

## REVIEW

# Urine-sampling methods for environmental chemicals in infants and young children

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This review paper examines and evaluates urine-sampling methodologies in infants and young children, to determine which methods are suitable for use in large biomonitoring surveys or studies of environmental chemicals in children younger than 6 years. Methods for non-toilet-trained children include the use of urine bags, collection pads (e.g., cotton or gauze inserts), disposable diapers, cotton diapers, and the clean catch method. In toilet-trained children, collection methods include use of a commode insert pan as well as specimen collection cups. The advantages and disadvantages of these various methods need to be evaluated with respect to the target population, timing and frequency of collection, minimum sample volume required, method of urine extraction, potential for contamination of the sample, stability of the analyte of interest, and burden on participants and research team. Collection methods must not introduce contamination or affect the integrity of the sample, should be logistically practical, and should minimize discomfort experienced by the child. Although collection of urine samples from children who are not toilet-trained is more challenging than collection from older toilet-trained children, the vulnerability of younger children to the exposure to and health effects of environmental chemicals makes finding suitable methods a priority.

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Children differ from adults in the nature of their exposures to environmental chemicals as well as their susceptibility, because of various factors including diet, behavior, physiology, and metabolism (Needham and Sexton, 2000). Thus, there is a clear need to develop appropriate methods to evaluate exposures in children (Fenske et al., 2000). A method that is best for assessing exposure to a given chemical at one life stage (e.g., *in utero*) may not be the best method for assessing exposure to that same chemical at a different life stage (e.g., 2 years of age) (Needham et al., 2005).

The purpose of this review is to examine and evaluate urine-sampling methodologies in young children, to determine which methods would be suitable for use in large surveys or studies that would obtain biomonitoring data in children younger than 6 years. Methods must be both acceptable to the participants and cost-effective on a large scale. Furthermore, the approach taken for collecting the

urine must not introduce any contamination or affect the integrity of the sample.

The focus of this review is appropriate and reliable urine collection methods for biomonitoring of environmental chemicals in young children; however, urine collection methods used for the diagnosis of clinical illness have also been included to supplement the review. Each urine collection methodology must be evaluated in terms of target population (i.e., toilet-trained *versus* non-toilet-trained children), timing and frequency of collection (i.e., single spot urine voids *versus* 24-h urine collection), minimum sample volume required, method of urine collection (i.e., ease of field collection for both boys and girls) (Matt et al., 1999), potential for contamination of the sample, stability of the analyte(s) of interest, and burden on participants, and the research team.

Evaluating whether urine is the most appropriate matrix for assessing body burden to a particular chemical is beyond the scope of this review.

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## Urine sampling

The younger the child, the more difficult it is to conduct biomonitoring studies. Compared with other matrices such as blood, urine collection is generally more acceptable to parents and children, less invasive, and relatively easy to

collect (Matt et al., 1999; Bradman and Whyatt, 2005; Fenske et al., 2005). Urine samples are routinely collected easily and usually without incident from infants and young children in clinical settings, such as hospital neonatal intensive care units (Burke, 1995).

Daily urine outputs for various age groups are as follows: infants and toddlers up to 2 years of age rarely greater than 500 ml; children 3–5 years up to 700 ml; and 5–8 year olds 1000 ml per day (Bolte, 2005). Infants void six to thirty times per day with the number of voids decreasing and volume increasing as the child ages (Bolte, 2005). From birth to 1 year of age, the volume of urine available for collection in a single void is generally less than 10 ml per sample, and is somewhat larger (10–20 ml) for 2 to 3 year olds (Barr et al., 2005a). Once children begin school, urine sample collections become a little easier with between 30 and 50 ml reasonably obtainable for those aged 4–11 years in a single void (Barr et al., 2005a). Daily estimated volumes of urine output in children have been discussed further by Baker et al., 2005; however, these authors note that a normal range is difficult to estimate as it depends on age, kidney function, water and liquid intake, and other factors.

Collection of complete 24-h urine samples has generally been viewed as impractical for small children (Fenske et al., 2005; Kissel et al., 2005) and presents problems such as contamination, leakage, and may result in dilution of the analyte if excreted immediately after exposure. Reported losses have ranged up to 40% (Bakker et al., 1999). Spot urine samples are the easiest to collect for biomonitoring purposes as it places low burden on study participants and sample processing and analyses are manageable and affordable (Fenske et al., 2005). However, spot urine samples show high intra-individual variability, both in terms of urine volume obtained and concentrations of endogenous and exogenous chemicals captured from void to void, particularly when compared with estimates based on 24-h samples (Kissel et al., 2005; Barr et al., 2005a; Scher et al., 2007). If a spot sample is obtained, a first-morning void is often preferred because the urine is more concentrated, represents a longer window of accumulation (usually greater than 8 h) and is more representative of the daily average (Fenske et al., 2005; Kissel et al., 2005; Barr et al., 2005a). However, a first-morning collection may not always be ideal because the timing for capturing the exposure is “off”; this collection may not correspond to time-dependent variations in the analyte concentrations and could represent an underestimation of exposure. Investigators often settle for taking a sample whenever it is convenient (Needham and Sexton, 2000).

#### *Urine Dilution Adjustment*

The degree of dilution of the urine sample is critical in interpreting results of tests performed on the specimen. Measurement of osmolality has been identified as the gold

standard for urine concentration (Chadha et al., 2001); however, in practice other methods such as specific gravity adjustment, timed excretion, and creatinine ratios are often used to adjust for effects of urinary flow (Vij and Howell, 1998). Intra-individual variability may be reduced for very young children (e.g., <12 months) because they feed and urinate frequently; however, variability in urinary dilution has not been evaluated for this very young age group (Bradman and Whyatt, 2005).

Creatinine adjustment using a colorimetric assay has been the standard method for accounting for urine dilution for environmental chemicals; however, as a number of factors such as age, gender, race/ethnicity, meat intake and body mass index affect creatinine levels, results must be interpreted with caution (Curl et al., 2003; Suwazono et al., 2005; Barr et al., 2005b). Creatinine adjustment to account for dilution may not be appropriate for some chemicals in young children (or pregnant women) and creatinine-adjusted metabolite concentrations should never be compared among individuals of vastly different age groups (i.e., children *versus* adults) (Fenske et al., 2005; Pearson et al., 2009). Creatinine excretion during the newborn period is reportedly higher and more variable compared with later infancy (Dorey and Zimmermann, 2008) and the limitations of standardizing urinary excretion of solutes during this period has been previously documented (Matos et al., 1999). Creatinine measurements also consume more (minimum 200  $\mu$ l) of the very limited volume of urine available for infants than specific gravity (minimum 100  $\mu$ l) and are more costly to perform.

