The Director General

Maisons-Alfort, 12 July 2017

OPINION
of the French Agency for Food, Environmental and Occupational Health & Safety

on "the development of reprotoxic oral TRVs for three phthalates: diisobutyl phthalate (DIBP), diisooctyl phthalate (DIOP) and di-n-octyl phthalate (DnOP)"

ANSES undertakes independent and pluralistic scientific expert assessments.
ANSES primarily ensures environmental, occupational and food safety as well as assessing the potential health risks they may entail.
It also contributes to the protection of the health and welfare of animals, the protection of plant health and the evaluation of the nutritional characteristics of food.

It provides the competent authorities with the necessary information concerning these risks as well as the requisite expertise and technical support for drafting legislative and statutory provisions and implementing risk management strategies (Article L.1313-1 of the French Public Health Code).

Its opinions are published on its website. This opinion is a translation of the original French version. In the event of any discrepancy or ambiguity the French language text dated 12 July 2017 shall prevail.

On 9 June 2015, ANSES issued an internal request to carry out the following expert assessments: development of oral toxicity reference values (TRVs) for the reprotoxic effects of the following chemicals: diisobutyl phthalate (DIBP) (CAS 84-69-5), diisooctyl phthalate (DIOP) (CAS 27554-26-3) and di-n-octyl phthalate (DnOP) (CAS 117-84-0).

1. BACKGROUND AND PURPOSE OF THE REQUEST

A toxicity reference value, or TRV, is a toxicological indicator for qualifying or quantifying a risk to human health. It establishes the link between exposure to a toxic substance and occurrence of an adverse health effect. TRVs are specific to a duration (acute, subchronic or chronic) and route (oral or respiratory) of exposure. The way TRVs are established differs depending on the knowledge or assumptions made about the substances' mechanisms of action. Currently, the default assumption is to consider that the relationship between exposure (dose) and effect (response) is monotonic. In the current state of knowledge and by default, it is generally considered that for non-carcinogenic effects, toxicity is only expressed above a threshold dose (ANSES, 2015a).

In practice, establishing a threshold TRV involves the following four steps:

- choice of the critical effect;
- choice of a good quality scientific study generally enabling establishment of a dose-response relationship;
- choice or establishment of a critical dose from experimental doses and/or epidemiological data;

- application of uncertainty factors to the critical dose to account for uncertainties.

TRVs are established according to a highly structured and rigorous approach involving collective assessments by groups of specialists.

This opinion follows the Agency's expert appraisal work on phthalates published in a 2015 report (ANSES, 2015a, b and c), in response to a formal request from the Directorate General for Health in June 2009 (2009-SA-0331) regarding Category 2 reprotoxic and/or endocrine-disrupting (ED) substances. This report had highlighted the need to propose reprotoxic TRVs for diisobutyl phthalate (DIBP), diisooctyl phthalate (DIOP) and di-n-octyl phthalate (DnOP).

2. ORGANISATION OF THE EXPERT APPRAISAL

ANSES entrusted examination of this internal request to the Expert Committee (CES) on "Characterisation of substance hazards and toxicity reference values" and the Working Group on "Endocrine disruptors" reporting to this CES. The work was presented to the CES between January 2016 and March 2017. It was adopted by the CES at its meeting on 30 March 2017.

The expert appraisal was carried out in accordance with French Standard NF X 50-110 "Quality in Expert Appraisals – General Requirements of Competence for Expert Appraisals (May 2003)".

ANSES analyses interests declared by experts before they are appointed and throughout their work in order to prevent risks of conflicts of interest in relation to the points addressed in expert appraisals. The experts' declarations of interests are made public via the ANSES website (www.anses.fr).

3. ANALYSIS AND CONCLUSIONS OF THE CES

Diisobutyl phthalate (DIBP) - CAS No. 84-69-5

- Toxicokinetics

Few data are available on DIBP. A study in rats dermally exposed to DIBP indicated high dermal or percutaneous absorption and elimination in urine and faeces, with no significant accumulation in the organs. The main metabolites found in humans are MIBP (monoisobutyl phthalate) and OH-MIBP (3OH-mono-methylpropyl phthalate) (CPSC, 2011; ANSES, 2015c).

