Collective expert appraisal: summary and conclusions

Regarding the expert appraisal on setting occupational exposure limits for chemical agents

Evaluation of biomarkers of exposure and recommendations for biological reference values for

Butylbenzyl-phthalate (n° CAS 85-68-7)

This document summarises and presents the work of the Expert Committee on expert appraisal for recommending occupational exposure limits for chemical agents (OEL Committee) and the Working Group on biomarkers of exposure.

Presentation of the issue

On 12 June 2007, AFSSET, which became ANSES in July 2010, was requested by the Directorate General for Labour to conduct the scientific expert appraisal work required for setting occupational exposure limit values (OELVs) for butylbenzyl-phthalate (BBzP).

France does not currently have any occupational exposure limit values (8-hour or 15-minutes) for butylbenzyl-phthalate.

The Directorate General for Labour asked ANSES to assess this substance and, if necessary, propose occupational exposure limits based on health considerations for butylbenzyl-phthalate.

ANSES OEL Committee decided to conduct the assessment of the biological monitoring data in occupational environment, in order to establish the relevance of recommending monitoring of one or more biomarkers in addition to the OEL and the establishment of biological limit values for the selected biomarker(s).

Scientific background

Biological monitoring of exposure in workplaces has emerged as a complementary method to atmospheric exposure measurement for assessing exposure to chemical agents. Biological monitoring assesses a worker's exposure by including all the routes by which a chemical penetrates the body (lung, skin, digestive tract). It is particularly worthwhile when a substance has a systemic effect, and:

- when routes other than inhalation contribute significantly to absorption,
- and/or when the pollutant has a cumulative effect,

- and/or when the working conditions (personal protection equipment, inter-individual differences in respiratory ventilation, etc.) determine large differences in internal dose between individuals that are not taken into account by atmospheric metrology.

With regard to prevention of chemical risk in the workplace, the French Labour Code authorises the use of biological monitoring of exposure and biological limit values.

OEL Committee definitions

Biomarker of exposure: parent substance, or one of its metabolites, determined in a biological matrix, whose variation is associated with exposure to the agent targeted. Biomarkers of early and reversible effects are included in this definition when they can be specifically correlated to occupational exposure.

Biological limit value (BLV): This is the limit value for the relevant biomarkers.

Depending on the available data, the recommended biological limit values do not all have the same meaning:

- if the body of scientific evidence is sufficient to quantify a dose/response relationship with certainty, the biological limit values (BLVs) are established on the basis of health data (no effect for threshold substances or risk levels for non-threshold carcinogens);
- in the absence of such data for substances with threshold effects, BLVs are calculated on the basis of the expected concentration of the biomarker of exposure (BME) when the worker is exposed to the 8-hour OEL. For carcinogens, in the absence of sufficient quantitative data, the biological limit value is calculated on the basis of another effect (pragmatic BLV). These last values do not guarantee the absence of health effects, but aim to limit exposure to these substances in the workplace.

Whenever possible, the OEL Committee also recommends biological reference values (BRVs). These correspond to concentrations found in a general population whose characteristics are similar to those of the French population (preferentially for biomarkers of exposure) or failing that, a control population not occupationally exposed to the substance under study (preferentially for biomarkers of effects).

These BRVs cannot be considered to offer protection from the onset of health effects, but do allow a comparison with the BME concentrations measured in exposed workers. These values are particularly useful in cases where it is not possible to establish a BLV.

Organisation of the expert appraisal

ANSES entrusted examination of this request to the Expert Committee on expert appraisal for recommending occupational exposure limits for chemical agents (OEL Committee). The Agency also mandated the Working Group on biomarkers of exposure for this expert appraisal.

The methodological and scientific aspects of the Working Group's work were regularly submitted to the OEL Committee. The report produced by the Working Group takes account of observations and additional information provided by the Committee members.

This expert appraisal was therefore conducted by a group of experts with complementary skills. It was carried out in accordance with the French Standard NF X 50-110 "Quality in Expertise Activities".

Preventing risks of conflicts of interest

ANSES analyses interests declared by the experts before they are appointed and throughout their work in order to prevent potential conflicts of interest in relation to the points addressed in expert appraisals.