In some cases, specific gravity assessments may be a more robust measure to correct for urinary output as it introduces less variability (appears to be independent of age and seasonal variation) and is very easily analyzed using a handheld refractometer (Pearson et al., 2009). Specific gravity is the ratio of the density of the urine to that of water and is affected by the number and weight of solute particles (urea, chloride, sodium, potassium, phosphate, uric acid, and sulfate) and on the temperature of the sample, in contrast to urine osmolality which depends solely on the number of solute particles (Pradella et al., 1988). Specific gravity may not be appropriate for study participants with certain conditions (e.g., diabetes and nephrotic syndrome (Voinescu et al., 2002)) where heavy molecules such as glucose and protein may disproportionately increase the measurement (Pearson et al., 2009) resulting in an underestimate of the urine analyte concentration. Good correlations ( $r = 0.933$ ) have been observed between urine specific gravity and osmolality in neonates (Leech and Penney, 1987). A modified specific gravity adjustment method has been proposed that applies weights for each substance depending on how the excretion rate varies with changes in urinary flow (Vij and Howell, 1998). The conventional method requires the assumption that a change in urine flow

preserves the relative ratio between the mass of the chemical substance and the mass of total dissolved solids (Vij and Howell, 1998) which has been shown to result in over-compensation, at least for cadmium (Sorahan et al., 2008).

The robustness of both the creatinine and specific gravity readings to freeze–thaw cycles (Anestis et al., 2009; Pearson et al., 2009) and to temperature fluctuations has been debated (Alessio et al., 1985; Riboli et al., 1995). Urine specific gravity does appear to be more stable than creatinine over storage temperatures ranging from  $-20$  to  $93^{\circ}\text{C}$  (Cook et al., 2007). More research is needed to evaluate various approaches for accounting for urine dilution in neonates and young children.

### Non-toilet trained children

Young children, particularly those in the first 2 years of life who are not toilet trained are a particularly difficult subpopulation to sample as they are unable to void on command (Schlager et al., 1995), it can be a challenge to obtain parental consent (Needham and Sexton, 2000), the collection procedures can be problematic and infants tend to soil and urinate at the same time. Five different methods that have been used to successfully obtain urine samples from non-toilet trained children are described below.

#### *Urine Bag*

A common method used in pediatric hospitals and a number of clinical studies is a collection bag attached over the genitals to the perineal skin using adhesive tape. In the CHAMACOS study, staff washed the pudendum, attached the urine bag, slit a diaper and pushed the bag through the diaper slit so that urine was collected in the urine bag but staff could see when the bag was full (Eskenazi et al., 2003). Urine bags commercially available include: “LilKatch” (General Medical Corp., Richmond, VA, USA) (Loewenherz et al., 1997; Fenske et al., 2002); “U-Bag” (Hollister Inc., Libertyville, IL, USA) (Schlager et al., 1995; Brock et al., 2002; Royster et al., 2002), and “Pediabags” (Kendall Medical Products, Mansfield, MA, USA) (Matt et al., 1999). Clearly, urine bags and transfer containers should be pre-screened and found to be devoid of the contaminant or metabolite of interest (Brock et al., 2002).

The use of urine bags is relatively simple, reliable, non-invasive, and inexpensive for obtaining a urine specimen (Schlager et al., 1995). However, at times, the adhesive may not adhere adequately or may cause discomfort to infants with skin disorders (Cohen et al., 1997). A lively infant can also displace the bag. In addition, urine bags are seldom useful in children who have diarrhea, incontinence, poor hygiene, or where the bag must remain attached for a considerable length of time (Cohen et al., 1997). Other disadvantages include

possible allergic reactions to the adhesive solutions and other irritating effects on the skin (Burke, 1995).

Obtaining a sufficient volume of sample may also be a concern. In one study (Schlager et al., 1995), 17% of the neonates were not able to provide a urine sample after two attempts of 30 min each, whereas in another (Royster et al., 2002) only 70% of those contacted were both willing to participate and produced sufficient urine volumes. Therefore, tactics to encourage urination within a given time frame may be required, such as offering the child a beverage (Royster et al., 2002; Barr et al., 2005a); however, encouraging urination with drinks will usually dilute the urine and make the analytical measurement more difficult (Barr et al., 2005a).

If children are not able to produce a void during scheduled contacts, the staff can train parents to attach the urine bag at home, transfer the urine to the appropriate container, and refrigerate or freeze the sample until delivered to or retrieved by the field staff (Loewenherz et al., 1997; Fenske et al., 2002, 2005). Eskenazi et al. (2003) reported that using a combination of urine collections at the clinic visit and, in very rare instances, sending the infant home with the urine bag attached and providing instructions for the mother to collect the sample herself resulted in missing less than 2% of the urine samples.

#### *Clean Catch*

The clean catch method has been defined as the gold standard in non-invasive methods for collecting urine for urinary tract diagnosis in non-toilet-trained children (Rogers and Saunders, 2008). With this method, a urine sample is collected by holding a sterile specimen bottle in the urine stream. The technique depends on inducing a voiding reflex by applying gently repetitive suprapubic pressure (Morris et al., 2007). Given the human resource requirements to collect the urine from the infant, this method would not be practicable for a large-scale study; however, in a study of 191 children with a median age of 2 months, urine was collected successfully by clean catch in 88%, and by urine bags and pads in 96% of the infants (Alam et al., 2005). These three methods were used consecutively with the parents trained to collect the clean catch sample (parents had a 30 ml urine container ready to collect urine from the infant who sat on their lap without a diaper) and study nurses assisted with collecting urine from the pads and bags (Alam et al., 2005).

#### *Urine Collection Pads: Cotton or Gauze Insert*

The addition of a cotton or gauze insert into an infant's disposable diaper is a relatively non-invasive method that does not cause adverse effects on the skin (Burke, 1995). The pad should be checked frequently (every 10 min) until the pad is wet with urine but not soiled by feces (Dorey and Zimmermann, 2008). Use of a cotton insert is relatively simple as the urine may be expressed on-site and the pad may be removed fairly easily from the diaper for analysis

(Hu et al., 2000). In addition, cotton will maintain the stability of the urine sample for at least 24 h at 37°C (Hu et al., 2000), and will not interfere/react with urinary creatinine concentration nor the concentrations of most chemicals used for clinical diagnoses (Roberts and Lucas, 1985; Hu et al., 2000).

