Scientific Committee on Emerging and Newly-Identified Health Risks

SCENIHR

Opinion

on

The safety of medical devices containing DEHP-plasticized PVC or other plasticizers on neonates and other groups possibly at risk (2015 update)

Adopted by the SCENIHR during the plenary meeting of 25 June 2015

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ABSTRACT

The present Opinion is an update carried out by the SCENIHR on its previous 2008 Opinion on the safety of medical devices containing di(2-ethylhexyl) phthalate (DEHP) or alternative plasticizers for their polyvinylchloride (PVC) based components. Even though most of the 2008 opinion remains valid, new studies on DEHP activity and alternative agents are available. Therefore, the scientific information published from 2008 and onwards was reviewed and evaluated by the SCENIHR. The Opinion is mainly concerned with the potential health risks for patients with high exposure to DEHP or related plasticizing compounds leaching from medical devices.

Food is the primary source of exposure to DEHP for the general population. The range of body burden values from all sources excluding medical and occupational exposure is estimated at 1-30 µg/kg bw/d (probabilistic calculations). For infants and children, the 95th percentile is estimated at 6-17 µg/kg bw/d. The most recent biomonitoring studies (measuring primary and secondary excreted DEHP metabolites) suggest a current median exposure of 2 to 5 µg/kg bw/d, whereas the 95th percentile is estimated to be between 6 and 17 µg/kg bw/d.

Use of PVC medical devices may lead to a higher exposure to DEHP compared to everyday sources affecting the general population. Several procedures such as exchange transfusion of blood in neonates, extracorporeal membrane oxygenation (ECMO) treatment of neonates and adults, total parenteral nutrition (TPN) in neonates, haemodialysis, enteral nutrition in neonates and adults, heart transplantation or coronary artery bypass graft surgery, massive blood transfusion of red blood cells and plasma or peritoneal dialysis may lead to high exposure to DEHP potentially leaching from the device used. The extent of exposure largely depends upon the type of device, the number and duration of medical procedures.

In adults, the highest acute/short term exposure may result from transfusions of blood components, reaching DEHP doses up to approximately 8000-10000 µg/kg bw/d in trauma patients and in patients undergoing ECMO, whereas the highest chronic treatment is represented by haemodialysis, during which the maximum reported exposure is 2200 µg/kg/d. Premature neonates in intensive care units (NICU), being dependent on multiple medical procedures, may receive even higher DEHP exposures than adults relative to their kg bw. Such exposures may occur for a period of weeks or even months. The estimate exposure for newborns in NICU is up to 6000 µg/kg bw/d.

In human studies, a maximal and rapid oral absorption of 50-75% was estimated; however in view of underestimation of metabolites and bile excretion, an almost complete absorption can be considered (ECHA,2013). The bioavailability following parenteral exposure is considered 100%. DEHP metabolic profiles in humans are different when compared to rats. DEHP metabolites (MEHP [mono(-2-ethylhexyl) phthalate] and oxidised derivatives), which are biologically active, are excreted in rat urine mostly as unconjugated forms (87.4% in free form). In contrast, in humans, DEHP is excreted mostly as glucuronides (87.7%), which lack toxic effects. This suggests that measuring concentrations of free and conjugated forms will be useful for estimating exposure and can also explain at least partially some differences in susceptibility among species. The metabolic pathway as well as the excretion pattern of DEHP in humans is qualitatively independent on the exposure routes (oral or i.v.).

DEHP has a low oral acute toxicity (LD_{50}>25 g/kg in rats and mice). Lower LD_{50} values were obtained after parenteral exposure (200-250 mg/kg in rats). Oral repeated toxicity in rodents indicated that DEHP induces toxicity in the kidney, liver and testis. The hepatocyte proliferation, hypertrophy and hepatocellular tumours are mediated by the agonistic interaction

of DEHP and its metabolite MEHP with the peroxisome-proliferator activated receptor alpha (PPARα). This mechanism is considered to be rodent-specific and not relevant for humans. However, new studies seem to indicate that other pathways may also be involved in hepatic tumour induction, thus the relevance of liver cancer in rodents cannot be completely ruled out. However, DEHP and its major metabolites are considered to be non-mutagenic substances, implying that a threshold mechanism is involved and the doses inducing hepatic tumours are higher than those eliciting non-neoplastic effects. Recently, the International Agency for Research on Cancer (IARC) indicated that there is sufficient evidence in experimental animals for the carcinogenicity of DEHP. Thus, DEHP has been classified since the 2008 Opinion as possibly carcinogenic to humans (Group 2B).

The reproductive and developmental toxicity of DEHP has been studied in rats, mice, hamsters, ferrets and marmosets: on the basis of these studies DEHP is classified as category 1B for reproductive toxicity according to the CLP-Regulation (EC) No 1272/2008. The testis toxicity of DEHP is age dependent, with immature young animals being more susceptible to testicular toxicity by DEHP than older mature animals. The lowest NOAEL is derived from a multigenerational study in rats for testicular toxicity and developmental toxicity equal to 4.8 mg/kg bw/d. Rodents are more susceptible to male reproductive toxicity than non-human primates. In addition, in xenogeneic transplantation models there was no effect of DEHP and DBP (di-n-butyl phthalate) metabolites on testosterone production in human foetal testis tissue, while rat foetal testis tissue was affected.

A review of the recent epidemiological studies investigating DEHP exposure associated with effects on testosterone production, breast tumour, hypospadias and cryptorchism, decreased anogenital distance, childhood growth and pubertal development, endometriosis, effect of DEHP metabolites on neurobehaviour, obesity, insulin resistance and type 2 diabetes, were either inconclusive or inconsistent.

The Tolerable Daily Intake (TDI) value of DEHP was previously established (RAR 2008 and ECB 2008) at 48 µg per kg bw per day, based on a NOAEL of 4.8 mg/kg/d for reproductive toxicity in rats and applying an assessment factor of 100. Based on the same studies, the EFSA rounded the TDI to 50 µg/kg bw/d (EFSA 2005). The SCENIHR supports the previously derived TDI value, considering that the new studies are in line or not sufficiently robust to justify the derivation of a new TDI.

Notably, the TDI is a value set up for a lifelong continuous exposure, in contrast with transient acute or subacute exposure produced by DEHP-containing medical devices. Therefore, the use of a TDI value for risk assessment associated to exposure via medical devices represents a conservative approach, as exposure to medical devices is generally higher but limited over time (corresponding to acute/sub-acute/subchronic scenarios from a toxicological point of view, depending on the medical device used). The exception is dialysis patients, whose regimen of treatment can be considered chronic.

Exposure to DEHP may significantly exceed the TDI in some specific groups, including adult patients undergoing haemodialysis: their median exposure levels reported in various studies exceed the TDI by 2-12 fold with peak values (up to 2200 µg/kg/d) >40 fold higher than the TDI. These exposure values thus have a small Margin of Safety (MoS) (lower than 100) using the NOAEL in rodents for induction of kidney toxicity (around 30 mg/kg/day), which is particularly relevant for that kind of patient. Therefore patients subject to haemodyalisis procedure may be at risk of DEHP induced effects.

Premature neonates in intensive care units (NICU), being dependent on multiple medical procedures, may receive DEHP levels (6000 µg/kg bw/d) similar to the No Observed Adverse Effect Level (NOAEL) (4.8 mg/kg/d) for reproductive toxicity and for this group of patients there is no MoS. In infants and neonates ECMO is the medical treatment which may give the highest daily exposure over repeated exposure for a short period of time (up to 35000 µg/kg over 10 days treatment in 4 kg bw infants: assuming an equal distribution over time, this would correspond approximately to 3500 µg/kg bw/d). Therefore, premature neonates in NICU and infants subjected to ECMO represent a high-risk population to DEHP exposure.

DEHP causes the most severe reproductive toxicity in animal studies, when compared to other plasticizers such as di(2-ethylhexyl) adipate (DEHA), tris(2-ethylhexyl)benzene-1,2,4-tricarboxylate (TOTM), 1,2-Cyclohexanedicarboxylic acid, diisononylester (DINCH) and di-iso-nonyl phthalate (DINP). For the alternative plasticisers, the critical endpoint for toxicity is generally different from reproductive effects. However, the paucity of data on their release from medical devices and consequent human exposure does not allow an appropriate risk assessment to be carried out. In addition aggregate exposure should be taken into account because these plasticisers are used in many other consumer products (including food contact material and toys) and dust and air samples may also contain these plasticizers. Glycerides, Castor-oil-mono-, hydrogenated, acetates (COMGHA) and TOTM could not be properly evaluated, since the only data available are from oral studies and since they are very poorly absorbed via the g.i. tract, these data are of limited use for the parenteral route of exposure. Therefore information is insufficient to identify the hazards and limits an evaluation of alternative plasticizers.

It should be realised that the benefit of medical devices has also to be considered: the survival of premature infants often depends on the availability of the same medical devices which result in a relatively high DEHP exposure due to treatment. Whenever possible, material with low release potential should be used.

The potential for replacement of DEHP in these products should be considered against their efficiency in the treatment, as well as the toxicological profile and leaching properties of the alternative materials. There is a strong need to develop and collect data on exposure of alternative materials in the actual conditions of use, to refine the knowledge on their toxicological profile and to develop other alternative materials with a favourable profile both for efficiency and safety.

Keywords: SCENIHR, scientific opinion, DEHP, phthalates, medical devices, neonates, alternative plasticizers, health risks

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EXECUTIVE SUMMARY

The safety of medical devices containing DEHP [di-(2-(ethylhexyl) phthalate] as plasticizer of their PVC-based components was previously considered by EU Scientific Committees (SCMPMD 2002 and the SCENIHR 2008). Alternative PVC softening agents were also assessed. Similarly, several scientific organisations have examined the possible health risks posed by exposure to DEHP (e.g. FDA 2002; EFSA 2005; ECB, 2004, 2008; CSTEE 2004; IARC 2000, 2012; BfR 2013). This Opinion is an update of the 2008 SCENIHR Opinion with the addition of information and literature published from 2008 and onwards. The main focus is on the potential risk for patients exposed to DEHP or similar plasticising compounds leaching from medical devices. However, exposure of the general population to plasticizers has also been addressed. The assessment includes information on currently available plasticizers as well as some proposed alternatives of DEHP in medical devices for neonates and for other patient groups. Importantly, a focus is on clinical procedures resulting in high DEHP exposure. This Opinion does not address non-PVC-based materials that are effective materials in medical devices, PVC-related environmental aspects or potential health risks associated with stabilisers, other additives and contaminants leaching out of PVC medical devices.

The general population is exposed to DEHP through a variety of routes, with food being the primary source. There are important differences among populations and individuals associated with various dietary habits and lifestyle, the exposure range from all sources being estimated to be between 1 and 30 µg/kg bw/d, excluding medical and occupational exposure. In general, exposure assessments from probabilistic calculations from DEHP measurements in environmental media and dose re-calculation (reverse dosimetry) from urinary metabolite levels are quite similar. The most recent studies suggest a current median exposure of 2-5 µg/kg bw/d, whereas the 95th percentile is estimated to be between 6 and 17 µg/kg bw/d. Children may have somewhat higher body burden of DEHP than adults, with a median exposure of around 4-8 µg/kg bw/d. There are indications that exposure to DEHP in the general population has decreased over the last few years.

Medical procedures using PVC medical devices can lead to DEHP exposures much higher than the levels presented above for the general population. The following procedures with a potential for high exposure to DEHP are: Exchange transfusion of blood in neonates; Extracorporeal membrane oxygenation (ECMO) treatment of neonates and of adults; Total Parenteral Nutrition (TPN) in neonates; Multiple procedures in preterm neonates; Haemodialysis; Enteral nutrition in neonates and adults; Heart transplantation or coronary artery bypass graft surgery; Massive blood transfusion of red blood cells and plasma; and Peritoneal dialysis. The exposure during the listed procedures is caused by the use of various types of medical devices including blood bags, tubing like catheters, intubation tubes and intravenous catheters and other medical devices made of PVC. The extent of exposure (both as peak levels and as total dose received) largely depends upon the medical treatment and its duration. In adults, the highest short term exposure may result from transfusions of blood components reaching DEHP doses up to approximately 8000-10000 µg/kg bw/d in trauma patients and in patients undergoing Extracorporeal Membrane Oxygenation (ECMO), whereas the highest chronic treatment occurs in haemodialysis, during which the maximum reported exposure is 2200 µg/kg/d. Voluntary medical treatments such as the apheresis procedure during blood donation can cause transient elevated exposure to DEHP (up to 38 µg/kg/d). However voluntary donations are not provided by groups deemed to be at risk for reproductive toxicity (pregnant and nursing mothers and neonates).

The long-term total parenteral nutrition corresponds to higher exposure for infants and children, leading to a maximum exposure of 2000 µg/d, implying that the lower the body weight, the higher the dose per kg b.w. (i.e. for an infants of 2.5 kg bw the exposure is 800 µg/kg/d).

The FDA (2002) has estimated an upper-bound daily DEHP dose of the order of 3000 µg/kg/d for a newborn (4 kg) in the neonate intensive care unit (NICU), calculated by considering exposure from multiple devices. However, most newborns requiring medical intensive care are premature babies whose weight is significantly lighter, in general between 0.5 and 2.5 kg. Therefore, the DEHP exposure in relation to bw may be even higher in premature newborns (i.e. 8000 µg/kg/d for a neonate of 1.5 kg bw).

In human studies, a maximal and rapid oral absorption of 50% was estimated. However, since the amount recovered in the urine depends on the number of urinary metabolites measured, and the amount of excretion via bile is unknown, an almost complete absorption can be used in risk characterisation (ECHA, 2013). The bioavailability following parenteral exposure is 100%. Distribution studies in rodents indicate that DEHP is widely distributed in the tissues without evidence of accumulation. In mammals, including man, DEHP is converted into a variety of metabolites by lipases and by cytochrome P450. The first and fastest stage in the metabolism of DEHP is the hydrolytic cleavage catalysed by acid lipases to mono(2-ethylhexyl) phthalate (MEHP) and 2-ethylhexanol (2-EH). Further metabolism takes place in the liver to secondary and tertiary metabolites. Human urine contains several of these primary, secondary and tertiary metabolites with excretion occurring mostly (more than 90%) within 24 hours post dose.

There are important differences in the DEHP metabolic profile between species : MEHP and its oxidised derivatives are excreted in rat urine mostly as unconjugated forms whereas in non-human primates and in humans the metabolites are mostly glucuronide conjugates (87.7% in males at 24 hour). It is suggested that measuring the concentrations of free and conjugated forms separately may be important for estimating their toxicity, since conjugates are not biologically active. Glucuronidation can be lower in infants at birth (especially in pre-term neonates), due to lower glucuronyl transferases activities; these are at the same levels as adults after the first year of life. The metabolic pathway as well as the excretion pattern of DEHP in humans is qualitatively independent on the exposure routes (oral or intra venous).

DEHP has a low oral acute toxicity (LD_{50}>25 g/kg in rats and mice). Lower LD_{50} values were obtained after parenteral exposure (200-250 mg/kg in rats). The acute toxicity of MEHP is about 5 times higher than that of DEHP. Oral repeated toxicity experiments in rodents indicated that DEHP induced toxicity occurs in the kidney, liver and testis. The NOAEL derived from a two-year study in rats for kidney toxicity is 29 and 36 mg/kg/day in males and females, respectively. The lowest NOAEL for non-neoplastic effects was associated with damage in the liver of mice at 19 mg/kg bw/d, with hepatomegaly due to hepatocyte proliferation (characterised by increased replicative DNA synthesis/cell division and hypertrophy) and peroxisome proliferation.

Using a Weight of Evidence (WoE) approach, it can be considered that DEHP and its major metabolites are non-mutagenic substances.

Several studies on the carcinogenicity (and mechanisms of carcinogenicity) of DEHP have been performed in rats and mice with oral administration and demonstrate the induction of hepatocellular neoplasms in rodents. The NOAEL for tumour induction in male mice was 98

mg/kg bw/d, thus higher than the NOAEL derived from the same study for non-neoplastic effects (i.e. 29 mg/kg/d).

The mechanisms for liver carcinogenesis in rodents is mediated by PPARα-activation and consequent peroxisome proliferation, a mode of action which has a threshold and is generally considered not to be relevant in the human liver. This is because (i) human PPARα is expressed at a lower level in human liver than in rats and mice, (ii) no studies have reported any evidence that DEHP activates PPARα in human liver *in vivo,* and (ii) marked species differences with respect to hepatic response to peroxisome proliferation have been demonstrated. However, multiple molecular signals and pathways in several cell types in the liver other than the PPARα-activation have been suggested to be involved in hepatic tumour induction, so that according to the IARC evaluation, the relevance to human cancer of the molecular events that lead to cancer elicited by DEHP in several target tissues (e.g. the liver and testis) in rats and mice cannot be definitely ruled out. On this basis IARC has considered that there is sufficient evidence in experimental animals for the carcinogenicity of DEHP. Thus, DEHP has been classified as possibly carcinogenic to humans (Group 2B).

The reproductive or developmental toxicity of DEHP has been studied in rats, mice, hamsters, ferrets and marmosets. On the basis of the obtained results, DEHP is classified under Regulation (EC) No 1272/2008 as toxic to reproduction category 1B. The testis toxicity of DEHP in rodents is age dependent. The NOAEL for both testicular toxicity and developmental toxicity has been derived from a multigenerational reproductive toxicity study of DEHP in rats (the most susceptible species) equal to 4.8 mg/kg bw/d. For male reproductive toxicity caused by DEHP, there is a difference in sensitivity between various animal species. Rodents are more susceptible than non-human primates, with cynomolgus monkeys and marmosets showing no effect on testicular function after high pre-natal DEHP exposure or mono-butyl phthalate (MBP) (the active metabolite of di-n-butyl phthalate (DBP)) exposure. These two chemicals, MBP and DBP, showed potency in inducing reproductive effects similar to DEHP in rodents. In marmoset, after postnatal exposure, some effects were noted similar to those in rodents. These observations are of importance for extrapolation to humans, because in terms of perinatal testis development and function and spermatogenesis in adulthood, the marmoset is considered to be a suitable model for studies relevant to humans.

In vitro studies using human foetal testis tissue showed no effect of the metabolites on testosterone production, whereas *in vitro* studies using testis tissue from adult men indicate that DEHP suppresses testosterone production. Altogether, these studies show that the human prenatal testis is not sensitive to the anti-steroidogenic effects of DEHP. In contrast, there are some indications about the possibility of some alterations in foetal germ cells but the meaning of this has not been elucidated and it is difficult to extrapolate from in vitro results to the in vivo situation. Effects of DEHP on human germ cells need further investigation.

DEHP in experimental systems has shown the potential to interact with the immune system depending on the exposure conditions. Interestingly for a medical device perspective, immune effects were reported when parenteral routes of administration were used.

A review of the recent epidemiological studies investigating the effect of DEHP exposure on testosterone production, breast tumour, hypospadias and cryptorchism, decreased anogenital distance, childhood growth and pubertal development and endometriosis, as well as the effect of DEHP metabolites on neurobehaviour, obesity, insulin resistance and type 2 diabetes, were either inconclusive or inconsistent. One of the major problems concerning the association between exposure and human health effects is related to the correct identification of the level of exposure to DEHP and other phthalates. The exposure assessment can be biased depending

on various factors: the detection of the parent compound alone, the variable number of metabolites and how the information from a snap shot urine sample can be affected by the short half-life of these chemicals (being therefore representative of a short-time exposure and consequently difficult to associate with a causality relationship to long-term pathologies or disease requiring a long lag time before their onset). The choice of exposure metric can introduce significant bias of varying magnitude and direction into the calculation of epidemiologic associations. This can at least partly explain the contrasting results observed in the available epidemiological studies.

The Tolerable Daily Intake (TDI) value of DEHP was established at 48 µg per kg bw per day, which was based on the above-mentioned No Observed Adverse Effect Level (NOAEL) of 4.8 mg/kg/d for reproductive toxicity in rats. Based on the same studies, the EFSA rounded the TDI value to 50 µg/kg bw/d. It should be noted that TDI is a value set up for a lifelong continuous exposure, in contrast with the transient acute or subacute exposure by most of the DEHP-containing medical devices, with some exceptions (i.e. dialysis or prolonged intensive care treatment). However, if such an exposure is below the TDI, the risk can be considered extremely low, so the TDI can be a useful starting point for the risk assessment. In cases where high levels of DEHP during certain medical procedures are attained, specific considerations regarding subacute/subchronic effects should be applied.

Adult patients undergoing haemodialysis are considered to have the highest exposure, due to the chronic nature of the treatment. Long-term haemodialysis may result in the highest cumulative dose of DEHP (up to 2200 µg/kg/d). The TDI can be appropriately used for the haemodialysis scenario. Median exposure levels reported in various studies exceed the TDI by 2-12 fold with peak values >40 fold higher than the TDI.

Children are potentially at higher risk, particularly neonates and infants due to their low body weight thus being particularly prone to high level of exposure on a body weight basis. Neonates in the Neonatal Intensive Care Unit (NICU) subjected to exposure up to 6000 µg/kg bw/d are also a potentially vulnerable population due to their physical conditions and the immaturity of many systems and organs, as well as their small size. The exposures estimated for neonates in NICU are in the same range as the doses inducing developmental and reproductive toxicity in animal studies, without any margin of safety. In infants and neonates, ECMO is the medical treatment which may give the highest daily exposure over repeated exposure for a short period of time (up to 35000 µg/kg over 10 days treatment in 4 kg bw infants: assuming an equal distribution over time, this would correspond approximately to 3500 µg/kg bw/d). Therefore, premature neonates in NICU and infants subjected to ECMO and repeated medical treatment with medical devices represent the most vulnerable population, both due to the levels of DEHP exposure and to their phases of life, particularly with regard to developmental and reproductive toxicity.

There is evidence suggesting that DEHP causes the most severe reproductive toxicity in animal studies, when compared to other alternative plasticizers such as di(2-ethylhexyl) adipate (DEHA), di-iso-nonyl phthalate (DINP), 1,2-Cyclohexanedicarboxylic acid, diisononylester (DINCH) and tris(2-ethylhexyl)benzene-1,2,4-tricarboxylate (TOTM). For the alternative plasticizers the critical endpoint for toxicity is generally different from reproductive effects. However, the paucity of data on their release from medical devices and consequent human exposure does not allow an appropriate risk assessment to be carried out. In addition, aggregate exposure should be taken into account because these plasticiser are used in many other consumer products (including food contact material and toys) and dust and air samples may contain these plasticizers. Castor-oil-mono-, hydrogenated, acetates (COMGHA) and TOTM could not be properly evaluated, since the only data available are from oral studies and

since they are very poorly absorbed via the gastro-inestinal tract, these data are of limited use for the parenteral route of exposure. Therefore there is a strong need to develop and collect data on exposure of alternative materials in the actual conditions of use in order to refine the knowledge of their toxicological profile. The possibility of replacing DEHP with these products could then be considered, taking account the efficacy of the treatment as well as the toxicological profile and leaching properties of the alternative materials.

1. BACKGROUND

According to Council Directive 93/42/EEC, Medical Devices may only be placed on the market if they meet the essential requirements laid down in the Annex I of the Directive. For certain medical procedures such as blood transfusion, haemodialysis, parenteral nutrition or endotracheal tubing, the flexibility of certain parts of a medical device is essential. Various substances are used to ensure this flexibility, among which DEHP [di-(2- ethylhexyl) phthalate] is the most frequently used plasticizer in PVC medical devices. DEHP may migrate from the device to the human body, resulting in a certain degree of patient exposure.