The experts' declarations of interests are made public on ANSES's website (www.anses.fr).

Description of the method

Two rapporteurs from this working group were appointed by the Agency to produce a summary report on biomarkers of exposure (BMEs) and the recommendation of biological limit values (BLVs) and biological reference values for the BME(s) considered relevant. An ANSES employee also contributed to this report.

The summary report on the BMEs for butylbenzylphthalate was based on bibliographical information taking into account the scientific literature published on this substance until march 2013.

The bibliographical research was conducted in the following databases: Medline, Toxline, HSDB, ToxNet (CCRIS, GENE-TOX, IRIS) and ScienceDirect. The rapporteurs reassessed the source articles or reports cited as references whenever they considered it necessary, or whenever the Committee requested it.

The collective expert appraisal work and its conclusions and recommendations were adopted on 13 December 2013 by the OEL Committee (term of office 2010-2013).

The collective expert appraisal work and the summary report were submitted to public consultation from 01/10/2014 to 01/12/2014. The people or organizations who contributed to the public consultation are listed in appendix of the report (only available in French). The comments received were reviewed by the OEL Committee (term of office 2014-2017) who adopted this version on 12 May 2015.

Result of the collective expert appraisal

Introduction

The scientific articles selected for evaluating biomonitoring data on butylbenzylphthalate were identified using the following keywords: "butylbenzylphthalate", "biomarker", "biomonitoring", "biological monitoring", "urine", "blood" and "occupational", while limiting the search to human data.

Toxicokinetics data

Absorption by inhalation has not been described in the literature for either animals or humans. Exposure by inhalation can result from aerosols generated when producing or using products containing BBzP[1] (CE, 2007).

There is little information regarding the distribution of BBzP in the body.

After BBzP is broken down into monoesters by intestinal esterases, mono-n-butyl-phthalate (MnBP) and mono-benzyl-phthalate (MBzP) can be metabolised, oxidised and conjugated with glucuronic acid. Anderson et al. (2001) undertook a study in human volunteers exposed by ingestion to set quantities of stable isotope-labelled phthalates (Anderson et al., 2001). The authors showed that 67% and 78% (molar fraction, depending on the administered dose: 253 and 506 µg respectively) of the ingested BBzP was eliminated in the urine as MBzP (free or conjugated)[2]. While MnBP (free and conjugated) was not found at the lowest dose, it was only excreted at a rate of 6 % at the highest dose (molar fraction). It should be noted that the proportions of MnBP and MBzP in the rats are reversed. MnBP is found in greater quantities than MBzP (Sipes[3] and al., cited by Agarwal et al., 1985).

In humans, unlike in rats, it appears that conjugation with glucuronic acid is a significant and major phenomenon, since 93% of the MBzP found in urine is in conjugated form (Silva et al., 2003). These differences in toxicokinetics should be taken into account when interpreting experimental data.

Urinary excretion appears to be the major route in humans. According to the data of the study by Anderson et al. (2001), up to 84% of the administered dose of BBzP can be found in the urine as the two metabolites. This urinary excretion appears to occur quickly, since the majority of metabolites are found within 24 hours.

Choice of biomarkers of exposure and effect

Two metabolites can be detected in human urine: MBzP and MnBP.

MnBP is not detected with low exposure to BBzP in volunteers and is only seldom detected with single high exposure. Moreover, it is not specific to BBzP exposure. In fact, it is also a metabolite of DnBP. It was therefore not chosen as a biomarker of exposure. Urinary MBzP is the main specific metabolite of BBzP. Furthermore, its half-life is compatible with end-of-shift urine sampling.

Therefore, urinary MBzP is the only biomarker of exposure used for monitoring exposure to BBzP.

