grandjean and Landrigan, 2014), metabolic syndrome (Behl et al., 2013; La Merrill and Birnbaum, 2011; Wang et al., 2014), and immune function (Grandjean and Budtz-Jorgensen, 2013). Teeth are uniquely positioned to capture early life chemical exposures in continuum starting prenatally (second trimester in utero) to progressive life stages (until deciduous teeth are shed around 6–12 year's age), unlike the common choice of biological matrices such as urine and blood that are limited by a short half-life for some chemicals (Arora and Austin, 2013). A method that utilizes micro-spatial chemical measurements of specific growth rings that correspond to specific critical developmental windows is required to exploit the archival nature of teeth. Extensive research is available in applying laser ablation technology to map trace metal concentrations in teeth at micrometer resolution (Arora et al., 2014; Arora et al., 2012; Arora et al., 2004; Arora et al., 2005; Arora et al., 2011; Arora et al., 2006; Arora et al., 2007). However, no such studies are available for organic chemicals in teeth.

Unlike previous analyses of teeth that digest entire samples and provide averaged concentration of organic chemicals where all temporal exposure information is lost, the proposed methodology will provide trimester-specific exposure information for organic toxicants. Further analyses are in progress to confirm the validity and applicability of teeth to identify trimester-specific exposures to environmental organic contaminants. Because of the widely accepted notion that prenatal life stage is a critical susceptibility window to environmental chemical exposures and adverse later life health effects, we believe that there is an unprecedented need to determine the timing of exposure. Hence we propose sample preparation methodologies that do not pulverize whole teeth or large fragments but rather analyze the complex microstructure of teeth and isolate developmental stage-specific fractions for analysis, thus retaining temporal exposure information. Beyond sample preparation we also propose the need for an integrated analytical workflow that is designed to extract both polar and nonpolar environmental organics and their metabolites, and is based on (i) a three stage extraction methodology, (ii) a simultaneous pre-concentration step

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

employing a polymeric solid-phase extraction sorbent, and (iii) targeted and non-targeted analysis using both liquid and gas chromatography technology coupled with a suitable mass spectrometer for detection and quantitation. Once validated, this work will be transformative in developmental environmental epidemiology as one could estimate longitudinal data on organic chemical exposure retrospectively, even in case-control studies.

Acknowledgments

M.A. is supported by grants from the National Institute of Environmental Health Sciences: DP2ES025453-01 (New Innovator Award), 5R00ES019597-05.

References

Adams J, et al. Workshop to identify critical windows of exposure for children's health: neurobehavioral work group summary. Environ Health Perspect. 2000; 108(Suppl 3):535–44. [PubMed: 10852852]

Altshuller LF, et al. Deciduous teeth as an index of body burden of lead. The Journal of Pediatrics. 1962; 60:224–229. [PubMed: 13860784]

Arora M, Austin C. Teeth as a biomarker of past chemical exposure. Curr Opin Pediatr. 2013; 25:261–7. [PubMed: 23429707]

Arora M, et al. Determining prenatal, early childhood and cumulative long-term lead exposure using micro-spatial deciduous dentine levels. PLoS One. 2014; 9:e97805. [PubMed: 24841926]

Arora M, et al. Determining fetal manganese exposure from mantle dentine of deciduous teeth. Environ Sci Technol. 2012; 46:5118–25. [PubMed: 22455722]

Arora M, et al. Spatial distribution of lead in the roots of human primary teeth. J Trace Elem Med Biol. 2004; 18:135–9. [PubMed: 15646259]

Arora M, et al. Spatial distribution of lead in enamel and coronal dentine of wistar rats. Biol Trace Elem Res. 2005; 105:159–70. [PubMed: 16034161]

Arora M, et al. Spatial distribution of manganese in enamel and coronal dentine of human primary teeth. Sci Total Environ. 2011; 409:1315–9. [PubMed: 21211818]

Arora M, et al. Spatial distribution of lead in human primary teeth as a biomarker of pre- and neonatal lead exposure. Sci Total Environ. 2006; 371:55–62. [PubMed: 16950500]

Arora M, et al. The application of synchrotron radiation induced X-ray emission in the measurement of zinc and lead in Wistar rat ameloblasts. Arch Oral Biol. 2007; 52:938–44. [PubMed: 17521603]

Austin C, et al. Barium distributions in teeth reveal early-life dietary transitions in primates. Nature. 2013; 498:216–9. [PubMed: 23698370]

Aylward LL, et al. Relationships of chemical concentrations in maternal and cord blood: a review of available data. J Toxicol Environ Health B Crit Rev. 2014; 17:175–203. [PubMed: 24749481]

Barbosa F Jr, et al. A critical review of biomarkers used for monitoring human exposure to lead: advantages, limitations, and future needs. Environ Health Perspect. 2005; 113:1669–74. [PubMed: 16330345]

Behl M, et al. Evaluation of the association between maternal smoking, childhood obesity, and metabolic disorders: a national toxicology program workshop review. Environ Health Perspect. 2013; 121:170–80. [PubMed: 23232494]

Bellinger DC. Prenatal Exposures to Environmental Chemicals and Children's Neurodevelopment: An Update. Saf Health Work. 2013; 4:1–11. [PubMed: 23515885]

Berkovitz, BKB.; HG; MoxhaM, BJ. Oral Anatomy, Histology and Embryology. 2009.

Berkovitz, BKB. Oral Anatomy, Histology and Embryology. 2002. p. 392

Bertelsen SL, et al. Evaluation of log KOW and tissue lipid content as predictors of chemical partitioning to fish tissues. Environmental Toxicology and Chemistry. 1998; 17:1447–1455.

Bouchard MF, et al. Prenatal exposure to organophosphate pesticides and IQ in 7-year-old children. Environ Health Perspect. 2011; 119:1189–95. [PubMed: 21507776]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Bradman A, et al. Measurement of pesticides and other toxicants in amniotic fluid as a potential biomarker of prenatal exposure: a validation study. Environ Health Perspect. 2003; 111:1779–82. [PubMed: 14594631]

Braun JM, et al. Prenatal bisphenol A exposure and early childhood behavior. Environ Health Perspect. 2009; 117:1945–52. [PubMed: 20049216]

Bruin JE, et al. Long-term consequences of fetal and neonatal nicotine exposure: a critical review. Toxicol Sci. 2010; 116:364–74. [PubMed: 20363831]

Burke H, et al. Prenatal and passive smoke exposure and incidence of asthma and wheeze: systematic review and meta-analysis. Pediatrics. 2012; 129:735–44. [PubMed: 22430451]

Camann DE, et al. Acetaminophen, pesticide, and diethylhexyl phthalate metabolites, anandamide, and fatty acids in deciduous molars: potential biomarkers of perinatal exposure. J Expo Sci Environ Epidemiol. 2013; 23:190–6. [PubMed: 22805989]

Cattaneo C, et al. The detection of morphine and codeine in human teeth: an aid in the identification and study of human skeletal remains. J Forensic Odontostomatol. 2003; 21:1–5. [PubMed: 12793124]

Chen A, et al. Prenatal polybrominated diphenyl ether exposures and neurodevelopment in U.S. children through 5 years of age: the HOME study. Environ Health Perspect. 2014; 122:856–62. [PubMed: 24870060]

Cory-Slechta DA, et al. Lifetime consequences of combined maternal lead and stress. Basic Clin Pharmacol Toxicol. 2008; 102:218–27. [PubMed: 18226077]

Ding G, Bao Y. Revisiting pesticide exposure and children's health: focus on China. Sci Total Environ. 2014; 472:289–95. [PubMed: 24291629]