Safety concerns have been expressed for high-risk patients groups, such as neonates, infants, pregnant and breast-feeding women exposed to DEHP. In September 2002, the Scientific Committee on Medicinal Products and Medical Devices adopted an Opinion according to which "there is no evidence that any of these groups do experience DEHP related adverse effects". However, "a lack of evidence of causation between DEHP-PVC and any disease or adverse effect does not mean that there are no risks".

In February 2008, the Scientific Committee on Emerging and Newly-Identified Health Risks (SCENIHR) adopted a revised Opinion on "The safety of medical devices containing DEHP plasticized PVC or other plasticizers on neonates and other groups possibly at risk". The conclusion was that "So far, there is no conclusive scientific evidence that DEHP exposure via medical treatments has harmful effects in humans. However, further studies are required to confirm or reject the suggestions of adverse effects of DEHP in humans. Patient groups with relatively high DEHP exposures, which may result in some risk, are those requiring repeated medical procedures, including male foetuses of pregnant women.

Recently, a Tolerable Daily Intake (TDI) value of DEHP was established and published in the EU Risk Assessment Report (RAR 2006). The TDI for DEHP is 48 µg per kg bw per day, which was based on a No Observed Adverse Effect Level (NOAEL) for reproductive effects in rats. In view of the potential high exposure to DEHP during certain medical procedures and a very special group of patients involved, the use of TDI is not considered appropriate in these procedures".

A project entitled, 'PVCfreeBloodBag', which the European Commission (EC) is funding through the LIFE+ programme, has recently issued a press release asserting that "blood bags made of DEHP-plasticised PVC pose a significant risk to human health, due to both DEHP and PVC". This statement is based on a new life cycle Assessment (LCA) report. In the light of this new study, the SCENIHR was asked to review and update the Opinion adopted in 2008.

In view of possible safety concerns linked to the use of DEHP in PVC plasticized medical devices, it is essential to review and evaluate available scientific data from 2008 related to the safety of possible alternatives for patients and, in particular, for high-risk groups.

2. TERMS OF REFERENCE

Update of the scientific Opinion adopted in February 2008 on DEHP plasticized medical devices. Taking into consideration recent scientific developments, the SCENIHR is requested to review and update, if appropriate, the scientific Opinion adopted in February 2008 on "The safety of medical devices containing DEHP plasticized PVC or other plasticizers on neonates and other groups possibly at risk".

In particular, the Scientific Committee is requested to evaluate:

• If DEHP in PVC plasticized medical devices is a cause for concern to neonates and children in paediatric care, in particular in relation to male fertility and tissue development,

• If there are other patient groups at risk, in particular in view of clinical procedures resulting in high exposure,

• If it is possible to establish Tolerable Intake Values (TIV) of DEHP leaching from soft PVC as a basis for risk assessment for high risk patient groups, taking into account the route of exposure,

• If it is possible to propose possible alternative approaches that could reduce potential risks either by identifying alternative practices or by identifying alternatives to the use of DEHP in PVC plasticized in medical devices. If no clear answer can be provided on this point, the SCENIHR is asked to formulate recommendations for research that could help provide scientific evidence to that end.

3. SCIENTIFIC RATIONALE

3.1. Introduction

Polyvinylchloride (PVC) is used extensively for a very wide range of purposes and materials, from linings for landfill waste disposal sites to food packaging. One of the key attributes of PVC that has led to its widespread use is its stability and flexibility, which is achieved by the incorporation of plasticizers in particular phthalates.

The use of PVC in medical devices represents a very minor percentage of the total amount of PVC manufactured each year. Nonetheless, the use of plasticized PVC in a wide range of medical devices has been very important for a number of reasons:

- flexibility in a variety of physical forms from tubes to membranes
- chemical stability and possibility to sterilise
- low cost and wide availability
- lack of evidence of significant adverse consequences in patients

A plasticizer is a substance that, when added to a material, usually a polymer, makes it flexible, resilient and easier to handle. There are more than 300 different types of described plasticizers, of which between 50 and 100 are in commercial use. The most commonly used plasticizers are phthalates, the most common being: di-iso-nonyl phthalate (DINP), di-iso-decyl phthalate (DIDP) and di(2-ethylhexyl) phthalate (DEHP). Plasticizers are used in a variety of PVC-based products such as electrical cables, toys, footwear, packaging, building materials, paints, rubber products, adhesives and cosmetics. PVC-containing plasticizers are also used for the production of medical devices such as medical tubing and blood bags. In general, there is a trend for a reduction in the use of DEHP as plasticizer in PVC (ECPI 2007).

Secondary plasticizers, also known as extenders, also play a role in flexible PVC formulations. Chlorinated paraffins (CPs), epoxidised soya bean oil (ESBO) and epoxidised linseed oil (ELO) are commonly used secondary plasticizers. CPs also act as flame retardants, ELO and ESBO as lubricants and also as secondary stabilisers to PVC due to their epoxy content, which can remove hydrochloric acid from the degrading polymer. Plasticizers are not chemically bound to PVC and may therefore leach, migrating into the surrounding environment. In this Opinion, the term leach will be used for consistency.

The biological properties of the phthalate plasticizers used in PVC, especially DEHP, have been the subject of a substantial number of research studies. Experimental animals/other experimental systems have revealed the potential for 1) Reproductive and developmental effects; 2) Testes toxicity; 3) Endocrine disruption; and 4) Peroxisome proliferation-related liver cancer in rodents. As a result, there has been a move to replace DEHP with alternative plasticizers. Environmental problems associated with the incineration of PVC and consequent production of dioxins is an additional concern, though improvements in incineration technologies in Europe minimises dioxin emission (Danish EPA, 2003).

In 2002, Health Canada (2002) recommended that available alternative products should be utilised for all ECMO (extracorporeal membrane oxygenation) procedures in newborns and infants. Tubing and storage bags used for administration of lipophilic drugs or drug formulations should not contain DEHP, and strategies to decrease DEHP exposure should be employed, particularly when administering lipophilic drugs to infants and children. Available alternative products were recommended for total parenteral nutrition solutions administered to

newborn and infants instead of DEHP-containing products (Health Canada, 2002). The US-Food and Drug Administration (FDA) recommended that manufacturers of medical devices consider eliminating DEHP in devices that cause high exposure in sensitive patients and that certain products be labelled with their DEHP content (FDA 2002). In the European Union (EU), the use of DEHP in toys for children under 3 years of age is not allowed.[1] Some specific National provisions are also in place (e.g. in France, Article 3 of Law No 2012-1442 of 24 December 2012 – which bans the use of pipes containing DEHP in children's, neo-natal and maternity wards from 1 July 2015).

According to European Pharmacopoeia, DEHP, ESBO and ELO can be used as plasticizers in medical devices (Medical Devices Directive 93/42/EEC) and DEHP is the main plasticizer used in PVC-based medical devices. A number of other substances are also available as alternatives to DEHP-PVC and used as plasticizers in medical devices (e.g., butyl trihexyl citrate in blood bags) as well as some non-PVC-based materials (e.g., enteral feeding bags made of ethyl vinyl acetate).

In 2008, a SCENIHR Opinion was published on the risks and benefits of the use of PVC incorporating DEHP in medical devices including the evaluation of possible alternative plasticizers, namely:

- Glycerides, Castor-oil-mono-, hydrogenated, acetates (COMGHA, CAS 736150-63-3)
- Acetyl-tri-n-butyl citrate (ATBC, CAS 77-90-7)
- n-Butyryl-tri-n-hexyl citrate (BTHC, CAS 82469-79-2)
- Di-iso-nonyl-1,2-cyclohexanedicarboxylate (DINCH, CAS 166412-78-8)
- Dioctyl terephthalate (DOTP, CAS 6422-86-2)
- Trioctyl trimellitate (TOTM, CAS 3319-31-1)
- Di-iso-nonyl phthalate (DINP, CAS 68515-48-0 and 28553-12-0)
- Di(2-ethylhexyl) adipate (DEHA, CAS 103-23-1)
- Polymeric plasticizers such as aliphatic polyesters can also potentially be used as alternative plasticizers in PVC medical devices.

In the 2008 Opinion it was concluded that some alternative plasticizers could replace DEHP in PVC in those conditions for which evaluation of risk and benefits should be done on a case-by-case basis (SCENIHR 2008). However, for most of the alternative plasticizers, risk assessment was not possible due to limited available information.

In 2012, a report was published from the PVC-Free Blood Bag Project (Carlson, 2012) describing the life cycle assessment (LCA) of a PVC blood bag and a hypothetical non-existing blood bag, made from high-density polyethylene (HDPE). The study authors considered the

[1]Directive 2009/48/EC of the European Parliament and of the Council of 18 June 2009 on the safety of toys. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:170:0001:0037:en:PDF

PVC blood bag to have a higher potential to harm human health in view of its release of DEHP and dioxin emissions at waste incineration. However, the SCENIHR considers that the study has some serious limitations in the study design and methodology, also related to the use of a 'fictional' blood bag as reference.

The focus of this Opinion is on the update of the previous 2008 Opinion about risk for patients exposed to medical devices. Therefore, new information and literature available after publication of the SCENIHR Opinion in 2008 was evaluated: all the data available were considered together and evaluated based on the WoE approach.

The safety assessment also includes currently available and proposed DEHP alternatives in medical devices for neonates and other patient groups undergoing clinical procedures with high exposure including blood bags, catheters, dialysis equipment, enteral feed containers, gastrointestinal tubes, I.V. solution storage and administration sets, tubing used in neonates, tubing used for respiratory therapy and containers for total parenteral nutrition (TPN). Whilst recognising that there are several non-PVC based materials that could be effective in medical devices production and use, this Opinion does not address these materials.

It must also be emphasised that in the following evaluation, only the health risks to patients exposed to plasticizers in PVC medical devices are considered. The following risk considerations are excluded from our consideration:

- Health, safety and environmental aspects of PVC manufacture and incorporation into medical devices (i.e. occupational exposure in the manufacturing areas).
- Health and safety of medical and ancillary staff handling or otherwise exposed to PVC medical devices and any substances released from them.
- Environmental risks associated with disposal of PVC containing medical devices and consequent human exposure via the environment.
- Health risks from other substances that might leach out of a PVC medical device, such as stabilisers, other additives and contaminants.

The Working Group has applied a weight of evidence approach, in which lines of evidence or hypothesis for causality are evaluated based on the supportive studies. When a line of evidence is consistently supported by various studies (i.e. evidence is independently reproduced in different studies) causality is likely between the observed effect and exposure to the substance. Relevance, strength and weaknesses of the studies evaluated are considered, according to the SCENIHR Opinion on the issue (SCENIHR, 2012).

The evidence for the presence of a causal relationship between exposure to DEHP and possible alternatives due to the use of medical devices and adverse effects are discussed in the chapters below.

3.2. Present use of plasticized PVC in medical devices

The worldwide PVC use was 2.94×10^7 tonnes in 2004 with a 4.3% annual growth rate. At EU level 341,000 tonnes in total were produced in 2007 (Maag *et al.*, 2010). A part of this was

exported and around 291,000 tonnes DEHP were used for manufacturing processes in the EU in 2007.

According to the EU life cycle assessment report[2], medical applications account for 0.5% of the PVC used in Europe. Thus, approximately 3×10^4 tonnes of plasticized PVC is used for medical applications annually in Europe. The major use (more than 95%) is soft medical grade PVC in containers, flexible tubing and medical gloves. The typical concentration of DEHP in plasticized PVC is 30% (ECB 2008).

DEHP is used in PVC not only for its action as a plasticizer to manufacture blood bags. DEHP is indeed released from the plastic into the blood components during storage of such blood components (Labow *et al.* 1986). DEHP is then integrated in the red blood cell membranes during storage stabilising cell membranes, thus avoiding disintegration of cells in the storage media and prolonging the possibilities of blood storage up to 6 weeks after collection. Similar effects have also been demonstrated with some other alternative plasticizers in PVC blood bags. This effect may need to be taken into account in the risk-benefit evaluations of the PVC plasticizers. Platelets are generally stored in other types of plastics, e.g. PVC-BTHC or polyolefin. Different plastic can be used for transfusion sets, flexible tubing for pumps and for dialysis. Transfusion sets are available in PVC-free material.

The use of plastics in medical applications is increasing. However, there is considerable interest from medical plastic producers in developing alternative materials to plasticized PVC. And indeed, it has been demonstrated that it is possible to reduce the use of DEHP-PVC in hospital procedures as demonstrated around Europe (Chiellini *et al.*, 2013; Van der Meer *et al.*, 2014). This might be achieved by using PVC-containing alternative plasticizers or using alternative materials. However, this probably cannot be achieved for all medical procedures.

3.3. Physicochemical properties of plasticizers

The most important physical parameters for evaluating potential human and environmental exposures to plasticizers are water solubility, octanol/water partition coefficient and leaching data. Furthermore, the vapour pressure of the plasticizers at the use temperature may in some cases be important. Whereas the solubility and vapour pressure data are available to some extent, very little information is available on leaching.

Table 1 summarizes important physico-chemical characteristics, some of which have been estimated (not experimentally determined), limiting their validity. It is possible to predict the relative exposure to be expected from the use of different plasticizers. The rate of leaching is dependent on the lipophilicity of the compound and of the material stored, duration of storage, storage temperature, contact area and, in some cases, agitation. In general, the plasticizers show a higher extent of leaching in lipophilic solutions. The clearest conclusion that can be drawn is that there is a severe lack of data on solubility, water/oil partition coefficients and especially leaching of the plasticizers under conditions relevant to the usage in plasticized products.

[2]Final Report of EU-Contract No. ETD/FIF.20020892: Life Cycle Assessment of PVC and of principal competing materials

Table 1. Overview of some physical properties of the assessed plasticizers.

Substance	Vapour pressure at 20°C (Pa)	Water Solubility (µg/L)	log K_{ow}	Water extractability (%)[a]	Kerosene extractability (%)[b]
COMGHA	<2.8 x 10^{-4} at 100°C (4)	7 x 10^{3} (4)	6.0 – 7.7 (4)		
ATBC	6 x 10^{-4} (3)	6 x 10^{2} (3)	4.3 (3)		
BTHC	8 x 10^{-8} (3)	6 x 10^{-2} (3)	8.2 (3)		
DEHA	4 x 10^{-4} (3)	0.5 (3)	8.1 (3)	0.10	>70
DEHP	3.4 x 10^{-5} (1)	3.0 (1)	7.5 (1)	0.01	44.3
DINCH	<2.8 x 10^{-4} at 100°C (4)	<20 (4)	10.0 (4)		
DINP	6 x 10^{-5} (2)	0.6 (2)	8.8 (2)	0.07	77
DEHT	3 x 10^{-3} (3)	1 (3)	8.3 (3)	0.09	71
TOTM	8 x 10^{-6} (3)	6 x 10^{-3} (3)	11 (3)	0.0	>70

a: Loss of plasticizers from a 1 mm PVC sheet containing 40% plasticizer when extracted with water at 50°C for 24h (ASTM D1239-55 (from Sears, 1989).

b: Loss of plasticizers from a 1 mm PVC sheet containing 40% plasticizer when extracted with kerosene at 23°C for 24h (ASTM D1239-55 (from Sears, 1989). The kerosene extractability is an indicator of lipid solubility.

(1): ECB 2001: (http://ecb.jrc.it/DOCUMENTS/Existing-Chemicals/RISK_ASSESSMENT/DRAFT/R042_0310_env_hh_combined.pdf)

(2): ECB 2003: (http://ecb.jrc.it/DOCUMENTS/Existing-Chemicals/RISK_ASSESSMENT/REPORT/dinpreport046.pdf)

(3): Estimated with EPISUITE 3.20 (http://www.epa.gov/opptintr/exposure/pubs/episuite.htm)

(4): see Annex 1

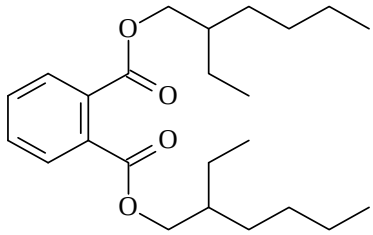
As can be seen in Table 1, the assessed plasticizers are lipophilic and all of them, except ATBC, have log K_{ow} values above 7 and low water solubility. In this respect, the alternatives are not very different from DEHP. The leaching of these substances from PVC to body fluids/tissues can be expected to be of similar magnitude compared with DEHP with the possible exception of ATBC.

3.4. DEHP (di(2-ethylhexyl) phthalate)

3.4.1. Physico-chemical properties

The evaluation of DEHP is included in this Opinion and is used as a basis for comparison with the different alternatives. The chemical characteristics of DEHP are presented below.

CAS Reg. No.: 117-81-7
Synonyms:
Empirical formula: C_{24} $H_{38}O_4$
Structure:



Molecular weight: 390.6
Melting point: -50°C
Boiling point: 385°C
Vapour pressure: 0.000034 Pa (20°C)
Solubility in water: 0.003 mg/L
Log K_{ow}: 7.5
Purity: 99.7%
Impurities: Other phthalates. Up to 0.5% Bisphenol A is added to some products[3].

3.4.2. Toxicokinetics of DEHP in humans

In rats and non-human primates, absorption rates of around 50% for doses up to about 200 mg/kg have been estimated (ECB 2004). Some saturation of absorption may occur at high doses (2000-2500 mg/kg, single or repeated daily dosing for 14 days) in non-human primates (Rhodes *et al.* 1983, Rhodes *et al.* 1986; Kurata et al, 2012a). Although in these conditions only a minor fraction of the administered dose (10%) is excreted in urine (Kurata *et al.* 2012a), the absorption appears to be quantitatively relevant. When 100 mg/kg bw was orally given to juvenile (3 months old) and adult marmosets, there were no clear age-related differences in the area under curve (AUC) (Kurata *et al.*, 2012a).

In human studies, oral absorption of 50% was also estimated on the basis of urinary excretion rates between 10 and 31% after oral DEHP administration (ECB 2004). The values are supported by further studies. A study on a single volunteer found that > 65% of orally administered DEHP is systemically absorbed in humans and excreted via urine (Koch *et al.*, 2005a) as oxidative metabolites. The toxicokinetics of DEHP was further investigated in 20 volunteers (10 per gender) receiving 2 different DEHP doses per person (0.31 and 2.8 mg deuterated DEHP, the highest dose corresponding to 0.047 mg/kg bw, that is the TDI for DEHP): the use of deuterium-labelled phthalates circumvents many of the problems that can arise from background levels of phthalate metabolites (e.g. presence of phthalate in the diet). The absorption, estimated by measuring the major excretion metabolites, was around 50% (Anderson et al, 2011). The time courses of DEHP were also measured in the blood of four male volunteers aged 28 – 61 years (Kessler *et al.*, 2012) orally ingesting a single dose (645±20 µg/kg bw) of deuterated DEHP (DEHP-D4), indicating a very rapid absorption (first peak in blood within half an hour). The bioavailability of DEHP-D4 was high with an area under the curve (AUC) until 24 h accounting for 50% of that of free MEHP-D4 (its major first step

[3] ECPI informed that DEHP formulations used for medical devices do not contain bisphenol A

metabolite). The DEHP-D4 AUC at 24h was 50 and 100 times higher than the one measured in rats and marmosets, respectively, upon oral administration of 30 mg/kg DEHP-D4 (Kessler *et al.*, 2004). Recently ECHA-RAC (2013) considered that a 50% absorption can be estimated from DEHP data obtained in adults as described above (Koch *et al.*, 2005; Anderson *et al.*, 2011; Kessler *et al.*, 2012). However, these studies indicate a rather high absorption rate in adults taking into account that the amount recovered in the urine depends on the number of urinary metabolite measured, and the unknown amount of excretion via bile. Therefore an almost complete absorption can be used in risk characterisation. The RAC also considers there is no indication that adults absorb less phthalate esters than children (ECHA, 2013).

Excretion rates were in the same range (30-35%). Terminal elimination half-lives were short (4-8h) (Kessler *et al.*, 2012; Anderson et al, 2011).

Recently ECHA (2013) reported that phthalates, including DEHP, can also be well absorbed through the lungs 75-100%), whereas absorption through the skin appears to be limited. For the exposure scenarios related with the use of medical devices, parenteral exposure is also relevant, for which a 100% bioavailability is generally assumed.

In mammals, including man, DEHP is converted into a variety of metabolites (Figure 1) by lipases, ubiquitous enzymes in various tissues, and by cytochrome P450. Pancreatic lipase plays a major role, especially after oral exposure. Species differences in lipase activity between tissues have been identified and may play a role in species differences in the effects of DEHP. The forms of cytochrome P450 or other enzymes responsible for oxidative metabolism are not well understood (IARC, 2012).

The first and fastest stage in the metabolism of DEHP is the hydrolytic cleavage catalysed by acid lipases (Carrière *et al.*, 1993) to mono(2-ethylhexyl) phthalate (MEHP) and 2-ethylhexanol (2-EH). After oral ingestion, enzymatic hydrolysis already occurs in the mouth (Niino *et al.*, 2001, 2003), but mostly in the gastrointestinal tract (Albro *et al.*, 1982, Albro and Thomas, 1973), therefore, after oral exposure DEHP is mainly absorbed as MEHP. Indeed, the early occurrence (half an hour) of the metabolite MEHP-D4 in blood (Kessler et al, 2012) is likely the result of the hydrolysis of the parent compound (DEHP-D4) in the stomach.
After oral exposure, a large species difference was observed in the AUC of DEHP-D4 (Kessler et al, 2012), which can be explained by the species-dependent hydrolysis of the compound and also by intestinal resorption. Besides metabolism and intestinal resorption, DEHP binds like lipids partly to lipoproteins (Griffiths *et al.*, 1988), which are formed in the enterocytes and transported in the lymph of the thoracic duct. The scoring for AUC values for DEHP (normalised to DEHP-D4 dose and body weight) is humans > marmoset > rat (Kessler et al, 2012). DEHP hydrolysis in the intestinal tissue is 10 times faster in rats than in marmosets. The intestinal resorption in marmosets seems to be lower than in rats because at a single oral dose of 100 mg/kg of radioactive labelled DEHP, marmosets excrete 47% of the dose in the faeces as the parent compound and rats only 13% (Kurata *et al.*, 2012a). On the other hand, DEHP hydrolysis in the intestinal tissue is ten times faster in rats than in marmosets (Ito *et al.*, 2005).

DEHP hydrolysing enzymes are found in many tissues (especially in the pancreas, intestinal mucosa, liver) and in blood plasma of rats (Albro and Thomas, 1973; Daniel and Bratt, 1974) and although quantitatively lower, this step also occurs after parenteral exposure.