- Toxicity

Only effects on reproduction and development were analysed, with the aim of proposing a reprotoxic TRV for DIBP. Among the available studies, two studies of good quality exposing pregnant rats to DIBP showed foetal mortality, impairment of the reproductive system, delayed puberty and nipple retention in male offspring (Saillenfait *et al*., 2006, 2008). These effects are also regularly described in animal studies with medium-chain phthalates. This led to some of these compounds, including DIBP, being assigned a harmonised European classification as Category 1B reprotoxic substances by the Risk Assessment Committee of the European Chemicals Agency (ECHA). To date, the data in humans are still contradictory.

- Establishing the TRV
    - Choice of the critical effect

The set of studies currently available on DIBP is relatively limited, particularly in terms of dose levels, exposure times and effects analysed. The substance has not been tested at doses below 100 mg/kg/day. The possibility that some critical effects could occur at lower doses cannot be ruled out. There would therefore be considerable uncertainty if a TRV was established on the basis of data specific to DIBP.

In view of the structural, physico-chemical and toxicological similarities between DIBP and DnBP (di-n-butyl phthalate, CAS 84-74-2), the CES experts believe that DIBP could induce effects comparable to DnBP under the same conditions. Although there appear to be differences in toxicity between these two substances, these are considered minor in view of the results of the study by Saillenfait *et al.* (2008). A read-across between the data on DIBP and DnBP was therefore considered appropriate by the CES in order to derive a reprotoxic TRV for DIBP.

- Analysis of existing TRVs for DnBP and proposed TRV for DIBP

Several reprotoxic TRVs for DnBP were found in the literature. The TRV for DnBP established by AFSSET (2009) was considered appropriate by the CES experts and was therefore selected as the TRV for DIBP.

Table 1: Reprotoxic TRV for DnBP

Critical effect (key study)	Critical concentration	UF	TRV
Reduction of testicular spermatocyte development and nipple retention observed in offspring Prenatal developmental toxicity study in Sprague-Dawley rats by oral route (GD15-PND21) Lee *et al.*, 2004	LOAEL[1] = 2 mg/kg/d No NOAEL[2] BMDL[3] of little relevance	1000 UF_A: 10 UF_H: 10 UF_L: 10	**TRV = 0.002 mg/kg/d** **Confidence level:** Data collection: high Study: moderate (+ uncertainty) Critical dose: moderate (no NOAEL) TRV: moderate

In conclusion: the TRV for DnBP developed by AFSSET (2009) is 0.002 mg/kg/d and is considered to be applicable to DIBP.

[1] Lowest Observed Adverse Effect Level

[2] No Observed Adverse Effect Level

[3] Lower limit of the confidence interval of the benchmark dose

Table 2: Reprotoxic TRV for DIBP

Critical effect (key study)	Critical concentration	UF	TRV
Reduction of testicular spermatocyte development and nipple retention observed with DnBP Lee *et al*., 2004	LOAEL = 2 mg/kg/d	1000 UF_A: 10 UF_H: 10 $UF_{B/L}$: 10	**TRV = 0.002 mg/kg/d** **Confidence level: moderate**

- Confidence level

The overall confidence level was assigned to the oral TRV for the reprotoxic effects of DIBP based on the following criteria:

- Level of confidence in the nature and quality of the data: **moderate**, considering that the TRV for DIBP is based on a comparative reading with the data on DnBP.
- Level of confidence in the choice of the critical effect and the mode of action: **high**; in view of the available knowledge on phthalates, the critical effects selected are considered appropriate and seem to be particularly sensitive. The effects are consistent with the reprotoxicity mode of action attributable to phthalates. Lastly, these effects were deemed to be extrapolable to humans.
- Level of confidence in the choice of the key study: **moderate**, considering the absence of a NOAEL and a limited duration of exposure from the 15th day of gestation to the 21st postnatal day.
- Level of confidence in the choice of the critical dose: **moderate**, considering the use of a LOAEL.

Thus, the overall level of confidence for this TRV is moderate.

The CES points out that regarding the effects on development, it is generally accepted that a single exposure may be sufficient to induce the effect if this exposure occurs during a critical phase of embryo-foetal development. This type of TRV is applicable for short exposure durations (from a few hours to a few days).