Certain effects of exposure to BBzP and more broadly to phthalates have been studied and particularly effects on reproduction in adults (changes in hormone levels, impaired sperm quality in men, endometriosis and uterine leiomyomata in adult women) and development (decreased anogenital distance at birth in boys born to mothers exposed to certain phthalates). It is not possible to establish a causal link between specific exposure to BBzP and these effects in humans in that all of the studies identified in the literature report complex exposure to mixtures of phthalates. Moreover, studies in humans rarely show a statistically

[1] Products carried out at high temperatures or use of spraying technique in occupational setting

[2] The origin of the beta glucuronidase used to treat urinary samples is not specified in this study.

[3] Personal communication

significant relationship between potential reprotoxicity markers and urinary concentrations of MBzP.

Therefore, the results do not highlight any relevant indicators of effects for biological monitoring.

Information on biomarkers of exposure identified as relevant for the biological monitoring of exposed workers

Name	**URINARY MONO-BENZYL PHTHALATE (MB_ZP)**
Other substances giving rise to this biomarker	not specified
Concentrations found in exposed workers or volunteers	Field studies: Corresponding atmospheric exposure levels not specified – various industry sectors urinary [MBzP] at end of shift: from <LD to 438 $\mu g.g^{-1}$ creatinine (cr) Studies in volunteers: Anderson et al. (2001) - Oral exposure - 253 µg of BBzP: 140 µg of MBzP.$24h^{-1}$ - 506 µg of BBzP: 323 µg of MBzP.$24h^{-1}$
Conversion factor	Molecular weight (MW): 256.26 1 $\mu g.L^{-1}$ = 0.004 $\mu mol.L^{-1}$ 1 $\mu mol.L^{-1}$ = 256.26 $\mu g.L^{-1}$ 1 $\mu g.g^{-1}$ cr = 0.44 $\mu mol.mol^{-1}$ cr 1 $\mu mol.mol^{-1}$ = 2.26 $\mu g.g^{-1}$ cr
Concentrations in the general population	USA-NHANES (2009-2010)[4] - 95^{th} percentile: (20 years and over, 1914 samples) 39.6 $\mu g.L^{-1}$ and 29.1 $\mu g.g^{-1}$ cr (CDC, 2013)
Recommended limit values for exposed workers	None

Study of relationships between concentrations of BMEs for BBzP and certain health effects

Studies in human adults

The epidemiological studies in which BMEs for BBzP have been assessed in relation to health effects have primarily involved the general population. It should be noted that four publications (team of Duty et al.: Duty et al., 2003; Duty et al., 2004; Duty et al., 2005 and Hauser et al., 2006) refer to a cross-sectional study (consultation for infertility in the same clinic) that appears to have been reproduced several times using the results of the previous study each time, thus increasing the population size with each publication but not showing independent results.

In 2003, Duty et al. found a relationship between increased urinary MBzP concentrations and lower sperm concentration. The latter was 5 times more common in the highest tertile of urinary concentrations (13.4 to 540.2 $\mu g.L^{-1}$). However, they did not see any relationship between increased MBzP concentrations and lower sperm motility.

The study was repeated with a larger population size in 2006 in order to increase its statistical power (Hauser et al., 2006). This publication confirmed the lack of a relationship between increased urinary concentrations of MBzP and lower sperm motility. However, the authors did not confirm the relationship that had been found between urinary concentrations

[4] *National Health and Nutrition Examination Survey - Centers for Disease Control (USA)*

of MBzP and sperm concentrations. They also did not highlight any changes in sperm morphology.

Using the same criteria, another team (Wirth et al. 2008) confirmed the lack of a relationship between urinary concentrations of MBzP and changes in sperm parameters.

Two studies did not report any changes in sperm parameters or in hormone levels in men whose median urinary concentrations of MBzP were 16 and 34 $\mu g.L^{-1}$ (Jönsson et al., 2005 and Joensen et al., 2012).

In 2004, the team of Duty et al. carried out a study showing a relationship between increased urinary concentrations of MBzP and changes in sperm motility (linear and curvilinear velocity) parameters but the results were not statistically significant.

The same team also tested dose-response relationships between hormone levels and MBzP concentrations (Duty et al., 2005). Follicule stimulating hormone (FSH) concentrations were significantly lowered when urinary MBzP concentrations increased. But according to the authors, it is difficult to know whether these results reflected biological alterations or if they were the result of making multiple (statistical) comparisons.