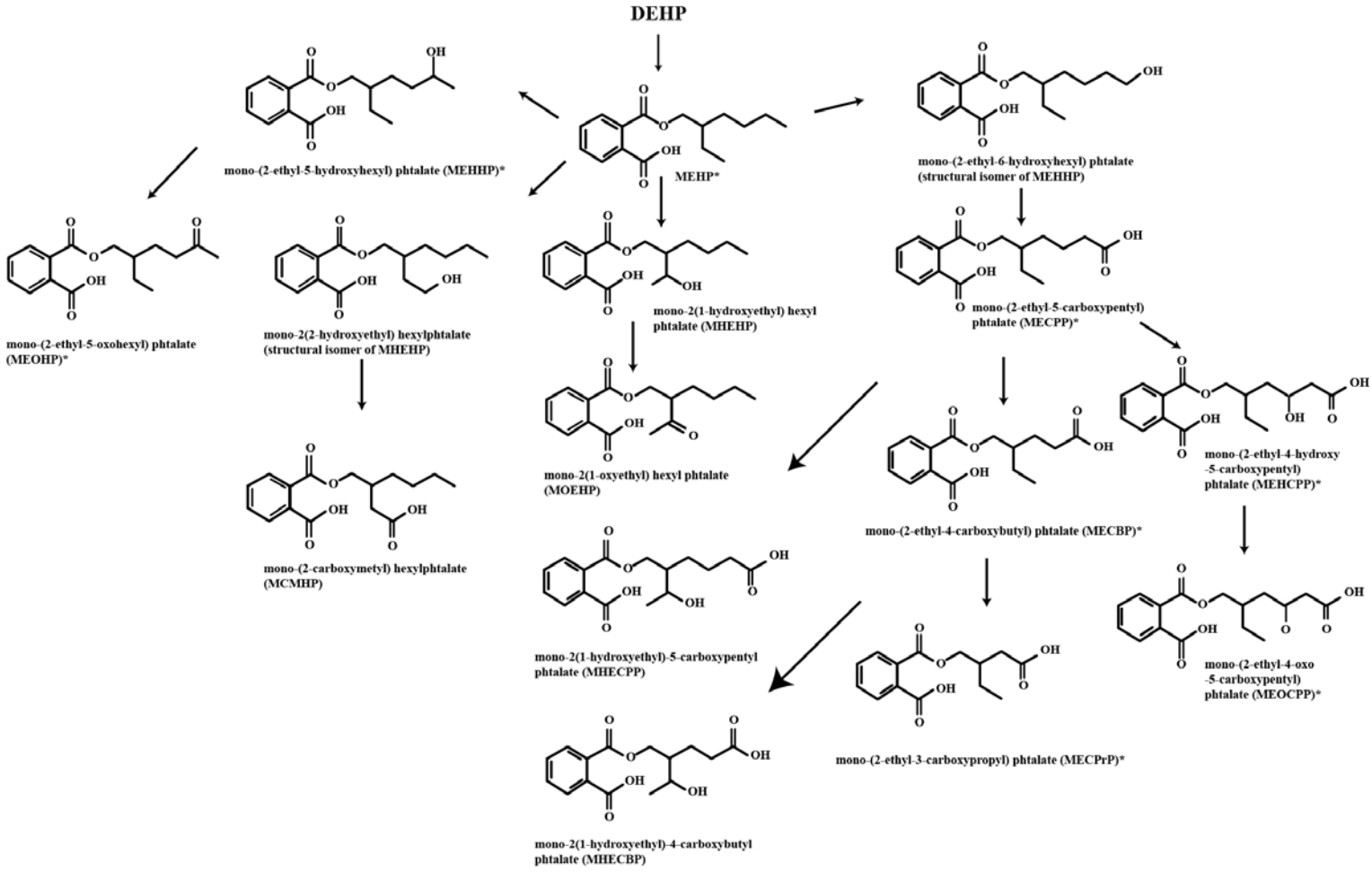


Figure 1. **Metabolic pathways of di(2-ethylhexyl) phthalate (DEHP) in humans.** *Adapted from: Toxicology, vol no 219, Silva MI et al., Urinary Oxidative metabolites of di(2-ethylhexyl) phthalate in humans, pp 22-32. Copyright (2006)*

Further metabolism takes place in the liver (Albro, 1986). In rats, the formed 2-EH is rapidly metabolised to 2-ethylhexanoic acid, which is further oxidised by ω- and (ω-1)-oxidation and subsequent β-oxidation to acetate and CO_2 (Albro, 1975). Human urine contains several of these oxidative metabolites as well (Wahl *et al.*, 2004, Wahl *et al.*, 2001).

MEHP metabolism leads to a large number of secondary and tertiary oxidative metabolites (Figure 1), the major being OH-MEHP, COOH-MEHP, 5cx-MEPP and oxo-MEPH, which together with DEHP and MEHP are considered to be biologically active and responsible for the reproductive effects. Oxidative metabolism of MEHP begins with hydroxylation of the alkyl chain at various positions and the formation of primary (ω-oxidation) and secondary alcohols (ω-n-oxidation). These hydroxylated products can undergo further oxidative reactions to the respective ketones and carboxylic acids. After that, the carboxylated alkyl chain can be subject to α- or β-oxidation to yield shorter carboxylated alkyl chains (Albro *et al.*, 1982; Albro *et al.*, 1983; Peck and Abro, 1982; Schmid and Slatter, 1985).

The primary, secondary and tertiary metabolites are all excreted rapidly in the urine, with excretion occurring mostly (more than 90%) within 24h post dose (Schmid and Schlatter, 1985; Anderson *et al.*, 2011). After a single administration of a dose corresponding to the TDI value to humans (10 males and 10 females), the metabolites in an enzymatically deconjugated urine sample were, in the order of abundance, -monoester < -oxo < -carboxy < -hydroxy

(Anderson *et al.*, 2011). The carboxy metabolites, having a longer half-life than the other metabolites, had the highest excretion on the 2nd day of collection (Anderson *et al.*, 2011).

In line with this finding, after repeated exposure, the ratios among the DEHP metabolites seem to be shifted in favour of the metabolites with longer half-lives. In population studies, 5cx-MEPP was found to be the principal urinary metabolite, followed by 5OH-MEHP, 5oxo-MEHP, 2cx-MEHP and MEHP (Preuss *et al.*, 2005; Silva *et al.*, 2006b).

Some species differences were described in the production of oxidised MEHP metabolite formation: in rats, the tertiary metabolite 5cx-MEPP was found to be the predominant DEHP metabolite in urine, whereas in mice it seems to be only a minor metabolic product (Peck and Albro, 1982). In contrast, rats excrete much lower amounts of MEHP compared to other mammals including primates (Peck and Albro, 1982).

However, the most relevant difference is that in rats, oxidised metabolites are present in plasma and then excreted in urine as free unconjugated forms (Albro *et al.* 1982, Kluwe 1982). At variance, glucuronidation is the major conjugation pathway in mice, guinea pigs, non-human primates (Albro *et al.*, 1982; Egestad *et al.*, 1996; Frederiksen *et al.*, 2007; Kurata *et al.*, 2012a) and in humans (Kurata *et al.*, 2012b). The ratio between the glucuronides vs. the unconjugated (free) metabolites forms (G/F) is 0.13, 7.14 and 3.5-5.3 in rats, marmoset and human, respectively (Kurata *et al.*, 2012a, 2012b).

The metabolic profile of DEHP in humans with glucuronides as the major excretion form for DEHP metabolites has been recently confirmed in urine samples collected from healthy male (n=10) and female (n=10) volunteers after oral administration of 3 mg/person of deuterium labelled DEHP (Kurata *et al.*, 2012b). The metabolites were analysed using high performance liquid chromatography/radioisotope detection/mass spectrometry (HPLC-RID/MS). Since the standards for glucuronide conjugates of DEHP metabolites are commercially unavailable they were synthetised in ^{14}C-DEHP treated human hepatocytes *in vitro*: the methodology used allowed the direct quantification of glucuronides without enzymatic hydrolysis. The ratios of the glucuronide conjugate forms in the total DEHP urinary metabolites were remarkably high from the initial stage after administration until 36h post dose (77.6% in males and 84.2% in females, 24h post dose).

Although inter-individual variations in the glucuronidation were observed for some DEHP metabolites (Dirven *et al.*, 1993; Silva *et al.*, 2006b), all the studies considered provided evidence that most of the oxidative metabolites (> 65%) are excreted in human urine as glucuronides, independently of the route of administration (oral or i.v.) (Albro *et al.*, 1982; Bronsch, 1987; Schmid and Slatter, 1985; Calafat *et al.*, 2006). While the carboxylic acid metabolites were found to be excreted only partially in their glucuronidated form, the alcohol and ketone metabolites are excreted mainly as glucuronic acid conjugates (Silva *et al.*, 2006b).

This information is quite relevant for the interpretation of the toxicological studies, since glucuronides are not biologically active; therefore, the bioavailability of active compounds is highly reduced, unless a specific cleavage of the molecule occurs, catalysed by β-glucuronidases. Therefore, the measurement of the ratio free/conjugated DEHP metabolites can be an important parameter possibly accounting for some species-differences in DEHP-induced effects on the testis.

Some age-related differences in glucuronidation have been described in infants having (especially in the case of pre-term neonates) lower levels of glucuronyl transferases at birth (Frederiksen *et al.*, 2014). Carboxylated metabolites decreased and oxidised metabolites

increased during the first year of life, at the end of which the urinary metabolite distribution pattern for DEHP and DiNP was comparable with the pattern in older children and adults (Frederiksen *et al.*, 2011; Frederiksen *et al.*, 2014).

The metabolic pathway of DEHP in humans is qualitatively independent of the exposure routes. After intravenous exposure to DEHP via a voluntary platelet donation, the secondary metabolites 5OH-MEHP, 5cx-MEPP and 5oxo-MEHP were the major urinary metabolites followed by the simple monoester MEHP and 2cx-MMHP (Koch *et al.*, 2005a; Koch *et al.*, 2005b). Furthermore, the elimination characteristics and relative distribution of the DEHP metabolites in urine were found to be similar to that observed after oral administration.

After i.v. administration of DEHP, a very similar profile of the urinary metabolites was determined in Green monkeys and humans by Albro *et al.* (1981) and Peck *et al.* (1978).

Distribution studies in rodents indicate that DEHP is widely distributed in the tissues without evidence of accumulation (Daniel and Bratt, 1974; Gaunt and Butterworth, 1982; Pollack *et al.*, 1985a). After oral administration of ^{14}C-DEHP, rats and marmosets showed qualitatively similar distribution patterns (liver>kidney>plasma>testes) (Rhodes *et al.*, 1986). The radioactivity concentrations in rat tissue were remarkably higher than in marmoset (Kurata et al, 2012a) DEHP and its metabolites may be secreted into the milk of lactating rats (Dostal *et al.*, 1987; Parmar *et al.*, 1985) and additionally pass into human milk (Bruns-Weller and Pfjordt, 2000; Calafat *et al.*, 2004b; Gruber *et al.*, 1998; Mortensen *et al.*, 2005; Zhu *et al.*, 2006). In rodents, ^{14}C-DEHP was found to cross the placenta and distribute into foetal tissues (Lindgren *et al.*, 1982; Singh *et al.*, 1975; Srivastava *et al.*, 1989). The monoester MEHP was found in rat and human amniotic fluid (Calafat *et al.*, 2006; Silva *et al.*, 2004b).

The data for bioavailability and metabolism following inhalation and dermal exposure are limited. Dermal absorption appears to be limited in humans. Wester *et al.* (1998) estimated that dermal absorption amounts to approximately 1.8% of a 24h applied dose of ^{14}C-DEHP solubilized in ethanol. In rats, bioavailability of DEHP after dermal exposure was estimated at 10% (Elsisi *et al.*, 1989; Melnick *et al.*, 1987). However, the results of *in vitro* studies (Scott *et al.*, 1987) indicate that the rat skin is about 4-fold more permeable for DEHP than human skin. Thus, approximately 2.5% of a dermal dose may be absorbed by human skin. Recently an *in vitro* study using excised human viable skin in a flow-through diffusion cell system measured DEHP (both deuterated and not-deuterated) and its possible metabolites dermal absorption (Hopf *et al.*, 2014). The skin permeation resulting from applying neat DEHP was extremely low and occured only after 30 h, while the aqueous DEHP solution permeated skin after 8 h as MEHP: only the metabolite was indeed detected in the receptor fluid. *In vitro* skin permeation (Kp) for DEHP as aqueous solution 15×10^{-5}cm/h was in line with previously reported results on di-n-butylphthalate (Beydon *et al.*, 2010).

3.4.3. DEHP exposure of the general population

DEHP is physically dispersed in PVC and therefore can leach, migrate or gas out from PVC articles. Thus, DEHP might be present in air, dust, water, soils, sediments and food and has become an ubiquitous environmental contaminant (Clark *et al.*, 2003b; Abb *et al.*, 2009; Guo and Kannan, 2011; Kang *et al.*, 2012). Diet is the main source of DEHP exposure for the general population, with fatty foods (e.g. dairy, fish, oils) containing the highest DEHP levels (Clark *et al.*, 2003b, ECB 2004, Meek and Chan 1994, Peterson and Breindahl 2000, Wormuth *et al.*, 2006, Chen *et al.*, 2008, Cirillo *et al.*, 2011). Food has been demonstrated to account for >90% of total DEHP exposure of the German population (Heinemeyer *et al.*, 2013). This

finding seems to be also confirmed in children. Langer *et al.* (2014) determined the concentrations of the metabolites of many phatalates, namely DEP, DnBP, DiBP, BBzP and DEHP, in urine samples from 441 Danish children (3–6 years old). The levels of DEP, DnBP, DiBP and BBzP in dust collected from the children's bedrooms and daycare centres significantly correlated with the concentrations of these phthalates' metabolites. However, DEHP levels in dust were not correlated with DEHP metabolites in children's urine, which suggests that the main DEHP exposure source in these children was other than air and dust, likely being diet. It has been reported that in 10 boys (aged 5 to 8 years) 58 % of the daily DEHP intake originates from foodstuff, whereas dust contributes to 18% of the daily DEHP intake (model calculations), based on content of DEHP in the air and dust from their homes and in their food and drinks, compared with the content of 5 DEHP metabolites in urine (ECHA, 2011). In other situations, using biomonitoring data and modelling, Shin *et al.* (2014) estimated that up to 39% of DEHP levels were attributable to indoor dust ingestion, and in residents in China and in Albany, New York, house dust was estimated to contribute in the range 2-5% and 10-58%, respectively to total DEHP exposure, although dietary intake was the main contributor to exposure for all age groups (>86%) in both countries, with contribution increasing with age (Guo and Kannan, 2011).
DEHP contamination of food may occur due to bioaccumulation in certain foods as well as during processing, handling, transportation, packaging and storage. DEHP dietary exposure was substantially reduced when diets were restricted to food with limited packaging (Rudel *et al.*, 2011). Other sources of DEHP exposure for the general population are indoor air, household dust, consumer products and medical procedures, although these appear to be less relevant when compared with diet, and this finding also applies to children (Langer *et al.*, 2014). Increased excretion of DEHP metabolites in urine is an indication for potential occupational exposure (Gaudin 2008, Fong *et al.*, 2014, Guadin 2011, Hines *et al.*, 2011).

To discriminate dietary and non-dietary phthalate exposure, Koch *et al.* (2013) studied phthalate metabolites in the urine of 5 volunteers who fasted under controlled conditions for 48 h. The study revealed that the exposure to DEHP, DiNP and DiDP were driven by dietary intake, while non-dietary routes such as use of personal care products and ubiquitous sources including dust and indoor air appear to explain exposure to low molecular weight phthalates. This is consistent with analyses of National Health and Nutrition Examination Survey (NHANES) data (Aylward *et al.*, 2011), data for Parisian adults (Blanchard *et al.*, 2013) and data for children (Langer *et al.*, 2014). This relationship is important when considering human diseases associated with DEHP exposure (section 3.4.7), because DEHP exposure could be a surrogate measure for differences in diet.

3.4.3.1. DEHP exposure assessment from probabilistic calculations

Exposure estimates based on probabilistic calculations from DEHP levels in environmental media and food are given in Table 2. The range of median DEHP exposure in the general population from all sources excluding medical and occupational exposure has been estimated to be 1 to 30 µg/kg bw/d (CERHR 2005, Doull *et al.* 1999, Huber *et al.* 1996), although the upper bound can be much higher. Children are estimated to have higher exposures to DEHP than adults (Clark *et al.*, 2003a, Meek and Chan 1994, Müller *et al.*, 2003, UBA, 2012; IARC, 2012). However, the deduction of DEHP exposure from concentrations in environmental media is difficult due to the numerous sources and routes that have to be considered and due to the uncertainties in assumptions made for the exposure assessment. Moreover, since DEHP is omnipresent in the environment, contamination can easily occur during analytical procedures (David *et al.*, 2003b). Finally, one has to consider that the calculated DEHP exposure via food might be based on out-dated DEHP contents in food or that the DEHP burdens have not been

corrected for background contamination (Clark *et al.*, 2003a), which would lead to an overestimation of the DEHP exposure.

Table 2. DEHP exposure for the general population (µg/kg bw/d) estimated from DEHP contents in environmental media and food (modelling studies)

Study	Age group	Median	Upper bound (P 95, max)
Meek *et al.* (1994) [a]	20-70 years	5.8	
	12-19 years	8.2	
	5-11 years	14	
	0.5-4 years	19	
	0-0.5 years	9	
MAFF (1996) [b]	Adults	2.5	5
Clark *et al.* (2003a) [c]	Adult (20-70 years)	8.2	
	Teen (12-19 years)	10	
	Child (5-11 years)	18.9	
	Toddler (7 months-4 years)	25.8	
	Infant (0-6 months)	5-7.3	
Müller *et al.* (2003) [d]	Adults		26
	children (7-14 years)		49
	children (1-6)		151
	infant 6-12 months		285
Wormuth *et al.* (2006) [e]	Children	1.8	15.8
	Adults	2.7	15.5
UBA (2009)[f]		(mean: 5.6)	11.33
Koch *et al.* (2006)[f]		4.8	16.7
Calafat & McKee (2006)[f]		(mean: appr. 5-7)	appr. 15 - 30
Fromme *et al.*, (2007)[f]		2.43	3.95
UBA (2012)[g]	children	Median max. 25 Mean max 44	
	adults	(mean: 11.0 – 25.5)	
IARC (2012)[h]	0 – 0.5 years 0.5 – 4 years 5 – 11 years 12 – 19 years 20 – 70 years	Mean: 8.9 – 9.1 Mean: 19.0 Mean: 14.0 Mean: 8.2 Mean: 5.8	

a estimated daily DEHP exposure from air, food, drinking water by the population of Canada
b dietary exposure in UK
c considering all exposure pathways excluding children's and other consumer products
d combined oral, inhalation and dermal exposure via several pathways in Denmark
e scenario-based approach including oral, dermal and inhalation pathways for Europeans
f taken from UBA (2012)
g population of Germany
h population of Canada

More recently, average DEHP intake in the German population was estimated deterministically using data on measured concentrations in food and food consumption. A total dietary median exposure to DEHP of 3.6 and 9.3 µg/kg bw per day was estimated deterministically; once data of distributions of concentrations and consumption figures were fitted for probabilistic estimations, a similar range of values was obtained (median= 10.2, arithmetic mean =14.0 and the 95th percentile = 28.6 µg/kg bw/d (Heinemeyer *et al.*, 2013). Although consistent with previous data, the authors underlined the uncertainties of the estimation due to insufficient analytical data on concentrations of DEHP in food, however considering it to be robust enough to draw conservative conclusions on DEHP exposures due

to food consumption for adults. For children, food has also been identified as an important source of exposure, and additional exposure via house dust and mouthing may play an important role for total DEHP exposure.

3.4.3.2. DEHP exposure assessment from urinary metabolite excretion/biomonitoring

The individual and actual internal exposure to DEHP can be determined by measuring DEHP metabolites in urine (Blount *et al.*, 2000, Koch *et al.*, 2006, Koch *et al.*, 2003b), the preferred matrix for phthalate determination in humans (Calafat and McKee, 2006). Metabolites can serve as biomarkers of DEHP exposure covering all sources and routes of exposure. However, the study design of any general population survey for exposure to phthalate should take into account that all DEHP metabolites have been demonstrated to have a short half-life (Preau *et al.*, 2010). Pharmacokinetic modelling also pointed out that the time between exposure and urine sampling is a major information requirement in order to characterise DEHP exposure in a proper way (Lorber *et al.* 2011). This can be one of the reasons for a high inter-individual variation in bio-monitoring studies: concentrations in body fluids vary substantially, and the 95th percentile can be 10-fold or higher than the median (CDC, 2009). Another important issue is the correct handling of samples: phthalates including DEHP are ubiquitous and have been detected even in the cleanest laboratory chemicals, sampling equipment and analytical apparatus: therefore data have to be interpreted with utmost caution because of possible external contamination (Wittassek et al, 2011).

For spot urine samples, depending on the time of day and on the times of eating occasions, the phthalate metabolites will at best reflect only short term exposure (i.e. the last two meals at best). Ideally, 24h urine samples are collected for a daily DEHP intake estimation as the absolute amount of the excreted DEHP metabolites during a whole day is directly accessible (Wittassek *et al.*, 2007a). However, this is laborious and not a realistic approach for children, for example.

In most cases, especially for more dated studies, data are available as spot urine concentrations, therefore DEHP metabolite excretion data in 24h urine samples have been extrapolated by using reference values for the daily creatinine excretion (23 and 18 mg/kg/d for men and women, respectively). Alternatively, a volume-based calculation model may be applied (Wittassek *et al.*, 2007b).

To date, urinary levels of DEHP metabolites have been measured in several studies in Germany and the USA that revealed ubiquitous exposure of the general population to DEHP (Table 3). The data from both countries are in accordance and lie within the same order of magnitude. While in the first studies only the simple monoester MEHP was determined in urine, the parameter spectrum has been steadily increasing, since the secondary metabolites were recognised as more reliable biomarkers for DEHP exposure (Koch *et al.*, 2006, Koch *et al.*, 2003b). They are excreted to a higher extent than MEHP and are more specific because they are not as susceptible to contamination as MEHP is, since it is possible that it may be formed by hydrolysis of DEHP during sample handling and processing. Mono(2-ethyl-5-carboxypentyl) phthalate (5cx-MEPP) is the main urinary metabolite measured in the general population, followed by mono(2-ethyl-5-hydroxyhexyl) phthalate (5OH-MEHP), mono(2-ethyl-5-oxohexyl) phthalate (5oxo-MEHP), mono(2-ethylhexyl) phthalate (MEHP) and mono(2-carboxy-methylhexyl) phthalate (2cx-MMHP) (Table 3). This is in line with the observation that carboxy metabolites have the longest half-lives, and as a consequence following chronic exposure of the general population, they are more abundant than short-living metabolites. Therefore, the ratio of primary to secondary metabolites can be used to identify recent or past exposure, with

the primary metabolites being more abundant following very recent exposure (Zeman et al, 2013).

The table reports data from the 2008 SCENIHR Opinion, as well as more recent ones for comparison: data seems to be quite similar.

