Maisons-Alfort, 14 November 2012

The Director General

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

on the development of a chronic toxicity reference value for di(2-ethylhexyl)phthalate (DEHP) via the oral route

(CAS No. 117-81-7)

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 all necessary information concerning these risks as well as the requisite expertise and scientific 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 made public.

1. BACKGROUND AND PURPOSE OF THE REQUEST

The development of toxicity reference values (TRVs) is one of the Agency's permanent missions, and draws on the previous experience of the French Agency for Environmental and Occupational Health Safety (AFSSET). Methodological work has thus been under way for several years, especially under the first and second French National Environment and Health Action Plans (PNSE1 from 2004-2008 and PNSE2 from 2009-2013) and the Cancer Plan (2003-2007). This led to the drafting of several methodological guides for the production of TRVs for chemicals based on reprotoxic effects on the one hand and carcinogenic effects on the other.

This approach was then extended to the field of chemical carcinogens, which in 2007 led to the development of a method for establishing TRVs based on carcinogenic effects. A pilot phase was conducted to validate the implementation of the proposed method. Benzene, cadmium, ethanol, naphthalene and vinyl chloride were selected as the substances to be studied during this pilot phase. This current Opinion concerns the TRVs for di(2-ethylhexyl)phthalate (DEHP).

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.

"Threshold dose" TRVs are established for substances that cause, above a certain dose, damage whose severity is proportional to the absorbed dose, while "non-threshold dose" TRVs are established for substances for which there is a probability, however small, that even a single molecule entering the body will cause harmful effects for the organism. Threshold TRVs are usually expressed as acceptable or tolerable daily doses or

concentrations (Acceptable Daily Intake: ADI, Tolerable Daily Intake: TDI, Tolerable Concentration in Air: TCA, etc.), or reference doses or concentrations (Reference Dose: RfD or Reference Concentration: RfC). Non-threshold TRVs are generally expressed as excess risk per unit (Excess Risk per Unit: ERU, Drinking Water Unit Risk: DWUR, Inhalation Unit Risk: IUR, Reference Concentration: RC, etc.).

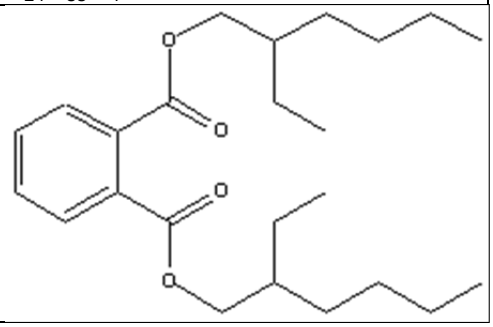
In practice, establishing a TRV involves the following four steps:

- choice of the critical effect;
- choice of a good quality scientific study generally enabling establishment of a dose-response relationship;
- choice or development of a critical dose from experimental doses and/or epidemiological data;
- application of uncertainty factors (UFs) to the critical dose to take uncertainties into account, or a linear extrapolation to the origin derived from the critical dose for non-threshold TRVs.

TRVs[1] are established according to a highly structured and rigorous approach involving collective assessments by groups of specialists.

Di(2-ethylhexyl)phthalate (DEHP) is part of the phthalate family. Phthalates have applications mainly as plasticisers for plastics and polymers, especially polymers of vinyl chloride, vinyl acetate and cellulose.
DEHP is being or has been assessed in various expert appraisals by the French Agency for Food, Environmental and Occupational Health & Safety (ANSES), mainly as part of the development of indoor air quality guidelines (IAQGs), Occupational Exposure Limits (OELs) and the assessment of the risks of Category 2B reprotoxic substances and/or substances suspected of being endocrine disruptors.

