Collective expert appraisal: summary of discussion with conclusions

Regarding the "expert appraisal on recommending occupational exposure limits for chemical agents"

Evaluation of di(2-ethylhexyl)phthalate (DEHP) biomarkers

[CAS no.117-81-7]

This document summarises 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 (biomarkers WG).

Presentation of the issue

On 12 June 2007, AFSSET received a solicited request from the French Directorate General for Labour to conduct the scientific expert appraisal work required for setting occupational exposure limit values (OELVs) for DEHP.

As set by a Circular[1], France has established an indicative 8h-OELV of 5 $mg.m^{-3}$ for DEHP. The Directorate General for Labour asked AFSSET to reassess this value and, if necessary, to propose new occupational exposure limit values based on health considerations.

This request was entrusted to AFSSET's OEL Committee which, in June 2010, issued a report making the following recommendations for DEHP:

- to set an 8h-OEL of 0.8 $mg.m^{-3}$;
- no assignment of "skin notation";

ANSES decided to supplement its expert appraisal with an assessment of the biological monitoring data on DEHP in occupational environment, in order to establish the relevance of recommending monitoring of one or more biomarkers in addition to the atmospheric 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 metrology for assessing exposure to chemical agents. Biological monitoring assesses a worker's exposure by including all the routes by which a chemical penetrates the

[1] Circular of 19 July 1982 supplemented and amended by the Circular of 13 May 1987 on the acceptable values for concentrations of certain hazardous substances in workplace atmospheres

body (lung, skin, digestive tract). It is particularly worthwile 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 (wearing of respiratory protection, 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 provides for the use of biological monitoring of exposure and biological limit values.

OEL Committee definitions

Biological limit value (BLV): This is the limit value for the relevant biomarkers As for the 8h-OEL, it aims to protect workers exposed to the chemical agent in question regularly and over the course of a working life from the adverse effects associated with medium- and long-term exposure. Two types of biological limit values can be recommended depending on the available data:

- BLV based on a health effect: the level of a biomarker for which the scientific data do not report any health effects;
- BLV based on exposure to the 8h-OEL: average level of a biomarker corresponding, according to the scientific data, to exposure to the 8h-OEL.

Biological reference values from:

- the general population: the closest value to the 95th percentile of the distribution of biomarkers concentrations found in a general adult population whose characteristics are similar to those of the French population;
- otherwise a control population not occupationally exposed to the substance under study: the closest value to the 95th percentile of the distribution of biomarkers concentrations found in a control population not occupationally exposed to the substance under study.

These values cannot be considered to offer protection from the onset of health effects, but do allow a comparison with the concentrations of biomarkers assayed in exposed workers. These values are of particular interest 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 for this expert appraisal.

The methodological and scientific aspects of this group's work were regularly submitted to the OEL Committee. The report produced by the working group takes account of the 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".

Description of the method

A rapporteur in the biomarkers WG was mandated by the Agency to produce a summary report on biomarkers of exposure and the recommendation of biological limit values (BLVs) and biological reference values for the biomarkers considered as relevant. An ANSES officer also contributed to this report.

The summary report on the biomarkers for DEHP results from bibliographical information taking into account the scientific literature published on this substance until 2011. The bibliographical research was conducted in the following databases: Medline, Toxline, HSDB, ToxNet (CCRIS, GENE-TOX, IRIS), ScienceDirect. The rapporteur reassessed the original articles or reports cited as references whenever he considered it necessary, or whenever the Committee requested it.

The Expert Committee on expert appraisal for recommending occupational exposure limits for chemical agents adopted the summary report on the biomarkers at its meeting on 12 January 2012.

The summary and conclusions of the collective expert appraisal were adopted by the Committee on expert appraisal for recommending occupational exposure limits for chemical agents on 12 January 2012.

The collective expert appraisal work and the summary report were submitted to public consultation from 18/10/2012 to 20/12/2012. No comments were received. The OEL Committee adopted this version on 04 April 2013.

Result of the collective expert appraisal

Introduction

DEHP is a phthalate used as a plasticiser for plastics and elastomers (PVC). It is a ubiquitous pollutant and internal concentrations may be high in the general population since it is found in both homes and food.

In Europe, it is classified CMR, toxic to reproduction category 2.