Studies in adult women

Few studies have assessed the possible role that exposure to phthalates may play in female reproductive toxicity. The study by Weuve et al. (2010) including 1200 women did not show any relationship between increased urinary concentrations of MBzP and the prevalence of uterine diseases (endometriosis and leiomyomata). In another study with a smaller study population, Itoh et al. (2009) did not find any relationship between urinary concentrations of MBzP and endometriosis.

Studies in newborns and children in relation to maternal exposure

A research team studied the possible relationship between decreased anogenital distance at birth and concentrations of phthalate metabolites in the urine of mothers (Swan et al., 2005). This first study showed a (statistically significant, $p < 0.05$) increase in the frequency of decreased anogenital distance in male newborns associated with an increase in maternal urinary concentrations of MBzP. However, in a second study, these results were not confirmed with a larger study population (Swan et al. 2008). It should be noted that it is difficult to use the results of the 2008 study in that the exposure levels of the mothers were not reported.

Table 1: Summary of the data of epidemiological studies comparing urinary concentrations of MBzP and effects on reproduction in adults and development

Reference	Population and size	Urinary concentrations of MBzP **Median** (95th percentile) µg.L^{-1}		Reproduction parameter	Levels of exposure associated with a significant effect (µg.L^{-1})
		Without adjustment	Adjusted to urinary density[5]		
Duty et al., 2003	143 men, infertile couples	**10.3** (49.6)	**9.3** (32.8)	Decrease in sperm concentration Frequency multiplied by 5	13.04 – 540.24 (adjusted for specific gravity)
				No decrease in sperm motility	
Duty et al., 2004	220 men, infertile couples	**9.9** (49.7)	**9.4** (46.8)	Changes in sperm motility parameters (results not statistically significant)	
Duty et al., 2005	295 men, infertile couples	**6.9** (37.1)	**7.9** (38.4)	Increase in FSH	
Hauser et al., 2006	463 men, infertile couples		**8** (40.6)	No dose-response relationship with sperm parameters (sperm concentration, morphology and motility)	
Jönsson et al., 2005	234 Swedish men (military service)	**16** (74)		No association with sperm counts or hormones	
Joensen et al., 2012	881 young men	**34 (164)**		No association with sperm counts or hormones	
Wirth et al., 2008	45 infertile men	**17.4 (**166.6)		No relationship with sperm parameters (sperm concentration, motility and morphology)	
Swan et al., 2005	85[6] mothers	**8.3** (75th p 23.5)		Reduced anogenital distance in male newborns Frequency multiplied by 3	≥ 3.5 and < 23.5
				Reduced anogenital distance in male newborns Frequency multiplied by 4	≥ 23.5

[5] 1.024 as reference value

[6] The article does not clearly state if the population size is 85 or fewer male newborns

Studies of the relationship between MBzP concentrations and BBzP exposure

Field studies (correlation)

No field studies have examined the correlation between BBzP exposure by inhalation and biological levels of MBzP in urine.

Only two studies in the workplace were identified. These report urinary concentrations of MBzP but not the corresponding atmospheric concentrations.

Table 2: Urinary concentrations of MBzP in workers in various sectors (according to Hines et al., 2009 and Martens and Martens 2002)

		MBzP		*References*
		Median - maximum value, *in µg/L and [µg/g of creatinine]*		
Business sector	n	Start of shift (for Martens 2002) and mid shift (for Hines 2009)	End of shift	
Floor tiles	3	**<60 [<43]** 75 [70]	**150 [150]** 1020 [560]	*Martens and Martens 2002*
Phthalate manufacturing	9	**22 [9]** 45 [21]	**25 [13]** 736 [386]	*Hines et al. 2009*
PVC film manufacturing	25	**34 [14]** 130 [51]	**32 [16]** 156 [69]	
Vehicle filters	18	**23 [16]** 70 [87]	**21 [17]** 72 [50]	
PVC compounding	12	**18 [15]** 119 [52]	**32 [22]** 170 [61]	
Rubber hoses	25	**16 [10]** 107 [54]	**17 [11]** 55 [33]	
Rubber boots	21	**39 [34]** 207 [127]	**74 [32]** 473 [125]	
Rubber gaskets	20	**101 [107]** 747 [272]	**167 [139]** 689 [438]	
Nail-only salons	25	**2 [3]** 32 [29]	**6 [5]** 54 [90]	

Experimental data

Due to the lack of relevant studies in humans, the OEL Committee used a study in animals to establish an 8h-OEL. A critical effect (alteration of reproductive organs and impaired fertility in male rats) transposable to workers was identified in this study undertaken in a population of adult rats.