The overall reliability of cotinine measurements is reportedly good and no bias was introduced from the use of cotton rolls placed in a syringe and expressed, compared with a standard method where urine was transferred by a standard pipette (Matt et al., 1999). Furthermore, the percentage recoveries of creatinine and metabolites have been shown to be acceptable, ranging from 70–130% in the laboratory, although recoveries depend on the analyte of interest (Hu et al., 2000). Additional advantages of a cotton ball or gauze insert include cost effectiveness of supplies and a substantial decrease in the time spent by a health care professional to apply the material, as compared with the use of a urine collection bag (Burke, 1995).

Some investigators have incorporated a sensor for a personal enuresis alarm buried in the matrix of the urine collection pad to try to reduce potential contamination of the urine by contact with the infant's skin and fecal matter, with limited success (Rao et al., 2003). The only apparent advantage was that it reduced the need to disturb the child for frequent checking of the pad. Replacing a urine collection pad every 30 min until urine is passed can reduce bacterial contamination of the urine; however, this method does increase the workload of staff and add to the costs, thus reducing feasibility (Rao et al., 2004).

There are a number of limitations to the cotton insert. Obtaining a sufficient volume of urine may be problematic if the pad is not saturated sufficiently—only 3–12 ml of urine can be extracted from the pad even though up to 170 ml of urine is voided by a 1- to 3-year-old child at any one time (Hu et al., 2004). Dorey and Zimmermann (2008) were able to extract approximately 2 ml of urine from cotton pads. To some extent the insufficient sample obtained can be corrected with the addition of water to the cotton pad and successive extractants, with back-calculations of creatinine concentration used to ensure unbiased normalization (Hu et al., 2000).

Several methods for extracting urine from the cotton insert have been used. The cotton insert may be frozen and shipped to the laboratory, where it is centrifuged to extract urine, although the volume recovered is often small (Calafat et al., 2004). Urine may also be extracted from the cotton pad through use of a disposable syringe on-site (Hu et al., 2000; Shalat et al., 2003; Zohouri et al., 2006) by placing the gauze into an empty syringe, replacing the plunger and squeezing urine into vials (Weuve et al., 2006). Alternatively, the urine can be aspirated from the pad using a syringe (Shalat et al., 2003; Dorey and Zimmermann, 2008).

Finally, contamination of the cotton pad by fecal material or by any lotions or powders applied to the baby has not been sufficiently addressed by researchers. Burke (1995) has

suggested that a thorough cleaning of the diaper area should be performed to minimize contamination with stool and other materials to improve accuracy of results.

Cotton inserts that have been used include: “Surgipad” (Johnson & Johnson, Skillman, NJ, USA), “Organic Diaper Doublers” cotton terry cloth inserts, and pads from Ontex Ltd. Northamptonshire, UK (Hu et al., 2000; Shalat et al., 2003; Zohouri et al., 2006).

#### *Disposable Diapers*

The third method to obtain urine samples in non-toilet-trained children includes extraction of urine directly from the infant diaper. A wet diaper may be brought in by a parent on the day of the study (Sathyanarayana et al., 2008), or the diaper may be wet on-site.

Hu et al. (2004) reported that pyrethroid metabolites were stable in diapers during a period of overnight urine collection (37°C for at least 8 h) and during storage at 1°C for at least 3 days. However, the method of extraction from disposable diapers is more problematic. The absorbent pad in the diaper consists of polyacrylate powder, which when wetted, uncoils to absorb water and swell into a three-dimensional gel-like material which makes extraction and analysis of urine very difficult (Hu et al., 2004). Extraction of urine can be done by either squeezing the wet diaper into a receptacle, or using a syringe to aspirate the sample from the diaper. In addition, diaper fibers can be inserted into the barrel of a syringe and compressed to extract urine (Ahmad et al., 1991; Cohen et al., 1997; Sathyanarayana et al., 2008). Moreover, in the laboratory, the addition of calcium salts has been shown to be effective in shrinking or collapsing these polymers and extracting the urine (Hu et al., 2004).

The brand of disposable diapers can affect the ease of urine extraction. Hu et al., (2004) observed more difficulty in separating the absorbent pads from top and bottom sheets for Pampers Premium, compared with Huggies Supreme. Furthermore, the increased absorbency of super-absorbent diapers limits the ability to extract the urine. Absorbent matter in super-absorbent diapers may come through with the urine during syringe aspiration, possibly compromising the analysis (Burke, 1995). Ahmad et al. (1991) deemed ultra-absorbent diapers unsuitable for urine extraction and similarly Cohen et al. (1997) excluded the use of ultra-absorbent diapers because of the difficult and time-consuming task of urine extraction from the gel matrix. Kirkpatrick et al. (1997) noted that urine recovered from ultra-absorbent diapers had significantly higher specific gravity values, pH and protein compared with cotton balls and non-absorbent gelling material diapers. Furthermore, the specific gravity of urine may also vary with the brand of diaper used, urine volume, and amount of time spent in the diaper before extraction (Gammage and Yarandi, 1993).

Authors have published on various techniques used when conducting direct urine extraction from diapers. Soden et al.

(2007) used untreated wood pulp-based study diapers (TenderCare) to collect urine from children who were not fully toilet-trained. Hu et al. (2004) noted that the best type of disposable diaper to use for analysis was the style that permits the absorbent pads to be easily separated from the porous polypropylene top and back sheets, such as Huggies Supreme diapers. Lombardero et al. (1993) extracted urine from Ultra Pampers diapers by immersing the absorbent gel layer in solvent and removing the liquid with a pipette. Cohen et al. (1997) removed the lining layer of the diaper using sterile tweezers, and pushed the damp fibers of the diaper into the barrel of a standard 20 ml disposable syringe from which the plunger had been removed. By replacing the plunger and compressing the fibers, the authors reported that urine was easily obtained from the diapers. Other methods for urine extraction include using a specially constructed hydraulic press applying a maximum of 120 kPa/cm<sup>2</sup> (Heckmann et al., 2005).