For the assessment of the biological monitoring data on DEHP, 35 scientific papers were selected from the *Medline* database using the following keywords:

- di(2-ethylhexyl)phthalate and biomarker
- di(2-ethylhexyl)phthalate and biological monitoring

Three reports (by the Agency for toxic substances and disease registry, ATSDR - USA, Centers for Disease Control and Prevention, CDC - USA and European Chemicals Bureau, ECB - European Union) were also considered.

Toxicokinetics data

Dermal absorption of DEHP is between 2 and 5% and increases to 75% for the pulmonary route (ECB, 2008).

In rats, after absorption, DEHP is distributed mainly in the liver, kidneys, testes and blood (ATSDR, 2002). It seems that distribution differences in humans and animals are mainly quantitative.

In its free form, DEHP has a relatively short half-life in blood, of around 28 minutes. Initially, DEHP is metabolised mainly in the pancreas but also in the lungs, skin, adipose tissue and

kidneys, to mono-(2-ethylhexyl)phthalate (MEHP, the major metabolite in blood), then forms 2-ethylhexanol (ECB, 2008). MEHP has two phases of elimination in blood with an initial half-life of about 30 minutes and a second half-life of more than 3 hours. MEHP can be oxidised to form primary or secondary alcohols, which in turn are oxidised to carboxylic acids (ATSDR, 2002). To a lesser extent (unquantified) MEHP can be hydrolysed to phthalic acid. According to Koch *et al.* (2004 and 2005) four secondary metabolites of MEHP are found in blood in humans (volunteer study, after ingestion):

- mono[(2-carboxymethyl)hexyl]phthalate (2cx-MMHP),
- mono(5-carboxy-2-ethylpentyl)phthalate (5cx-MEPP),
- mono(2-ethyl-5-oxo-hexyl)phthalate (5oxo-MEHP),
- mono(2-ethyl-5-hydroxyhexyl)phthalate (5OH-MEHP).

Peak blood concentrations of the various DEHP metabolites are reached 2 to 4 hours after the start of ingestion. The half-lives of the oxidised metabolites (secondary metabolites) are between 2 and 5 hours with monophasic elimination kinetics in blood (Koch *et al.*, 2004 and 2005). These compounds can be conjugated to glucuronic acid. Humans excrete 60% of the metabolites as conjugates (ATSDR, 2002).

All the metabolites identified in urine have biphasic elimination (Koch *et al.*, 2005). In 2011, Anderson *et al.* published a study involving 20 volunteers (10 men and 10 women) who ingested a dose (0.31 or 0.78 mg randomly) of DEHP (radiolabelled). Over a 48-hour period, urine was collected at several intervals. The molar excretion fractions were determined at 24 and 48 hours and are summarised in the table below.

The kinetic parameters are summarised in the following table (from Anderson *et al.*, 2011):

Urinary metabolite	$T_{1/2(1)}$* (h)	$T_{1/2(2)}$* (h)	T_{max}* (h)	Molar excretion fraction (%) at 24h	Molar excretion fraction (%) at 48h
MEHP	2	5	2	6.2	6.3
5OH-MEHP	2	10	4	14.9	15.6
5oxo-MEHP	2	10	4	10.9	11.3
5cx-MEPP	3	12 to 15	4	13.2	13.9
2cx-MMHP	3	24	9 and 24	NR	NR

* $T_{1/2}$: half-life; T_{max}: time to obtain peak concentration

The authors state that the concentrations of the different metabolites are normally distributed and that the dispersion of the results is equivalent for the three secondary metabolites (between 20 and 25%). The exposure dose and gender do not significantly influence the excretion fractions.

Choice of biomarkers

The DEHP biomarkers identified in the scientific literature are the following (abbreviations given in brackets):

- Mono(2-ethylhexyl)phthalate — Urine — (MEHPu)
- Mono(2-ethyl-5-hydroxyhexyl)phthalate — Urine — (5OH-MEHP)
- Mono(2-ethyl-5-oxo-hexyl)phthalate — Urine — (5oxo-MEHP)
- Mono(5-carboxy-2-ethylpentyl)phthalate — Urine — (5cx-MEPP)
- Mono[(2-carboxymethyl)hexyl]phthalate — Urine — (2cx-MMHP)
- Di-2-ethylhexylphthalate — Blood — (DEHPb)

- Mono(2-ethylhexyl)phthalate Blood (MEHPb)

Given DEHP's short half-life in blood (estimated at 30 minutes), it is difficult to use this biomarker for biological monitoring in workplaces. No studies in workplaces using DEHP or MEHP in blood were identified. The studies in workplaces mainly report measurements of urinary metabolites. Consequently, blood biomarkers were not selected.