Identification of DEHP

CAS No., EINEICS No., etc.	CAS: 117-81-7 EINECS: 204-211-0 INDEX: 607-317-00-9
Name	Di(2-ethylhexyl)phthalate
Synonyms	Bis(2-ethylhexyl)phthalate Phthalic acid dioctyl ester DEHP DOP
Molecular formula	$C_{24}H_{38}O_4$
Structural formula	

[1] Method of establishing toxicity reference values for carcinogenic chemicals, ANSES Scientific Edition, March 2010

Regulations

Regulation (EC) No 1272/2008 on classification, labelling and packaging, known as the CLP Regulation, classifies DEHP as reproductive toxicity Category 1B (Safety Phrases: 53 – 45; Identified Hazard: T).
Under the REACH Regulation (EC) No. 1907/2006, the European Chemicals Agency (ECHA) has proposed adding DEHP to the list of substances for inclusion in Annex XIV for authorisation procedures[2]. A restriction dossier was therefore submitted in 2011.
Furthermore, DEHP is subject to a ban on use (0.1%) in toys and childcare articles, in cosmetics and in plastic materials and articles intended to come into contact with foodstuffs.

DEHP is being assessed in various expert appraisals by ANSES, mainly as part of the development of indoor air quality guidelines (IAQGs) and the assessment of the risks of Category 2B reprotoxic substances and/or substances suspected of being endocrine disruptors. This work is being supervised by respectively the Expert Committee (CES) on Assessment of the risks related to air environments and the CES on Assessment of the risks related to chemical substances.

2. ORGANISATION OF THE EXPERT APPRAISAL

ANSES entrusted the development of an oral TRV for DEHP to the Expert Committee (CES) on Assessment of the risks related to chemical substances.

This work drew on the expert appraisal conducted by the Working Group on Indoor Air Quality Guidelines II (IAQG II WG), reporting to the CES on Assessment of the risks related to air environments, on the process for establishing indoor air quality guidelines (IAQGs) and indoor dust guidelines (IADGs) for DEHP. This work was also presented for comments to the WG on Toxicity Reference Values on 19 November 2010.

The expert work by the Working Group was submitted to the CES on Assessment of the risks related to chemical substances on 15 September 2011, and 31 May, 28 June and 20 September 2012.

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” to ensure compliance with the following points: competence, independence, transparency and traceability.

[2] The authorisation scheme intends each use of certain substances among those of greatest concern for health or the environment to be subject to an authorisation that would enable it to be strictly controlled.

3. ANALYSIS AND CONCLUSIONS OF THE CES

Collection of toxicological data

Toxicokinetics

After oral administration, DEHP is rapidly hydrolysed to a mono-ethyl metabolite (MEHP) by digestive hydrolases and esterases. Consequently, it is absorbed mainly in hydrolysed form. The bioavailability of DEHP varies according to species and physiological stage.

It is distributed widely in tissue (liver, kidneys, testes, etc.) without preferential tissue accumulation. There are numerous sites of biotransformation. Besides the digestive tract, lipases have been found in the pancreas, lungs, skin, adipose tissue and kidneys. Microsomal oxidation followed by conjugation also contribute to the formation of around twenty metabolites (alcohols, acid, etc.), and monitoring of exposure to some of these has been proposed.

Elimination occurs via urine and faeces. Excretion in breast milk and placental distribution (detection in foetal tissues) have also been reported. The pathways and rates of elimination vary according to the dose levels.

PBPK models have been published for the ingestion of DEHP in humans and rats, but their use during this expert appraisal did not yield sufficiently reliable results for establishing a TRV.

Health effects

In general, the human epidemiological data on DEHP have limitations relating mainly to the estimation of exposure (no direct measurement of DEHP, indirect estimation via the use of plastic materials, high co-exposure in the workplace, etc.), or other study weaknesses (confounding factors not taken into account, low numbers studied, etc.). This limits the scope of these studies' conclusions and more importantly makes it difficult to identify a causal link between levels of DEHP exposure and a health effect.

The numerous animal studies have come to similar conclusions, especially regarding effects on reproduction. It should be noted that even for these studies, there may be some experimental bias making it difficult to interpret the studies. In particular, contamination from phthalates in laboratory equipment can bias the measurement of exposure.

Non-carcinogenic effects

Acute toxicity

In humans and animals, no acute toxicity has been observed following ingestion of DEHP.

Subchronic and chronic toxicity other than toxicity to reproduction

The data in humans are rare and inconclusive.