The OEL was established based on an NOAEL in animals (oral route) of 200 $mg.kg^{-1}.day^{-1}$. This critical dose in animals was extrapolated to humans through allometric adjustment. An equivalent oral dose for humans of 49 $mg.kg^{-1}.day^{-1}$ was obtained. In 2001, Anderson et al. reported data on urinary excretions of MBzP in humans that could be used in a mass conservation equation. This type of equation can calculate urinary concentrations of MBzP based on ingested doses (Kohn et al, 2000).

Establishment of BLVs and choice of biological reference values

The epidemiological studies in which BMEs for BBzP have been assessed in relation to health effects have primarily involved the general population.

Of the 11 available studies that have described the statistical relationship between urinary concentrations of MBzP and the occurrence of health effects (effects on fertility and development), only two report statistically significant results (effect observed when urinary concentrations of MBzP are increased).

The team of Duty et al. first found a statistically significant decrease in sperm concentration when urinary concentrations of MBzP increased (Duty et al., 2003) but was unable to confirm this result with a larger study population (Duty et al., 2004; Hauser et al., 2006). Using the same study parameters, another team confirmed the lack of a statistical relationship between urinary concentrations of MBzP and changes in sperm parameters in a cohort of infertile men (Wirth et al., 2008).

Two studies sought to describe statistical relationships between effects observed in adult women (endometriosis, uterine leiomyomata). They did not show a statistical relationship between urinary concentrations of MBzP and these effects (Itoh et al., 2009 and Weuve et al., 2010).

Swan et al. (2005) reported an effect on foetal development (decreased anogenital distance in male newborns) related to maternal urinary MBzP concentrations. In 2008, the same team was unable to confirm these results and did not specify the mothers' exposure levels.

In conclusion, studies in humans (general population) rarely show a statistical relationship between reprotoxicity parameters and urinary concentrations of MBzP.

Furthermore, no epidemiological studies in the workplace are available linking urinary concentrations of MBzP and the occurrence of health effects.

It is therefore not possible, in the current state of knowledge, to recommend a biological limit value on the basis of health effects.

Since no field studies linking atmospheric concentrations of BBzP and its potential biomarkers of exposure (MBzP in particular) are available, it is also not possible to establish a BLV based on exposure to the OEL.

The calculations proposed by Kohn et al. (2000) cannot be used to extrapolate the oral human equivalent dose to urinary concentrations because they include too many uncertainties. The kinetic parameters of BBzP and its metabolites were described for oral absorption of only 2 different doses of BBzP, and excretion fractions were reported over 24 hours (Anderson et al. 2001). Therefore, no biological value can be established based on the experimental data.

Thus, since it is not possible to recommend a biological limit value, biological reference values can be proposed.

<u>Proposed biological reference values</u>

There are no French data reporting urinary levels of MBzP for large samples of the general population[7].

[7] There are no results involving BME assays for phthalates in French national studies (ENNS and Esteban). One cohort (ELFE) is intended to measure BME concentrations (particularly for phthalates) in young children (from birth to adulthood) and their mothers, but the results have not yet been published.

Only the data in the American national NHANES survey can be used to establish biological reference values. The data reported in this survey show that urinary concentrations of MBzP vary significantly depending on age. This criterion was therefore used to analyse the results of the NHANES survey. The over-20-years population was one of the age groups specified.

The urinary MBzP concentration of 40 µg.L^{-1} or 30 µg.g^{-1} creatinine corresponding to the 95th percentile of the distribution of urinary concentrations in men and women (over the age of 20 years), taken from the 2009-2010 campaign, has been proposed as the biological reference value.