Table 3. Median body burden to DEHP of the general population, indicated by urinary concentrations of DEHP metabolites (in µg/l)

Study	Year of sampling	n (age)	5cx-MEPP	5OH-MEHP	5oxo-MEHP	2cx-MMHP	MEHP	FOD*	DEHP+[µg/kg bw/day]
Blount et al (2000)[1]	1988-1994	298 (20-60)	n.d.	n.d.	n.d.	n.d.	2.7	>75	1.3
Koch et al (2003b)[2]	2002	85 (7-63)	n.d.	46.8	36.5	n.d.	10.3	100	5.8
Barr et al (2003)[1]	n.s.	62 (n.s.)	n.d.	35.9	28.3	n.d.	4.5	96	4.3
Silva et al (2004a)[1]	1999/2000	2541 (>6)	n.d.	n.d.	n.d.	n.d.	3.2	78	1.6
Becker et al (2004)[2]	2001/2002	254 (3-14)	n.d.	52.1	41.4	n.d.	7.2	100	(6.3)
Koch et al (2004a)[2]	2003	19 (2-6) 36 (adults)	n.d.	49.6 32.1	33.8 19.6	n.d.	6.6 9.0	100 100	(5.6) 3.8
Kato et al (2004)[1]	2001	127 (n.s.)	n.d.	17.4	15.6	n.d.	<LOD	95	2.4
CDC (2005)[1]	2001/2002	393 (6-11) 742 (12-19) 1647 (>20)	n.d.	32.9 25.2 17.7	22.6 18.5 12.2	n.d.	4.4 4.5 4.1	NA	(3.7) 3.0 2.1
Swan et al, (2005)[1]	1999-2002	85 (>18) pregnant women	n.d.	11.4	11.1	n.d.	3.3	98	1.4
Silva et al (2006)[1]	2003/2004	129 (adults)	15.6	15.3	7.1	5.9	3.1	100	1.9
Wittassek et al (2007a)[2]	2001/2003	120 (20-29)	19.5	14.6	13.4	5.8	5.0	100	2.3
Becker et al (2009)	2003/2006	599 (3-14)	61.4	46.0	36.3	20.4	6.7	100	
Boas et al (2010)	2006-2007	845 (4-9) 503 male 342 female	- -	37 31	19 16	30 27	4.5 3.6	100	1.8 1.9
Koch et al (2011)	2007	108 (5-6)	MEHP/MEHHP/MEOHP/ MECPP/2cx-MMHP						4.5
Frederiksen et al (2011)	2006/2008/2011	129 (6-21)	Individual values not reported						4.04

1 US population
2 German population
* Frequency of detection for at least one DEHP metabolite in%
\+ Median daily intake estimation applying equation (1) assuming that creatinine related concentrations are equal to volume related concentrations and a mean creatinine excretion of 21 mg/kg/day (men and women); values for children in parentheses
n.d.: not determined
NA: not available

Daily DEHP intake evaluations coming from the more dated biomonitoring studies were based on the excretion of the simple monoester MEHP only (David *et al.*, 2000, Kohn *et al.*, 2000), which led to substantial differences in the resulting daily intake values depending on the

excretion factor used. After 2003, daily intake calculations include secondary DEHP metabolites (Koch *et al.*, 2003a, Wittassek *et al.*, 2007a, Wittassek *et al.*, 2007b; Becker et al, 2009, Boas et al, 2010, Frederiksen et al, 2011). Estimations based on 3 or 5 DEHP metabolites may lead to more reliable estimations of the daily DEHP intake.

In general, children showed higher concentrations of DEHP metabolites than adults with higher ratios of the secondary oxidative metabolites compared to MEHP (Becker *et al.*, 2004, CDC 2005, Koch *et al.*, 2004a, Becker *et al.*, 2009). The concentrations of the phthalate metabolites decreased with increasing age (Becker *et al.*, 2009). However, Frederiksen *et al.* (2011) found that 6–10 yr old children and adolescents seem to take up similar absolute amounts of DEHP. In addition, declining levels of DEHP (as well as for other phthalates), were found in Germany over time, in agreement with the restrictions of use of certain phthalates in toys and other products within recent years (Koch & Calafat, 2009).

Many other papers detecting DEHP metabolites have been published in the past years in many countries and populations, often reporting a different way to normalise results from spot urine samples: in 108 Mexican women (Romero-Franco *et al.*, 2011), in 430 pregnant women aged between 25 and 35 years, from Central Taiwan (Lin *et al.*, 2011), in 111 German primary school starters (Koch *et al.*, 2011), in 183 volunteers in three Chinese cities (Guo *et al.*, 2011), in 60 premenstrual girls from Egypt (Colacino *et al.*, 2011), in a population of Spanish pregnant women and children (Casas *et al.*, 2011), in not occupationally exposed 157 healthy subjects (74 males and 83 females) in Central Italy (Tranfo et al, 2013) and in 110 infertile and 43 fertile women in Italy (La Rocca *et al.*, 2014). This latter study, although limited by the measurement of DEHP and MEHP only, shows a mean higher levels of phthalates in infertile women (37.9 ng/mL blood) when compared to fertile ones (13.1 ng/mL blood).

By expressing the geometric mean of excretion of two metabolites (namely MEHP and MEHHP) as µg/g creatinine, very limited differences were noted in comparable populations (in the case of the above mentioned studies the female populations) in China, Mexico, Italy and the United States (Tranfo *et al.*, 2013). The ranges were: 3.37-5.16 µg MEHP/g creatinine and 10.7-40.8 µg MEHHP/g creatinine. Significant differences in phthalates concentrations between genders were not identified.

The urinary concentration of some DEHP metabolites in pregnant French women measured in a recent study (Zeman *et al.*, 2013) was shown to be higher compared to previous European and US studies. By using data as an input for a pharmacokinetic model as proposed by Lorber *et al.* (2010), DEHP exposure was estimated by reverse dosimetry. The mean exposure to DEHP was estimated at 4.2 µg/kg bw/d; the median and the 95th percentile exposure to 1.3 and 15.6 µg/kg bw/d; these estimates are lower than predicted with the daily intake estimates (median value= 5.8 µg/kg bw/d and the 95th percentile value= 65.1 µg/kg bw/d) (Zeman *et al.*, 2013).

In general, daily DEHP intake estimations based on urinary biomarkers generate similar values, slightly lower but in the same order of magnitude as those based on probabilistic calculations (SCENHIR, 2008). The current median DEHP exposure for the German general population was estimated between 2 and 5 µg/kg bw/d (Koch *et al.*, 2003a, Wittassek *et al.*, 2007a). Children seemed to have higher exposures in relation to kg bw/ with a median exposure of around 4 to 8 µg/kg/d (Wittassek *et al.*, 2007b). The comparison of exposure estimates based on bio-monitoring data and indirect estimates lead Calafat & McKee (2006) to geometric mean DEHP exposures of around 2-3 µg/kg bw/d (95th percentile=12 to 17 µg/kg bw/day) for children and adults. The exposure seems therefore to be independent of age, based on bio-monitoring, whereas indirect estimates are higher for children than for adults (Calafat & McKee (2006).

The results of a retrospective biomonitoring study (Wittassek *et al.*, 2007a) indicated that the inner burden to DEHP has decreased during the last 20 years in Germany by a factor of nearly 2.

DEHP is not the only phthalate found in biomonitoring studies (Frederiksen *et al.*, 2010; Tranfo *et al.*, 2013, Zeman *et al.*, 2013, ECHA, 2011). Generally, when present, the different metabolite levels correlated significantly, indicating high concurrent exposure to several phthalates. Frederiksen *et al.* (2010) showed that in the urine samples of 60 young Danish men (age 18.2-16.2 yrs), summed DEHP metabolites were excreted in urine in the highest amount (median = 91.1 ng/mL), followed by monoesters of other phthalates. Several metabolites were also detectable in serum and in seminal plasma, although in much lower levels. For DEHP, a correlation between urine and serum levels was observed only for MECPP. In seminal plasma, only MEP levels correlated significantly to levels in urine and in serum.

The presence of phthalate metabolites as estimates of phthalate exposures was investigated in a large study carried out by Trasande *et al.* (2013, 2013a, 2014). They examined associations between urinary phthalate metabolites and body mass measurements (as marker of obesity and overweight) in a representative sample of USA children and adolescents. The study was performed on a stratified and whole-sample cross-sectional analysis of 2884 children (6-19-year-old) who participated in the United States of America (USA) 2003-2008 National Health and Nutrition Examination Survey. A large series of parameters were considered in a multivariable linear and logistic analyses, especially body mass index, overweight and obesity against concentrations in urine of phthalate low-molecular weight, high-molecular weight and DEHP metabolites. A 3-fold increase in low-molecular weight phthalate metabolites was associated with 21-22% increases in odds ratios (95% CI: 1.05-1.39) of overweight and obesity among non-Hispanic blacks (a category of population only defined in the USA). Significant associations were not identified in any other racial/ethnic subgroup of the study sample. Interestingly enough, in this study DEHP metabolites and high-molecular weight phthalate metabolites were not associated with body mass outcomes (Teitelbaum *et al.*, 2012). However, it has been recently reported, using the 2009/2010 cycle of the NHANES and DEHP exposure as a case-study and as the data source for this analysis, that the choice of exposure metric can introduce significant bias of varying magnitude and direction into the calculation of epidemiologic associations (Christensen *et al.*, 2014).

3.4.3.3. Mother/infant exposure levels measured by biomonitoring

Adibi *et al.* (2008) measured phthalate metabolite concentrations in spot urine samples collected from 246 pregnant Dominican and African-American women. Analysis was performed on 28 repeat urine samples collected over a 6-week period, 48h personal air samples (n = 96 women) and repeated indoor air samples (n = 32 homes) for 5 phthalate diesters, including DEHP. The geometric mean (CI 95%) DEHP concentration in 48h personal air samples was 0.18 (0.16–0.21µg/m^3) and that in indoor air was 0.09 (0.08–0.10µg/m^3).The geometric mean of urinary DEHP metabolites were 4.8 ng/ml MEHP, 17.5 ng/ml MEOHP, 19.9 ng/ml MEHHP and 37.1 ng/ml MECPP. Most metabolite concentrations in the newborns were consistently lower than maternal concentrations based on the geometric mean. However, the median concentration of MECPP was higher in the newborns than in the mothers (56.9 vs 37.1 ng/mL).

The correlation between mother and child DEHP and its metabolites (MEHP, 5-OH-MEHP, 5-oxo-MEHP) urinary excretion was also studied in South Korea (Song *et al.*, 2013). A total of 258 mother and child pairs, plus another 297 adults age-matched to the mothers and an additional 134 children were enrolled. Among the three DEHP metabolites, only the MEHP of children was significantly correlated to that of paired mothers (p-value≤0.01). The relative

metabolic rate (RMR) of DEHP metabolism was faster in children (especially in infants <2 years age) than in mothers and adults, specifically in the step related to MEHP hydroxylation to 5-OH-MEHP (Song *et al.*, 2013). This finding is in line with other studies, indicating that the oxidative pathway in DEHP metabolism is a function of age. In several studies, higher ratios of the oxidative metabolites 5OH-MEHP, 5oxo-MEHP and 5cx-MEPP to the simple monoester MEHP were found in children in comparison to adults (CDC, 2005, Koch *et al.*, 2004a, Silva *et al.*, 2006b). Additionally, among children, increasing ratios with decreasing age were observed (Becker *et al.*, 2004). In neonates, there is a higher capacity for oxidation of MEHP with 5cx-MEPP being the main metabolite (Egestad *et al.* 1996, Koch *et al.*, 2006).

In the UK phthalate exposure in children and their mothers were measured, as part of the European biomonitoring pilot study, Demonstration of a Study to Coordinate and Perform Human Biomonitoring on a European Scale (DEMOCOPHES) (Exley et al., 2015). Very low levels of the phthalate metabolites were detected in 21 school children aged 6–11 years old and their mothers. The geometric means in mothers: 5OH MEHP 8.6 µg/L, 5oxo-MEHP 5.1 µg/L, MEHP 1.2 µg/L, MEP 26.8 µg/L, MiBP 17.0 µg/L, MBzP 1.6 µg/L and MnBP 13.5 µg/L; and in children: 5OH-MEHP 18.4 µg/L, 5oxo-MEHP 11.4 µg/L, MEHP 1.4 µg/L, MEP 14.3 µg/L, MiBP 25.8 µg/L, MBzP 3.5 µg/L and MnBP 22.6 µg/L). The levels were similar to or below population based reference values published by the US National Health and Nutrition Examination Survey (NHANES) and Germany's GerES surveys. The authors concluded that results were of no concern with regards to health (Exley et al., 2015).

Using multivariate analysis, Sathyanarayana *et al.* (2008) showed that a mother's DEHP metabolite concentration significantly predicted infant DEHP metabolite concentrations. Regarding glucuronidation, due to lower glucuronyl transferases activities at birth, neonates showed lower conjugation metabolites in their urine during the first year of life, at the end of which the urinary metabolite distribution pattern for DEHP and DiNP was comparable with the pattern in older children and adults (Frederiksen *et al.*, 2014).

To assess maternal–foetal exposure to phthalates and investigate whether in utero phthalate exposure is associated with low birth weight (LBW), Zhang *et al.* (2009) studied a total of 201 newborn–mother pairs (88 LBW cases and 113 controls) in China. Maternal blood, cord blood and meconium specimens were collected and analysed for phthalates, including DEHP. More than 70% of the biosamples had quantifiable levels of phthalates, with higher levels in the LBW infants compared with the controls. In utero, DEHP exposures were associated with LBW in a dose-dependent manner.

Lin *et al.*(2011) evaluated the association between maternal phthalate exposure and cord sex steroid hormones in 155 maternal and infant pairs from the general population. In female newborns, the maternal urinary levels of MEHP and 5OH-MEHP were negatively correlated with free testosterone (fT) and fT/ E2 (estradiol) levels in cord serum. Additionally, after gestational age was adjusted, the maternal urinary level of DEHP was negatively correlated with the free testosterone (fT) and fT/E2 levels in cord serum.

Enke *et al.* (2013) determined 21 urinary phthalate metabolites (indicating exposure to 11 parent phthalates) in the urine of 20 healthy newborns at days 2–5 post-partum, 47 urine samples of 7 women during pregnancy and in first urine samples of 9 healthy newborns together with their mothers' urine shortly before the mothers gave birth. All urine samples revealed ubiquitous exposures to phthalates comparable to other populations. Metabolite levels in the newborns' first-day urine samples were generally lower than in all other samples. However, newborn urine samples (both first and day 2–5 samples) showed a metabolite pattern distinctly different from the maternal and general population samples: in the urine of

newborns, the carboxy-metabolites of the long chain phthalates (DEHP, DiNP, DiDP) were by far the dominant metabolites with a relative share in the metabolite spectrum up to 6 times higher than in maternal urine. For the short chain phthalates (DBP, DiBP) oxidised metabolites appeared to be less favoured than the simple monoesters in the urine of newborns.

Lactational exposure

Latini *et al.* (2009) measured several phthalate metabolites in breast milk from 62 healthy mothers living in southern Italy. The simple monoesters MiBP (median 18.8 µg/l) and MEHP (median 8.4 µg/l) were present in all milk samples; MnBP (median 1.5 µg/l) and MBzP (median 0.3 µg/l) were found in 64.5% and 43.5% of the samples, respectively. Among the oxidative metabolites of DEHP and DiNP, only 5cx-MEPP and monoisononyl phthalate with one hydroxyl group (OH-MiNP) were detectable in 1 and 13 samples (21%), respectively. These findings indicate that exposure to phthalates through breast milk in infants from southern Italy is comparable to that of other countries, thus confirming that human milk may represent an additional potential source of phthalate exposure in a population at increased risk.

Fromme *et al.* (2011) analysed 78 breast milk samples to characterise the exposure of infants to phthalates including 3 metabolites of DEHP (MEHP MEOHP; MEHHP). Median concentrations for DEHP were 3.9 ng/g. For MEHP, the median value in breast milk was 2.3 µg/l, but MEOHP and MEHHP were not detected. In infant formula (n=4), mean values of 19.7 ng/g DEHP was measured using median and 95th percentile values, an "average" and "high" daily intake for an exclusively breast-fed infant of 0.6 µg/kg bw and 2.1 µg/kg bw, respectively, for DEHP was estimated.

Conjugated metabolites in breast milk were not analysed (Kavlock et al, 2006). However, data from Calafat et al (2004) show that oxidative metabolites are present in free (unconjugated) form both in breast milk and amniotic fluid.

3.4.3.4. Exposure to DEHP following medical procedures

DEHP is currently the primary plasticizer used in PVC-containing medical devices such as containers for blood or nutrients, tubings and catheters. Thus patients undergoing medical treatment can be exposed to DEHP released from PVC medical devices (FDA 2002, Health Canada 2002). The following procedures with a potential for high exposure to DEHP are identified:

- Exchange transfusion of blood in neonates
- Extracorporeal membrane oxygenation (ECMO) treatment of neonates and of adults
- Total Parenteral Nutrition (TPN) in neonates
- Multiple procedures in sick neonates
- Haemodialysis in peripubertal males
- Haemodialysis in pregnant or lactating women
- Enteral nutrition in neonates and adults
- Hearth transplantation or coronary artery bypass graft surgery
- Massive blood transfusion of red blood cells and plasma

Patients undergoing haemodialysis are considered to have the highest exposure, due to the chronic nature of the treatment.

Depending on the medical procedure, exposure to DEHP varies widely and is a function of the lipophilicity of the fluid that is in contact with the medical devices, the PVC surface size, the

temperature, the flow rate and the contact time (Haishima *et al.*, 2005, Hanawa *et al.*, 2003, Hanawa *et al.*, 2000, Kambia *et al.*, 2003, Loff *et al.*, 2002, Loff *et al.*, 2000, Loff *et al.*, 2004). In addition, DEHP is converted by a plasma enzyme to MEHP, its biologically active metabolite (Rock *et al.*, 1978). Polyethylene linings of PVC articles (e.g. tubing) do not seem to substantially prevent the release of DEHP (Bourdeaux *et al.*, 2004, Demore *et al.*, 2002).

3.4.3.5. DEHP leaching *in vitro* from medical devices

DEHP was detected in whole blood at levels ranging from 16.8 to 46.1 µg/mL and in packed cells at levels ranging from 32.6 to 55.5 µg/mL in PVC blood bags stored at 5 °C. These levels increased with storage time (Sasakawa and Mitomi, 1978).

Dine *et al.* (1991) shows the accumulation of DEHP in platelet-poor plasma stored for one or two weeks in PVC bags sterilised by steam, ethylene oxide or irradiation at levels of 378 ± 19, 362 ± 10 and 275 ± 15 mg/L, respectively after 1 week. The levels were 432 ± 24, 428 ± 22 and 356 ± 23 mg/L respectively after two weeks storage, indicating that the sterilisation process is not a significant factor in DEHP leaching from PVC plasma bags.

Some other *in vitro* studies have been published since the last SCENIHR Opinion, further substantiating the relevance of DEHP leaching from medical devices and the consequent exposure of patients undergoing medical treatments.

Rael *et al.* (2009) assessed the leaching of DEHP and MEHP in donated packed red blood cells (PRBC) during 42 days of storage in PVC-bags. The supernatants of PRBC were analysed on storage day 1 and day 42. The DEHP content significantly increased from 34.3 µM (±20.0 SD) on day 1 to 433.2 µM (±131.2 SD) on day 42. The MEHP content increased from 3.7 µM (±2.8 SD) on day 1 to 74.0 µM (±19.1 SD) on day 42. The authors also demonstrated that DEHP and MEHP increased the release of IL-8 from human umbilical vein endothelial cells (HUVEC). Thus, the transfusion of older units of PRBC might lead to an accumulation of phthalate esters, possibly resulting in inflammation and other effects.
Bagel *et al.* (2011) showed that the type of lipids used in parenteral nutrition admixtures influence the quantity of DEHP leached out from PVC-based tubing: release of DEHP was highest when olive-oil based emulsions were used, the second highest was when soybean oil-based emulsions were used and the least leaching of DEHP was found when a fish oil-based emulsion was used.
Chiellini *et al.* (2011) studied DEHP leakage from endotracheal tubes and correlated the leaching of the plasticiser and the time of intubation of the tubes. The study revealed that the release of DEHP occurred within the first 24h that the tubes were in use.
PVC tubing released significantly higher amounts of DEHP when blood was circulated through 3 different types of tubing: traditional DEHP-PVC, dioctyl phthalate (DOP) PVC and heparin-coated PVC tubing (Greiner *et al.*, 2012).

Rose *et al.* (2012) determined the amount of DEHP that leached from commercial infusion sets into lipid and non-lipid infusates. Based on the results of the study, exposure to DEHP for a 3-kg neonate was calculated to be 24.9±2.5 µg/kg when infusion simulation was performed at 32°C. Higher exposures would occur if the infusate was at 37 °C.

In a simulated study, Takatori *et al.* (2008) determined the leaching of di(2-ethylhexyl)phthalate (DEHP) and mono(2-ethylhexyl)phthalate (MEHP) from medical products made of PVC to enteral nutrition (EN) for neonatal patients. The worst-case daily exposures of the neonatal patient to DEHP and MEHP by the administration of EN were estimated to be 148 and 3.72 µg/kg/day.

3.4.3.6. Adult exposure during medical procedures

Exposure to DEHP due to the usage of PVC medical devices can be short- or long-term. Long-term exposures in adults are caused by haemodialysis, continuous ambulatory peritoneal dialysis (CAPD), transfusions of blood components and blood products to patients with leukaemia, aplastic anaemia, sickle cell anaemia, clotting disorders, administration of total parental nutrition (TPN) and enteral nutrition of critically ill patients. Short-term DEHP exposures include blood transfusions e.g. to patients with haematological diseases, in trauma patients, patients undergoing surgical procedures or extracorporeal membrane oxygenation (ECMO) procedures and intravenous infusion of drugs. There are at least 3 factors that are known to increase the release of DEHP from the plastic of the blood containers into blood components:

(1) The content of plasma; plasma lipids will increase the release,

(2) The temperature; DEHP will be released at significantly higher rate at room temperature than in the cold,

(3) Release of DEHP will be essentially linear over time.

Reported DEHP exposures estimated due to medical procedures for adults are summarised in Table 4.

The reported data are based on measurements of DEHP blood levels in patients before and after specific medical procedures, area under curve (AUC) calculations and DEHP levels in stored blood and blood components together with different scenario assumptions (e.g. rate extraction of DEHP). Long-term haemodialysis is the continuously repeated procedure, which may result in the highest cumulative dose of DEHP (up to 2200 µg/kg/d). Blood transfusions to trauma patients or during ECMO may be the short-term procedure that gives the highest acute DEHP exposure in adults (up to 10 mg/kg/d).

Table 4. Daily DEHP exposure of adults due to medical procedures using PVC medical devices calculated from measurement of DEHP in patient's blood or calculated from the leaching rate of DEHP from the medical apparatus (Health Canada 2002)

Medical procedure	Daily DEHP dose (µg/kg/d)	Reference
Long-term exposures		
Haemodialysis	640[a,b,c] (150-2200) (corresponding to approximately median 16 g DEHP over the course of a year (range 3.7–56 g) 450[a,b,c] (270-1210) – delivered dose 100[a,b,c] (20-360) – retained dose 230[c] (50-850) – retained dose	Pollack (1985) Faouzi (1999) Dine (2000)
Continuous ambulatory peritoneal dialysis	20[e]	Mettang (1996)
Long-term transfusion of blood and blood products	6-90[f]	Jacobson (1977) Doull (1999) Plonait (1993) Health Canada (2002)
Long-term total parenteral nutrition	130-280[d] 800-2000 µg/day[d] (infants/children)	Mazur (1989) Loff (2000) Kambia (2003)
Short-term exposures		
Transfusions of blood components Trauma patient	 8500[f] (63 units whole blood) 1300-2600[b] (2.5l whole blood)	 Jaeger and Rubin(1972)

		Sjoberg (1985b)
Transfusions of blood components Red blood cells, one unit, fresh Red blood cells, one unit stored 35 days Platelets in plasma, one unit, fresh Platelets in plasma, one unit stored 7 days Plasma, one unit, thawed apheresis, fresh Plasma, one unit, thawed apheresis stored 5 days During ECMO process	38 [f] 114 [f] 64 [f] 130 [f] 71 [f] 311 [f] 3000-10000[f] (21-46 units combined blood products)	Sampson 2011 Sampson 2011 Sampson 2011 Sampson 2011 Sampson 2011 Sampson 2011 Butch (1996)
Cardiopulmonary bypass During artificial heart transplant At the end of cardiopulmonary bypass	2400[e] 81 ± 40	Barry (1989) Takahashi (2008)
IV Infusion of drugs Non-liphophilic drugs Lipophilic drugs	< 5[f] up to 1500[f]	Health Canada (2002) Pearson (1993)

a assuming 3 dialysis sessions per week for a 70 kg patient
b area under curve (AUC) calculations
c estimated by DEHP blood levels coming to and/or from the patient, 4h-dialysis treatment
d based on estimated rates of DEHP extraction from PVC storage bags and infusion lines
e calculated from DEHP serum concentrations measured in patients
f based on DEHP concentrations in stored blood and blood components or infusion solutions

The estimated DEHP doses given in Table 4 are based on measurements of DEHP alone. However, analytical determination of DEHP is prone to contamination during sample handling and processing. This is to be kept in mind when assessing the estimated DEHP exposure levels. On the other hand, depending on the presence of plasma enzymes, DEHP can be hydrolysed to MEHP, therefore these two factors influence the results in the opposite direction.