■ Diisooctyl phthalate (DIOP) - CAS No. 27554-26-3

- Toxicokinetics

Experimental data in different animal species show that DIOP is readily absorbed orally. According to a study on volunteers exposed to a mixture of phthalates including DIOP, monoesters of DIOP were excreted in urine in 24 hours. The metabolites found in rats receiving DIOP by gavage were mono-(3-carboxypropyl) phthalate (MCPP), mono-n-octyl phthalate (MnOP) and mono-(3-methyl-5-dimethylhexyl) phthalate (MiNP). However, the presence of MCPP and MnOP may actually be due to contamination with DnOP (CPSC, 2011).

- Toxicity

Only effects on reproduction and development were analysed, with the aim of proposing a reprotoxic TRV for DIOP. Among the available studies, a study of good quality exposing pregnant rats to DIOP (Saillenfait *et al*., 2013) showed a decrease in foetal weight, skeletal variations, foetal mortality and impairment of the reproductive system in male offspring. These effects are also frequently described in animal studies with medium-chain phthalates. This led to some, including DIBP, DnBP (di-n-butyl phthalate), DEHP (di-(2-ethylhexyl) phthalate), DnPP (di-n-pentyl phthalate) or DnHP (di-n-hexyl phthalate), being assigned a harmonised European classification as Category 1B reprotoxic substances by the Risk Assessment Committee of the European Chemicals Agency (ECHA). Concerning DIOP, a proposal for classification as a Category 1B reprotoxic substance was submitted by France to ECHA in 2016. The Risk Assessment Committee is expected to take a decision on this proposal in 2017 or 2018. To date, the data in humans are still contradictory.

- Establishing the TRV
  - Choice of the critical effect

The set of studies currently available on DIOP is very limited in terms of dose levels, exposure times and effects analysed. There would therefore be considerable uncertainty if a TRV were established on the basis of data specific to DIOP.

In view of the toxicological similarities between DnBP and DIOP, the CES experts consider that DIOP could induce effects comparable to DnBP under the same conditions but at doses twice as high. A read-across between the data on DIOP and DnBP was therefore considered appropriate by the CES in order to derive a reprotoxic TRV for DIOP after adjustment of their respective toxicities.

  - Analysis of existing TRVs for DnBP and proposed TRVs for DIOP

Several reprotoxic TRVs for DnBP were found in the literature. The TRV for DnBP established by AFSSET (2009) was considered appropriate by the CES experts and was therefore selected as the TRV for DIOP.

Table 3: Reprotoxic TRV for DnBP

Critical effect (key study)	Critical concentration	UF	TRV
Reduction of testicular spermatocyte development and nipple retention observed in offspring Prenatal developmental toxicity study in Sprague-Dawley rats by oral route (GD15-PND21) Lee *et al*., 2004	LOAEL = 2 mg/kg/d No NOAEL BMDL little relevance	1000 UF_A: 10 UF_H: 10 UF_L: 10	**TRV = 0.002 mg/kg/d** **Confidence level:** Data collection: high Study: moderate (+ uncertainty) Critical dose: moderate (no NOAEL) TRV: moderate

In conclusion: the TRV for DnBP developed by AFSSET (2009) is 0.002 mg/kg/d. After adjustment, the TRV for DIOP is 0.004 mg/kg/d.

Table 4: Reprotoxic TRV for DIOP

Critical effect (key study)	Critical concentration	UF	TRV
Reduction of testicular spermatocyte development and nipple retention observed with DnBP Lee *et al.*, 2004	$LOAEL_{DnBP}$ = 2 mg/kg/d $LOAEL_{DIOP}$ = 4 mg/kg/d (after taking into account the difference in toxicity between DIOP and DnBP)	1000 UF_A: 10 UF_H: 10 $UF_{B/L}$: 10	**TRV = 0.004 mg/kg/d** **Confidence level: moderate**

- Confidence level

The overall confidence level was assigned to the oral TRV for the reprotoxic effects of DIOP based on the following criteria:

- Level of confidence in the nature and quality of the data: **moderate**, considering that the TRV for DIOP is based on a comparative reading with the data on DnBP.
- Level of confidence in the choice of the critical effect and the mode of action: **high**; in view of the available knowledge on phthalates, the critical effects selected are considered appropriate and seem to be particularly sensitive. The effects are consistent with the reprotoxicity mode of action attributable to phthalates. Lastly, these effects were deemed to be extrapolable to humans.
- Level of confidence in the choice of the key study: **moderate**, considering the absence of a NOAEL and a limited duration of exposure from the 15th day of gestation to the 21st postnatal day.
- Level of confidence in the choice of the critical dose: **moderate**, considering the use of a LOAEL.