Eaton DL, et al. Review of the toxicology of chlorpyrifos with an emphasis on human exposure and neurodevelopment. Crit Rev Toxicol. 2008; 38(Suppl 2):1–125. [PubMed: 18726789]

Ericson JE, et al. Measurements of manganese with respect to calcium in histological enamel cross sections: toward a new manganese biomarker. Environ Res. 2001; 86:46–50. [PubMed: 11386740]

Eskenazi B, et al. In utero and childhood polybrominated diphenyl ether (PBDE) exposures and neurodevelopment in the CHAMACOS study. Environ Health Perspect. 2013; 121:257–62. [PubMed: 23154064]

Eskenazi B, et al. Organophosphate pesticide exposure and neurodevelopment in young Mexican-American children. Environ Health Perspect. 2007; 115:792–8. [PubMed: 17520070]

Florek E, et al. Effect of maternal tobacco smoking or exposure to second-hand smoke on the levels of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) in urine of mother and the first urine of newborn. J Physiol Pharmacol. 2011; 62:377–83. [PubMed: 21893699]

Fos PFL, Llena C, Anadon A. Analysis of clarithromycin in dental pulp with and without inflammation. International Dentistry SA. 2011; 13:50–54.

Garcia-Algar O, et al. Nicotine concentrations in deciduous teeth and cumulative exposure to tobacco smoke during childhood. Jama. 2003; 290:196–7. [PubMed: 12851273]

Gareri J, Koren G. Prenatal hair development: implications for drug exposure determination. Forensic Sci Int. 2010; 196:27–31. [PubMed: 20074880]

Gascon M, et al. Effects of pre and postnatal exposure to low levels of polybromodiphenyl ethers on neurodevelopment and thyroid hormone levels at 4 years of age. Environ Int. 2011; 37:605–11. [PubMed: 21237513]

Grandjean P, Budtz-Jorgensen E. Immunotoxicity of perfluorinated alkylates: calculation of benchmark doses based on serum concentrations in children. Environ Health. 2013; 12:35. [PubMed: 23597293]

Grandjean P, Landrigan PJ. Developmental neurotoxicity of industrial chemicals. Lancet. 2006; 368:2167–78. [PubMed: 17174709]

Grandjean P, Landrigan PJ. Neurobehavioural effects of developmental toxicity. Lancet Neurol. 2014; 13:330–8. [PubMed: 24556010]

Guilarte TR, et al. Is lead exposure in early life an environmental risk factor for Schizophrenia? Neurobiological connections and testable hypotheses. Neurotoxicology. 2012; 33:560–74. [PubMed: 22178136]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Gulson BL, et al. Stable lead isotopes in teeth as indicators of past domicile--a potential new tool in forensic science? J Forensic Sci. 1997; 42:787–91. [PubMed: 9304823]

Gundacker C, et al. The relevance of the individual genetic background for the toxicokinetics of two significant neurodevelopmental toxicants: mercury and lead. Mutat Res. 2010; 705:130–40. [PubMed: 20601101]

Gunier RB, et al. Determinants of manganese in prenatal dentin of shed teeth from CHAMACOS children living in an agricultural community. Environ Sci Technol. 2013; 47:11249–57. [PubMed: 24053404]

Hackenberg RSA, Ballschmiter K. High-resolution gas chromatography retention data as basis for the estimation of KOW values using PCB congeners as secondary standards. Environ Sci Technol. 2003; 37:2274–9. [PubMed: 12785536]

Hamada S. Study of trace elements in bovine permanent teeth germ. Kanagawa Shigaku. 1989; 24:24–37. [PubMed: 2489644]

Harari R, et al. Neurobehavioral deficits and increased blood pressure in school-age children prenatally exposed to pesticides. Environ Health Perspect. 2010; 118:890–6. [PubMed: 20185383]

Harley KG, et al. Prenatal and early childhood bisphenol A concentrations and behavior in school-aged children. Environ Res. 2013; 126:43–50. [PubMed: 23870093]

HerbstmanJB SA, Kurzon M, Lederman SA, Jones RS, Rauh V, Needham LL, Tang D, Niedzwiecki M, Wang RY, Perera F. Prenatal exposure to PBDEs and neurodevelopment. Environ Health Perspect. 2010; 118:712–9. [PubMed: 20056561]

Herrmann M, et al. Prenatal tobacco smoke and postnatal secondhand smoke exposure and child neurodevelopment. Curr Opin Pediatr. 2008; 20:184–90. [PubMed: 18332716]

Hinners T, et al. Chronologically matched toenail-Hg to hair-Hg ratio: temporal analysis within the Japanese community (U.S.). Environ Health. 2012; 11:81. [PubMed: 23113987]

Humphrey LT, et al. Unlocking evidence of early diet from tooth enamel. Proc Natl Acad Sci U S A. 2008; 105:6834–9. [PubMed: 18458343]

Jan J, et al. Distribution of organochlorine pollutants in ovine dental tissues and bone. Environ Toxicol Pharmacol. 2006; 21:103–7. [PubMed: 21783645]

Jan J, et al. Levels and distribution of organochlorine pollutants in primary dental tissues and bone of lamb. Environ Toxicol Pharmacol. 2013; 36:1040–5. [PubMed: 24100271]

Jan J, Vrbic V. Polychlorinated biphenyls cause developmental enamel defects in children. Caries Res. 2000; 34:469–73. [PubMed: 11093020]

Jan J, et al. Bioconcentration of lipophilic organochlorines in ovine dentine. Arch Oral Biol. 2001; 46:1111–6. [PubMed: 11684030]

Kanjanawattana S, et al. Determination of lidocaine in dental pulp by high-performance liquid chromatography. J Endod. 2001; 27:31–5. [PubMed: 11487160]

Kobrosly RW, et al. Prenatal phthalate exposures and neurobehavioral development scores in boys and girls at 6–10 years of age. Environ Health Perspect. 2014; 122:521–8. [PubMed: 24577876]

La Merrill M, Birnbaum LS. Childhood obesity and environmental chemicals. Mt Sinai J Med. 2011; 78:22–48. [PubMed: 21259261]

Landrigan PJ, et al. Children's health and the environment: public health issues and challenges for risk assessment. Environ Health Perspect. 2004; 112:257–65. [PubMed: 14754581]

Leonardi-Bee J, et al. Secondhand smoke and adverse fetal outcomes in nonsmoking pregnant women: a meta-analysis. Pediatrics. 2011; 127:734–41. [PubMed: 21382949]

Lorber M. Exposure of Americans to polybrominated diphenyl ethers. J Expo Sci Environ Epidemiol. 2008; 18:2–19. [PubMed: 17426733]

Marchei E, et al. Ultrasensitive detection of nicotine and cotinine in teeth by high-performance liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom. 2008; 22:2609–12. [PubMed: 18646249]

Marks AR, et al. Organophosphate pesticide exposure and attention in young Mexican-American children: the CHAMACOS study. Environ Health Perspect. 2010; 118:1768–74. [PubMed: 21126939]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Mazdai A, et al. Polybrominated diphenyl ethers in maternal and fetal blood samples. Environ Health Perspect. 2003; 111:1249–52. [PubMed: 12842781]

Moller SE, et al. Risk of childhood overweight after exposure to tobacco smoking in prenatal and early postnatal life. PLoS One. 2014; 9:e109184. [PubMed: 25310824]

Müller JF, Hawker DW, Connell DW. Calculation of bioconcentration factors of persistent hydrophobic compounds in the air/vegetation system. Chemosphere. 1994; 29:623–640.