Conclusions of the collective expert appraisal

The biological values proposed for monitoring exposure to butylbenzyl-phthalate are:

Urinary mono-benzyl phthalate

BLV based on health effect	None
BLV based on exposure to the 8h-OEL	None
Biological reference value	30 µg.g^{-1} creatinine or 40 µg.L^{-1}

Sampling method and factors that may affect the interpretation of results

Samples can be taken at end of shift.

Urine samples must be frozen after they are collected at –20°C (Blount et al. 2000a) and –70°C (Silva et al. 2008) in order to be stored for several years.

The use of polypropylene bottles is recommended since they are free of contamination by phthalates (Blount et al. 2000b, Koch et al. 2003b).

The ubiquitous nature of BBzP in the non-occupational environment (food, cosmetics, plastic flooring, etc.) is undoubtedly a factor in the variability of urinary assay results for MBzP.

Biomonitoring

Urinary mono-butylbenzyl-phthalate		
Inter-laboratory quality control	Not specified	
	Method 1	**Method 2**
Analytical technique	Liquid chromatography coupled to tandem mass spectrometry with electrospray ionisation LC-ESI-MS/MS	Liquid chromatography coupled to tandem mass spectrometry with electrospray ionisation LC-ESI-MS/MS
Limit of detection	Not specified	0,3 µg.L^{-1}
Limit of quantification	0,5 µg.L^{-1}	Not specified
Repeatability and trueness	Coefficient Variation (CV): 3,9 et 3,6 % à 9 µg.L^{-1}	CV: 5,4 et 6,8 % à 73 µg.L^{-1}
Precision	Not specified	3 %
Reference standard	Standard solution: D_4-MBzP	Standard solution of: $^{13}C_4$-MBzP
Treatment before analysis	1:00 treatment at 37°C with ß-glucuronidase[8]. Solid-phase extraction (SPE) of metabolites.	1:30 treatment at 37°C with ß-glucuronidase[8]. Solid-phase extraction (SPE) of metabolites.
Références	Koch et al 2003b	Silva et al. 2007

Inter-laboratory quality control	Intercomparison Programme (G-EQUAS) organized by the University of Erlangen-Nuremberg
Méthode 3	
Analytical technique	LC-ESI-MS/MS
Limit of détection	Not specified
Limit of quantification	0,2 µg.L^{-1}
Repeatability and trueness	CV : 5,7 et 3,8 % à 100 µg.L^{-1}
Precision	Not specified
Reference standard	Standard solution:$^{13}C_4$-MBzP
Treatment before analysis	1:30 treatment at 37°C with ß-glucuronidase[8] Direct injection in urine (10 µl).
Références	Servaes et al. 2013

8 ß-glucuronidase d'Escherichia Coli, car sans activité lipasique sur les diesters de phtalate. Le contrôle de la dé-conjugaison est réalisé par incorporation dans les échantillons de 4-méthyl-umbelliferryl-glucuronide ($^{13}C_4$).

References

Anderson WA, Castle L, Scotter MJ, Massey RC and Springall C. (2001). "A biomarker approach to measuring human dietary exposure to certain phthalate diesters." Food Addit Contam 18(12): 1068-74.

Agarwal DK, Maronpot RR, Lamb JC and Kluwe WM. (1985). "Adverse effects of butyl benzyl phthalate on the reproductive and hematopoietic systems of male rats." Toxicology 35(3): 189-206

Blount BC, Milgram KE, Silva MJ, et al. (2000a). "Quantitative detection of eight phthalate metabolites in human urine using HPLC-APCI-MS." Anal Chem. 72: 4127-34.

Blount BC, Silva MJ, Caudill SP, et al. (2000b). "Levels of seven urinary phthalate metabolites in a human reference population." Environ Health Perspect 108(10): 979-82.

CDC. Fourth National Report on Human Exposure to Environmental Chemicals, Updated tables. Atlanta: Center for Disease Control; March 2013.

CDC. Fourth National Report on Human Exposure to Environmental Chemicals. Atlanta: Center for Disease Control; 2009.