As with the use of cotton or gauze inserts, there is a risk of contamination from fecal material when using infant diapers, or from the use of diaper creams or ointments on the infant's skin which could change the measurement of chemicals in urine (Hu et al., 2000). Although there still exist concerns regarding the collection of urine directly from diapers and its ability to isolate a broad array of target analytes, it would be the most attractive method because it is the least burdensome on the participant and the most logistically practical (Barr et al., 2005a), providing there are validated methods to analyze the chemicals of interest.

#### *Cotton Diapers*

An alternative method that to our knowledge has not been tested would be to use cotton diapers (as opposed to cotton pad inserts in disposable diapers) along with a disposable liner to separate urine from fecal matter (e.g., polypropylene liners available from <http://www.merehelene.com/fr/couche-accessoires-jetable.aspx>). Thin gauze has been used to reduce contamination of the urine with meconium or stool for studies of cortisol production rates (Heckmann et al., 2005).

#### *Additional Considerations*

For those methods that use absorbent collection materials (i.e., cotton or gauze inserts, disposable diapers, and cotton diapers), the issue of chemical extraction efficiency has not been discussed in detail. Many studies have discussed methods of extracting the liquid (urine) from the particular matrix, but have not quantified percentage recoveries of the chemical from the actual matrix itself. Roberts and Lucas (1985) found that most biochemical parameters (e.g., sodium, potassium, and creatinine) extracted from nappies that had been dosed with urine were within 2% of actual concentrations, provided that evaporation of water in the urine was kept to a minimum. However, two other studies examining environmental pesticides have found greater variability in percentage recovery of

chemical metabolites from cotton matrices. Hu et al. (2000) found recoveries of certain pesticide analytes to be 66–131% from cotton gauze pads, and 63–133% for three pyrethroid metabolites from disposable diapers using calcium chloride salts (Hu et al., 2004). Percentage recovery of creatinine in the Hu et al. (2004) study ranged from 71 to 133% and had greater variability than urine samples that did not come into contact with diapers, although relative SD for pesticide analytes and creatinine were deemed to be in an acceptable range of  $\pm 25\%$  by authors. Further laboratory analyses should be performed for those collection methods that use absorbent materials, to establish whether chemical analytes of interest are adequately being extracted from the collection matrices and whether extracted volumes for analysis are representative of the urine sample absorbed. The method of development and validation would likely be cost-intensive at the outset, and therefore use of absorbent collection materials may be better suited for studies examining a smaller number of analytes in urine.

#### **Toilet-trained children**

A number of studies have been conducted examining pesticide exposure in children's urine as a biomarker of exposure. These studies often involve older children from 2 to 5 or 6 years old, obtain urine samples over a period of 24 h, and require the children to be toilet-trained to be eligible for the study (Lu et al., 2001; Curl et al., 2002; Becker et al., 2008). When collecting urine on-site, Needham and Sexton (2000) note that it is important for children to feel comfortable and have adequate privacy. In addition, the presence of a familiar and trusted person, such as a parent or school nurse, often reassures younger children and can provide a validity check on the samples (Needham and Sexton, 2000). Obtaining a sample from young children can also be more difficult because they void only when they feel the urge and not on command (Pillitteri, 2006).

Two methods have been used to collect the entire urine void from toilet-trained children. One such method is the use of a specimen cup that is provided to the parents of the child, or the children themselves (if they are old enough) who are then instructed to collect the entire void (Kissel et al., 2005; Becker et al., 2006). The specimen cups provided are typically 100 or 118 ml polypropylene containers with screw-cap lids (Curl et al., 2002; Kissel et al., 2005) or polyethylene containers (TYCO Healthcare, Kautex) (Becker et al., 2006). Samples have also been obtained in larger containers, such as the 500 ml high-density polyethylene wide-mouth containers used in the Farm Family Study (Acquavella et al., 2004) or 1 l polyethylene wide-neck flasks as used in GerES IV from children 5 years and older (or in some girls older than 7 years) (Becker et al., 2008). Plastic collection jugs as well as wide-mouth beakers have also been used (Valcke et al., 2006; Soden et al., 2007).

A second collection method used is that of a commode pan insert which fits into a standard toilet. This insert is typically used for children who are very young and/or who find it difficult to urinate directly into the specimen cup, for example, girls (Valcke et al., 2006). Some specific brands of commode, "potty" or "hat" inserts used in previous studies include "Specipan" (Baxter Scientific, McGraw Park, IL, USA), lined potty chairs, 750 ml toilet seat inserts and inserts from Sage Products Inc., Crystal Lake, IL, USA (Loewenherz et al., 1997; Curl et al., 2002; Fenske et al., 2002; Shalat et al., 2003; Becker et al., 2008). Again, it should be ensured that sample cups and commode inserts are pre-screened and found to be devoid of the contaminant or metabolite of interest, such as phthalates (Brock et al., 2002).

Once the sample is obtained, the commode insert is either sealed using the provided snap top and placed in plastic Ziplock bags for transport (Loewenherz et al., 1997), or the urine is transferred immediately to a polypropylene sample cup (Sarstedt Group) before shipping and storage (Lu et al., 2001; Koch et al., 2007). If a sample is not obtained during a home visit by field staff, collection apparatus and instructions can be left with the parent. Once the urine sample has been produced, it can be picked up by field staff shortly after the void (Loewenherz et al., 1997; Lu et al., 2001; Fenske et al., 2002; Kissel et al., 2005) or refrigerated or frozen until delivered to the lab. To ensure that the sample collected is representative of the full void, the sample must be well mixed before making aliquots for chemical analysis.

It has been recognized that insufficient urine void volumes (less than 15 ml per sample) is one of the major limitations in collecting urine from young children (Curl et al., 2002). Some children at this age may not be fully toilet-trained and may still wet the bed at nighttime, which was noted in the study by O'Rourke et al. (2000). Curl et al. (2003) also noted problems with occasional accidents or contaminating the urine samples with feces. With girls reaching menarche at increasingly younger ages (Aksglaede et al., 2009), there is also the potential for contamination of the urine sample with blood (false-positive hematuria) when collecting from young girls during their menstrual period. Menstrual blood can change the specific gravity, protein and red blood cell analyses of urine (Pillitteri, 2006) and contains lysosomes and hydrolytic enzymes, white blood cells, and vaginal secretions (Hahn, 1980). As little has been reported on the potential impact of blood contamination on urine analyses for environmental chemicals, it is advisable to note the potential for contamination, have the girl wash the perineum well with soap and water, rinse and dry and then place a sterile cotton ball into her vagina just before voiding (Pillitteri, 2006).