Muñoz-Quezada MTLB, Barr DB, Steenland K, Levy K, Ryan PB, Iglesias V, Alvarado S, Concha C, Rojas E, Vega C. Neurodevelopmental effects in children associated with exposure to organophosphate pesticides: a systematic review. Neurotoxicology. 2013; 39:158–68. [PubMed: 24121005]

Nanci, A. Ten Cate's Oral Histology: Development, Structure, and Function. Mosby Elsevier; St. Louis: 2008.

Needham LL, et al. Partition of environmental chemicals between maternal and fetal blood and tissues. Environ Sci Technol. 2011; 45:1121–6. [PubMed: 21166449]

Needleman HL, et al. Subclinical lead exposure in philadelphia schoolchildren. Identification by dentine lead analysis. New England Journal of Medicine. 1974; 290:245–248. [PubMed: 4808928]

Needleman HL, Shapiro IM. Dentine lead levels in asymptomatic Philadelphia school children: subclinical exposure in high and low risk groups. Environ Health Perspect. 1974; 7:27–31. [PubMed: 4831144]

Needleman HLSI. Dentine lead levels in asymptomatic Philadelphia school children: subclinical exposure in high and low risk groups. Environ Health Perspect. 1974; 7:27–31. [PubMed: 4831144]

Pascual JA, et al. A simple and reliable method for the determination of nicotine and cotinine in teeth by gas chromatography/mass spectrometry. Rapid Commun Mass Spectrom. 2003; 17:2853–5. [PubMed: 14673838]

Pellegrini M, et al. Development and validation of a gas chromatography-mass spectrometry assay for opiates and cocaine in human teeth. J Pharm Biomed Anal. 2006; 40:662–8. [PubMed: 16076541]

Perera F, et al. Prenatal bisphenol a exposure and child behavior in an inner-city cohort. Environ Health Perspect. 2012; 120:1190–4. [PubMed: 22543054]

Pichini S, et al. The analysis of nicotine in infants' hair for measuring exposure to environmental tobacco smoke. Forensic Sci Int. 1997; 84:253–8. [PubMed: 9042731]

Polanska K, et al. Review of current evidence on the impact of pesticides, polychlorinated biphenyls and selected metals on attention deficit/hyperactivity disorder in children. Int J Occup Med Environ Health. 2013; 26:16–38. [PubMed: 23526196]

Polanska K, et al. Phthalate exposure and child development: the Polish Mother and Child Cohort Study. Early Hum Dev. 2014; 90:477–85. [PubMed: 25038557]

Rabinowitz MB, et al. Relationships between serial blood lead levels and exfoliated tooth dentin lead levels: models of tooth lead kinetics. Calcif Tissue Int. 1993; 53:338–41. [PubMed: 8287322]

Rabinowitz MB, et al. Kinetic analysis of lead metabolism in healthy humans. J Clin Invest. 1976; 58:260–70. [PubMed: 783195]

Rauh VA, et al. Impact of prenatal chlorpyrifos exposure on neurodevelopment in the first 3 years of life among inner-city children. Pediatrics. 2006; 118:e1845–59. [PubMed: 17116700]

Rice D, Barone S Jr. Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. Environ Health Perspect. 2000; 108(Suppl 3):511–33. [PubMed: 10852851]

Ricketts SA, et al. Reducing low birthweight by resolving risks: results from Colorado's prenatal plus program. Am J Public Health. 2005; 95:1952–7. [PubMed: 16195530]

Rodriguez-Barranco M, et al. Association of arsenic, cadmium and manganese exposure with neurodevelopment and behavioural disorders in children: a systematic review and meta-analysis. Sci Total Environ. 2013; 454–455:562–77.

Rudge CV, et al. The placenta as a barrier for toxic and essential elements in paired maternal and cord blood samples of South African delivering women. J Environ Monit. 2009; 11:1322–30. [PubMed: 20449220]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Sabel N, et al. Neonatal lines in the enamel of primary teeth--a morphological and scanning electron microscopic investigation. Arch Oral Biol. 2008; 53:954–63. [PubMed: 18589400]

Salquebre G, et al. Multi-class pesticide analysis in human hair by gas chromatography tandem (triple quadrupole) mass spectrometry with solid phase microextraction and liquid injection. Anal Chim Acta. 2012; 710:65–74. [PubMed: 22123113]

Schussl Y, et al. Concentrations of amoxicillin and clindamycin in teeth following a single dose of oral medication. Clin Oral Investig. 2014; 18:35–40.

Smith AJ, Lesot H. Induction and regulation of crown dentinogenesis: embryonic events as a template for dental tissue repair? Crit Rev Oral Biol Med. 2001; 12:425–37. [PubMed: 12002824]

Smith CE. Cellular and chemical events during enamel maturation. Crit Rev Oral Biol Med. 1998; 9:128–61. [PubMed: 9603233]

Tellez-Rojo MM, et al. Prenatal urinary phthalate metabolites levels and neurodevelopment in children at two and three years of age. Sci Total Environ. 2013; 461–462:386–90.

Uryu T, et al. Analysis of lead in tooth enamel by laser ablation-inductively coupled plasma-mass spectrometry. Anal Sci. 2003; 19:1413–6. [PubMed: 14596408]

Waliszewski SM, et al. Organochlorine pesticide levels in maternal adipose tissue, maternal blood serum, umbilical blood serum, and milk from inhabitants of Veracruz, Mexico. Arch Environ Contam Toxicol. 2001; 40:432–8. [PubMed: 11443377]

Wang G, et al. Early Life Origins of Metabolic Syndrome: The Role of Environmental Toxicants. Curr Environ Health Rep. 2014; 1:78–89. [PubMed: 24883264]

Whyatt RM, et al. A biomarker validation study of prenatal chlorpyrifos exposure within an inner-city cohort during pregnancy. Environ Health Perspect. 2009; 117:559–67. [PubMed: 19440494]

Yolton K, et al. Prenatal exposure to bisphenol A and phthalates and infant neurobehavior. Neurotoxicol Teratol. 2011; 33:558–66. [PubMed: 21854843]

Yoshimasu K, et al. A meta-analysis of the evidence on the impact of prenatal and early infancy exposures to mercury on autism and attention deficit/hyperactivity disorder in the childhood. Neurotoxicology. 2014; 44:121–31. [PubMed: 24952233]

Zeren C, et al. Demonstration of ethyl glucuronide in dental tissue samples by liquid chromatography/electro-spray tandem mass spectrometry. J Forensic Leg Med. 2013; 20:706–10. [PubMed: 23910866]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Highlights

- Teeth are an important matrix to reconstruct prenatal exposure
- Assessment of organic chemicals in teeth poses many technical challenges
- We reviewed the currently available literature on the topic
- Previously used analytical methods did not provide temporal exposure information
- We propose novel methods to obtain trimester-specific exposure information for organics