With a half-life of 24 hours, urinary 2cx-MMHP seems relevant as a biological indicator of exposure. However, very few data on it are available, and in particular no studies in workplaces provide information on this biological indicator. Urinary 2cx-MMHP was therefore not selected as a biological indicator of exposure to DEHP.

Urinary MEHP, 5OH-MEHP, 5oxo-MEHP and 5cx-MEPP are documented in some studies in workplaces. These biomarkers, specific to exposure to DEHP, have half-lives enabling samples to be taken at the end of the work shift. These biological indicators of exposure to DEHP can be selected for the biological monitoring of occupational exposure. However, urine samples collected for measuring MEHP require special measures to be taken at the time of collection to prevent transformation of DEHP to MEHP (leading to an overestimation of concentrations). Measuring MEHP alone can lead to an underestimation (low excretion) or overestimation (contamination) of exposure. This is the reason why the measurement of this metabolite has a greater inter-individual variability (30%) than the three secondary metabolites, 5OH-MEHP, 5cx-MEPP and 5oxo-MEHP (20 to 25%) (Anderson *et al.*, 2011). This biomarker was not selected for the biological monitoring of occupational exposure.

Determining the sum of several metabolites (MEHP, 5OH-MEHP, 5oxo-MEHP, 5cx-MEPP) can be considered because the individual variability from determining the sum of these metabolites would be slightly lower (18%) than the variability if each metabolite were measured separately. However, this difference does not seem significant and determining the sum of the four metabolites seems complex from an analytical point of view. Determining the sum of the four metabolites is no more advantageous than measuring a single metabolite.

5OH-MEHP and 5cx-MEPP account for the highest urinary fractions of the three secondary metabolites. Out of the three secondary metabolites in urine, 5oxo-MEHP is a more minor fraction and is therefore not the most suitable biomarker of exposure.

Studies in workplaces for all the secondary metabolites are extremely fragmented. Only one study establishes a relationship between 5OH-MEHP, 5oxo-MEHP or 5cx-MEPP with atmospheric concentrations of DEHP. Taking into account additional information from a field study conducted in France where concentrations of 5cx-MEPP were reported, **this biomarker can be selected as the most suitable for biological monitoring of exposure to DEHP.**

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

Name	**Urinary mono(5-carboxy-2-ethylpentyl)phthalate (5cx-MEPP)**	
Other substances giving rise to this BIE	None	
Conversion factor (with molecular weight)	MW: 308.37 1 µg.l^{-1} = 0.0032 µmol.l^{-1} 1 µmol.l^{-1} = 308.37 µg.l^{-1} 1 µg.g^{-1} creat = 0.364 µmol.mol^{-1} creat 1 µmol.mol^{-1} creat = 2.75 µg.g^{-1} creat	
Concentrations in the general population	USA-NHANES (2007-2008, 2604 people in the general population) - 95th percentile 20 years and older (1814 samples): 214 µg.g^{-1} creat (CDC, 2011)	
Recommended limit values for exposed workers	USA - ACGIH (BEI)	NR
	Germany - DFG (BAT)	
	Quebec - IRSST (BIE)	
	Finland - FIOH (BAL)	
	Other value(s) (Swiss, etc.)	

Study of the relationship between 5cx-MEPP concentrations and health effects

Most studies reporting reprotoxic effects of DEHP to humans concern the general population and have already been described in an AFSSET report (2010). They were not selected to establish the critical dose of DEHP but have been summarized in Appendix 1.

AFSSET's report (2010) concluded that only animal data were suitable to identify a critical dose for reproductive toxicity.

Study of the relationship between 5cx-MEPP concentrations and exposure to DEHP

Field studies

While exposure to DEHP in the general population is relatively well documented, occupational data on biological monitoring of exposure to this phthalate are currently scarce.