Indeed, it has been demonstrated that patients receiving blood and blood products are not only exposed to DEHP, but also to its hydrolysis product, mono(2-ethylhexyl) phthalate (MEHP), which is formed by plasma lipases (Albro and Thomas 1973, Peck *et al.*, 1979). The conversion increases with increasing storage time and temperature, while storage at low temperatures prevents it (Cole *et al.*, 1981, Rock *et al.*, 1978). MEHP has been measured in stored blood, blood products and peritoneal dialysate (Cole *et al.*, 1981, Labow *et al.*, 1986, Peck *et al.*, 1979, Rock *et al.*, 1978, Sjoberg *et al.*, 1985a, Sjoberg *et al.*, 1985b). Nevertheless, the data available are not sufficient to accurately calculate the *in vitro* conversion rates (Health Canada 2002). The MEHP exposure due to exchange transfusion has been estimated to be in the range of 5 to 680 µg/kg/d (Sjoberg *et al.*, 1985a, Sjoberg *et al.*, 1985b).

On the contrary, MEHP was never detected above the lod (0.4 mg/L) in patients undergoing ambulatory peritoneal dialysis (CAPD), whereas DEHP was found at concentrations ranging from 0.8 to 4.2 mg/L serum after dialysis. In the pre-dialysis serum samples from the same patients, DEHP was not detected (< 0.1 mg/L) (Nässberger *et al.*, 1987).

Exposure to DEHP may also occur through voluntary medical treatments such as the apheresis procedure to donate blood products (Table 5). Many disposables used in apheresis are manufactured from PVC containing DEHP. Highest DEHP exposure has been estimated for

continuous-flow plateletpheresis (dual needle technique). Based on urinary measurements of DEHP metabolites, Koch *et al.* (2005b) calculated for such donors (overall) daily DEHP intakes of 28.2-38.1 µg/kg/d. For platelet donors undergoing the single needle discontinuous-flow technique, values were somewhat lower with 14-24 µg/kg/d. The internal burden after plasma donation (3.1-9.6 µg/kg/d) was not elevated in comparison to controls (3-11.6 µg/kg/d), which indicates that the DEHP dose associated with plasmapheresis is not elevated above background. This may be because the lipid-rich plasma may contain most of the DEHP, which is removed from the body by the procedure. From serum, Buchta *et al.* (2003) estimated DEHP concentrations exposures of 1.8-20.3 µg/kg/d due to apheresis procedures.

Table 5. Daily DEHP exposure of adults due to apheresis procedure using PVC medical devices calculated from measurement of urinary DEHP metabolites or from serum DEHP concentrations

Donation procedure (apheresis technology used)	n	Mean daily DEHP dose (range) [µg/kg/d]	Reference
Controls	5	6.2 (3.0-11.6)	Koch et al, 2005b
Plasma	6	5.7 (3.1-9.6)	
Platelet (discontinuous)	6	18.1 (14.3-23.8)	
Platelet (continuous)	6	32.3 (28.2-38.1)	
Platelet (continuous)	1	31.6	Koch et al, 2005c
Platelet (discontinuous)	19	6.5 (1.8-20.3) 6	Buchta et al, 2003 Sampson et al, 2011
Platelet (continuous)	17	7.2 (2.0-20.3)	Buchta et al, 2003

3.4.3.7. Exposure of newborns during medical procedures

The developing foetus and the neonate represent the most vulnerable phases of life particularly with regard to developmental and reproductive toxicity based on animal studies. Neonates in the Neonatal Intensive Care Unit (NICU) environment may be especially vulnerable (CERHR 2005, FDA 2002, Health Canada 2002) due to their small body size, their physical condition and their multiple medical device-related DEHP exposure (feeding tubes, infusion tubing systems, umbilical catheters, PVC blood bags, transfusion tubing systems, hemodialysis systems, cardiopulmonary bypass, continuous peritoneal dialysis, extracorporeal membrane oxygenation circuits or endotracheal tubes). In addition, glucuronidation activity is lower at birth, and the pattern of metabolites in full-term and especially in pre-term neonates shows some age-related differences (Frederiksen *et al.*, 2014), eliminated only after the first year of life.

Green *et al.* (2005) studied 54 neonates admitted to NICU in the USA for at least 3 days, divided into 3 exposure groups based on medical products used: the low-exposure group included infants receiving primarily bottle and/or gavage feedings; the medium-exposure group included infants receiving enteral feedings, intravenous hyperalimentation and/or nasal continuous positive airway pressure; and the high-exposure group included infants receiving umbilical vessel catheterization, endotracheal intubation, intravenous hyperalimentation and in-dwelling gavage tube. Urinary MEHP median levels were 4, 28 and 86 ng/mL, in the three groups respectively (P = 0.004). After adjustment for institution and sex, urinary MEHP levels among infants in the high-exposure group were 5.1 times those among infants in the low-exposure group (P = 0.03).

In another study, when the levels of three DEHP metabolites were measured in the urine of the 54 infants, urinary concentrations stratified by intensiveness of product use (in ng/mL)

indicated that among infants in the high-intensiveness group, MEHHP and MEOHP levels (555-598 ng/mL) were 13–14 times those among infants in the low-intensiveness group ($P \leq 0.007$) (Weuve *et al.*, 2006).

Neonates have been reported to receive higher doses of DEHP than adults, in terms of bw, than the general population (Calafat *et al.*, 2004b, Green *et al.*, 2005) and their daily exposure to DEHP may exceed the tolerable daily intake.

Fifty-eight full-term (FT) and 67 pre-term (PT; gestational age, 24.7–36.6 weeks) infants were recruited at birth and followed until the age of 14 months (nine times). Urinary concentrations of metabolites of DEHP, diethyl phthalate (DEP), dibutyl phthalate isomers (DiBP and DnBP), butylbenzyl phthalate (BBzP) and diisononyl phthalate (DiNP) were measured (Frederiksen *et al.*, 2014). Metabolites of BBzP, DiNP, and DEHP were 5–50 times higher at day 7 and month 1 in PT than in FT healthy infants, in line with medical treatment received by PT babies in NICU. Thereafter and after discharge from hospital, metabolite concentrations were similar between the two groups.

Table 6 gives estimates of DEHP exposures in neonates resulting from medical treatments calculated from spot measurements of DEHP or delivered doses using AUC calculations. The values are related to a 4 kg infant. However, most newborns requiring medical intensive care are prematurely born babies whose weight is significantly lighter, in general between 0.5 and 2.5 kg. Therefore, the DEHP exposure in relation to bw may be even higher in premature newborns: for many procedures the exposure estimates reach the mg/kg bw/d range. Compared to adults undergoing the same medical procedures, the values are significantly higher and are several orders of magnitude above the exposure levels estimated for the general population.

Table 6. Estimated dose of DEHP received by neonates (4 kg bw) undergoing medical procedures calculated from measurement of DEHP in the patient's blood, calculated from the leaching rate of DEHP from the medical apparatus (Health Canada 2002) or measurement of DEHP in blood components (Sampson 2011)

Medical procedure	Daily DEHP dose (µg/kg/d) of neonate (4 kg)	Reference
Infusion of pharmaceuticals • Midazolam (24 ml) • Fentanyl (29 ml) • Propofol (1%, 10 ml, 24h)	 7 [a] 33[a] 1640 [a]	Loff (2000)
TPN	30 (free of lipid) [a] 2500 (lipid emulsion 20%, 27°C) 3250 (fat infusion, 33°C) [a]	Loff (2000) Loff (2002)
Exchange transfusion – short term	1200-22600 [c] 840-3300 [b] 1700-4200 [a]	Plonait (1993) Sjoberg (1985a) Sjoberg (1985b)
Reconstituted blood for exchange transfusion, fresh (800 mL) Reconstituted blood for exchange transfusion, stored 24h (800 mL) Single dose Red Blood Cells (20 ml) Single dose Red Blood Cells (20 mL), fresh Single dose Red Blood Cells (20 mL), stored 35 days Platelets in plasma, fresh (20 mL) Platelets in plasma, stored 7 days (20 mL)	36-152 [a] 1660 [a] 2660 [a] 232 [a] 40 [a] 125 [a]	Loff (2000) Sampson (2011) Loff (2000) Sampson (2011)

Single dose Fresh Frozen Plasma (20 ml)	75 [a] 121 [a] 78 [a]	
ECMO - sub-acute	Up to 14000 µg/kg/10 days[d, g, h] Up to 34900 µg/kg/ 10 days[e,h]	Schneider (1989) Karle (1997)
Respiratory therapy - oxygen therapy	< 130 [f]	Health Canada (2002)
Respiratory therapy using endotracheal tube	< 700 [f]	Health Canada (2002) Latini 1999
Aggregate exposures of NICU infants (iv administration of sedatives, TPN, replacement transfusion)	2830	FDA (2002)

a calculated from DEHP concentrations in the respective medium
b AUC calculations
c DEHP blood levels measured before (undetectable) and after medical procedure. The highest values correspond to transfusion of three consecutive blood units.
d based on blood levels and certain assumption
e based on blood levels and *in vitro* leaching rates measured
f calculated from DEHP vapour pressure
g DEHP undetected when heparin-coated PVC tubing was used
h If equally distributed over time, corresponding values are 1400 and 3490 µg/kg/d,

ECMO is the medical treatment which may give the highest daily exposure over repeated exposure for a short period of time (up to 34000 µg/kg over a 10-day treatment) (Karle et al, 1997). Moreover, critically ill neonates generally require not only a single medical treatment, but also a combination of several medical interventions, which may lead to even higher DEHP exposure. The FDA (2002) has estimated an upper-bound daily DEHP dose of the order of 3000 µg/kg/d for a newborn (4 kg) in the neonate intensive care unit (NICU) calculated by considering exposure from multiple devices. Such exposures may occur for a period of weeks or even months. However, the total DEHP exposure may vary dramatically from medical centre to centre, depending on the treatment protocols and specific medical devices used (Rosenberg *et al.* 1994; Calafat et al, 2004a).

The urinary concentrations of DEHP metabolites in neonates undergoing intensive medical interventions were found to vary widely and reach levels that are much higher than those found in the general population (SCENIHR, 2008). Compared to adults, the ratios among the metabolites are shifted in favour of the oxidative metabolites with MEHHP, MEOHP and 5cx-MEPP detected at higher levels than MEHP (Calafat *et al.*, 2004a, Koch *et al.*, 2006). Concentrations per gram of creatinine were approximately eightfold higher than results in nanograms per millilitre (Calafat *et al.*, 2004a).

Based on the urinary measurements, Koch (2006) estimated for 45 premature neonates a median daily DEHP dose of 42 µg/kg bw/d and a 95th percentile of 1780 µg/kg bw/d. The large difference between the median and the 95th percentile indicate a great variability in DEHP exposure for newborns in intensive care (with a maximum of 2300 µg/kg bw/d), which may reflect the variety and intensity of the medical procedures performed. The maximum estimated daily DEHP intake was based on the data of Calafat *et al.*, (2004a) giving rise to DEHP exposures up to 6000 µg/kg bw/d (CERHR 2005).

Kambia *et al.* (2011) measured circulating DEHP concentrations in plasma of 7 randomised infants and children on regular cyclic, long-term parenteral nutrition (PN). The circulating concentrations of DEHP before and after a 10 to 11h cyclic PN treatment in 7 infants and children under regular perfusion widely differed, showing a significant increase after the treatment among all the patients, although levels are considerably lower than those measured in newborns in NICU (Table 7).

Latini *et al.* (2009) assessed degradation of endotracheal tubes (n = 63) used for neonatal ventilation by: (1) analysis of colour and spectral changes in endotracheal tubes after use; (2) DEHP leakage assessed by thermal characterisation and thermal gravimetric analysis. Significant colour differences were evident in used endotracheal tubes, as compared to virgin samples. DEHP weight loss was found to be 1.5%.

Levels of DEHP ranging from < 1 to 4100 mg/L in the condensate from water traps of six respirators have been reported, leading to an estimate inhalation of DEHP of 1 and 4200 µg/h in 5 artificially ventilated preterm infants over a 24-h period. An amount of 0.23 mg DEHP/kg wet weight was found in the lung tissue of one infant who died of pneumothorax soon after birth following artificial ventilation (Roth *et al.*, 1988).

Table 7. Plasma concentration of DEHP before and after 10-11h parenteral nutrition (PN) according to the levels of perfused lipids (Kambia *et al.*, 2011)

Patient	Lipid Rate,%	DEHP ng/ml	
		Before PN treatment	After PN treatment
1	1.50	35.0	128.0
2	1.00	26.0	36.0
3	1.85	313.0	391.0
4	1.45	32.0	122.0
5	3.85	150.2	493,0
6	3.40	122.6	327.2
7	2.90	40.0	397.0

Further studies are needed to evaluate if less invasive medical treatments may reduce phthalate exposure risk (Latini *et al.*, 2003b).

3.4.3.8. Mother/infant exposure levels during medical procedures

The urinary concentration of some DEHP metabolites in pregnant French women measured in a recent study (Zeman et al, 2013) was shown to be higher compared to previous European and US studies. A higher concentration of MEHP than expected on the basis of data on secondary metabolites was attributed to a very recent exposure due to medical treatment, rather than to a contamination during sample handling (Vandentorren *et al.*, 2011). Indeed the urinary MEHP concentrations were significantly higher in women with caesarean section or forceps (69.02 µg/L) put on intravenous injection for glucose, water and electrolyte balance support before urinary sampling, when compared to those of women with natural delivery (44.64 µg/L).
This finding is in line with the report of Yan *et al.* (2009) in 150 pregnant American women and besides demonstrating the occurrence of a substantial exposure due to the use of DEHP-containing medical device, this further stresses the fact that gathering data on the time gap between exposure and bio-monitoring is an essential information requirement for reconstructing the dose of non-persistent pollutants.

In the Yan *et al.* (2009) study, several phthalate metabolites were measured in maternal urine, maternal serum and cord serum samples collected from pregnant women at the time of delivery. The urinary concentrations of most metabolites were comparable to or less than those among the U.S. general population, except for MEHP, MEHHP and MEOHP. The median urinary concentrations of MEHHP (109 µg/l) and MEOHP (95.1 µg/l) were more than 5 times their population-based concentrations, whereas the median urinary concentration of MEHP was more than 20 times higher.

Another bio-monitoring study measured the content of DEHP metabolites in 11 pairs of amniotic fluid and the corresponding maternal urine (Wittassek *et al.* 2009). The levels of DEHP metabolites were much higher in the maternal urine, which according to the authors was probably contaminated with DEHP from the PVC urine bags that contained 20-40% DEHP, indicating the importance for sample handling to get reliable results (Wittassek *et al.* 2009), but at the same time the exposure related to medical device use.

3.4.3.9. Summary and conclusions on the exposure to DEHP

The general population is exposed to DEHP through a variety of routes, with food being the primary source. Several biomonitoring studies (measuring primary and secondary excreted DEHP metabolites) indicate exposure to DEHP in the whole general population. Since all DEHP metabolites have been demonstrated to have a short half-life, the time between exposure and urine sampling is a major information requirement in order to characterize DEHP exposure. In adults the ratio between primary and secondary metabolites allow to distinguish short term exposures from less recent ones. However, generally but not always, children demonstrated higher concentrations of DEHP metabolites than adults, with higher ratios of the secondary oxidative metabolites compared to MEHP. In addition, during the first year of life, glucuronidation is generally lower when compared to the activity in older children and adults.
In general, DEHP exposure assessments from probabilistic calculations from DEHP measurements in environmental media and internal dose recalculation from urinary metabolite levels are in the same order of magnitude, with the indirect estimate being generally slightly higher than the values obtained via reverse dosimetry. Most recent studies suggest a current median exposure for the general population of 2 to 5 µg/kg bw/day, whereas the 95th percentile is estimated to be between 6 and 17 µg/kg bw/day. Children may have a somewhat higher body burden of DEHP than adults, with a median exposure of around 4 to 8 µg/kg/d. There are indications that exposure to DEHP in the general population has decreased during the last few years.

Medical procedures using PVC medical devices can lead to DEHP exposures much higher than the background levels. The extent of exposure largely depends upon the medical treatments given and the duration of the treatment, and in the case of plastic blood bags, by the length of storage as well as the storage temperature. In adults, highest short term exposure may result from transfusions of blood components reaching DEHP doses up to approximately 8000-10000 µg /kg bw/day during ECMO, where the highest chronic treatment is represented by haemodialysis, during which the maximum reported exposure is 2200 µg/kg/d. Voluntary medical treatments such as apheresis procedure to donate blood products can cause transient elevated exposure to DEHP (up to 38 µg/kg/d). However voluntary donations are not provided by groups deemed to be at risk for reproductive toxicity (pregnant and nursing mothers and neonates).

The long-term total parenteral nutrition corresponds to higher exposure for infants and children, leading to a maximum exposure of 2000 µg/d, implying that the lower the body weight, the higher the exposure (i.e. for an infants of 2.5 kg bw the exposure is 800 µg/kg/d). In infants and neonates, ECMO is the medical treatment that may give the highest daily exposure over repeated exposure for a short period of time (up to 35 mg/kg over 10 days treatment in a 4 kg bw infants: assuming an equal distribution over time, this would correspond approximately to 3500 µg/kg/d).

Premature neonates in intensive care units (NICU), being dependent on multiple medical procedures, may receive even higher DEHP exposures than adults relative to their kg bw. Such

exposures may occur for a period of weeks or even months. The FDA (2002) has estimated an upper-bound daily DEHP dose of the order of 3000 µg/kg/d for a newborn (4 kg) in the neonate intensive care unit (NICU) calculated by considering exposure from multiple devices. However, most newborns requiring medical intensive care are prematurely born babies whose weight is significantly lighter, in general between 0.5 and 2.5 kg. Therefore, the DEHP exposure in relation to bw may be even higher in premature newborns (i.e. 8000 µg/kg/d for a neonate 1.5 kg bw). The estimate is in line with experimental data showing DEHP exposures up to 6000 µg/kg bw/d for newborn in NICU.

3.4.4. Toxicity

Comprehensive reports provide an in-depth evaluation of the toxicity of DEHP. In particular, the European Union Risk Assessment Report of 2006 (draft version, an update of the final report published in 2004, while the final report was published in 2008 in the framework of the Existing Chemicals program at http://ecb.jrc.it) and the NTP-CERHR Expert Panel Update on the Reproductive and Developmental Toxicity published in 2006 (available at http://cerhr.niehs.nih.gov), the IARC evaluation (IARC, 2012) as well as the ECHA Opinions (ECHA, 2008, 2012, 2013). SCENIHR has carefully considered these summary documents along with new pertinent original publications.

3.4.4.1. Animal Studies

Acute toxicit

Acute toxicity studies indicate low acute toxicity of DEHP, with an LD_{50} of >25 g/kg in rats and mice. The i.v. acute toxicity of DEHP is higher, with an LD_{50} in the region of 200-250 mg/kg in rats. The acute toxicity of MEHP is about 5 times higher than that of DEHP (ECB 2008, NTP-CEHRHR, 2005).

Repeated dose toxicity

Toxicity of orally administered DEHP to experimental animals (e.g. rats) in short-term and repeated dose studies compliant to GLP indicates DEHP induced toxicity in the kidney, liver and testis. The effects on the kidneys included increased absolute and relative organ weights, increased incidence and severity of mineralisation of the renal papilla, increased incidence and/or severity of tubule cell pigment and increased incidence and/or severity of chronic progressive nephropathy.

In long-term studies in rats and mice, there was no indication that DEHP-related changes in the kidney were reversible upon cessation of DEHP-exposure. The lowest NOAEL for kidney toxicity is 500 mg/kg DEHP in the feed (corresponding to approximately 29 and 36 mg/kg/d in males and females, respectively) derived from a well-performed 104-week-study in rats (Moore 1996, David *et al.*, 2000a) and based on increased absolute and relative kidney weight in both sexes at the next higher dose level (LOAEL = 146.6 mg/kg bw/d). More severe kidney lesions were observed at the highest dose level. The most striking effect is hepatomegaly due to hepatocyte proliferation (characterised by increased replicative DNA synthesis/cell division and hypertrophy), peroxisome proliferation and hepatocellular tumours. The NOAEL for non-neoplastic effects on the liver in mice was 100 ppm DEHP in the diet (that is 19 mg/kg bw/d).
Hepatomegaly has been associated to peroxisome-proliferator receptor (PPARα) activation by DEHP and its metabolite MEHP. Indeed, a chronic (22-month) study conducted in groups of

wild-type (Sv/129 strain) and PPARα-null mice fed diets containing 0, 0.01 or 0.05% DEHP, failed to induce any significant effect on the body or liver weights, or non-carcinogenic liver function in all the treatment groups (Ito *et al.*, 2007a).

Sub-chronic liver toxicity has been studied in rhesus monkeys, subjected to repeated transfusions through PVC tubing containing DEHP, mimicking conditions of patients undergoing repeated blood or platelet transfusion. Hepatic dysfunctions and cholestasis have been reported in the group for which PVC tubing was used, but not observed in the group for which polyethylene containers and tubes were used. The average cumulative amount of DEHP infused over 1 year was 69.3 mg (or 21.3 mg/kg bw), which the authors found to be comparable or even lower than that in humans on repeated transfusion therapy (Jacobson *et al.*, 1977). Some functional and histological abnormalities in the liver were still present up to 26 months after cessation of transfusions (Kevy & Jacobson, 1982).

The effects on the peroxisome of DEHP were evaluated in four young adult male cynomolgus monkeys, to compare the hepatic effects seen in rats and mice after treatment with high doses of phthalates. DEHP (500 mg/kg bw/d) was administrated by intragastric intubation for 14 consecutive days. Negative control groups of monkeys received vehicle (0.5% methyl cellulose, 10 mL/kg bw), whereas clofibrate (250 mg/kg bw/d), known to be a peroxisome proliferator in rodents, was used as reference chemical. None of the test substances had any effect on body weight or liver weights: no treatment-related histopathological or functional effects were observed in the liver, kidney or testes (Pugh *et al.*, 2000).