In animals, the organs affected by oral exposure to DEHP are mainly the liver, kidneys and to a lesser degree the nervous system.

In the liver, exposure to DEHP causes hyperplasia (the first effect observed in rats). In rodents, chronic ingestion of DEHP also causes necrosis, lipid infiltration, spongiosis hepatitis and neoplastic lesions.

In the kidneys, increased kidney weight and mineralisation of the renal papilla have mainly been observed after chronic oral exposure to DEHP in rats, mice and rabbits. In monkeys, however, a decrease in relative kidney weight was observed in females.

Reprotoxicity

In humans, several epidemiological studies have identified a relationship between exposure to DEHP (estimated from the concentration of urinary or blood metabolites) and the occurrence of testicular function disorders. In particular, they have shown a decrease in the concentration of spermatozoa in ejaculate, a negative association between the concentration of urinary MEHP and sperm velocity, and a correlation between the excretion of DEHP metabolites and changes in sperm quality (sperm motility, concentration and morphology). Some authors have also linked DEHP exposure to a decrease in serum testosterone concentrations. Epidemiological studies assessing the reprotoxic effects of DEHP are however limited by the low numbers of study subjects or confounding biases.
These effects have also been observed in rodents (rats and mice), and to a lesser extent, in primates (a few studies available). Many effects, including in particular a decrease in testicular weight, degeneration of seminiferous tubules, testicular atrophy leading to aspermatogenesis and variations in serum testosterone levels, have been observed in animals.

There are few data in women. A significant correlation between high blood levels of DEHP and MEHP and premature breast development (thelarche) has been observed in young girls aged 2 to 8 years.

In animals, despite the few publications available, the data confirm the observations made on the effects of DEHP on the female reproductive system. Reported effects include in particular suppression of ovulation or a decrease in the granulosa cells of the preovulatory follicle and in serum oestradiol.

In animals, other effects on endocrine function have been reported following ingestion of DEHP. For example, in female Fischer 344 rats, metabolism of oestradiol and oestrogen receptors were affected following subchronic exposure via feed.

In rats and mice, teratogenic effects, including especially an increase in foetal mortality, the number of resorbed foetuses or the number of malformations in survivors, have also been described following ingestion of DEHP.

Consistency between animals and humans

The main reprotoxic effects observed in rodents are changes in the male reproductive tract, with inhibition of steroidogenesis and gametogenesis functions (greater when exposure occurs during certain periods of life). It is likely that phthalates, including DEHP, also exert an anti-androgenic effect directly on the reproductive organs. Although these effects have not (or rarely) been observed in laboratory primates, the results in rats are consistent with the studies conducted in humans. The observation of a decreased anogenital distance in male infants, related to high phthalate and metabolite levels in the mother at 29 weeks of gestation, suggests insufficient androgenisation during foetal development. Moreover, in adult men, the majority of studies show a link between phthalate levels and changes in certain sperm characteristics.

Changes in sperm count or viability may have more severe consequences in humans, in whom fertility is more susceptible to small changes to these gametes. There are differences in metabolism and physiology between rodents and humans. It is difficult, however, to determine the importance of this effect without knowing precisely which effects on human cells correspond to the most vulnerable types and stages in rodents.

Consequently, exposure to phthalates in humans can result in changes to certain parameters of reproductive function, especially if this exposure takes place during sensitive periods, such as intrauterine life and the neonatal period.

Carcinogenicity

The results of studies with or without metabolic activation (studies on *S. typhimurium, S. cerevisiae,* mouse lymphoma cells, human lymphoblasts, etc.) did not indicate any genotoxic activity of DEHP.

Concerning the carcinogenic effects, in 2011 the International Agency for Research on Cancer (IARC) reclassified DEHP as possibly carcinogenic to humans (from Group 3 to Group 2B) based on animal data and assumptions about its mechanisms of action. The liver tumours observed in rats and mice are partly due to a mechanism of action specific to rodents that cannot be transposed to humans. However, the IARC has raised the possibility of other mechanisms of action concerning liver and testicular tumours observed in rats. As DEHP is not genotoxic, the assumption of a threshold dose mechanism could be retained, but the current data appear insufficient in quantitative terms for establishing a TRV.