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

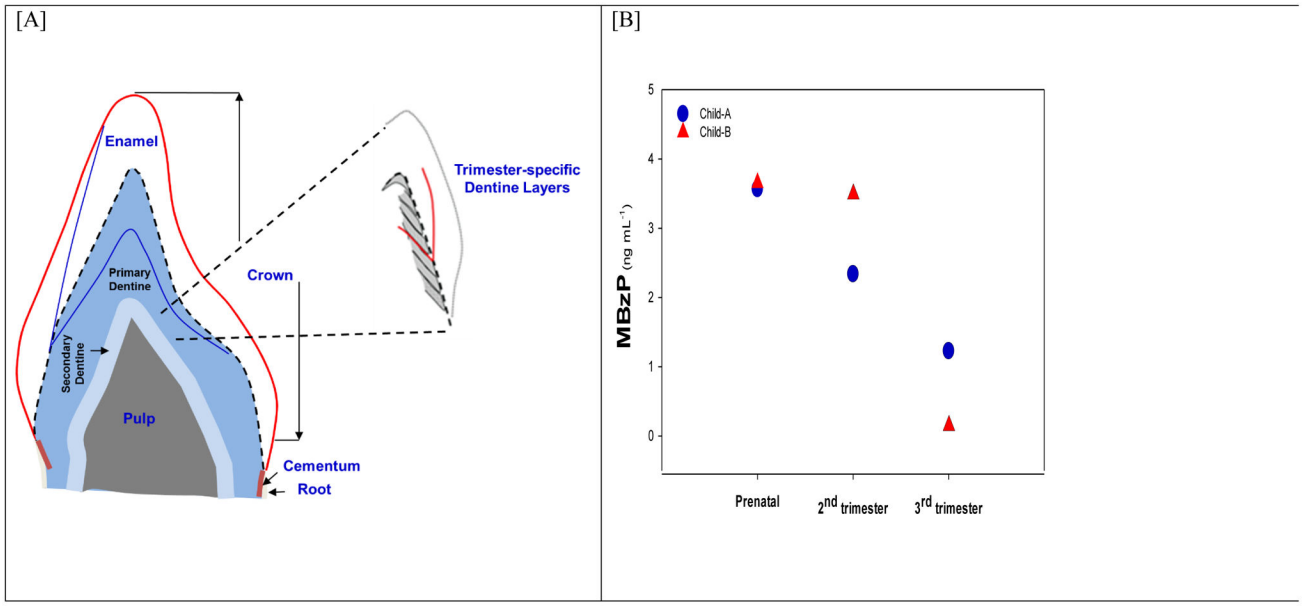

Figure 1.

[A] Representation of trimester-specific dentine layers. Details on tooth structure and development aspects are provided in (Arora et al., 2014) and (Arora and Austin, 2013), and [B] typical case of exposure misclassification: Cumulative vs. time-specific mono-benzyl phthalate (MBzP) levels in the second and third trimester tooth layers. Both participants appear to have identical prenatal exposures, but detailed trimester-specific analysis reveals marked differences in exposure profile over the second and third trimesters, Child B experiencing bulk of their exposure in the second trimester.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 1

Teeth as a bio-matrix of organic chemical exposure

#	Analyte	Chemical class	Study country	Study population, sample size, & age	Exposure source/route & detection window	Teeth (Analyzed part)	Detection rate (%)	Mean (SD) or Range	Reference
1	Polychlorinated biphenyls (PCB)	Persistent environmental contaminant	Slovenia	Children N = 68 (Cases: n=36; Controls: n=32) 8–15 yrs	PCBs contaminated agricultural soil	Permanent teeth (Dentine)	-	Total PCB (sum of congeners PCB- 28, 66, 101, 105, 118, 138, 153, 180) Cases: 38 ng/g; Controls: 7 ng/g	(Jan and Vrbic, 2000)
2	Polychlorobiphenyls (PCB) Hexachlorobenzene (HCB) Dichlorodiphenyl-dichloroethylene (4,4′DDE)	Organochlorines	Slovenia	Sheep (Lambs) N = 4 (Cases: n=2; Controls: n=2; 34 permanent teeth overall from lambs)	Lactation	Permanent dental tissues: Maxillae and Mandibles (Dentine)	PCBs: 100% in cases and 0% in controls HCB and 4,4′DD E: ~50 times lower in controls compared to cases (baseline exposure)	Dentine PCB-54: 0.20 ng/g PCB-80: 0.16 ng/g PCB-155: 0.30 ng/g PCB-169: 0.21 ng/g HCB: 0.12 ng/g 4,4′DDE: 0.87 ng/g	(Jan et al., 2001)
3	Lidocaine (LID)	Anesthetic	Thailand	Dogs N = 2 (Upper canine: n=2; Lower canine: n=2)	Local application	Canine tooth (Dental pulp)	100%	Upper canine: 0.21 mg/g wet pulp Lower canine: 0.17 mg/g wet pulp	(Kanjanawattana et al., 2001)
4	Morphine (MOR) Codeine (COD)	Illegal drugs	Italy	Deceased people (forensic study) N = 4 (Molar: n=4; Premolar: n=3; Incisor: n=1)	Drug abuse (died from overuse)	Molar, premolar and incisor (Whole)	100%	Morphine Molar: 8.24–83.0 ng/g; Premolar: 6.94–35.1ng/g; Incisor: 76.8 ng/g Codeine Molar: 5.47–28.0 ng/g; Premolar: 2.99–15.8ng/g; Incisor: 8.88 ng/g	(Cattaneo et al., 2003)
5	Nicotine (NIC) Cotinine (COT)	Tobacco smoke	Rome	Children N = 35 (Cases: n=8; Controls: n=11) 7.4 (0.6) yrs	Passive exposure from environment	Deciduous teeth, Superior incisor (Whole)	100%	Nicotine Cases: 44 ng/g; Controls: 15 ng/g Cotinine Cases: 29 ng/g; Controls: 14 ng/g	(Garcia-Algar et al., 2003); (Pascual et al., 2003)
6	Nicotine (NIC) Cotinine (COT)	Tobacco smoke	Rome	Children N = 35 (Cases: n=8; Controls: n=11) 7.4 (0.6) yrs	Passive exposure from environment	Deciduous teeth, Superior incisor (Whole)	100%	Nicotine Cases: 44 ng/g; Controls: 15 ng/g Cotinine Cases: 29 ng/g; Controls: 14 ng/g	(Pascual et al., 2003); (Garcia-Algar et al., 2003)
7	Polychlorobiphenyls (PCB) Hexachlorobenzene (HCB) Dichlorodiphenyl-dichloroethylene (4,4′DDE)	Organochlorines	Slovenia	Sheep (Lambs) N = 4 (Cases: n=2; Controls: n=2; 34 permanent teeth overall from lambs)	Lactation	Permanent dental tissues: Maxillae and Mandibles (Pulp, Dentine, and Enamel)	-	PCB-54 (pmol/g dry wt) Pulp: 0.28 (0.06), Dentine: 0.18 (0.02), Enamel: 0.54 (0.03) PCB-80 (pmol/g dry wt) Pulp: 0.52 (0.12), Dentine: 0.29 (0.02), Enamel: 0.62 (0.05) PCB-155 (pmol/g drywt) Pulp: 0.50 (0.02), Dentine: 0.25 (0.02), Enamel: 0.72 (0.05) PCB-169 (pmol/g drywt)	(Jan et al., 2006)