Genotoxicity/mutagenicity

DEHP has been studied extensively in a wide range of *in vitro* and *in vivo* assays for detection of gene mutations, DNA damage and chromosomal effects. Most of the studies are performed in compliance or according to GLP principles and are comparable to guideline studies for mutagenicity or genotoxicity. The results have been negative in the majority of assays with DEHP and metabolites (MEHP and 2-EH) (IARC, 2012). Positive results were obtained in human and other mammalian primary cells or established cell lines assays on DNA strand breaks, cell transformation, induction of aneuploidy and cell proliferation. However, it is not clear enough if the positive results are associated with a direct interaction of DEHP or its metabolite with DNA or rather to secondary oxidative stress or other events. In addition, these test systems are also sensitive to several non-genotoxic substances such as tumour promoters and/or peroxisome proliferators. *In vivo* studies of mutagenicity in two different transgenic mouse models have also been conducted, but IARC considered their results conflicting and not conclusive (IARC, 2012). Thus, in a WoE approach, it can be considered that DEHP and its major metabolites are non-mutagenic substances.

Carcinogenicity

Several studies on the carcinogenicity (and mechanisms of carcinogenicity) of DEHP have been performed in rats and mice with oral administration and an inhalation study in Syrian golden hamsters. These studies are summarised in the RAR report of 2008 and other summary documents (IARC, 2000; IARC 2012).

The results of four different peroral long-term carcinogenicity studies in rats and mice indicate clearly that DEHP is a hepatocarcinogen in both males and females of the 2 species. In the NTP studies (1982a), the LOAEL for tumour induction in mice was 3000 mg/kg DEHP in the feed (corresponding to 670 mg/kg bw/d for male mice). A NOAEL for DEHP-induced tumour

development in the rat has not been identified, as the lowest dose in the study resulted in an increase of the incidence of liver tumours. The LOAEL for tumour induction in rats was 6000 mg/kg DEHP in the feed (320 mg/kg bw/d for male rats).

Two more long-term carcinogenicity studies in rats and mice have been conducted by Moore (1996, 1997) and reported by David *et al.* (2000a and 2000b). The LOAEL and the NOAEL for tumour induction (male mice with hepatocellular neoplasms) in this study was 1500 and 500 mg/kg DEHP in the feed, respectively (corresponding to 292 and 98 mg/kg bw/d for males respectively).

Intrauterine oral DEHP exposure in mice has been reported to alter liver programming and delays the hepatocyte maturation (proliferation/differentiation balance) in post-natal offspring by eliciting a cell phenotype characterised by glycogen accumulation, intracytoplasmic localization of beta-catenin and increased AFP gene expression. The authors hypothesised a potential developmental effect increasing the predisposition of liver tissue to tumours later in life (Maranghi *et al.*, 2010), although the real meaning of these alterations for human health has not been demonstrated so far.

Mechanism of action of carcinogenigity

Several studies demonstrated that liver carcinogenesis in rodents is mediated by PPARα-activation and consequent peroxisome proliferation, a mechanism generally considered not to be relevant in the human liver. Markers of PPARα activation have been quantitated in mice and rats after exposure to DEHP and compared with increases in the incidence of liver tumours by IARC, establishing a good correlation between liver tumour induction and several well characterized indicators of PPARα activation (IARC, 2012). The induction of the markers of PPARα occurred at doses usually lower than those that induce liver cancer, supporting a role for PPARα in liver tumour induction by DEHP.

Mice and rats express PPARα at high levels in the liver, whereas human PPARα is expressed at a lower level in human liver, although IARC underlined that the expression of human PPARα has not been determined in a sufficient number of samples to conclude unequivocally that all populations express less PPARα than responsive rodents (IARC, 2012). In addition, human and rodent PPARα differ in their ability to be activated by PPARα agonists, such as DEHP and/or MEHP, since the amino acid sequences within the ligand-binding domains differ between species. No studies have reported any evidence that DEHP activates PPARα in human liver *in vivo* (IARC, 2012), although some *in vitro* studies showed that all three human PPAR subtypes (alpha, beta and gamma) were activated by MEHP but not by DEHP (IARC, 2012).
As a consequence of differences in the expression and molecular signalling for PPARα, marked species differences with respect to hepatic response to peroxisome proliferation have been demonstrated. Rats and mice seem to exhibit the highest sensitivity. Guinea pigs and monkeys are relatively insensitive. This ranking in responsiveness was also shown by comparing the hepatic peroxisomal proliferation in primary hepatocyte cultures from rats, guinea pigs, marmosets and humans (Elcombe and Mitchell, 1996). Cynomolgus monkeys and marmosets treated orally with DEHP for 14 days and 65 weeks, respectively, showed no treatment-related hepatic alteration (Pugh et al, 2000; Tomonari *et al.* 2006), although the relevance of studies on marmosets may be limited, due to the poor ability of this species to metabolise DEHP (Rhodes *et al.* 1986).
These significant differences in species were considered to be of critical importance for the evaluation of human cancer risk from DEHP in the 2000 IARC evaluation (IARC, 2000).

Since then, transgenic mice with hepatocyte-specific constitutively active PPARα in the absence of ligand were generated and used to evaluate the role of PPARα in responses to peroxisome proliferators, including DEHP (Eveillard *et al.*, 2009; Ren *et al.* 2010). Neither study reported on liver weight or liver histopathology, but focused on gene expression profiling, suggesting that some other target genes were induced by DEHP in PPARα-null mice, such as CAR receptors and other genes involved in the expression of drug-metabolising enzymes. These findings were shown by a number of other studies, summarised by IARC in its recent evaluation (IARC, 2012).
These studies, along with results in humans exposed to DEHP from the environment, suggest that multiple molecular signals and pathways in several cell types in the liver, rather than a single molecular event, may contribute to the induction of cancer in rats and mice. Thus, IARC considers that the relevance to human cancer of the molecular events that lead to cancer elicited by DEHP in several target tissues (e.g. the liver and testis) in rats and mice cannot be definitely ruled out (IARC 2012). On this basis, IARC indicated that there is sufficient evidence in experimental animals for the carcinogenicity of DEHP. Thus, DEHP has been classified as possibly carcinogenic to humans (Group 2B).

Immunotoxicity

Larsen and colleagues (2001a, 2001b) studied adjuvant effects of DEHP and MEHP and other phthalate monoesters in a subcutaneous injection model in BALB/c mice. Ovalbumin (OVA) was used as the model antigen and OVA-specific IgE, IgG_1 and IgG_{2a} antibodies were measured as indicators of allergic response. MEHP produced a significant increase in both IgE and IgG_1 levels and DEHP increased IgG_1 levels, these antibodies being related to a Th_2 response predominant in Type I allergy. The adjuvant activity was noted when DEHP was mixed with OVA. When a mixture of DEHP and OVA was administered intraperitoneally (i.p.) in PPAR-alpha knockout mice OVA-specific IgE, IgG1 and IgG2a, responses were similar to responses in the wild-type mouse strain, indicating that the adjuvant activity of DEHP is mediated by a PPAR-alpha receptor-independent mechanism (Larsen and Nielsen 2007). Airborne exposure to DEHP and OVA induced an increase in serum IgG1 and inflammatory cells in the lung, but only at rather high concentrations of 13 mg/m^3. Lower DEHP airborne exposure comparable to levels measured in ambient air did not show an adjuvant effect or induce allergic lung inflammation in the mouse model used (Larsen *et al.*, 2007). Similar results were obtained for the DEHP metabolite MEHP, and thus it was speculated that the airway effects of DEHP were mediated by MEHP (Larsen *et al.*, 2007, Hansen *et al.*, 2007). Although the induction of OVA-specific IgG1 antibodies is an indicator for immunogenicity and adjuvanticity in experimental mouse systems, it is not clear whether this response should be considered a protective or a risk factor for the development of IgE and immediate type hypersensitivity (Larsen *et al.*, 2007). For some other routes and combinations of DEHP (topical) and OVA (subcutaneous) administration, no effect on anti-OVA antibody production was noted (Dearman *et al.*, 2008).

In a model for atopic dermatitis, combined i.p. administration of DEHP and antigen was found to exacerbate skin responses to the antigen (Takano *et al.*, 2006).

One of the metabolites of DEHP, MEHP, induced immunosuppression, i.e. reduced antibody titres, when the same protocol was used (Larsen *et al.* 2001b), indicating that DEHP and its metabolites have the potential to interact with the immune system in various ways, although it is unknown whether such effects are observed in humans after oral or parenteral exposure to DEHP.

Some monophthalates promote cytokine IL-6 and IL-8 production in the human epithelial cell line A549, indicating a potential role in inflammatory process (Jepsen *et al.*, 2004).

Phthalate plasticisers, including DEHP, have been suggested as possible contributor to the increasing prevalence of atopic (IgE-mediated) allergic diseases and asthma in Europe and the US. The evidence for the ability of phthalates to impact immune and allergic responses has been revised (Kimber et al, 2010): the epidemiological data provide some evidence that exposure to phthalates may be associated with increased risk of development of allergies and asthma, however, the lack of objective exposure information limits the interpretation. A variety of studies have been performed in mice measuring antibody responses and other parameters of inflammation such as eosinophil infiltration and cytokine production: no consistent pattern has emerged, since results ranged from potentiation of immune or inflammatory responses, to the absence of any effect, to inhibitory or immunosuppressive activity, depending on the chemical and the route of exposure. Interestingly, immune effects were reported when parenteral routes of administration were used (Kimber and Dearman, 2010).

In conclusion, DEHP in experimental systems has the potential to interact with the immune system depending on the exposure conditions.

Reproductive and developmental toxicity

The reproductive or developmental toxicity of DEHP has been studied in rats, mice, hamsters, ferrets and marmosets. Based on the available data, which varies in both study designs and number of animals included, testicular effects were demonstrated in both male rodents and non-rodents and on this basis, DEHP is classified under Regulation (EC) No 1272/2008 as toxic to reproduction category 1B.

The testis toxicity of DEHP is age dependent (Sjoberg *et al.* 1985b). The lowest NOAEL is seen in the range from 3.5-4.8 mg/kg bw in rats. The females need to be exposed in the most critical period of gestation day (GD) 12-21 to see testicular effects at low doses (<10 mg/kg bw) (Fabjan *et al.*, 2006). In mice, after continuous exposure during breeding, a NOAEL for maternal and developmental toxicity of respectively 20 and 600 mg/kg bw/d can be identified (Lamb et al, 1987). In another study in mice, a NOAEL for maternal toxicity was 200 mg/kg bw/d and for developmental toxicity a NOAEL of 40 mg/kg bw/d (Huntington et al, 1997). In ferrets a LOAEL is 1200 mg/kg bw/d (Lake 1976). In animal experiments, DEHP is embryotoxic and causes malformations in mice, but not in rats, when given orally in doses close to the maternal toxic dose (Sullivan *et al.*, 1993).

For male reproductive toxicity caused by DEHP, there is a difference in sensitivity between various animal species; rodents are more susceptible than non-human primates (Rhodes et al 1986). The same dose (2000 mg/kg for 14 days orally) induced testis atrophy and liver enlargement in rats, but failed to do so in marmosets, although this may be due to the poor ability of marmosets to metabolise DEHP to its active metabolites (Rhodes *et al.* 1986). In another study, adult male marmosets treated with up to 2500 mg/kg DEHP for 13 weeks failed to show evidence of testicular toxicity (Kurata *et al.* 1998). After short-term exposure of young adult cynomolgus monkeys for 14 days to di-isonyl phthalate (DINP) or DEHP at 500 mg/kg daily, there were no treatment-related effects observed for liver, kidney and testis (Pugh *et al.*, 2000). In addition, when marmoset monkeys were exposed to high doses of DEHP up to 2500 mg/kg daily for 65 weeks, no changes were noted in the testis (Tomonari *et al.*, 2006). In this study, the animals were exposed continuously in the pre-adolescent period starting at approximately day 100 after birth until the peri-adolescent period at the age of almost 18 months. Thus, in studies using marmosets and cynomolgus monkeys, no effect on testicular function was observed after high DEHP exposure. However, studies on marmosets may be of

limited human relevance, because of the poor capacity of this species to metabolise parent phthalates to their active metabolites, thus, there may not have been effective exposure to the generated metabolites (Rhodes *et al.* 1986).

Of more direct human relevance, since the metabolic step was by-passed, may be studies that have used monobutyl phthalate (MBP), the active metabolite of dibutyl phthalate (DBP), which has similar bioactive effects and potency in rodents as DEHP. Administration of 500 mg/kg/d MBP to pregnant marmosets for 7 weeks did not result in any adverse effects on masculinisation of the resulting male offspring, which is consistent with absence of effect on steroidogenesis by the foetal marmoset testis (McKinnell *et al.*, 2009); this treatment did not result in any discernible long-term consequences for reproductive function in adulthood. However, similar treatment of male marmosets shortly after birth (when the testes are actively secreting testosterone) with 500 mg/kg/d MBP causes ~50% reduction in blood testosterone levels after a single treatment and 14 days of continuous treatment with this high dose of MBP resulted in Leydig cell hyperplasia/hypertrophy, consistent with compensated Leydig cell steroidogenic suppression by MBP (Hallmark *et al.*, 2007). There is no current explanation for this foetus-neonate difference in susceptibility of the marmoset testis to MBP, but it is presumed that the same will apply to MEHP (DEHP). These observations are of importance for extrapolation to humans, because in terms of perinatal testis development and function and spermatogenesis in adulthood, the marmoset is a suitable model (Millar *et al.*, 2000; McKinnell *et al.*, 2001, 2013).

In contrast to rats, treatment of mice during pregnancy with MEHP, DBP or MBP at doses up to 1500mg/kg/d had no or minimal effects on testosterone production by the foetal testis, but did affect germ cells, similarly to the rat (Guido *et al.*, 2007).

In a previous CSTEE Opinion (CSTEE, 1998), testicular toxicity was identified as the critical endpoint for DEHP from a 13-week dietary study in Sprague-Dawley rats and a NOAEL was set at 3.7 mg/kg bw/d based on mild Sertoli cell vacuolation (Poon *et al.* 1997). Since that time, the result of a new multigenerational reproductive toxicity study of DEHP in Sprague-Dawley rats has become available (Wolfe and Layton, 2003). The European Chemical Bureau (ECB) 2008 evaluated the study in which 3 generations were fed DEHP in the diet corresponding to doses of 0.1, 0.5, 1.5, 4.8, 14, 46, 359 and 775 mg/kg bw/d. There were dose-dependent effects on numerous testis-related parameters (decreased testicular weight, small or aplastic testes, seminiferous tubular atrophy, infertility at high doses) The NOAEL for both testicular toxicity and developmental toxicity from this experiment was determined at 4.8 mg/kg bw/d, which is very similar to the previously identified one. Since the endpoints seen in the Wolfe and Layton (2003) study are more robust and the study was well performed, SCENIHR in the previous Opinion agreed with the Risk Assessment Report (RAR) to use this NOAEL (SCENIHR, 2008).

More recent studies on human foetal testicular tissue have provided new insights on the potential testicular toxicity of DEHP. Studies using human foetal testis tissue for short-term *in vitro* culture (Lambrot *et al.*, 2009, Hallmark *et al.*, 2007) investigated the effect of DEHP and DBP metabolites, MEHP and MBP, respectively. Neither study showed any effect of the metabolites on testosterone production. In addition, xenografting of human foetal testicular tissue into immune-compromised (nude) rodents (Mitchell *et al.*, 2012; Heger *et al.*, 2012), showed an absence of an effect of DBP on testosterone production or on relevant steroidogenic enzyme expression, even at doses of 500 mg/kg/d in the xenograft studies. Aggregation of germ cells was described, although the meaning of these effects has yet to be determined (Mitchell *et al.*, 2012). In these studies using xenograft rat foetal testicular tissue in immune-compromised (nude) rodents, the rat foetal testicular tissue was similarly affected by DBP as *in*

situ in male foetuses. The human foetal testes xenograft results were consistent with the absence of effect on male offspring in marmoset studies involving exposure during pregnancy to 500 mg/kg/d MBP (McKinnell *et al.*, 2009). These studies should be considered with the human association studies as discussed in section 3.4.7.

In contrast to the foetal human testes studies, *in vitro* studies using either testis tissue from adult men or a steroidogenically active human adrenocortical cell line have shown that 10^{-5}M DEHP or MEHP can suppress testosterone production without affecting other Leydig or Sertoli cell secretory functions (Desdoits-Lethimonier *et al.*, 2012). These authors also measured intra-tissue MEHP content in their cultures after 24h phthalate exposure and concluded that effective exposure was very low and might be within the range of normal human exposure, implying that similar suppressive effects on testicular steroidogenesis might occur in the normal male population.

One aspect of male foetal testis development that appears to be affected in a species-independent way is the foetal germ cells. In *in vitro* studies using human (1^{st} trimester), rat and mouse foetal testes, the addition of 10^{-5}M MEHP increased apoptosis of foetal germ cells (Chauvigne *et al.*, 2009; Lambrot *et al.*, 2009; Muczynski *et al.*, 2012), under conditions in which species-dependent effects on steroidogenesis occur (discussed above). Based on mouse studies, the effect on foetal germ cells is independent of androgen or oestrogen action (Lehraiki *et al.*, 2009) and appears to be attributable to the 5-OH metabolite of MEHP (Chauvigne *et al.*, 2009). Based on earlier studies of rat foetal testis cell co-cultures, the MEHP-driven increase in foetal germ cell apoptosis may result from the loss of normal adhesion contacts with the somatic Sertoli cells (Li *et al.* 1998; Iona *et al.*, 2002). This sort of change may also underlie the widely reported ability of DEHP or DBP *in vivo* to cause aggregation of foetal germ cells in the centre of seminiferous cords in the rat and mouse, a change also associated with occurrence of multinucleated germ cells (e.g. Parks *et al.*, 2000; Barlow & Foster 2003; Fisher *et al.*, 2003; Boekelheide *et al.*, 2009); the latter has also been observed in human foetal testis xenografts after exposure to DBP (Heger *et al.*, 2012). Administration of 500mg/kg/d DBP to pregnant rats also results in loss of foetal germ cells, an effect that is restricted to the stage when the germ cells are pluripotent and actively proliferating (Jobling *et al.*, 2011) and this also appears to be the case for the mouse (Lehraiki *et al.*, 2009). Perhaps of more concern from a human health point of view is the demonstration that DBP exposure can alter the timing of differentiation of foetal germ cells in the rat (Jobling *et al.*, 2011), as adverse changes in this process underlie the foetal origins of testicular germ cell cancer in humans (Rajpert-de Meyts 2006). In this regard, Yao *et al.* (2012) showed that MEHP promotes invasion and migration of an embryonal carcinoma cell line (derived from a testis germ cell cancer).

In the female reproductive system, some ovarian effects related to DEHP exposure have been described (Lovekamp-Swan and Davis, 2003) as well as some effects on the hypothalamus-pituitary-ovarian axis in adult female rats (Liu *et al.*, 2014) or *in vitro* in cultured rat ovarian follicles following MEHP exposure (Inada *et al.*, 2012). However, relevant information concerning the female reproductive system is limited, especially when compared to effects on males.

MEHP induces oxidative stress in human placental cells (Tetz *et al.*, 2013). In addition, MEHP at high doses has cytotoxic effects and negatively affects the development of human embryonic cell lines, thus indicating the potential toxicity in human embryos (Shi et al, 2013). These observations support the potential role of antenatal DEHP exposure to the development of chorioamnionitis, the foetal inflammatory response syndrome and the leading cause of foetal/neonatal morbidity and mortality (Latini *et al.*2005, 2006). However, maternal DEHP

exposure in relation to birth outcomes have yielded inconsistent results for associations with birth weight (see below) and no reports of association with mortality.

An emerging line of research of developmental toxicity is aimed to highlight the possible effects of phthalates in relation to brain structure and function with particular emphasis on developmentl of hippocampal structural and functional plasticity and has been recently reviewed (Holahan and Smith, 2015; Miodovnik et al., 2014). For the moment the observed associations are based on limited studies with a broad range of endpoints, and pre- and post-natal exposure to phthalates. Whether these changes occur as a direct neurotoxic effect of phthalates or an indirect effect through disruption of endogenous endocrine functions is not fully understood. However, this kind of outcomes could be of concern and merit further investigation given the exposure of infants, especially in NICU.

Mechanisms of toxic action to male reproductive organs

The mechanisms underlying the toxic effect of DEHP on male reproductive organs have been investigated in several animal studies. In the testis, peroxisome proliferators-activated receptors PPAR and their subtypes may explain some of the reproductive effects of phthalates. The alpha and beta subtypes are expressed in adult rat testis, as well as in neonatal and adult Sertoli and Leydig cells although the literature shows significant discordance in results concerning the role of PPAR (Corton and Lapinski 2005, Latini *et al.*, 2006).

The anti-androgenic effects of some phthalates on the developing male rat foetus are a direct result of reduced androgen availability in target organs, causing malformations of male reproductive organs and low adult sperm production (Gray *et al.*, 2000, Barlow *et al.*, 2003). Maternal DEHP treatment from gestational day 14 to postnatal day 3 results in greatly reduced testosterone synthesis by the foetal rat testis (Parks *et al.*, 2000). This indicates that DEHP has an effect on rat male development by reducing the testosterone levels in the foetal male during a critical stage of reproductive tract differentiation (Parks *et al.*, 2000). In contrast, DEHP and its metabolite MEHP do not have an affinity for the human androgen receptor in an *in vitro* assay (Parks *et al.*, 2000). The phthalates with side-chain length C4 to C6 produce similar severe reproductive effects in experimental animals. Steroidogenesis in foetal rats is reduced by DEHP *ex vivo* and DINP, DBP, DIBP and DEHP reduce testicular testosterone production by a similar mechanism of action (Barlow and Foster 2003, Borch *et al.*, 2004, Borch *et al.*, 2006; Plummer *et al.*, 2007; van den Diesche *et al.*, 2012).

Treatment of rats during pregnancy with DEHP or DBP resulted in a similar reduction in the expression of genes (and their encoded proteins) that play key roles in cholesterol transport and metabolism and steroidogenesis (Thompson *et al.*, 2004; Borch *et al.*, 2006; Plummer *et al.*, 2007), which suppresses testosterone production. All of the affected genes are regulated by the nuclear receptor steroidogenic factor-1 (SF-1). Immunohistochemistry for SF-1 showed a small reduction in expression after gavage administration of 300 mg/kg bw/d DEHP (Borch *et al.*, 2006b), but similar treatment with 500 mg/kg/d DBP had no effect on expression of SF1 at the mRNA or protein level, despite identifying down-regulation of 7 SF-1 dependent genes (Plummer *et al.*2007). An explanation for this has emerged recently in a study demonstrating that DBP exerts its effects on foetal rat testis steroidogenesis by preventing the normal age-dependent lifting of repression of SF-1 by chicken OVA upstream promoter transcription factor-II (COUP-TFII), which competes with SF-1 for binding to SF-1 elements in the target genes (van den Diesche *et al.*, 2012). Additionally, a recent study showed that in DBP-exposed foetal rat testes, something(s) prevents binding of SF-1 to the promoter regions of target genes in Leydig cells, although the authors also propose that this may work indirectly via PPARalpha as well as via COUP-TFII (Plummer *et al.*, 2013). Phthalates are PPAR agonists and administration

of 300 mg/kg bw/d DEHP reduces immune-expression of PPARgamma in foetal rat testes (Borch *et al.*, 2006b).

A difference was observed in the toxicity of MEHP, DBP or MBP in foetal testes responses between mice and rats (Guido *et al.*, 2007). Consistent with this, DBP exposure at 500 mg/kg/d does not affect normal loss of expression of COUP-TFII in mouse foetal Leydig cells as it does in rats and does not reduce intratesticular testosterone (van den Diesche *et al.*, 2012).