Susceptible population groups

Reproductive toxicity studies conducted in animals show that the young are more susceptible to the testicular effects than adults. DEHP affects fertility and development of rodents of both sexes and induces postnatal developmental effects in juveniles or animals exposed *in utero*. In juvenile males, DEHP causes severe testicular effects in several species (rats, mice, ferrets and hamsters) including atrophy. The European Chemical Bureau has stated that developing male rats are more susceptible to the testicular toxicity induced by DEHP than sexually mature animals. After exposure to DEHP, lesions appear more rapidly in juvenile rats than in adult rats. Juvenile rats respond at lower doses than adults or develop more severe lesions than adult rats at equivalent doses. The studies indicate that these effects may be irreversible, thus affecting the animal's development and particularly the reproductive organs.
INSERM's collective expert report on "Reproduction and Environment" (2011) indicates that the most vulnerable periods of exposure are those during intrauterine and neonatal life. However, it is unclear whether the mechanisms involved during these two periods are identical.

Review of existing TRVs

The following table lists the various oral threshold TRVs for chronic exposure.

Organisation	Critical effect (animal species)	Critical dose (BMD/NOAEL/LOAEL)	UF	TRV ($mg.kg^{-1}.d^{-1}$)	Source study
ATSDR (2002)	Aspermatogenesis (F344 rats)	NOAEL = 5.8 $mg.kg^{-1}.d^{-1}$ Via feed 104 weeks	100	MRLc = 0.06	David *et al.*, 2000
Health Canada (1994)	Developmental toxicity (mice)	NOAEL = 44 $mg.kg^{-1}.d^{-1}$ Via feed	1000	TDI = 0.044	Wolkowski-Tyl *et al.*, 1984
RIVM (2000)	Sertoli cell vacuolation (SD rats)	NOAEL = 3.7 $mg.kg^{-1}.d^{-1}$ Via feed 13 weeks	1000	TDI = 0.004	Poon *et al.*, 1997
US EPA (1987)	Increase in liver weight (guinea pigs)	LOAEL = 19 $mg.kg^{-1}.d^{-1}$ Via feed 1 year	1000	RfD = 0.02	Carpenter *et al.*, 1953
EFSA (2005)	Testicular and developmental toxicity (SD rats)	NOAEL = 5 $mg.kg^{-1}.d^{-1}$ Via feed 3 generations	100	TDI = 0.05	Wolfe and Layton, 2003

Analysis and assessment of the choices for selecting a TRV

Choice of the critical effect

Many animal studies have demonstrated impaired reproduction after ingestion of DEHP at doses of around 1 $mg.kg^{-1}.d^{-1}$. These results are supported by certain human epidemiological studies suggesting a link between DEHP exposure and the occurrence of reprotoxic effects. The most relevant and most sensitive effects are observed in the male reproductive tract following exposure during gestation. Impairment is both histological and functional.
The critical effect selected, i.e. histological and functional impairment of the male reproductive system, is consistent with all the literature.

Choice of the TRV

The CES adopted the TRV proposed by the European Food Safety Authority (EFSA) on the basis of the criteria explaining the approach, along with the quality of the key study, the choice of the critical dose and the uncertainty factors.

It relates to a multi-generational study (Wolfe and Layton, 2003; NTP, 2004) in Sprague-Dawley rats (17 per sex and per group). This study follows OECD guideline 416 on testing two-generation reproductive toxicity and the tests were conducted in accordance with good laboratory practices. It should be noted that this study differed slightly from the guideline recommendations, mainly regarding the number of animals per group, 17 males and 17 females (F0) instead of 20, as well as the administration of DEHP *via* the diet for 6 weeks prior to mating of the F0 generation instead of 10 weeks. However, these differences are

unlikely to compromise the results, because in this three-generation study, the F1 and F2 males were treated for the entire cycle of spermatogenesis.
This robust study, rated 1 according to the Klimisch classification, is one of the few three-generation studies available and its results enabled identification of two doses without effect on fertility and development. Moreover, the authors took into account background levels of exposure to DEHP in the control group.