The difficulty of collecting a 24-h urine sample from children has been noted in the Farm Family Exposure Study on account of the number of samples required from children (Baker et al., 2005). However, generally, collection of urine from older children who are toilet-trained presents fewer

difficulties than those involved when sampling urine from non-toilet-trained children.

## Summary and conclusions

In summary, the collection of urine samples from children who are toilet-trained is generally more simple and straightforward than obtaining urine from those wearing diapers, and the older the child, the easier the biomonitoring and sampling activities (Barr et al., 2005a). Although younger children urinate more frequently, older children are able to control timing of urination and produce a urine sample within a given time frame. Older children are also able to produce greater volumes of urine (Matt et al., 1999; Fenske et al., 2005), which is necessary in a national survey where numerous (bio)chemical analytes of interest will be examined. Furthermore, older children are more able to communicate and understand instructions from caregivers, teachers, or study technicians, thus improving compliance with research protocols (Needham and Sexton, 2000).

Obtaining urine specimens from children who are not toilet trained presents a number of challenges. For example, the pilot study of the German Environmental Survey on Children (GerES IV) reported that the volume of urine obtained from many children was too small, and collection of standardized urine specimens free from external contamination from children still wearing diapers at night was deemed impossible under the given conditions. The GerES IV survey therefore restricted participation to a subsample of children ages 3–14 who were toilet trained, using methods appropriate for toilet-trained children (Becker et al., 2006, 2008).

As collection methods used for toilet-trained children involve use of containers and materials that do not have to be attached or even contact the child's body, this increases the ease of collection and minimizes the invasiveness and discomfort suffered by the child. These methods also ensure minimal risk of contamination of the urine sample with feces, and the risk of sample "loss" or leakage to diapers and other surrounding materials is low (however, some sample loss and inconvenience may be a factor for young girls). In addition, as the sample is produced intact in a collection container, there are no challenges regarding extraction of a sample from an absorbent matrix.

For infants and toddlers there are more options for urine collection; however all suffer from some limitations (Table 1). Urine microbial contamination rates have been reported to be significantly lower with the clean catch method and similarly higher for pads and urine collection bags (Alam et al., 2005). The National Institute for Clinical Excellence (2009) suggests that urine collection pads are the next best option, if the clean catch is not suitable.

Urine collection pads may be preferable because of their cost-effectiveness, similar or lower contamination rates to urine collection bags, ease of use and parental preference (Lewis, 1998; Rao et al., 2004; Rogers and Saunders, 2008).

**Table 1.** Urine collection methods for infants and young children

Method	Age of children	Urine volume collected	Advantages	Disadvantages	Example references
Urine bags	Neonates to 24 months	Minimum 5 ml	Routine use in hospitals; 75–98% success in obtaining urine; easy to aliquot; limited interference with analytes	May cause discomfort; may leak; more difficult to attach; may be insufficient volume	Marbury et al. (1993); Schlager et al. (1995); Royster et al. (2002); Eskenazi et al. (2003)
Cotton/gauze inserts	Neonates (including preemies) to 36 months	1–12 ml	Easy to install and remove; limited interference with analytes	Requires expression/extraction; may be insufficient volume; potential for contamination by feces	Hu et al. (2000); Calafat et al. (2004); Weuve et al. (2006); Dorey and Zimmermann, (2008)
Disposable diapers	Neonates (including preemies) to $\geq 3$ years	Not reported	Readily available; acceptable to parents	Requires expression/extraction; potential for contamination by diaper debris, feces; may be insufficient volume; may interfere with analytes	Hu et al. (2004); Soden et al. (2007); Sathyanarayana et al. (2008)
Clean catch	Neonates to <3 years	Not reported	Minimal contamination	Resource intensive	Alam et al. (2005)
Specimen cups and commode inserts	2 to 8+ years	15–55 ml	Minimal contamination; easy to aliquot; limited interference with analytes	Child must be toilet trained; requires urination on command	Matt et al. (1999); Curl et al. (2002); Koch et al. (2002); Kissel et al. (2005)

It is recommended that if pads are used, that they be changed every 30–45 min to reduce contamination risks (Rogers and Saunders, 2008). The disadvantage of the pads is the difficulty of aspirating urine, especially when only a small amount has been voided (Alam et al., 2005), and the difficulty in analyzing for a large number of analytes (Barr et al., 2005a). Clean catch methods are often time-consuming and impractical. With disposable diapers, although more convenient for the parent, there can be difficulties in extracting urine as well as the potential for changing the actual urine composition due to extraction losses of analytes, salts and other solutes. As new diaper products emerge, methods will have to be re-evaluated.

The best method of collection will be the least burdensome on the participants and the most logistically practical in a field setting without introducing contamination provided the laboratory methods are adequately validated and costs are manageable. A survey of parents' preferences for home collection of urine for culture found that pads and bags were easy to use and were preferred to clean catch collections for both sexes; however, problems were reported with leakage and irritation upon removal of the bags, and extraction from the pad or emptying the bag were found to be awkward (Liaw et al., 2000).

Many studies have used a combination of the above urine collection methods to collect urine samples from a wide range of children (Fenske et al., 2002; Arcury et al., 2007; Bradman et al., 2007; Curwin et al., 2007). Generally,

studies of toilet-trained children aged 6 years and over have been performed according to procedures used by the US Centers for Disease Control and Prevention in the National Health and Nutrition Examination Survey (Bradman et al., 2007; Arcury et al., 2007).

Although urine sampling in older children who are toilet trained appears to be the most feasible and practical method of sampling, particularly in a national survey, it must be recognized that younger children may be more vulnerable to the effects of environmental chemicals. Therefore, the unique vulnerability of this age group must be balanced against the feasibility of urine collection for this population in a national survey. To overcome volume shortages and account for within-day variability in exposure, multiple urine voids on the same day could be collected and pooled for each infant.

The National Children's Study in the United States, which has begun to recruit participants and will be underway in 2008–2010, is intending to use cotton inserts to obtain urine samples in young children, although there are still a number of uncertainties regarding sources of potential contamination and use of this protocol (K Schoendorf, 2008—personal communication). Diaper inserts are currently being used in the HOME Study (Health Outcomes and Measures of the Environment) in Cincinnati to obtain urine samples from children aged 12–36 months. Researchers in this study report that abdominal gauze pads work as well as cotton diaper inserts (Kendall Curity Abdominal Pad) (S Liddy, 2008—personal communication).