Only the study by Dirven *et al.* in the Netherlands reports both concentrations of urinary metabolites of DEHP and atmospheric concentrations of DEHP (individual samples) in several sectors of the PVC industry (Dirven *et al.*, 1993). Other studies in the workplaces (Gaudin *et al.* in France and Hines *et al.* in the United States) only report the urinary concentrations of biomarkers (Gaudin *et al.*, 2008 and 2011; Hines *et al.*, 2009).

As in the environmental field, workers primarily excrete the secondary metabolites, of which the most abundant is 5cx-MEPP (Preuss *et al.*, 2005). The tables below only list biomonitoring studies that used, in addition to MEHP, the secondary oxidative metabolites, such as 5cx-MEPP, to assess DEHP exposure.

5cx-MEPP (urinary) at the end of the work shift		
Sector	BIE concentration **Median** - maximum value $\mu g.l^{-1}$ and [$\mu g.g^{-1}$ of creatinine]	References
Footwear	**124.7 [91.6]** - NR	Dirven *et al.*, 1993
Cables	**48.4 [35.6]** - NR	
DEHP manufacture	NR	Hines *et al.*, 2009
PVC film	**283.0 [142.0]** - 2030 [625]	
Automotive filters	NR	
PVC compounding	**391.0 [200.0]** - 1080 [444]	
Tubing	**51.4 [31.0]** - 497 [53]	
Footwear	**132.0 [69.7]** - 3520 [1180]	
DEHP manufacture	**18.8 [14.3]** - 219 [122]	Gaudin *et al.*, 2011
Plastisol coatings	**103.7 [63.0]** - 961 [533]	
PVC granules 1	**166.4 [105.1]** - 1320 [372]	
PVC granules 2	**57.6 [26.9]** - 488 [579]	
Moulding polymers	**34.3 [27.7]** - 529 [177]	
Wall coverings	**134.6 [78.6]** - 1410 [600]	

No study reported any correlation equation between atmospheric concentrations of DEHP and urinary biomarkers concentrations.

Experimental data

The OEL Committee's calculations of atmospheric concentrations, according to the risk level, are based on the study by David *et al.* (2000). This is a chronic oral (diet) toxicity study in rats, involving male and female groups. Dose levels tested were 0; 100; 500; 2500 and 12,500 ppm for 104 weeks. These doses correspond to 0; 5.8; 28.9; 146.6 and 789.0 $mg.kg^{-1}.d^{-1}$ for males and 0; 7.3; 36.1; 181.7 and 938.5 $mg.kg^{-1}.d^{-1}$ for females. There was no significant change in the weight of the animals at the end of the study regardless of the exposure dose.

Bilateral aspermatogenesis was directly observed in groups exposed to 500, 2500 and 12,500 ppm, defining a NOAEL at 100 ppm, or 5.8 $mg.kg^{-1}.d^{-1}$, with a significant difference compared to the study's control group.

The study was performed in adult rats and therefore can potentially be transposed to workers. This was a long-term study (2 years in rats), which virtually corresponds to lifetime exposure in accordance with an occupational exposure scenario. The critical effect chosen, aspermatogenesis, is consistent in transposition from animals to humans.

A NOAEL of 5.8 $mg.kg^{-1}.d^{-1}$ for a critical effect corresponding to the onset of aspermatogenesis was determined in this study (David *et al.*, 2000).

To extrapolate this NOAEL to humans taking into account the same route of exposure (oral), it was decided to apply an allometric adjustment factor, thereby determining a human equivalent dose from the dose determined in rats.

Pulmonary absorption of DEHP in humans reaches 75 to 100% in adults (ECB, 2008). It seems that the rate of oral absorption of DEHP is at least equal to 50%, even 75% in humans. The OEL Committee selected an absorption fraction by oral route of 50% and by inhalation of 100% (the most protective assumption).

The daily dose, after allometric adjustment, corresponding to 100% absorption is equal to 0.75mg.kg^{-1}.d^{-1}. Several authors present an equation based on an equation of mass conservation (Kohn *et al.*, 2000; Koch *et al.*, 2003a; Wittassek *et al.*, 2007) such that:

$$\text{Ingested dose (mg.kg}^{-1}\text{.d}^{-1}\text{)} = \frac{\text{[5cx-MEPP] x CE x M(DEHP)}}{\text{f x M(5cx-MEPP)}}$$

- CE: creatinine excretion rate normalised to body weight (g.kg^{-1}.d^{-1}) 0.02
- f: molar excretion fraction of the urinary metabolite over 24 hours (%) 13.2
- M(DEHP): molecular weight of DEHP 391
- M(5cx-MEPP): molecular weight of 5cx-MEPP 294
- [5cx-MEPP]: urinary concentration of 5cx-MEPP (mg.g^{-1} creat)

Calculating these concentrations presents many uncertainties. The kinetic parameters of DEHP and of the metabolites were measured for oral absorption of a single dose of DEHP. The molar excretion fraction of 5cx-MEPP is not determined:

- for continuous exposure, with the 5cx-MEPP concentration at equilibrium;
- for exposure by inhalation.