#	Analyte	Chemical class	Study country	Study population, sample size, & age	Exposure source/route & detection window	Teeth (Analyzed part)	Detection rate (%)	Mean (SD) or Range	Reference
								Pulp: 3.50 (0.45), Dentine: 0.31 (0.02), Enamel: 0.63 (0.02) Pulp: 3.50 (0.45), Dentine: 0.31 (0.02), Enamel: 0.63 (0.02) HCB (pmol/g dry wt) Pulp: 3.60 (0.21), Dentine: 0.61 (0.04), Enamel: 0.63 (0.05) 4,4′DDE (pmol/g drywt) Pulp: 4.60 (0.35), Dentine: 1.40 (0.14), Enamel: 1.60 (0.11)	
8	Opiates Cocaine	Illegal drugs	Spain	Former drug addicts N = 24 (Controls: n=10; Former chronic drug users: n=14)	Illicit drug consumption	Extracted teeth (Whole)	Controls: 0% Cases (based on LOD): 6-MAM (biomarker for heroin use): 64% BEG (biomarker for cocaine use): 79%	6-monoacetylymorphine (6-MAM): 43.3–570 ng/g Morphine: 8.7–154.8 ng/g Codeine: 7.9–127.9 ng/g Cocaine: 5.6–57.2 ng/g Benzoylecgonine (BEG): 11.7–81.7 ng/g Cocaethylene: 10 ng/g	(Pellegrini et al., 2006)
9	Nicotine (NIC) Cotinine (COT)	Tobacco smoke	Spain	Children N = 64 (Cases: n=16; Controls: n=25) 6–8 yrs	Passive exposure from environment	Shed teeth (Whole)	100%	Nicotine Cases: 27 ng/g; Controls: 14 ng/g Cotinine Cases: 12 ng/g; Controls: 9 ng/g	(Marchei et al., 2008)
10	Clarithromycin (CLR)	Antibiotics	Spain	Adult patients N = 47 (Healthy pulp: n=24; Inflamed pulp: n=11) 18–50 yrs	Clinical administration	Extracted teeth (Pulp)	74% (12 were <LOD)	Healthy pulp: 0.23–1.30 ng/mg Inflamed pulp: 0.24–4.40 ng/mg	(Fos P, 2011)
11	Acetaminophen (ACM) Ibuprofen (IBP) 3,5,6-trichloro-2-pyridinol (TCPy) (metabolite of Chlorpyrifos) 2-isopropyl-6-Methyl-4-pyrimidinol (IMPy) (metabolite of Diazinon) Monoethylhexyl phthalate (MEHP) (metabolite of DEHP)	Analgesics, pesticides, and plastics additive	USA	Children N = 21	Perinatal exposure	Exfoliated or extracted deciduous molars (Tooth crown)	ACM: 43% IBP: 5% TCPy: 10% IMPy: 10% MEHP: 29%	Acetaminophen (ng/g) Median: <0.5, Max: 17.3 Ibuprofen (ng/g) Median: <98, Max: 111 TCPy (ng/g) Median: <5.6, Max: 46 IMPy (ng/g) Median: <2.5, Max: 3.1 MEHP (ng/g) Median: <4.9, Max: 2460	(Camann et al., 2013)
12	Polychlorobiphenyls (PCB) Hexachlorobenzene (HCB) Dichlorodiphenyl-dichloroethylene (4,4′DDE)	Organochlorines	Slovenia	Sheep (Lambs) N = 4 (Cases: n=2; Controls: n=2; 20 primary teeth from each lamb)	Lactation	Primary dental tissues: Maxillae and Mandibles (Pulp, Dentine, and Enamel)	-	PCB-54 and PCB-80: Below limit of detection (0.01 pmol/g dry wt.) in pulp, dentine and enamel, respectively. PCB-155 (pmol/g drywt) Pulp: 0.03 (0.005), Dentine: 0.07 (0.01), Enamel: 0.05 (0.004) PCB-169 (pmol/g drywt) Pulp: 4.85 (0.36), Dentine: 2.13 (0.18), Enamel: 1.62 (0.02) HCB (pmol/g drywt)	(Jan et al., 2013)

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

#	Analyte	Chemical class	Study country	Study population, sample size, & age	Exposure source/route & detection window	Teeth (Analyzed part)	Detection rate (%)	Mean (SD) or Range	Reference
								Pulp: 0.60 (0.04), Dentine: 0.71 (0.06), Enamel: 0.17 (0.008) Pulp: 0.60 (0.04), Dentine: 0.71 (0.06), Enamel: 0.17 (0.008) 4,4′DDE (pmol/g drywt) Pulp: 1.90 (0.14), Dentine: 1.15 (0.11), Enamel: 1.29 (0.08)	
13	Ethyl glucuronide (EtG)	Metabolite of ethyl alcohol	Turkey	Men N = 29 (Controls: n=6; Light users: n=14; Abusers: n=9) 35–60 yrs	Alcohol consumption	Teeth (Whole)	79% (6 were <LOD)	Controls: <0.48 (<LOD) pg/mg Light users: 6.20 (2.32) pg/mg Abusers: 21.6 (1.07) pg/mg	(Zeren et al., 2013)
14	Amoxicillin (AMC) Clindamycin (CLM)	Antibiotics	Germany	Adult patients N = 25 (Amoxicillin-group1: n=11, 25–75 yrs; Clindamycin-group2: n=11, 19–84 yrs; Controls: n=3, 20–29 yrs)	Clinical administration of a single dose	Teeth (Crown and Root)	Group 1: 100% for AMC Group 2: 100% for CLM Controls: 0% for both	Amoxicillin Crown: 0.17 (0.15) μg/g Root: 0.50 (0.30) μg/g Clindamycin Crown: 0.06 (0.03) μg/g Root: 0.27 (0.26) μg/g	(Schussl et al., 2014)

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Environ Res. Author manuscript; available in PMC 2016 October 01.

Table 2

Sample preparation protocols used for the quantification of organic chemicals in tooth using chromatography and mass spectrometry tools.