As with most studies, a successful outcome will only be accomplished by a team approach including laboratory scientists, researchers, study nurses and research assistants, and prospective study participants from the outset to develop the most appropriate procedures. No single set of procedures will be applicable to all situations.

It is hoped that new research will develop improved approaches to collect urine from infants and young children and to adjust for urine dilution. Microanalytical-based sensors that can accurately and precisely process small amounts of urine are showing some promise (Barry et al., 2009). These new methods and approaches should be non-invasive, inexpensive and not introduce contamination of the sample by feces or by collection materials, so that this vulnerable population will not be ignored in future national biomonitoring surveys.

### Conflict of Interest

The authors declare that they have no competing financial interests. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of Health Canada, nor does mention of trade names or commercial products constitute endorsement or recommendations for use.

### References

Acquavella J.F., Alexander B.H., Mandel J.S., Gustin C., Baker B., and Chapman P., et al. Glyphosate biomonitoring for farmers and their families: results from the Farm Family Exposure Study. *Environ Health Perspect* 2004; 112(3): 321–326.

Ahmad T., Vickers D., Campbell S., Coulthard M.G., and Pedler S. Urine collection from disposable nappies. *Lancet* 1991; 338: 674–676.

Aksglaede L., Sorensen K., Petersen J.H., Skakkebaek N.E., and Juul A. Recent decline in age at breast development: the Copenhagen Puberty Study. *Pediatrics* 2009; 123: e932–e939.

Alam M.T., Coulter J.B.S., Pacheco J., Correia J.B., Ribeiro M.G.B., and Coelho M.F.C., et al. Comparison of urine contamination rates using three different methods of collection: clean-catch, cotton wool pad and urine bag. *Ann Trop Paediatr* 2005; 25: 29–34.

Alessio L., Berlin A., Dell'Orto A., Toffoletto F., and Ghezzi I. Reliability of urinary creatinine as a parameter used to adjust values of urinary biological indicators. *Int Arch Occup Environ Health* 1985; 55: 99–106.

Anestis S.F., Breakey A.A., Beuerlein M.M., and Bribiescas R.G. Specific gravity as an alternative to creatinine for estimating urine concentration in captive and wild chimpanzee (*Pan troglodytes*) samples. *Am J Primat* 2009; 71: 130–135.

Arcury T.A., Grzywacz J.G., Barr D.B., Tapia J., Chen H., and Quandt S.A. Pesticide urinary metabolite levels of children in Eastern North Carolina farmworker households. *Environ Health Perspect* 2007; 115(7): 1254–1260.

Baker B.A., Alexander B.H., Mandel J.S., Acquavella J.F., Honeycutt R., and Chapman P. Farm Family Exposure Study: methods and recruitment practices for a biomonitoring study of pesticide exposure. *J Expo Anal Environ Epidemiol* 2005; 15(6): 491–499.

Bakker B., Vulsma T., de Randamie J., Achterhuis A.M., Wiedijk B., and Oosting H., et al. A negative iodine balance is found in healthy neonates compared with neonates with thyroid agenesis. *J Endocrinol* 1999; 161: 115–120.

Barr D.B., Wang R.Y., and Needham L.L. Biologic monitoring of exposure to environmental chemicals throughout the life stages: requirements and issues for consideration for the National Children's Study. *Environ Health Perspect* 2005a; 113: 1083–1091.

Barr D.B., Wilder L.C., Caudill S.P., Gonzalez A.J., Needham L.L., and Pirkle J.L. Urinary creatinine concentrations in the U.S. populations: implications for urinary biologic monitoring measurements. *Environ Health Perspect* 2005b; 113: 192–200.

Barry R.C., Lin Y., Wang J., Liu G., and Timchalk C.A. Nanotechnology-based electrochemical sensors for biomonitoring chemical exposures. *J Expo Sci Environ Epidemiol* 2009; 19: 1–18.

Becker K., Müssig-Zufika M., Conrad A., Lüdecke A., Schulz C., and Seiwert M., et al. German Environmental Survey for Children 2003/06—GerES IV. Human biomonitoring: levels of selected substances in blood and urine of children in Germany, 2008. Ed: UmweltBundesamt. German Federal Environment Agency, Robert Koch Institute. Research report 202 62 219. Available online at: [www.umweltbundesamt.de/survey](http://www.umweltbundesamt.de/survey).

Becker K., Seiwert M., Angerer J., Kolossa-Gehring M., Hoppe H-W., and Ball M., et al. IV GerES Pilot study: assessment of the exposure of German children to organophosphorus and pyrethroid pesticides. *Int J Hyg Environ Health* 2006; 209: 221–233.

Bolte R.G. Urinary frequency in childhood. In: Fleisher G.R., Ludwig S., Henretig F.M., Ruddy R.M., and Silverman B.K., (Eds.). *Textbook of Pediatric Emergency Medicine*, 5th edn. Lippincott Williams & Wilkins, Philadelphia, PA, 2005, pp 663–668.

Bradman A., Whitaker D., Quiros L., Castorina R., Henn B.C., and Nishioka M., et al. Pesticides and their metabolites in the homes and urine of farmworker children living in the Salinas Valley, CA. *J Expo Sci Environ Epidemiol* 2007; 17(4): 331–349.

Bradman A., and Whyatt R.M. Characterizing exposures to nonpersistent pesticides during pregnancy and early childhood in the National Children's Study: a review of monitoring and measurement methodologies. *Environ Health Perspect* 2005; 113(8): 1092–1099.

Brock J.W., Caudill S.P., Silva M.J., Needham L.L., and Hilborn E.D. Phthalate monoesters levels in the urine of young children. *Bull Environ Contam Toxicol* 2002; 68: 309–314.

Burke N. Alternative methods for newborn urine sample collection. *Pediatr Nurs* 1995; 21: 546–549.

Calafat A.M., Needham L.L., Silva M.J., and Lambert G. Exposure to di-(2-ethylhexyl) phthalate among premature neonates in a neonatal intensive care unit. *Pediatrics* 2004; 113(5): e429–e434.

Chadha V., Garg U., and Alon U.S. Measurement of urinary concentration: a critical appraisal of methodologies. *Pediatr Nephrol* 2001; 16: 374–382.

Cohen H.A., Woloch B., Linder N., Vardi A., and Barzilai A. Urine samples from disposable diapers: an accurate method for urine cultures. *J Fam Pract* 1997; 44(3): 290–292.