Thus, the overall level of confidence for this TRV is moderate.

The CES points out that regarding the effects on development, it is generally accepted that a single exposure may be sufficient to induce the effect if this exposure occurs during a critical phase of embryo-foetal development. This type of TRV is applicable for short exposure durations (from a few hours to a few days).

■ Di-n-octyl phthalate (DnOP) - CAS No. 117-84-0

- Toxicokinetics

Oral absorption of DnOP has rarely been described in the literature. However, it is assumed to be rapid. DnOP is metabolised to MnOP (mono-n-octyl phthalate) and n-octanol after hydrolysis via esterases. The n-octanol is then oxidised to fatty acids. The MnOP is oxidised to MCPP (mono-(3-carboxypropyl) phthalate) but also to other minor compounds such as MCMP (mono-carboxymethyl phthalate), MCPeP (mono-(5-carboxy-n-pentyl) phthalate), MCHpP (mono-(7-carboxy-n-heptyl) phthalate), MOOP (mono-oxo-n-octyl phthalate) isomers such as mono-(7-oxo-n-octyl) phthalate and phthalic acid. MnOP and its oxidised metabolites are then conjugated to

glucuronic acid. DnOP is not found in urine or faeces. The major metabolite found in urine is MCPP (CPSC, 2010).

- Toxicity

Only effects on reproduction and development were analysed, with the aim of proposing a reprotoxic TRV for DnOP. Among the available studies, a study of good quality exposing pregnant rats to DnOP (Saillenfait *et al*., 2011) showed skeletal abnormalities and effects on the liver (increased liver enzymes and decreased weight).

- Establishing the TRV
    - Choice of the critical effect

The study by Saillenfait *et al.* (2011) does not have any methodological bias, but the observations made were essentially morphological and the study stops at the end of foetal life without any postnatal follow-up. It was able to identify a LOAEL of 250 mg/kg/d for DnOP with the presence of a supernumerary lumbar rib as the observed effect. Considering that this type of malformation can be frequent in rats, related to various deterministic factors, and reversible, it can be regarded as normal in relation to the developmental process and generally has no impact on health. The experts therefore considered that this effect was not appropriate for deriving a TRV for this substance.

Moreover, unlike other phthalates (especially medium chain), DnOP does not seem to induce any effect on the endocrine system. However, it should be noted that few studies in the literature on DnOP have investigated this type of effect.

In conclusion, no reprotoxic TRV was proposed for DnOP.

The CES recommends conducting toxicological studies on DnOP in order to establish a TRV for this compound.

Due to the massive and widespread presence of phthalates, a reprotoxic TRV for other phthalates should be established, if necessary by means of a comparative reading.

4. AGENCY CONCLUSIONS AND RECOMMENDATIONS

The French Agency for Food, Environmental and Occupational Health & Safety endorses the conclusions and recommendations of the CES on "Characterisation of substance hazards and toxicity reference values" on the formulation of oral toxicity reference values for the reprotoxic effects of the phthalates assessed.

Critical effect (key study)	Critical concentration	UF	TRV
DIBP			
Reduction of testicular spermatocyte development and nipple retention observed with DnBP (Lee *et al*., 2004)	LOAEL = 2 mg/kg/d	1000 UF_A: 10 UF_H: 10 $UF_{B/L}$: 10	**TRV = 0.002 mg/kg/d** **Confidence level: moderate**
DIOP			
Reduction of testicular spermatocyte development and nipple retention observed with DnBP (Lee *et al*., 2004)	$LOAEL_{DnBP}$ = 2 mg/kg/d $LOAEL_{DIOP}$ = 4 mg/kg/d (after taking into account the difference in toxicity between DIOP and DnBP)	1000 UF_A: 10 UF_H: 10 $UF_{B/L}$: 10	**TRV = 0.004 mg/kg/d** **Confidence level: moderate**
DnOP			
No reprotoxic TRV was proposed			

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KEYWORDS

Toxicity reference value, reprotoxicity, endocrine disruption, DIBP, DIOP, DnOP, phthalates, oral route