In laboratory animals, the metabolites are less studied, but one report suggests that at least in rats, the anti-androgenic effect is partly caused by 2 anti-androgenic metabolites 5OXO-MEHP and 5-OH-MEHP (Stroheker *et al.*, 2005). DBP and certain other phthalates can competitively inhibit activity of the steroidogenic enzyme 3β-HSD in microsomal preparations of adult rat and human testes (Yuan *et al.*, 2011), although the mechanistic relevance is uncertain.

In adult or prepubertal rats, mechanisms of action other than PPARs activation may be of importance. In the rat testis, the Sertoli cell may be the target for acute toxicity after exposure to high doses of DEHP. In Sertoli cells, the distribution of the cell structure protein vimentin and an increased caspase-3 level activity, may be early markers of MEHP testis toxicity, as these are already affected at 6h after one application of 400 mg/kg bw by gavage (Dalgaard *et al.*, 2001). The same effect of DEHP after oral doses of 5 and 10 g/kg bw for 4 weeks resulted in collapse of vimentin in the Sertoli cells (Dalgaard *et al.*, 2000). In addition, in human interactions of DEHP with other nuclear receptors such as human Pregnane X receptor (hPXR) or human Constitutive Androstane Receptor may play a role (Hurst and Waxman, 2004; Mnif *et al.*, 2007; DeKeyser *et al.*, 2011).

Conclusions on toxicity in experimental animals

The DEHP acute toxicity in animals is low by the oral route, with lower LD_{50} values obtained after parenteral exposure (200-250 mg/kg in rats). The MEHP acute toxicity is 5 times higher than that found in the parent.

Oral repeated toxicity in rodents indicates that DEHP induced toxicity in the kidney, liver and testis. The NOAEL derived from a two-year study in rats for kidney toxicity is 29 and 36 mg/kg/d in males and females, respectively. The lowest NOAEL for non-neoplastic effects was associated with damage in the liver of mice that is 19 mg/kg bw/d , with hepatomegaly due to hepatocyte proliferation (characterised by increased replicative DNA synthesis/cell division and hypertrophy) and peroxisome proliferation.

In rhesus monkeys, sub-chronic liver toxicity has been studied after repeated transfusions through PVC tubing containing DEHP, mimicking conditions of patients undergoing repeated blood or platelet transfusion. The average cumulative amount of DEHP infused over 1 year was 69.3 mg (or 21.3 mg/kg bow) and found to give hepatic dysfunctions and cholestasis.

DEHP has been studied extensively in a wide range of *in vitro* and *in vivo* assays for detection of gene mutations, DNA damage and chromosomal effects. The results have been negative in the majority of assays with DEHP and MEHP. Few positive results were obtained; however, it is not clear enough if they are associated with a direct interaction of DEHP or MEHP with DNA or rather to secondary oxidative stress or other events. Thus, in a WoE approach, it can be considered that DEHP and its major metabolites are non-mutagenic substances.
Several studies on the carcinogenicity (and mechanisms of carcinogenicity) of DEHP have been performed in rats and mice with oral administration. They demonstrate the induction of hepatocellular neoplasms in rodents. The NOAEL for tumour induction in male mice was 98

mg/kg bow/d, therefore higher than the NOAEL derived from the same study for non-neoplastic effects (i.e. 29 mg/kg/d).

The mechanisms for liver carcinogenesis in rodents is mediated by PPARα-activation and consequent peroxisome proliferation, a mode of action which has a threshold and is generally considered not to be relevant in the human liver. This is because human PPARα is expressed at a lower level in human liver than in rats and mice, no studies have reported any evidence that DEHP activates PPARα in human liver *in vivo,* and marked species differences with respect to hepatic response to peroxisome proliferation have been demonstrated. However, it has been suggested that multiple molecular signals and pathways in several cell types in the liver other than the PPARα-activation may be involved in hepatic tumour induction, so that according to the IARC evaluation, the relevance to human cancer of the molecular events that lead to cancer elicited by DEHP in several target tissues (e.g. the liver and testis) in rats and mice cannot be definitely ruled out.

The reproductive or developmental toxicity of DEHP has been studied in rats, mice, hamsters, ferrets and marmosets. For male reproductive toxicity caused by DEHP, there is a difference in sensitivity between various animal species, with rodents being more susceptible than non-human primates, and cynomolgus monkeys showing no effect on testicular function after high DEHP exposure. The testis toxicity of DEHP in rodents is age dependent. The NOAEL for both testicular toxicity and developmental toxicity has been derived from a multigenerational reproductive toxicity study of DEHP in rats (the most susceptible species), equal to 4.8 mg/kg bw/d. *In vitro* studies using human foetal testis tissue showed no effect of the metabolites on testosterone production, whereas *in vitro* studies using testis tissue from adult men indicate that DEHP suppress testosterone production. There is some indication about the possibility of some alterations in foetal germ cells but the meaning of this has not been elucidated and it is difficult to extrapolate from *in vitro* results to the *in vivo* situation (Habert *et al.*, 2014).

In the testis, peroxisome proliferators-activated receptors PPAR and their subtypes may explain some of the reproductive effects of phthalates. In addition, the anti-androgenic effects of some phthalates on the developing male rat feotus are a direct result of reduced androgen availability in target organs, causing malformations of male reproductive organs and low adult sperm production. In contrast, DEHP and its metabolite MEHP did not show an affinity for the human androgen receptor in an *in vitro* assay.

3.4.5. Evidence from epidemiological and clinical studies

Short-term effects

Short-term effects of DEHP exposure were evaluated in 28 term infants with respiratory failure, 18 of whom received ECMO being exposed to up to 2 mg/kg bw DEHP over 3–10 days (mean peak plasma concentration, 8 µg/mL) and were compared with 10 untreated infants. No differences between the groups were observed in various clinical parameters of liver, pulmonary and cardiac function which remained unaltered (Karle et al, 2007).
A retrospective analysis, before and after changing from PVC-containing to PVC-free infusion systems, was conducted on two groups of 30 and 46 patients respectively who were receiving parenteral nutrition. The development of cholestasis correlated strongly with (P = 0.0004) the use of PVC-containing lines; the incidence of cholestasis decreased from 50 to 13% after PVC-containing infusion systems were discontinued (von Rettberg *et al.*, 2009).

Epidemiological studies

One of the major problems related to the association between exposure and human health effects is related to the correct identification of the level of exposure to DEHP and other phthalates. It has been discussed in the biomonitoring paragraph how the exposure assessment can be biased depending on the detection of the parent compound alone, on the variable number of metabolites and considering how the information from a snap shot urine sample can be affected by the short half-life of these chemicals, being therefore representative of a short-time exposure and consequently difficult to associated with a causality relationship to long-term pathologies or disease requiring a long lag time before their onset. The choice of exposure metric can introduce significant bias of varying magnitude and direction into the calculation of epidemiologic associations (Christensen *et al.*, 2014).

The epidemiological studies that evaluated cancer risk and exposure specifically to DEHP are very limited, and include an occupational cohort study of DEHP production workers (Thiess *et al.* 1978) in which the exposure was not well established, the methods were poorly described and the power was inadequate to detect a potential excess risk, and a case–control study of breast cancer（López-Carrillo *et al.*, 2010).

This case–control study was conducted in northern Mexico to evaluate the association between urinary levels of nine phthalate metabolites and breast cancer in 233 women with breast cancer and 221 age-matched controls. After adjusting for risk factors and other phthalates, increased odds ratios for breast cancer were statistically significantly associated with urinary concentrations of one MECPP with a dose–response trend (P = 0.047) with lack of consistency in effect between the four DEHP metabolites measured and the lack of a dose–response for all metabolites. In addition, urinary concentrations of other phthalate metabolites were positively or negatively associated with breast cancer. In its evaluation, IARC expressed a concern related to the timing of exposure assessment, which occurred after diagnosis among cases and it is not known whether the disease status could have affected metabolite levels.

Hypospadias and cryptorchism

Potential male developmental effects in humans include hypospadias, cryptorchidism and decreased anogenital distance, which are part of the so-called testicular dysgenesis syndrome. There is limited epidemiologic evidence of the effects of phthalates on these health outcomes.

Van Tongeren and colleagues (2002) developed a job-exposure matrix (JEM) to assess exposure to potential endocrine disrupting agents, including phthalates. Vrijheid and colleagues (2003) applied this JEM in a study of 3471 hypospadias cases identified by the National Congenital Anomaly System of England and Wales in 1980-1996, which included a total of 35962 cases of congenital anomalies. The authors compared the prenatal exposures of hypospadia cases with exposures of all the cases. The risk of hypospadias was not related to estimated maternal occupational exposure to phthalates. For 1992-96, there was an increased risk of hypospadias related to probable exposure, mainly among hairdressers, with an adjusted odds ratio of 1.52 (1.05-2.20) without social class adjustment and 1.26 (0.81-1.97) after such adjustment. The JEM was also applied in a Dutch nested case-control study of 56 cases of hypospadias and 78 cases of cryptorchism and 313 controls selected from a cohort of 8,698 male newborns. No association was found between estimated occupational exposure to potential endocrine disrupting agents and these outcomes (Pierik *et al.*, 2004). In a study on contamination of breast milk with phthalates, no association was found between breast milk phthalate monoester levels and cryptorchidism, but other potential anti-androgenic metabolites were not measured (Main *et al.*, 2006). Most recently, a case-control study that

involved both JEM evaluation and direct measurement of the urinary levels of 11 phthalate metabolites in pregnant women found no association between pregnancy phthalate exposure and occurrence of hypospadias or cryptorchidism (Chevrier *et al.*, 2012).

Decreased anogenital distance

Swan *et al.* (2005) provided the first indications for the effects of phthalates on anogenital distance in a study of 134 male infants. Eighty-five of the participating pregnant women gave a prenatal urine sample, which was analysed for 9 phthalate metabolites commonly used as biomarkers of exposure to phthalates. Anogenital distance was measured in boys at various ages after the delivery. The 9 urinary metabolites measured were monomethyl phthalate, monoethyl phthalate, mono-n-butyl phthalate, mono-iso-butyl phthalate, monobenzyl phthalate, mono-3-carboxypropyl phthalate, mono-2-ethyl-5-hydroxyhexyl phthalate, mono-2-ethylhexyl phthalate and mono-2-ethyl-5-oxohexyl phthalate. Four of these were negatively associated with anogenital index (AGI=anogenital distance/kg bw cubed), being monoethyl phthalate, mono-n-butyl phthalate, monobenzyl phthalate and mono-iso-butyl phthalate. Boys with a reduced anogenital index (AGI) may have an increased likelihood of impaired testicular descent, reduced penile volume and testis size, although in the study itself, no diseases or malformations were identified. However, the study had several limitations, namely it was retrospective, AGI was measured across a wide age range in boys and the number of subjects was small. Another small, but better designed, Taiwanese study (prospective, phthalate measurements in amniotic fluid, anogenital distance measurements at a standard time) found no association between boys' anogenital distance and amniotic fluid levels of MEHP, MBP or MEP (Huang *et al.*, 2009); unexpectedly, a significant negative correlation was found between MBP exposure and anogenital distance in girls in this study. A third, Japanese, prospective study (Suzuki *et al.*, 2012) measured 11 phthalate metabolites in spot urine samples obtained at variable stages of pregnancy and related this to AGI in resulting male offspring (N=111). A significant negative correlation was found for MEHP levels in pregnancy and AGI, although this association was not strong (r=-0.189, p=0.05). In a multiple regression model, the log-transformed MEHP concentration was negatively significant and maternal smoking status was positively significant, in explaining anogenital index (AGI) when potential covariates were controlled for.

Overall, the 3 studies investigating the association between maternal phthalate exposure in pregnancy and anogenital distance (AGD) in resulting offspring have produced only limited and inconsistent evidence that supports the view that phthalate exposure reduced foetal androgen exposure. (In animal studies, treatment-induced reduction in AGD is associated with increased risk of most male reproductive abnormalities, including those present at birth, such as cryptorchidism, hypospadias and those present in adulthood, e.g. low sperm count or infertility.) Based on the absence of effect of DBP/MBP or MEHP on steroidogenesis by the human foetal testis *in vitro* and in xenografts models, discussed above, no association between phthalate exposure and anogenital distance in boys would be anticipated. More detailed, prospective studies are, therefore, required to clarify the present uncertainty. An additional issue to be considered regarding DEHP and its metabolites is that human exposure is determined largely by diet (Koch *et al.*, 2013), raising the possibility of confounding if (maternal) diet should prove to be influential on AGD. One recently published study based on 2 mother-child cohorts totalling 707 pairs (Papadopoulou *et al.*, 2013) showed that a high fat diet was significantly associated with reduced AGD in male offspring. The aim and context of the study was to show an association between exposure to lipophylic organochlorine compounds and reduced AGD and this was found, but in this context it is equally possible that it was the high fat diet itself that was causal. This also raises the concern that because DEHP

exposure is dietary-driven, then exposure to DEHP may also be a surrogate for other exposures, such as organochlorine compounds, which may have confounding effects.

Birth weight and gestational age

Latini and colleagues (2003a) measured serum DEHP and MEHP concentrations in the cord blood of 84 consecutive newborns. Detectable cord blood phthalate concentrations were found in almost 90% of these individuals. In this study, the mean gestational age was significantly lower among newborns with detectable cord blood MEHP compared with those without (38.2 vs. 39.4 weeks), although this is unlikely to be of any clinical significance. Mean birth weight was lower (3150 vs. 3475 g), although the difference was not statistically significant. In logistic regression analysis adjusting for potential confounders, the absence of MEHP was a significant determinant of gestational age. Meeker *et al.*, (2009) compared 30 women who delivered preterm with 30 who delivered full term in a large Mexican birth cohort and reported an association between urinary levels of MBP, MBzP and 4 DEHP metabolites and occurrence of preterm birth. Similarly, Zhang *et al.*, (2009) compared 88 low birth weight babies with 113 normal birth weight controls in a nested case-control study in China and found a significant negative association between maternal DEHP exposure and birth length and a significant negative association between maternal DBP exposure and birth weight, although neither of these 'effects' were large. Thus, associations were present for DEHP exposure and birth length and DBP exposure and birth weight, although this does not imply causality.

Whyatt *et al.* (2009) assessed the relationship between (DEHP) exposure during pregnancy and gestational age at delivery among 311 African American or Dominican women. DEHP levels were measured in forty-eight-hour personal air and/or spot urine samples and 4 DEHP metabolite levels were measured in urine, collected during the third trimester. DEHP was detected in 100% of personal air samples (geometric mean: 0.20 μg/m^3). Natural logarithms of air concentrations were inversely but not significantly associated with gestational age. Two or more of the DEHP metabolites were detected in 100% of urine samples (geometric mean: 4.8–38.9 ng/mL. Controlling for potential confounders, gestational age was shorter by 1.1 days (95% CI: 0.2–1.8 days) for each 1-logarithmic unit increase in specific gravity-adjusted MEHP concentrations (P = 0.01) and averaged 5.0 days (95% CI: 2.1– 8.0 days) less among subjects with the highest vs. lowest quartile concentrations (P=0.001). Results were similar and statistically significant for the other DEHP metabolites. Although the results indicated that prenatal DEHP exposure was associated with shorter gestation, this would not have been of any clinical significance.

Levels of 10 phthalate monoesters were determined in maternal urine from 404 women in New York in the third trimester, but none showed any relationship to birth weight in the offspring, with the exception of low molecular weight phthalates (e.g. MEP), which were positively associated with birth weight (Wolff *et al.*, 2008). Snijder *et al.*, (2012), using a questionnaire-based approach showed a significant negative association between deduced phthalate exposure, including occupational exposure, in the generation R study (N=4680 pregnancies) and foetal growth and placental weight. However, an earlier study of the same cohort did not find a significant association between maternal phthalate exposure and birth weight (Budorf *et al.*, 2011). Similarly, a case-control study of male reproductive abnormalities (N=287), in which 11 phthalate metabolites were measured in maternal urine, found no association with birth weight or foetal growth parameters (Philippat *et al.*, 2012).

Adibi *et al.* (2009) examined DEHP exposure in relation to the timing of labour in a pregnancy cohort study of 283 women recruited in 4 US states between 2000 and 2004. The authors estimated associations between concentrations of DEHP metabolites and gestational age at

delivery using linear regression models and associations between DEHP metabolites and clinical outcomes using logistic regression models. After covariate adjustment, women at the 75th percentile of DEHP metabolite concentrations had a 2-day-longer mean length of gestation than women at the 25th percentile (95% confidence interval: 1.4, 3.3). Log-unit increases in MEHP and 5-oxo-MEHP concentrations were associated with increased odds of caesarean section delivery (30% and 50% increased odds, respectively), increased odds of delivering at 41 weeks or later (100% and 120% increased odds) and reduced odds of preterm delivery (50% and 60% decreased odds). These data suggest that DEHP may interfere with signalling related to the timing of parturition, although it is noteworthy that no account was taken of maternal bodyweight/BMI and bodyweight gain during pregnancy as this information was unavailable.

In a prospective cohort study of pregnant women recruited early in gestation in northern Puerto Rico, the associations between urinary phthalate metabolites and biomarkers of inflammation were measured in plasma twice during pregnancy, and oxidative stress biomarkers in urine were measured three times per woman. Inflammation and oxidative stress can be related to gestational age and pre-term birth. Association of DEHP metabolites with biomarkers of inflammation was absent or not statistically significant; the only statistically significant association was between DEHP metabolites and increase of 8-hydroxydeoxyguanosine (Ferguson *et al.*, 2014).

Taken together, the association of phthalate exposure and preterm birth/birth weight is suggestive of an association, although this relationship is inconsistent. As DEHP exposure appears to be largely determined by diet, potential confounding effects due to differences in maternal diet should be kept in mind when considering studies that show a significant association between DEHP exposure and birth weight or other pregnancy-related endpoints.

Childhood growth and pubertal development

Three studies have investigated associations between pubertal development and phthalate exposure (Colon *et al.*, 2000, Rais-Bahrami *et al.*, 2004, Wolff *et al.*, 2010). The relation between serum phthalate concentrations and premature breast development was studied in a case-control study of 41 patients from an endocrinology division and 35 controls from the general paediatric care without signs of premature sexual development (Colon *et al.*, 2000). Higher serum levels of DMP, DEP, DBP and DEHP plus its metabolite MEHP were measured in cases than in controls. The average concentration of DEHP was 450 ppb in cases and 70 ppb in controls, the difference being statistically significant. This was not seen with other phthalates studied. There appears to be a correlation between DEHP exposure and breast development in young females. However, the quality of the data is incertain due to laboratory and/or diagnostic procedures performed (CERHR 2005), and there is no obvious mechanism to account for the association. However, a large (N=1151 girls) longitudinal US study that related 2 markers of pubertal development (pubic hair and breast development) to urinary levels of 9 phthalate metabolites, including MEHP and MBP, in girls aged 6-8 years at study onset, found no association with breast development (corrected for BMI), but did find a small negative association between high molecular weight phthalate exposure and pubic hair development (Wolff *et al.*, 2010). In the same study, a significant positive association was found between low molecular weight phthalate exposure (e.g. MEP) and breast and pubic hair development, but this is most probably explained by greater use of deodorants (that contain MEP) associated with onset of puberty in girls. A study of 440 girls aged 12-16 in the US, based on data from NHANES, evaluated the association between exposure to a range of endocrine-active chemicals, including phthalates and age at menarche based on reproductive health and

laboratory data (Buttke *et al.*, 2012); no association was found between total phthalate exposure and age at menarche.

Rais-Bahrami *et al.* (2004) reported a 14-16 year follow-up study to DEHP toxicity noted in adolescents after a high DEHP exposure as neonates during extracorporeal membrane oxygenation (ECMO) support. The onset of puberty and sexual maturity was evaluated in 19 adolescents (13 males and 6 females). There were no significant adverse effects on their physical growth and pubertal maturity. Thyroid, liver, renal and male and female gonadal functions tested were within normal range for age and sex distribution. A limitation of the study is the low number of individuals studied and the evaluation period of maximal 16 years.

In a 20-year follow up study, Hack *et al.* (2002) compared young adults with a normal birth weight (mean 3279 gram, n=233) with individuals with very low birth weight (mean 1179 gram, n=242) who were presumed to have had a high DEHP exposure as a consequence of their treatment/management. The very low birth weight individuals showed educational disadvantages persisting into early adulthood. There were no differences observed concerning male fertility. The confounding effects of birth weight render this study deeply problematic.

A study in 845 children aged 4-9 years revealed a negative relationship between urinary phthalate metabolites and certain growth-related parameters. In girls, there was a negative relationship between MBP and thyroid hormone levels and in boys a negative relationship between DEHP metabolites and levels of insulin-like growth factor-1 (Boas *et al.*, 2010). However, none of these 'effects' were large or of clinical significance.

Mieritz *et al.* (2012) investigated an association between concurrent measures of urinary phthalate metabolites and pubertal timing as well as the presence of gynaecomastia in otherwise 555 healthy boys (age 6.07–19.83 years). Anthropometry and pubertal stages were evaluated and the presence of gynaecomastia was assessed. The urinary levels of phthalate metabolites were not associated with age at pubertal onset, serum testosterone levels or presence of gynaecomastia. In conclusion, there was no evidence of anti-androgenic effects of phthalates in boys. Thus, current phthalate exposure was not associated with pubertal timing, testosterone levels or the presence of pubertal gynaecomastia in this cross-sectional study.

To investigate associations between phthalate exposure and changes in pubertal timing among girls, Frederiksen *et al.* (2012) determined the concentration of 12 phthalate metabolites in first morning urine samples from 725 healthy Danish girls (aged 5.6–19.1 years) in relation to age, pubertal development (breast and pubic hair stage) and reproductive hormone levels (luteinizing hormone, oestradiol and testosterone). Furthermore, urinary phthalates were determined in 25 girls with precocious puberty. In general, the youngest girls with less advanced pubertal development had the highest first morning urinary concentration of the monobutyl phthalate isoforms (PMBP(i+n)), monobenzyl phthalate (MBzP), metabolites of di-(2-ethylhexyl)phthalate (PDEHPm) and of di-iso-nonyl phthalate (PDINPm). After stratification of the urinary phthalate excretion into quartiles, it was found that the age at pubarche (pubic hair development), was increasing with increasing phthalate metabolite quartiles (except for MEP). This trend was statistically significant when all phthalate metabolites (except MEP) were summarised and expressed as quartiles. No association between phthalates and onset of breast development was observed. In addition, there were no differences in urinary phthalate metabolite levels between girls with precocious puberty and controls.

Yum *et al.* (2013) explored an association between precocious puberty and levels of certain endocrine disruptors, including DEHP and MEHP, in 190 female precocious puberty patients (age 8.91±1.40 years, range 6-12 years) who showed secondary sex characteristics under the

age of 8 or menarche that had occurred before 9.5 years. A parallel control group included 90 healthy children (age 8.5±1.68) who did not exhibit any evidence of endocrine disease or pubertal signs. Patients plasma levels of DEHP (143.8±85.3 ng/ml, range 2.1 – 489.3 ng/ml) and MEHP (8.0±7.1 ng /ml, range N.D. – 38.7 ng/ml) were not significantly different in girls with precocious development compared with controls (plasma levels of DEHP 209.0±105.3 ng/ml, range N.D. – 585.0 ng/ml and MEHP 13.1±7.4 ng /ml, range N.D. – 35.0 ng/ml). This indicates that precocious puberty is not associated with DEHP exposure.