Duty SM, Calafat AM, Silva MJ, Ryan L and Hauser R. (2005). "Phthalate exposure and reproductive hormones in adult men." Hum Reprod 20(3): 604-10.

Duty SM, Calafat AM, Silva MJ, et al. (2004). The relationship between environmental exposure to phthalates and computer-aided sperm analysis motion parameters. J Androl. 25(2):293-302.

Duty SM, Silva MJ, Barr DB, et al. (2003). "Phthalate exposure and human semen parameters." Epidemiology 14(3): 269-77.

EC (2007). European Union Risk assessment report. Benzyl Butyl Phtalate, volume 76. (European Commission, Luxembourg). 274 p.

Hauser R, Meeker JD, Duty S, Silva MJ, Calafat AM. (2006) “Altered semen quality in relation to urinary concentrations of phtalate monoester and oxidative metabolites”. Epidemiology.;17(6):682-91.

Hines CJ, Nilsen Hopf NB, Deddens JA, et al. (2009). "Urinary Phthalate Metabolite Concentrations among Workers in Selected Industries: A Pilot Biomonitoring Study." Ann Occup Hyg 53(1): 1-17.

Itoh H, Iwasaki M, Hanaoka T, Sasaki H, Tanaka T, Tsugane S (2009). “Urinary phthalate monoesters and endometriosis in infertile Japanese women”. Sci Total Environ 408: 37-42.

Joensen UN, Frederiksen H, Jensen MB, et al. (2012). “Phthalate excretion pattern and testicular function: a study of 881 healthy Danish men”. Environ Health Perspect. 120(10):1397-403. Epub 2012 Jul 23.

Jönsson BA., Richthoff J, Rylander L, Giwercman A and Hagmar L. (2005). "Urinary phthalate metabolites and biomarkers of reproductive function in young men." Epidemiology 16(4): 487-93.

Koch HM, Gonzalez-Reche LM and Angerer J. (2003). “On-line clean-up by multidimensional liquid chromatography-electrospray ionization tandem mass spectrometry for high throughput quantification of primary and secondary phthalate metabolites in human urine” J. Chromatogr. B 784:169-182.

Kohn MC, Parham F, Masten SA, Portier CJ, Shelby MD. (2000) “Human exposure estimates for phthalates”. Environ Health Perspect.;108(10):440-2.

Martens F and Martens M. (2002). "[Determination of monoester metabolites of butylbenzyl phthalate (BBP) by GC-MS in the urine of exposed workers]." Acta Clin Belg Suppl(1): 16-23.

Servaes K, Voorspoels S et al. (2013) “Direct analysis of phthalate ester biomarkers in urine without preconcentration: Method validation and monitoring” J. Chromatogr. A 1294:25-32.

Silva MJ, Preau JL Jr, Needham LL, Calfat AM (2008). Cross validation and ruggedness testing of analytical methods used for the quantification of urinary phthalate metabolites Chromatogr B Analyt Technol Biomed Life Sci. 873(2):180-6.

Silva MJ, Samandar E, Preau JL Jr, et al. (2007). Quantification of 22 phthalate metabolites in human urine. J Chromatogr B Analyt Technol Biomed Life Sci. 860(1):106-12.

Silva MJ, Barr DB, Reidy JA, et al. (2003). "Glucuronidation patterns of common urinary and serum monoester phthalate metabolites." Arch Toxicol 77(10): 561-7.

Swan SH, Main KM, Liu F, et al. (2005). "Decrease in anogenital distance among male infants with prenatal phthalate exposure." Environ Health Perspect 113(8): 1056-61.

Weuve J, Hauser R, Calafat AM, Missmer SA and Wise LA (2010) “Association of Exposure to Phthalates with Endometriosis and Uterine Leiomyomata: Findings from NHANES, 1999-2004”. Environ Health Perspect 118(6): 825-32.

Wirth JJ, Rossano MG, Potter R, et al. (2008) “A Pilot Study Associating Urinary Concentrations of Phthalale Metabolites and Semen Quality”. Systems Biology in Reproductive Medicine 54: 143-154.