The rats were exposed via feed to 1.5; 10; 30; 100; 300; 1000; 7500 and 10,000 ppm (0.1; 0.5-0.8; 1.4-2.4; 4.8-7.9; 14-23; 46-77; 359-592 and 543-775 $mg.kg^{-1}.d^{-1}$ on the basis of the amount of feed consumed by the F0, F1 and F2 generations) for two successive generations. The administration, initiated six weeks before mating, was continued throughout gestation and lactation. The F1 and F2 animals were treated after weaning. Treatment of the 10,000 ppm group was stopped due to the sterility of F1 animals.
The different groups were examined to assess fertility, animal behaviour, potential endocrine-disrupting effects (male and female) as well as the condition of the reproductive organs in the different groups. The examinations included statistical assessments using conventional tests (Shirley's or Dunn's, Wilcoxon, Cochran and Chi-squared). A macroscopic examination of the animals, histological examinations and biological analyses were also performed. In addition to the requirements of the OECD 416 guideline, crossover studies were performed between animals selected from the F1 and F2 groups.
In unmated F1 adult males from the group exposed to 300 ppm, the authors observed a slight increase in the number of rats (3/45) with testicular and/or epididymal atrophy (no observation in F0 males).
For developmental effects, a **NOAEL of 100 ppm (5 $mg.kg^{-1}.d^{-1}$)** and a **LOAEL of 300 ppm (14 $mg.kg^{-1}.d^{-1}$)** were observed for reduced testes size and slight atrophy of the seminiferous tubules in the F1 and F2 generations.
This study is one of the few to propose a detailed assessment of "phthalate syndrome" using a large number of young male rodents to detect reprotoxic effects at low levels of exposure and over several generations.

Adjustment

No temporal adjustment was applied by EFSA due to the animals' continuous exposure to DEHP via feed.

Choice of uncertainty factors

EFSA adopted an uncertainty factor of 100:

- UF_A (inter-species variability) of 10
- UF_H (intra-species variability) of 10

Chronic TRV by ingestion
After applying an uncertainty factor of 100, EFSA proposed a TDI (tolerable daily intake) of 5 $mg.kg^{-1}.d^{-1}$ /100 or 0.05 $mg.kg^{-1}.d^{-1}$.

4. CONCLUSIONS AND RECOMMENDATIONS OF THE COLLECTIVE EXPERT APPRAISAL

The CES selected the TRV proposed by EFSA on the basis of the criteria explaining the approach but also the quality of the key study, the choice of the effect, the critical dose and the uncertainty factors.
The chronic oral TRV for the reprotoxic effects of DEHP proposed at the end of the expert appraisal is:

Di(2-ethylhexyl)phthalate (DEHP) CAS No.: 117-81-7

Critical effect	Critical dose*	UF	TRV
Developmental abnormalities of the male reproductive organs Multi-generation study Wolfe and Layton (2003)	**LOAEL = 14 mg.kg^{-1}.d^{-1}** **NOAEL = 5 mg.kg^{-1}.d^{-1}**	**100** **UF_A 10** **UF_H 10**	**0.05 mg.kg^{-1}.d^{-1}**

Based on studies of reproductive toxicity in animals, the CES concluded that prepubertal children and pregnant women are most vulnerable to the reprotoxic effects of DEHP.

As exposure of the population to DEHP is permanent throughout life, the TRV is applicable to exposure of adults (including pregnant women) and children.

Given the lack of precise knowledge on the mechanism of action and the critical period of sensitivity, the CES recommends that the value calculated for long-term exposure and based on reprotoxic effects should also be complied with for short-term exposure.

5. AGENCY CONCLUSIONS AND RECOMMENDATIONS

The French Agency for Food, Environmental and Occupational Health & Safety endorses the conclusions and recommendations of the CES on Assessment of the risks related to chemical substances, concerning the development of toxicity reference values for di(2-ethylhexyl)phthalate (DEHP), and adopts these TRVs.

The Director General

Marc Mortureux

KEY WORDS

di(2-ethylhexyl)phthalate (DEHP), toxicity reference values, critical dose, uncertainty factors, general population.