#	Analyte	Sample collection	Decontamination & pre-treatment (key steps)	Sample weight	Extraction & Cleanup (key steps)	Derivatization	Extraction recovery (%) & Matrix effect	Injection volume (μL)	Reference
1	Polychlorinated biphenyls (PCB)	Teeth were extracted because of orthodontic and dental caries issues Enamel and cementum from dentine were removed with a diamond burr.	Pooled dentine sample of children from each area (meaning pooled sample for cases and controls) was homogenized	2–4 g	Incubation: Dentine treated ultrasonically treated with conc. H_2SO_4 and C_6H_{14} Extraction: The combined hexane extracts were (i) treated with conc. H_2SO_4 and (ii) saponification with KOH in C_2H_5OH Purification: Collected extract was purified on micro-columns of silica and Florisil. Extract was further separated for planar and non-planar PCB using carbon cartridges with C_6H_{14}-CH_2Cl_2 (1:1) and with $C_6H_5CH_3$	-	-	-	(Jan and Vrbic, 2000)
2	Polychlorobiphenyls (PCB) Hexachlorobenzene (HCB) Dichlorodiphenyl-dichloroethylene (4,4′DDE)	Maxillae and mandibles were dissected and frozen Enamel and cementum were extracted from the dentine using a diamond burr	Pulp was separated by cutting the tooth into two halves Dentine and pulp samples were homogenized Powdered samples were dried over P_2O_5 (desiccator, room temperature)	2–4 g of dentine 1 g pulp	Incubation: Powdered dentine sample was treated with conc. H_2SO_4 (30 mL) and hexane (10 mL) for 10 hours. Extraction: Pooled hexane extracts were (i) treated with and (ii) conc. H_2SO_4 saponification with 2.5% KOH in C_2H_5OH (80°C) Purification: The above extract was purified on micro-columns of silica (0.5 cm i.d. × 5 cm length), eluted with 15 mL C_6H_{14}, and passed through Florisil (deactivated with 1.25% H_2O), and eluted with 15 mL of 1.5% CH_2Cl_2 in C_6H_{14}	-	Recovery (%): 75–103%	-	(Jan et al., 2001); (Jan et al., 2006); (Jan et al., 2013)
3	Lidocaine (LID)	Mandibular canine teeth were extracted	Pulp was extripated and stored in a cryotube (frozen with liquid nitrogen)	15 mg	Spike: Internal standard (Bupivacine), 10 μL of 25 μL/mL per 15 mg sample Incubation: 2M NaOH (100 μL) Extraction method: (i) sample was homogenized (1 min), (ii) add $C_5H_{12}O$ (2 μL), (iii) vortex for 5 min, and centrifuge at 3000 rpm for 10 min (4°C). Evaporation: Collect the ether fraction (1–1.5 mL) and dry under nitrogen stream Reconstitution: Mobile phase (CH_3CN/5% (v/v) HCOOH, 25:75) (100μL)	-	Recovery (%): Lidocaine: 89 ± 4% (n=5) Bupivacaine (internal standard): 73 ± 6% (n=5)	20 μL	(Kanjanawattana et al., 2001)
4	Morphine (MOR) Codeine (COD)	Teeth extraction from human remains	Teeth washed and cleaned (to remove blood and soil residues) Crown and root were gently polished to remove external contamination Incubated in distilled water for 24 hours at room temp and rotation. Pulverized with a ceramic mill	1.8 g	Powdered tooth sample was (i) incubated ovemight in 0.25M HCl at 60C, and (ii) hydrolyzed in 0.25M HCl (2 mL) at 50°C for 18hours. Purification: Add 50 μL morphine-d3 (internal standard @ 1μg/mL), and purified with C_7H_{16} -isoamylalcohol (98.5:1.5). Extraction: Solvent discarded and the rest was mixed with $CHCl_3$: C_3H_8O: C_7H_{16} (50:33:17) (4mL). Separation and evaporation: Solvent was separated by centrifugation at 3500 rpm (10 min) and evaporated in a rotating evaporator.	Derivatization: 100 μL penta-fluoranhydride and 70 μL pentafluoro-propanol (15 min at 90C) Reconstitution: 50 μL $C_4H_8O_2$	-	2 μL	(Cattaneo et al., 2003)
5	Nicotine (NIC) Cotinine (COT)	Donated shed teeth	3 × 2mL CH_2Cl_2 Ball mill pulverization: 90amp.× 10min.	80–150 mg	Spike: Internal standards (i) 1′-N-ethylnotnicotine perchlorate (NENC) and (ii) 1′-N-ethylnorcotinine (NENN), 10 μL/sample Incubation: 1M NaOH (1mL), 80°C, 30min. Extraction: Liquid-liquid extraction Extraction method: (i) add 5 mL CH_2Cl_2, vortex for 1 min, centrifuge at 2000g for 5 min, (ii) collect organic layer and mix with 0.2M HCl (1.5 mL), vortex for 1 min, centrifuge at 2000g for 5 min, (iii) collect aqueous layer and mix with 6M NaOH (0.25 mL), extract with 5mL CH_2Cl_2, vortex for 1 min, centrifuge at 2000g for 5 min, and (iv) add 0.5mL methanolic-HCl (25mM) to prevent loss of nicotine. Evaporation: Nitrogen stream and 40°C bath Reconstitution: CH_3OH/$C_6H_5CH_3$, 7:3 v/v) (3 μL)	-	3 QC samples of 6, 12, and 30 ng/g spiked in both water and blank teeth (NIC and COT free) Recovery (%): NIC: 95% COT: 79% NENN: 87% NENC: 82%	3 μL	(Garcia-Algar et al., 2003); (Pascual et al., 2003)
6	Opiates Cocaine	Teeth extracted because of dental issues such as caries	Cleaned and washed in (i) hypochlorite, (ii) saline, and (iii) distilled water solutions to remove blood remains; dried and stored in plastic tubes at room temp Pulverized with a ball mill (30 freq/min, 3.5min)	1g	Spike: Internal standard (Nalorphine), 5 μL/1 g sample Incubation: 0.1M HCl (2mL), 37°C, 18 hrs. pH: centrifuged for 10 min at 3500 rpm and adjusted to 6.0 with 1N NaOH (20 μL) Extraction: Liquid-liquid extraction Extraction method: two volumes of 3 mL $CHCl_3$: C_3H_7OH (9:1), vortex for 2 min, centrifuge at 3500 rpm for 10 min Evaporation: Collect the organic layer and dry under nitrogen stream (40°C)	Derivatization: 50 μL BSTFA and 1% TMCS (30 min at 70C)	Recovery (%): 6-MAM: 89–92% Morphine: 73–75% Codeine: 91–92% Cocaine: 81–83% BEG: 80–86% Cocaethylene: 78–82% Nalorpine: 98% Neither matrix effect nor a carryover was observed for the study analytes	1 μL	(Pellegrini et al., 2006)
7	Nicotine (NIC) Cotinine (COT)	Donated shed teeth	3 × 2mL CH_2Cl_2 Ball mill pulverization: 30amp.× 3.5min.	20 mg	Spike: Internal standard (1′-N-ethylnorcotinine), 1 ng/20 mg sample Incubation: 1N NaOH (1mL), 80°C, 30min. pH: adjusted to 6.0 Clean-up: Solid phase extraction using Isolute HCX columns, conditioned with CH_3OH, deionized H2O, and conc. CH_3COOH (3/3/1 mL) Elution: CH_2Cl_2/C_3H_7OH (80:20, v/v) (3 mL) + 2% NH_4OH	-	3 QC samples of 15, 150, and 225 ng/g spiked in both water and blank teeth (NIC and COT free), 5 replicates Recovery (%): NIC: 75–78% COT: 87–88%	30 μL	(Marchei et al., 2008)