Cook J.D., Strauss K.A., Caplan Y.H., LoDico C.P., and Bush D.M. Urine pH: the effects of time and temperature after collection. *J Anal Toxicol* 2007; 31: 486–496.

Curl C.L., Fenske R.A., and Elgethun K. Organophosphorus pesticide exposure of urban and suburban preschool children with organic and conventional diets. *Environ Health Perspect* 2003; 111(3): 377–382.

Curl C.L., Fenske R.A., Kissel J.C., Shirai J.H., Moate T.F., and Griffith W., et al. Evaluation of take-home organophosphorus pesticide exposure among agricultural workers and their children. *Environ Health Perspect* 2002; 110(12): A787–A792.

Curwin B.D., Hein M.J., Sanderson W.T., Striley C., Heederik D., and Kromhout H., et al. Urinary pesticide concentrations among children, mothers and fathers living in farm and non-farm households in Iowa. *Ann Occup Hyg* 2007; 51(1): 53–65.

Dorey C.M., and Zimmermann M.B. Reference values for spot urinary iodine concentrations in iodine-sufficient newborns using a new pad collection method. *Thyroid* 2008; 18: 347–352.

Eskenazi B., Bradman A., Gladstone E.A., Jaramillo S., Birch K., and Holland N. CHAMACOS, a longitudinal birth cohort study: lessons from the fields. *J Child Health* 2003; 1: 3–27.

Fenske R.A., Bradman A., Whyatt R.M., Wolff M.S., and Barr D.B. Lessons learned for the assessment of children's pesticide exposure: critical sampling and analytical issues for future studies. *Environ Health Perspect* 2005; 113: 1455–1462.

Fenske R.A., Lu C., Barr D., and Needham L. Children's exposure to chlorpyrifos and parathion in an agricultural community in Central Washington State. *Environ Health Perspect* 2002; 110(5): 549–553.

Fenske R.A., Lu C., Simcox N.J., Loewenherz C., Touchstone J., and Moate T.F., et al. Strategies for assessing children's organophosphorus pesticide exposures in agricultural communities. *J Expo Anal Environ Epidemiol* 2000; 10: 662–671.