Establishment of BLVs and choice of biological reference values

In humans and at the workplace, the dose-response relationship between urinary concentrations of 5cx-MEPP and aspermatogenesis or any other effect has not been studied. In animals, calculating the concentrations of 5cx-MEPP depending on the study selected to establish the OEL presents many uncertainties. Thus, it was not considered relevant to recommend a biological limit value based on a reprotoxic effect or another health effect.

The field studies were unable to find a relationship between atmospheric concentrations of DEHP and urinary concentrations of 5cx-MEPP at the end of the work shift. It was therefore not possible to establish a BLV on the basis of exposure to the OEL.

In addition, the Committee wishes to reiterate that the ALARA[2] principle should be applied in the presence of a carcinogenic, mutagenic or reprotoxic substance. Thus, when it is not possible to calculate biomarker concentrations on the basis of a quantitative risk assessment or to recommend a pragmatic biological limit value, biological reference values may be proposed.

It should be noted that in 2011 the European Commission decided to prohibit six substances used in industry, including DEHP, because of their health hazards. An exemption may however be specified for companies which have been granted authorisation for use (under the REACh Regulation).

Proposed biological reference values

These values are not intended to protect from health effects but allow to assess worker's exposure.

Environmental data in the general population are relatively more abundant. The American NHANES studies with cohorts of over 1500 people (aged 20 years and over) are reference studies. Urine samples collected in 2007-2008 give a value for the 95th percentile of the

[2] As Low As Reasonably Achievable

distribution of urinary concentrations of 5cx-MEPP equal to 214 µg.g^{-1} of creatinine (CDC, 2011). Starting from the assumption that the characteristics of the US population are close to those of the French population, a concentration of 214 rounded to 200 µg.g^{-1} of creatinine for 5cx-MEPP can be proposed as the biological reference value.

Conclusions of the collective expert appraisal

Biological indicator of exposure: 5cx-MEPP (urine)

BLV based on a health effect: None

BLV based on exposure to the 8h-OEL: None

Biological reference values:

- 200 µg.g^{-1} of creatinine (irrespective from the smoking status)

Sampling method and factors that may affect the interpretation of 5cx-MEPP assays

Diet is a source of variability in the results since food is one of the main sources of DEHP in the general population (from laminated wrap when heated).

The kinetics of 5cx-MEPP can cause it to accumulate very slightly over the course of a working week. When monitored over several consecutive working days, samples taken at the beginning of the shift can provide information on whether or not there is an accumulation effect over the week and show evidence of the exposure at the very end of the previous day's shift. No particular requirements related to urine sampling for determining 5cx-MEPP are indicated in the literature.

Biometrology

Urinary 5cx-MEPP		
Analytical methods		
	Method 1	**Method 2**
Analytical technique Bibliographic references	Column switching - HPLC-MS/MS with ESI interface in negative ionisation mode Koch *et al.*, 2003b	Column switching - HPLC-MS/MS with ESI interface in negative mode Preuss *et al.*, 2005
Limit of detection	NR	0.25 µg.l^{-1}
Limit of quantification	NR	0.5 µg.l^{-1}
Fidelity	Repeatability (%CV): 2.5 – 8.3 for urine overloaded at concentrations of around 10 µg.l^{-1}	Repeatability (%RSD): 4.0 – 5.6 for urine overloaded at 10 µg.l^{-1}
Precision	NR	NR
Reference standard	Deuterium-labelled internal standards	Deuterium-labelled internal standards
Existence of an inter-laboratory quality control programme	NR	Inter-comparison programme (G-EQUAS) organised by the University of Erlangen-Nuremberg

Summary date validated by the Committee: 4 April 2013

On behalf of the Committee Experts

François Paquet,

Chairman of the Committee

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