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

#	Analyte	Sample collection	Decontamination & pre-treatment (key steps)	Sample weight	Extraction & Cleanup (key steps)	Derivatization	Extraction recovery (%) & Matrix effect	Injection volume (μL)	Reference
					Evaporation: Nitrogen stream Reconstitution: Mobile phase (0.01% HCOOH/CH_3OH/C H_3CN, 73:25:2 v/v/v) (100μL)				
8	Clarithromycin (CLR)	Extracted teeth were used to collect the pulp (inflamed or normal)	Extracted pulp was placed in Eppendorf tubes, weighed and frozen at −85°C.	Max wt. pulp: 85mg	Reconstitution: Extracts were dissolved in 500 μL (further details are unavailable)	-	-	-	(Fos P, 2011)
9	Acetaminophen (ACM) Ibuprofen (IBP) 3,5,6-trichloro- 2-pyridinol (TCPy) (metabolite of Chlorpyrifos) 2-isopropyl-6-Methyl-4-pyrimidinol (IMPy) (metabolite of Diazinon) Monoethylhexyl phthalate (MEHP) (metabolite of DEHP)	Extracted or shed teeth	Pulp was scraped out Crown was detached Removed any fillings, roots, cavities Crown was rinsed with CH_2Cl_2	50 mg	I. Sample preparation for LC-MS/MS analysis: Spike: Internal standard (Acetaminophen-d_4), 5 ng/sample pH of the extraction: Acidic, alkaline and neutral pH were used for extracting multiclass organic chemicals from pulp Extraction 1: Sample sonication with CH_3CN (0.5 mL), collect CH_3CN fraction, acidify with CH_3COOH, and equilibrate overnight Extraction 2: Sample sonication with CH_3CN (0.5 mL), collect CH_3CN fraction, mix with acidified extract from 1, and obtain a neutral extract Extraction 3: Sample sonication with CH_3CN (0.5 mL), collect CH_3CN fraction, add NH_4OH for alkalinity, and equilibrate overnight Pooled extract: Combine the extracts from 1, 2 and 3; and make up the volume to 100 μL for LC-MS/MS analysis II. Sample preparation for GC/MS analysis: Spike: Internal standards of PCBs were spiked to 50 mg of pulp sample Extraction: (i) Soxhlet-extraction with C_6H_{14}: C_2H_6CO (1:1), (ii) concentrated to 1 mL, (iii) Florisil column for cleaning, and (iv) nonane addition to concentrate the analytes (0.25 mL)	-	Matrix spike recovery (%) ACM: 53–69% IBP: 59–78% % TCPy: 104–22% IMPy: 19–36% MEHP: 81–126%	-	(Camann et al., 2013)
10	Ethyl glucuronide (EtG)	Extracted tooth separated from dentin-containing root	Pulverization: Extracted teeth plus steel ball bearings (5–6, 2mm dia) in a capped vial, and placed in a high energy cell disrupter (Mini-Bead-Beater-8) for 3 min.	50 mg	Extraction: Weighed sample powder was mixed with CH_3CN and H_2O (1:1), and sonicated for 2h at 25°C. Ethyl glucuronide-(internal d_5 standard) was spiked at 50 μL, vortexed, and centrifuged (4000 rpm, 10 min). Supernatant (2 mL) was further extracted with a mixture of CH_3CN and H_2O (1:1).	-	-	-	(Zeren et al., 2013)
11	Amoxicillin (AMC) Clindamycin (CLM)	Extraction of decayed tooth by periodontitis Deep frozen in liquid nitrogen and stored at −80°C	Cleaning: Teeth surface cleaned with sterile swabs for removing blood and saliva, and adherent tissue was removed with scalpels and bone curettes. Separation: Teeth were separated into the crown and root sections with a band saw. Heating was avoided during teeth sawing. Pulverization: Crown and root were powdered with a bone mill. Storage: Sample powder was frozen in liquid nitrogen and stored at −80°C	200 mg for AMC 100 mg for CLM	Extraction: Pulverized crown or root sample was extracted with a phosphate buffer (pH 7.4) for an hour in a cooled ultrasonic bath Clean-up: Solid phase extraction of the supernatant using Oasis HLB cartridges (60mg, 3mL)	-	-	10 μL for AMC 30 μL for CLM	(Schussl et al., 2014)

Abbreviations: BSFTA: N,O-Bis(trimethylsilyl)trifluoroacetamide; C_2H_5OH: ethanol; C_2H_6CO: acetone; C_3H_7OH: isopropanol; $C_4H_8O_2$: ethyl acetate; $C_5H_{12}O$: tert-butyl methyl ether; C_6H_{14}: hexane; $C_6H_5CH_3$: toluene; C_7H_{16}: n-heptane; CH_2Cl_2: dichloromethane; CH_3CN: acetonitrile; CH_3COOH: acetic acid; CH_3OH: methanol; $CHCl_3$: chloroform; H_2O: water; H_2SO_4: sulfuric acid; HCl: hydrochloric acid; HCOOH: formic acid; KOH: potassium hydroxide; P_2O_5: phosphorus pentoxide; NaOH: sodium hydroxide; NH_4OH: ammonium hydroxide; TMCS: trimethylchlorosilane

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Environ Res. Author manuscript; available in PMC 2016 October 01.

Table 3

Bioanalytical procedures based on chromatography and mass spectrometry principles for the determination of tooth bio-matrix for organic chemicals.

#	Analyte	Instrumentation & detection mode	Chromatography column	Chromatography conditions	Detector or Mass spectrometry conditions	Analytical performance	Reference
1	Polychlorinated biphenyls (PCB)	High resolution gas chromatography Electron capture detection	60m × 0.25mm (i.d.) fused silica capillary column coated wiSPB-5.	Split-splitless injection Flow gas: N_2. GC injector: 280°C, Oven temp program: 55C for 1.1 min, 200°C at 15°C/min, and 235°C at 1.5°C/min. Purge activation time: 1.5min.	63-Ni electron capture detector	-	(Jan and Vrbic, 2000)
2	Polychlorobiphenyls (PCB) Hexachlorobenzene (HCB) Dichlorodiphenyl-dichloroethylene (4,4′DDE)	High resolution gas chromatography Electron capture detection	SPB-Octyl and SPB-5 fused silica columns (60m × 0.25mm i.d.; 0.25 μm thickness)	Split-splitless injection Carrier gas: H_2 Makeup gas: N_2	63-Ni electron capture detector	Limit of detection: PCB-54, 80, 155, 155, and 169, HCB, and 4,4′-DDED: 0.01 pmol/g dry wt.	(Jan et al., 2001); (Jan et al., 2006); (Jan et al., 2013)
3	Lidocaine (LID)	HPLC-UV detector	μBondasphere C8 column	Mobile phase (CH_3CN/5% (v/v) HCOOH, 25:75) pH: The 5% glacial HCOOH was adjusted to pH 3.0 with 2 M NaOH 28C column temp Flow: 1.0 mL/min Retention time: Lidocaine: 3.54 min Bupivacaine: 8.79 min	Detector: Ultraviolet absorbance detector Detection wavelength: 254 nm Sensitivity: 0.02 absorbance units per 100% scale	Limit of detection: Lidocaine: 0.1 μg/mL Intra-day variation: 7.0% and 3.2% for 1 and 10 μg/mL Lidocaine spike Inter-day variation: 5.2% and 3.1% for 1 and 10 μg/mL Lidocaine spike	(Kanjanawattana et al., 2001)
4	Morphine (MOR) Codeine (COD)	GC/MS	MS5 (12m × 0.2mm i.d.; 0.33 μm thickness)	Pulsed splitless mode Injection port: 260°C Carrier gas: Helium, 1mL/min Oven temp program: 70°C for 2 min, 20°C/min to 200°C, successive 8 min increments up to 250°C, and a final 40°C/min to 280°C. 1 min at 60°C, 30°C/min to 300°C, 1 min at 300°C	Ion source: 230°C Quadrupole temp: 150°C Selected ion monitoring (SIM) m/z: Morphine: 414, 430, 577 Codeine: 266, 282, 445 Morphine-d3 (internal standard): 417, 433, 580	-	(Cattaneo et al., 2003)
5	Nicotine (NIC) Cotinine (COT)	GC/MS EI mode	Zerbon 5% dimethyl-polysiloxane capillary column (15m × 0.25mm i.d.; 0.25μm thickness)	Split mode: 1:10 ratio Carrier gas: Helium, 0.7 mL/min Injector temp: 280°C	Ion source: 230°C Quadrupole temp: 150°C Electron impact: 70eV	Limit of detection: NIC: 0.35 ng; COT: 0.31 ng Limit of quantification: NIC: 1.2 ng; COT: 1.1 ng RSD of precision:	(Garcia-Algar et al., 2003); (Pascual et al., 2003)