- Gammage D., and Yarandi H. The effects of diaper brands, urine volume, and time on specific gravity measurement. *J Pediatr Nurs* 1993; 8: 10–14.
- Hahn L. Composition of menstrual blood. In: Diczfalussy E., Fraser I.S., Webb F.T., (Eds.). *Endometrial bleeding and steroidal contraception: proceedings of a Symposium on Steroid Contraception and Mechanisms of Endometrial Bleeding*, Geneva, 12–14 September 1979. Pitman Press, Bath, England, 1980, pp 107–131.
- Heckmann M., Hartmann M.F., Kampschulte B., Gack H., Bödeker R.-H., and Gortner L., et al. Assessing cortisol production in preterm infants: do not dispose of the nappies. *Pediatr Res* 2005; 57: 412–418.
- Hu Y.A., Barr D.B., Akland G., Melnyk L., Needham L., and Pellizzari E.D., et al. Collecting urine samples from young children using cotton gauze for pesticide studies. *J Expo Anal Environ Epidemiol* 2000; 10: 703–709.
- Hu Y., Beach J., Raymer J., and Gardner M. Disposable diaper to collect urine samples from young children for pyrethroid pesticide studies. *J Expo Anal Environ Epidemiol* 2004; 14: 378–384.
- Kirkpatrick J.M., Alexander J., and Cain R.M. Recovering urine from diapers: are test results accurate? *MCN* 1997; 22: 96–102.
- Kissel J.C., Curl C.L., Kedar G., Lu C., Griffith W., and Barr D.B., et al. Comparison of organophosphorus pesticide metabolite levels in single and multiple daily urine samples collected from preschool children in Washington State. *J Expo Anal Environ Epidemiol* 2005; 15: 164–171.
- Koch H.M., Becker K., Wittassek M., Seiwert M., Angerer J., and Kolossa-Gehring M. Di-*n*-butylphthalate and butylbenzylphthalate—urinary metabolite levels and estimated daily intakes: pilot study for the German Environmental Survey on children. *J Expo Sci Environ Epidemiol* 2007; 17: 378–387.
- Koch D., Lu C., Fisker-Andersen J., Jolley L., and Fenske R.A. Temporal association of children's pesticide exposure and agricultural spraying: report of a longitudinal biological monitoring study. *Environ Health Perspect* 2002; 110: 829–833.
- Leech S., and Penney M.D. Correlation of specific gravity and osmolality of urine in neonates and adults. *Arch Dis Child* 1987; 62: 671–673.
- Lewis J. Clean-catch versus urine collection pads: a prospective trial. *Paediatr Nurs* 1998; 10: 15–16.
- Liaw L.C.T., Nayar D.M., Pedler S.J., and Coulthard M.G. Home collection of urine for culture from infants by three methods: survey of parents' preferences and bacterial contamination rates. *BMJ* 2000; 320: 1312–1313.
- Liddy S., (Cincinnati Children's Hospital Medical Center). Re: Urine collection-diaper inserts. 9 May 2008. Personal communication (e-mail) to Mandy Weselak (Health Canada). 2008.
- Loewenherz C., Fenske R.A., Simcox N.J., Bellamy G., and Kalman D. Biological monitoring of organophosphorus pesticide exposure among children of agricultural workers in central Washington State. *Environ Health Perspect* 1997; 105: 1344–1353.
- Lombardero N., Casanova O., Behnke M., Eyer F.D., and Bertholf R.L. Measurement of cocaine and metabolites in urine, meconium, and diapers by gas chromatography/mass spectrometry. *Ann Clin Lab Sci* 1993; 23: 385–394.
- Lu C., Knutson D.E., Fisker-Andersen J., and Fenske R.A. Biological monitoring survey of organophosphorus pesticide exposure among preschool children in the Seattle metropolitan area. *Environ Health Perspect* 2001; 109: 299–303.
- Marbury M.C., Hammond S.K., and Haley N.J. Measuring exposure to environmental tobacco smoke in studies of acute health effects. *Am J Epidemiol* 1993; 137: 1089–1097.
- Matos V., Drukker A., and Guignard J.P. Spot urine samples for evaluating solute excretion in the first week of life. *Arch Dis Child Fetal Neonatal Ed* 1999; 80: F240–F242.
- Matt G.E., Wahlgren D.R., Hovell M.F., Zakarian J.M., Bernert J.T., and Meltzer S.B., et al. Measuring environmental tobacco smoke exposure in infants and young children through urine cotinine and memory-based parental reports: empirical findings and discussion. *Tob Control* 1999; 8: 282–289.
- Morris C.B., Vince J.D., Ripa P., and Tefurani N. The clean catch technique for urine collection in infants and young children [letter]. *Trop Doctor* 2007; 37: 125.
- Needham L.L., Özkaynak H., Whyatt R.M., Barr D.B., Wang R.Y., and Naeher L., et al. Exposure assessment in the National Children's Study: introduction. *Environ Health Perspect* 2005; 113: 1076–1082.
- Needham L.L., and Sexton K. Assessing children's exposure to hazardous environmental chemicals: an overview of selected research challenges and complexities. *J Expo Anal Environ Epidemiol* 2000; 10: 611–629.
- NICE (National Institute for Clinical Excellence). Urinary tract infections in children: diagnosis, treatment and long-term management. Accessed online at: <http://guidance.nice.org.uk/CG54> (25 March 2009).
- O'Rourke M.K., Lizardi P.S., Rogan S.P., Freeman N.C., Aguirre A., and Saint C.G. Pesticide exposure and creatinine variation among young children. *J Expo Anal Environ Epidemiol* 2000; 10(6): 672–681.
- Pearson M.A., Lu C., Schmotzer B.J., Waller L.A., and Riederer A.M. Evaluation of physiological measures for correcting variation in urinary output: implications for assessing environmental chemical exposure in children. *J Expo Sci Environ Epidemiol* 2009; 19: 336–342.
- Pillitteri A. Nursing care of a child undergoing diagnostic techniques and other therapeutic modalities. In: *Maternal & Child Health Nursing: Care of the Childbearing & Childrearing Family*, 5th edn. Lippincott Williams & Wilkins, Philadelphia, PA, 2006, pp 1106–1138.
- Pradella M., Dorizzi R.M., and Rigolin F. Relative density of urine: methods and clinical significance. *CRC Crit Rev Clin Lab Sci* 1988; 26: 195–242.
- Rao S., Bhatt J., Houghton C., and Macfarlane P. An improved urine collection pad method: a randomised clinical trial. *Arch Dis Child* 2004; 89: 773–775.
- Rao S., Houghton C., and Macfarlane P.I. A new urine collection method; pad and moisture sensitive alarm [letter]. *Arch Dis Child* 2003; 88: 836.
- Riboli E., Haley N.J., De Waard F., and Saracci R. Validity of urinary biomarkers of exposure to tobacco smoke following prolonged storage. *Int J Epidemiol* 1995; 24: 354–358.
- Roberts S.B., and Lucas A. Measurement of urinary constituents and output using disposable napkins. *Arch Dis Child* 1985; 60: 1021–1024.
- Rogers J., and Saunders C. Urine collection in infants and children. *Nurs Times* 2008; 104: 41–42.
- Royster M.O., Hilborn E.D., Barr D., Carty C.L., Rhoney S., and Walsh D. A pilot study of global positioning system/geographical information system measurement of residential proximity to agricultural fields and urinary organophosphate metabolite concentrations in toddlers. *J Expo Anal Environ Epidemiol* 2002; 12: 433–440.
- Sathyanarayana S., Karr C.J., Lozano P., Brown E., Calafat A.M., and Liu F., et al. Baby care products: possible sources of infant phthalate exposure. *Pediatrics* 2008; 121: e260–e268.
- Scher D.P., Alexander B.H., Adgate J.L., Eberly L.E., Mandel J.S., and Acquavella J.F., et al. Agreement of pesticide biomarkers between morning void and 24-h urine samples from farmers and their children. *J Expo Sci Environ Epidemiol* 2007; 17: 350–357.
- Schlager T.A., Hendley J.O., Dudley S.M., Hayden G.F., and Lohr J.A. Explanation for false-positive urine cultures obtained by bag technique. *Arch Pediatr Adolesc Med* 1995; 149: 170–173.
- Schoendorf K., (NIH). Re: Urine collection from toddlers. 6 May 2008. Personal communication (e-mail) to Tye Arbuckle (Health Canada) 2008.
- Shalat S.L., Donnelly K.C., Freeman N.C.G., Calvin J.A., Ramesh S., and Jimenez M., et al. Nondietary ingestion of pesticides by children in an agricultural community on the US/Mexico border: preliminary results. *J Expo Anal Environ Epidemiol* 2003; 13(1): 42–50.
- Soden S.E., Lowry J.A., Garrison C.B., and Wasserman G.S. 24-H provoked urine excretion test for heavy metals in children with autism and typically developing controls, a pilot study. *Clin Toxicol* 2007; 45: 476–481.
- Sorahan T., Pang D., Esmen N., and Sadhra S. Urinary concentrations of toxic substances: an assessment of alternative approaches to adjusting for specific gravity. *J Occup Environ Hyg* 2008; 5: 721–723.
- Suwazono Y., Akesson A., Alfvén T., Järup L., and Vahter M. Creatinine versus specific gravity-adjusted urinary cadmium concentrations. *Biomarkers* 2005; 10: 117–126.
- Valcke M., Samuel O., Bouchard M., Dumas P., Belleville D., and Tremblay C. Biological monitoring of exposure to organophosphate pesticides in children living in peri-urban areas of the Province of Québec, Canada. *Int Arch Occup Environ Health* 2006; 79: 568–577.
- Vij H.S., and Howell S. Improving the specific gravity adjustment method for assessing urinary concentrations of toxic substances. *Am Ind Hyg Assoc J* 1998; 59: 375–380.
- Voinescu G.C., Shoemaker M., Moore H., Khanna R., and Nolph K.D. The relationship between urine osmolality and specific gravity. *Am J Med Sci* 2002; 323: 39–42.
- Weuve J., Sánchez B.N., Calafat A.M., Schettler T., Green R.A., and Hu H., et al. Exposure to phthalates in neonatal intensive care unit infants: urinary concentrations of monoesters and oxidative metabolites. *Environ Health Perspect* 2006; 114: 1424–1431.
- Zohouri F.V., Swinbank C.M., Maguire A., and Moynihan P.J. Is the fluoride/creatinine ratio of a spot urine sample indicative of 24-h urinary fluoride? *Community Dent Oral Epidemiol* 2006; 34: 130–138.