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

#	Analyte	Instrumentation & detection mode	Chromatography column	Chromatography conditions	Detector or Mass spectrometry conditions	Analytical performance	Reference
				Oven temp program: 1 min at 60°C, 30°C/min to 300°C, 1 min at 300°C Oven temp program: 1 min at 60°C, 30°C/min to 300°C, 1 min at 300°C Oven temp program: 1 min at 60°C, 30°C/min to 300°C, 1 min at 300°C	Selected ion monitoring (SIM) m/z: NIC: 84(quant), 133(qual) COT: 98(quant), 176(qual) NENN:98(quant), 161(qual) NENC: 112(quant), 190(qual)	Intra-assay: 2.8–9.1 Inter-assay: 3.6–9.6 Error (%) of accuracy: Intra-assay: 3.5–8.7	
6	Opiates Cocaine	GC-MS EI mode	HP-5MS column (30m × 0.25mm i.d.; 0.25μm thickness	Split mode: 1:15 ratio Carrier gas: Helium, 1.0 mL/min Injector temp: 260°C Oven temp program: 1 min at 80°C, 35°C/min to 230°C, 10°C/min t0 290°C, and held for 10 min	Ion source: 230°C Quadrupole temp: 150°C Interface temp: 280°C Selected ion monitoring (SIM) m/z: 6-MAM: 399 (quant), and 287 and 340 (qual) Morphine: 429 (quant), and 236 and 401 (qual) Codeine: 371 (quant), and 196 and 234 (qual) Cocaine: 303 (quant), and 82 and 182 (qual) BEG: 361 (quant), and 82 and 240 (qual) Cocaethylene: 196 (quant), and 272 and 317 (qual) Nalorphine (internal standard): 455 (quant), and 212 and 312 (qual)	Limit of detection and limit of quantification: 6-MAM: 2.5 ng/g,7.5 ng/g Morphine: 2.5 ng/g, 7.5 ng/g Codeine: 2.0 ng/g, 6.0 ng/g Cocaine: 2.5 ng/g, 7.5 ng/g BEG: 2.5 ng/g, 7.5 ng/g Cocaethylene: 2.5 ng/g, 7.5 ng/g RSD of precision: Intra-assay: 6-MAM: 5.7–12.1 Morphine: 1.0–14.8 Codeine: 2.4–7.2 Cocaine: 5.4–10.6 BEG: 3.8–12.6 Cocaethylene: 2.3–14.4 Inter-assay: 6-MAM: 3.7–7.3 Morphine: 0.6–7.2 Codeine: 1.2–4.4 Cocaine: 3.3–6.1 BEG: 1.4–8.2 Cocaethylene: 3.3–9.5 Error (%) of accuracy: Intra-assay: 6-MAM: 0.7–1.7 Morphine: 2.1–12.5 Codeine: 6.9–10.0 Cocaine: 3.7–9.2 BEG: 7.5–12.5 Cocaethylene: 7.5–11.8 Inter-assay: 6-MAM: 1.9–2.5 Morphine: 1.8–13.3 Codeine: 6.3–10.8 Cocaine: 4.2–10.0	(Pellegrini et al., 2006)

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

#	Analyte	Instrumentation & detection mode	Chromatography column	Chromatography conditions	Detector or Mass spectrometry conditions	Analytical performance	Reference
						BEG: 7.7–11.7 Cocaethylene: 6.6–11.5	
7	Nicotine (NIC) Cotinine (COT)	LC-MS/MS ESI mode	Eclipse XDB-C8 (100 x 3.0mm, 3.5 μm)	Mobile phase: 0.01% $HCOOH/CH_3OH/CH_3$ CN (73:25:2 v/v/v) Flow rate: 0.3mL/min	Collision energy (eV) Nicotine (NIC): 15; Cotinine (COT): 18; 1′-N-ethylnorcotinine (NENC): 20 Capillary voltage: 3.0 kV Cone voltage: 25V Source temp: 120C Desolvation temp: 400C Cone gas: 50L/h Desolvation gas: 400L/h Collision gas: Argon, 0.25 Pa m/z transitions: NIC: 163=>132 (quant), and 163=>120 and 163=>106 (qual) COT: 177=>80 (quant), and 177=>146 and 177=>98 (qual) NENC: 191=>120 (quant), and 191=>148 and 191=>80 (qual)	Limit of detection: NIC: 3.3 ng/g; COT: 1.6 ng/g Limit of quantification: NIC: 10.0 ng/g; COT: 5.0 ng/g RSD of precision: Intra-assay: 0.7–13.8 Inter-assay: 5.1–14.9 Error (%) of accuracy: Intra-assay: 7.0–14.4 Inter-assay: 4.6–14.5	(Marchei et al., 2008)
8	Clarithromycin (CLR)	RP-HPLC/MS ESI (at atmospheric pressure)	Zorbax Eclipse XDB-C8 column (4.6 x 150 mm, 5μm particle size)	Mobile phase: CH_3CN/ HCOOH 0.1% (70/30 v/v) 40C column temp Flow: 0.5 mL/min RT: 2.4 min	Extracted ion m/z: 748.5	Limit of quantification: 3ng/mL	(Fos P, 2011)
9	Acetaminophen (ACM) Ibuprofen (IBP) 3,5,6-trichloro-2-pyridinol (TCPy) (metabolite of Chlorpyrifos)	LC-MS/MS ESI mode (positive: ACM) (negative: IBP, TCPy, IMPy, MEHP)	NA	NA	NA	Quantification limit: ACM: 0.5 ng/g IBP: 98 ng/g TCPy: 5.6 ng/g IMPy: 2.5 ng/g MEHP: 4.9 ng/g	(Camann et al., 2013)

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

#	Analyte	Instrumentation & detection mode	Chromatography column	Chromatography conditions	Detector or Mass spectrometry conditions	Analytical performance	Reference
	2-isopropyl-6-Methyl-4-pyrimidinol (IMPy) (metabolite of Diazinon) 2-isopropyl-6-Methyl-4-pyrimidinol (IMPy) (metabolite of Diazinon) 2-isopropyl-6-Methyl-4-pyrimidinol (IMPy) (metabolite of Diazinon) Monoethylhexyl phthalate (MEHP) (metabolite of DEHP)						
10	Ethyl glucuronide (EtG)	RP-HPLC/MS/MS ESI mode (negative)	Two serially connected Zorbax Hilic Plus (4.6 × 100 mm, 3.5µm particle size)	Mobile phase: Solvent A: NH_4Ac (1mM) Solvent B: CH_3CN Flow: 0.8 mL/min 25°C column temp Isocratic: Solvent B at 65% from 0.0 min to 2.2 min.	Capillary voltage: 4000V Nozzle voltage: 0V Desolvation gas (also as sheath gas): N2, heated to 350°C, and flow rate 11 L/min Nebulizer gas pressure: 50 psi Multiple reaction monitoring (MRM) m/z: EtG: 221=>75 (quant) and 221=>85 (qual) EtG-d5: 226=>75 (quant) and 226=>85 (qual) Fragment voltage: 100V Collision energy: 12V	Limit of quantification: EtG: 1.61 ng/g Limit of detection: EtG: 0.48 ng/g Precision and accuracy: Within acceptable range (±15%)	(Zeren et al., 2013)
11	Amoxicillin (AMC) Clindamycin (CLM)	LC-MS/MS	Luna PFP2 column (150 × 3 mm, 100 A)	Mobile phase: Solvent A: HCOOH (0.1% v/v) with $HCOONH_4$ (1mmol/L) Solvent B: CH3CN + HCOOH (0.1%) (95:5 v/v) with $HCOONH_4$ (1mmol/L) Gradient: 12 mins Flow: 0.25 mL/min	Multiple reaction monitoring (MRM) m/z: AMC: 366=>114, 366=>134, and 366=>349 CLM: 425=>126, 425=>377, and 425=>425 Amoxicillin (internal standard): 350=>106	Limit of quantification: AMC: 5.0 ng; CLM: 0.5 ng	(Schussl et al., 2014)

Abbreviations:

EI: electron impact ionization; ESI: electrospray ionization; GC/MS: gas chromatograph coupled with a mass selective detector; GC-MS/MS: gas chromatograph coupled with a triple quadrupole mass spectrometer; LC-MS/MS: liquid chromatograph coupled with a triple quadrupole mass spectrometer.