There does not appear to be any consistent or strong association between exposure to DEHP or other phthalates and birth weight, childhood growth or reproductive development and timing of puberty in either sex. However, follow-up data for babies subjected to serious medical interventions perinatally, which will have resulted in very much higher exposures than the general population, is extremely limited. More detailed follow-up of such individuals would provide the best reassurance for absence of any DEHP effects on childhood growth and pubertal development.

DEHP and congenital hyperthyroidism

Jung *et al.*, (2013) determined plasma concentrations of several endocrine disruptors (including DEHP/MEHP) to investigate their possible associations with the occurrence of congenital hypothyroidism and passage of target compounds from the mother was investigated. Plasma levels of DEHP and MEHP (2.6-692.8 ng/ml and 2.4-95.8 ng/ml respectively) in 39 patients were not significantly different from DEHP and MEHP plasma levels (8.5-800.2 ng/ml and 7.4-182.5 ng/ml, respectively) of 20 controls. DEHP was not detected in mothers of patients and controls. No correlation was found between MEHP plasma levels of patient-mothers or control-mothers group.

Endometriosis

Two case-control studies have investigated the relations between biomarkers of DEHP exposure and the risk of endometriosis. A case-control study by Cobellis and colleagues (2003) provided the first evidence of an association between plasma and peritoneal fluid levels of DEHP and the risk of endometriosis. The 24 cases were patients who underwent diagnostic laparoscopy for ovarian cysts or chronic pelvic pain and dysmenorrhoea and who had a histological confirmation of endometriosis. The 35 controls were healthy age matched individuals without infertility or reproductive diseases. The cases had a higher plasma concentration of DEHP (median 0.57 µg/ml, interquartile range 0.06-1.23) than the controls (0.18 µg/ml 0-0.44, P=0.0047), but the plasma MEHP and peritoneal DEHP and MEHP concentrations were similar. However, certain limitations in these studies include possible exposure due to medical procedures, information on the selection of controls, evaluation of confounding factors and small sample size (CERHR Expert Panel 2005).

Reddy and colleagues (2006a) conducted a case-control study with 49 infertile women with endometriosis and 2 control groups. The first control group (I) included 38 age-matched women without endometriosis, but with infertility related to tubal defects, fibroids, polycystic ovaries, idiopathic infertility and pelvic inflammatory disease diagnosed by laparoscopy. The second control group (II) comprised 21 age-matched fertile women undergoing laparoscopic sterilisation. The endometriosis cases had a significantly higher concentration of DBP (mean 0.44 µg/ml, SD 0.41), BBP (0.66, 0.61), di-n-octyl phthalate (DOP)_(3.32, 2.17) and DEHP (2.44, 2.17) compared with both the first (DBP 0.08, 0.14; BBP 0.12, 0.20; DOP 0; DEHP 0.50, 0.80) and second control group (DBP 0.15, 0.21; BBP 0.11, 0.22; DOP 0; DEHP 0.45, 0.68). These studies indicate a correlation between the phthalate ester concentrations and the

severity of endometriosis for all compounds.

Kim *et al.* (2011a) showed that the plasma levels of MEHP, as well as DEHP, were significantly higher in 97 women with advanced-stage endometriosis compared to 169 controls (control MEHP 12.4 ±1.1 ng/mL, DEHP 92.5 ± 31.1 ng/mL, endometriosis MEHP 17.4 ± 1.5 ng/mL, DEHP 179.7 ± 32.5 ng/mL. This may indicate that that exposure to phthalates might play a role in the establishment of endometriosis.

Itoh *et al.* (2009) assessed the association between phthalate exposure and endometriosis in 166 women who were registered at a university hospital for consultation regarding infertility. They were then categorised by the severity of endometriosis as controls (stages 0–I) and cases (stages II–IV). Urinary concentrations of the phthalate metabolites including MEHP, 5-oxo-MEHP and MEHHP were measured in 57 cases and 80 controls. Adjusted odds ratios for endometriosis in relation to dichotomised individual phthalate metabolites (standardised for creatinine) were calculated. No significant association between endometriosis and any urinary creatinine-adjusted phthalate monoester was seen. Adjusted odds ratio (95% confidence interval) for higher dichotomised MEHP by endometriosis was 1.57 (0.74–3.30). No monotonic trend was seen in urinary creatinine-adjusted concentration of phthalate metabolites by endometriosis stage (p=0.23–0.90).

Based on increased plasma levels of DEHP in women with endometriosis, Kim *et al.*, (2011a) evaluated whether *in vitro* treatment with DEHP can increase viability of endometrial cells. Utilising 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide assay, fluorescent activated cell sorter analysis and microscopic evaluation after Hoechst staining, it was revealed that *in vitro* treatment with DEHP leads to increased viability of Ishikawa cells as well as endometrial stromal cells in serum-free condition and following exposure to hydrogen peroxide, which suggests that exposure to phthalates might play a role in the establishment of endometriosis.

IARC (2012) mentions a cross-sectional study of urinary concentrations of phthalate metabolites obtained from the US National Health and Nutrition Examination Survey (1999–2004) which examined their relation to self-reported history of endometriosis and uterine leiomyomata among 1227 women 20–54 years of age (Weuve *et al.*, 2010). Four phthalate metabolites, including MEHP, were examined. Eighty-seven (7%) and 151 (12%) women reported diagnoses of endometriosis and leiomyomata, respectively. After comparing the highest vs. lowest 3 quartiles of urinary MEHP, there were no significant associations with endometriosis or leiomyomata. A significant inverse association (OR, 0.59; 95% CI: 0.37–0.95) was found for both conditions combined.

Giovanna *et al.* (2012) compared concentrations of several phthalate monoesters in spot urine samples of 56 couples with infertility problems with those of 56 age-matched control couples living in the same area. The median concentration of MEHP+MEHHP in the group of infertility problems (17.20 µg/g creatinine) was not significantly different from that of control group (15.14 µg/g creatinine). No gender differences were observed in phthalate monoester concentrations. Median concentrations of MEP, MBzP and MnBP in urine of couples with infertility problems were significant higher than those in control group.

Buck Louis *et al.* (2013) investigated the relation between bisphenol A and 14 phthalate metabolites and endometriosis in a cohort comprised of 495 women undergoing laparotomy/laparoscopy and in a population cohort comprised of 131 matched on age and residence. Odds ratios (OR) and 95% confidence intervals (CIs) were estimated using logistic regression adjusting for age, body mass index and creatinine. In the population cohort, 6

phthalate metabolites-mono-n-butyl phthalate, mono-[(2-carboxymethyl) hexyl] phthalate, mono (2-ethyl-5-carboxyphentyl) phthalate, mono (2-ethylhexyl) phthalate, mono (2-ethyl-5-hydroxyhexyl) phthalate and mono (2-ethyl-5-oxohexyl) phthalate-were significantly associated with an approximately 2-fold increase in the odds of an endometriosis diagnosis. Two phthalates were associated with endometriosis in the operative cohort when restricting to visualised and histologic endometriosis (monooctyl phthalate; OR 1.38; 95% CI 1.10-1.72) or when restricting comparison of women to those with a postoperative diagnosis of a normal pelvis [mono (2-ethylhexyl) phthalate; OR 1.35; 95% CI 1.03-1.78].

Gonadal hormones and semen quality

Phthalate monoesters including MEHP, the initial metabolite of DEHP and DBP (MBP) are known testicular toxicants in rodents, with important negative effects on testosterone production by the foetal rat testis. Experimental data for newborn male marmosets and *in vitro* studies using testicular tissue from adult men both point towards the possibility that similar inhibition of testicular steroidogenesis could occur after birth and in adulthood. Several epidemiological studies have addressed this and/or have assessed in adult men if there is any association between phthalate exposure and semen quality or fertility.

No relationship was found between maternal exposure to DEHP in human pregnancy and free testosterone levels in cord blood of boys at birth, although there was a significant negative relationship for girls (Lin *et al.*, 2011). However, Main and colleagues (2006) studied 62 cryptorchid boys and 68 healthy boys from a prospective cohort of Danish and Finnish boys. As biomarkers of exposure, they analysed breast milk samples collected 1-3 months postnatally for phthalate monoesters including MMP, MEP, MBP, MBzP, MEHP and MINP. Serum samples were analysed for gonadotropins, sex-hormone binding globulin (SHBG), testosterone and inhibin B. No association was found between phthalate monoesters and cryptorchidism. Exposure to MEP and MBP was positively, but weakly, correlated with SHBG (Spearman correlation coefficient [r]=0.323, p=0.002 and r=0.272, p=0.01 respectively) whilst MMP, MBBEP and MBP exposure was correlated with the LH: free testosterone ratio and MINP with LH (r=0.243, p=0.019). MBP was negatively correlated with free testosterone (r=-0.22, p=0.033). These findings provide some evidence for an association between phthalate exposure and reproductive hormone levels in boys, but there was no consistent meaningful association when comparing exposure to different phthalates expected to have similar effects (e.g. DBP and DEHP).

With the aim of examining the associations between DEHP exposure *in utero* and reproductive hormone levels in cord blood, maternal blood samples were taken from 23–35 weeks of gestation in 514 pregnant women between 2002 and 2005 (Araki *et al.*, 2014). Maternal MEHP levels were found to be associated with reduced levels of estradiol (E2), total testosterone (T), and progesterone (P4), and inhibin B: reductions were quantitatively limited and statistically significant for males only. MEHP was the only DEHP metabolite measured, which is a strong limitation for this study.

Meeker *et al.* (2009) measured urinary concentrations of MEHP and other phthalate monoester metabolites and serum levels of testosterone, estradiol, sex hormone–binding globulin (SHBG), follicle-stimulating hormone (FSH), luteinizing hormone (LH), inhibin B and prolactin in urine and serum samples from 425 men recruited through a US infertility clinic. Two oxidised urinary metabolites of DEHP were also measured in urine from 221 of the men. In multiple regression models adjusted for potential confounders, MEHP exposure was inversely associated with testosterone, estradiol and the free androgen index (FAI). An interquartile range increase in MEHP was associated with a 3.7% (95% confidence interval [CI], -6.8% to -0.5%) and 6.8%

(95% CI, -11.2% to -2.4%) decline in testosterone and estradiol, respectively, relative to the population median hormone levels. There was limited evidence for effect modification of the inverse association between MEHP and FAI by the proportion of DEHP metabolites in the urine measured as MEHP (MEHP%), which is considered a phenotypic marker of less efficient metabolism of DEHP to its oxidized metabolites. Finally, the ratio of testosterone to estradiol was positively associated with MEHP (P = 0.07) and MEHP% (P =0.007), suggesting potential relationships with aromatase suppression. These results provide some evidence that urinary metabolites of DEHP are inversely associated with circulating steroid hormone levels in adult men. Results were supported by a more recent cross-sectional study on 2208 individual in the US general population, stratified by sex and age (6 –12, 12–20, 20–40, 40–60, and 60–80 y) within the US National Health and Nutrition Examination Survey, 2011–2012. In adult men, the only significant inverse association was observed among men ages 40–60 years between DEHP metabolites and T (around 10%). In boys 6–12 years old, an interquartile range increase in MEHP was associated with a 29% (95% confidence interval) reduction in T (Meeker and Ferguson, 2014).

Mendiola *et al.* (2011) measured 11 phthalate metabolites in urine samples provided by 425 men, who were partners of pregnant women. Serum samples provided on the same day were analysed for reproductive hormones, including FSH, LH, testosterone, inhibin B, estradiol and SHBG. Pearson correlations and parametric tests were used for unadjusted analyses and multiple linear regression analysis was performed controlling for appropriate covariates. All measures of testosterone [total, calculated free testosterone and the free androgen index (FAI)] were inversely correlated with the urinary concentrations of four DEHP metabolites. After adjustment by appropriate covariates, there was no longer an association between urinary DEHP metabolite concentrations and total testosterone levels; however, the FAI was significantly associated with the urinary concentrations of several DEHP metabolites. SHBG was positively related to the urinary concentrations of MEHP, but not with other DEHP metabolites, an association that was attenuated after adjustment. The authors suggested that DEHP exposure of fertile men is associated with minor alterations of markers of free testosterone.

Mendiola *et al.* (2012) followed up on the studies described in the preceding 2 paragraphs by undertaking a combined analysis of both study populations in order to increase the study power to detect 'effects'. Associations between urinary metabolites of DEHP and reproductive hormones — FSH, LH, testosterone, inhibin B, estradiol and SHBG were examined in the pooled population. In this combined analysis, they confirmed the association between phthalate metabolite exposure and reproductive hormone levels, despite the fact that these 2 populations spanned a range of fertility, urinary phthalate metabolites and reproductive hormone levels. The magnitude of the associations seen was similar to those reported for each population separately, but the effect estimates were more precise because of the increased sample size and the greater range of phthalate metabolite concentrations and hormone levels. Urinary concentrations of 3 metabolites of DEHP (MEHP, MEHHP and MEOHP) were inversely associated with the free androgen index (FAI; testosterone/SHBG) and calculated free testosterone. Urinary concentrations of MEHHP and MEOHP were positively associated with SHBG and MEHP was inversely associated with estradiol levels. No other phthalate metabolites were associated with serum hormones, consistent with results in each population. Results in this diverse population suggest that DEHP exposure is robustly associated with some male sex steroid hormones, but the changes observed are small and are not easily related to adverse health consequences. This is reinforced by other studies (below), in which similar associations were not found.

Pan *et al.* (2006) reported the effect of occupational exposures to high levels of the phthalate esters, DBP and DEHP, on the balance of gonadotropin and gonadal hormones including the

circulating concentration and/or balance of free testosterone (fT), LH, FSH and estradiol. They compared blood and urine concentrations of 74 male workers in a factory producing unfoamed PVC flooring and 63 men from a construction company matched for age and smoking status. The exposed workers had significantly elevated urinary concentrations of MBP (644.3 vs. 129.6 µg/g creatinine, p <0.001) and MEHP (565.7 vs. 5.7 µg/g creatinine, p<0.001). The fT concentration was significantly lower (8.4 vs. 9.7 µg/g creatinine. P=0.019) in the exposed workers compared with the unexposed, though this 'effect' was not large, considering the 100-fold difference in exposure. Among the exposed, fT had a negative correlation with MBP (r=-0.25, p=0.03) and MEHP (r=-0.19, p=0.095). In the regression analysis, fT decreased significantly with increasing total phthalate ester score.

Joensen *et al.* (2012) also investigated the association between phthalate exposure and reproductive hormone levels and semen quality in 881 Danish healthy men. Serum levels of testosterone, estradiol, SHBG, LH, FSH and inhibin-B, semen quality and urinary concentrations of 14 phthalate metabolites, including metabolites of DEHP, were assessed. The free androgen index was 15% lower [95% confidence interval (CI): −23, −8%] for men in the highest %MiNP quartile compared to the lowest quartile (p < 0.001) after adjusting for confounders and 9% lower (95% CI: −16, −1%) in the highest %MEHP quartile (p = 0.02). %MEHP and %MiNP were negatively associated with the ratio of testosterone/LH and testosterone/FSH. %MEHP was negatively associated with total testosterone, free testosterone and the ratio of testosterone/estradiol. %MiNP was positively associated with SHBG. Based on these results, the authors suggested that both testosterone production and pituitary–hypothalamic feedback may be altered in individuals who excrete a high proportion of primary metabolites of long-chained phthalates relative to the proportion of secondary metabolites. However, in this large study, there was little evidence of any association between the urinary level of individual phthalate metabolites, or the sum of phthalates, with reproductive hormones, in contrast to the findings in the US studies by Mendiola and co-workers described above. Another US study of adult men also failed to find any association (Duty *et al.*, 2006) and a large study of 555 boys going through puberty in Denmark found no relationship between urinary levels of 12 phthalate metabolites, including MEHP and associated metabolites and testosterone levels or occurrence of gynaecomastia (Mieritz *et al.*, 2012).

Duty *et al.* (2003a, 2003b, 2004, 2005) and Hauser *et al.* (2006) conducted a series of studies in male partners of sub-fertile couples recruited at an infertility clinic (US). They estimated associations between blood and urinary biomarkers of exposure to phthalates and various measures of semen quality and morphology. Sperm concentration, motility and motion parameters were measured using computer aided sperm analysis. Sperm DNA damage was measured using the comet assay. In an analysis of 168 males (Duty *et al.*, 2003b), there was an exposure-response relationship between MBP levels and sperm motility and concentration. Monobutyl benzyl phthalate (MBBP) levels were inversely associated with sperm concentration.

Hauser *et al.* (2006) studied 463 male partners of sub-fertile couples (including the 168 men in the previous study) who presented for semen analysis at the infertility clinic. They compared urine concentrations of phthalate esters between 76 men with subnormal sperm concentrations (<20 million/mL), 221 men with subnormal sperm motility (<50% motile) and 114 with subnormal sperm morphology (<4% normal) with 210 subjects whose sperm concentration, motility and morphology were normal (above the 3 cut points). There was a dose-response relationship between MBP and low sperm concentration (adjusted odds ratios per quartile: 1.00; 3.1; 2.5; 3.3, P for trend = 0.04) and suggestive evidence for a dose-response relationship between MBzP and low sperm concentration (adjusted odds ratios per quartile: 1.00; 1.1; 1.1; 1.9, P for trend = 0.13). No association was found between monoethyl phthalate, monomethyl phthalate or the DEHP metabolites and the 3 semen parameters.

In an analysis of 220 males, straight-line velocity (VSL), curvilinear velocity (VCL) and linearity (VCL/VCL) of sperm motion were inversely associated with levels of MBP, MBzP and MEHP (Duty *et al.*, 2004). The association between urinary concentration of phthalate metabolites and sperm DNA damage was reported in 2 analyses with partly same study subjects (Duty *et al.*, 2005, Hauser *et al.*, 2006). Various measures of sperm DNA damage were measured, including comet extent and tail distributed moment. The studied metabolites were MMP, MEP, MBzP, MEHP, mono(2-ethyl-5-hydroxyhexyl) phthalate and mono(2-ethyl-5-oxohexyl) phthalate. There was an association between MEP and sperm DNA damage. MEHP, a metabolite of DEHP, was associated with sperm DNA damage after adjustment for the oxidative DEHP metabolites mono(2-ethyl-5-hydroxyhexyl) phthalate and mono(2-ethyl-5-oxohexyl) phthalate. There was an indication of altered sperm motility and sperm DNA damage (as measured by chromosomal breaks) after exposure to DEHP and several other phthalates. A more detailed follow-up study of 379 men from an infertility clinic found essentially the same associations between phthalate metabolites and sperm DNA damage (Hauser *et al.*, 2007).

The association between exposure to phthalates and sperm DNA damage could be related to phthalate-induced lipid peroxidation and mitochondrial dysfunction, leading to the generation of reactive oxygen species (Pant *et al.*, 2008), to which sperm are especially susceptible. In this regard, Han *et al.* (2009) determined levels of DEHP, MEHP, DBP, MBP and phthalic acid (PA, a common metabolite of phthalates), in semen samples from 99 healthy volunteers (age 20-25 years, BMI 18.28-26.95) without known prior medicosurgical history. The mean concentrations in semen samples were 1.07 mg/ml for MEHP, 0.61 mg/ml for DEHP, 0.39 mg/ml for PA, 0.06 mg/ml for MBP and 0.003 mg/ml for DBP. The concentration of MEHP was the highest and the concentrations of the metabolites including MEHP, MBP and PA were higher than actual concentrations of parent DEHP and DBP. Since DEHP may reduce cellular levels of glutathione and other antioxidants in rats, the presence of high levels of DEHP in testes may produce oxidative stress (Kasahara *et al.*, 2002).

In a cross-sectional study of 589 male partners of pregnant women from Greenland, Poland and Ukraine, inverse associations between serum levels of DEHP and DiNP total metabolites and the serum concentrations of testosterone and semen volume was observed (although not statistically significant within each site). Sperm concentration, morphology and motility, LH, FSH, estradiol, inhibin B, and PSA were not associated with phthalate metabolites (Specht *et al.*, 2014).

Liu *et al.* (2012) investigated the general exposure of a Chinese reproductive age cohort to various phthalates to assess their potential effect on semen quality. A total of 125 men were recruited from couples seeking fertility assessment because of an inability to conceive. Six phthalate metabolites, including MEHP and MEOHP were measured in spot urines of the participants. The creatinine adjusted average concentrations for MEHP and MEOHP were 2.99 and 3.90 µg/g, respectively. After adjustment for age, body mass index (BMI), abstinence, smoking, drinking and education, there was a borderline-significant dose–response relationship only between MBP and sperm concentration, with odd ratios (ORs) of 1.0, 6.8 and 12.0 for increasing exposure tertiles (p=0.05).

In contrast to the studies described above, Herr *et al.* (2009) found no relationship between exposure to metabolites of DEHP and sperm concentration, motility or morphology. This study analysed urinary DEHP metabolites in 349 men (median age 34 years). Median concentrations ([µg/L) were MEHP 4.35, 5OH-MEHP 12.66, 5oxo-MEHP 9.02 and 5cx- MEPP 14.53.

Huang *et al.* (2011) investigated the association between semen quality in workers from PVC plants and the concentrations of DEHP in personal air. The study population comprised 80 men

who had worked for at least 1 year in one large scale and one medium scale PVC pellet manufacturing plant in Taiwan. The workers were divided into low- and high-DEHP-exposed groups according to the median levels of DEHP (23.7 mg/m3) in personal air. In the high DEHP-exposed group, significant increases were found in the tendency for sperm DNA denaturation (αT) induction, the DNA fragmentation index (DFI) and propensity for coffee drinking. After adjusting for coffee drinking, cigarette smoking and age, personal air concentrations of DEHP showed positive associations with αT (β = 0.038) and DFI (β = 0.140) and a negative association with sperm motility (β = -0.227).

In a biomonitoring study, Tranfo *et al.* (2013) compared excretion of urinary metabolites of phthalates, MEHP+MEHHP, MEP, MnBP and MBzP, of 56 couples with infertility problems with those of 56 couples of controls living in the same area. Urinary excretion of MEHP and MEHHP in couples with infertility problems (mean 30.62 µg/g creatinine, median 17.20 µg/g creatinine range 5.64–551.8 µg/g creatinine) was significantly higher than in control couples (mean 20.85 µg/g creatinine, median 15.14 µg/g, range creatinine 1.66–191.34 µg/g creatinine), but without statistically significant differences between genders. Urinary excretion of other measured phthalate metabolites of couples with infertility problems was also significantly higher than that of controls.

Male fertility

A Swedish epidemiologic study by Modigh and colleagues (2002) assessed the association between occupational exposure to DEHP and male fertility as determined by evaluating the time to pregnancy in 227 couples and their 397 pregnancies in which the male partner was working in a plant producing PVC plastics. Exposure assessment was based on air measurements at the work place and questionnaires on work tasks and locations. Time to pregnancy was compared in subjects who had no, low (<0.1 mg/m3) and high (>0.1 mg/m3) exposure. There was no association between exposure and time to pregnancy.

Testicular cancer

Two epidemiological studies of testicular cancer used source-based exposure assessment rather than measurements of specific phthalate concentrations (Hardell *et al.* 1997, Hansen 1999). Hardell and colleagues (1997) conducted a case-control study of the association between occupational exposure to PVC plastics and testicular cancer. They identified 148 testicular cancer cases and 315 controls from the Swedish Cancer Registry. Exposure assessment was based on questionnaires on occupations with probable PVC exposure. There were 6 exposed cases of seminoma and 2 exposed controls resulting in an adjusted odds ratio of 5.6 (1.1-196). No other association of cancer with plastics exposure was identified. Hansen (1999) conducted a case-control study of 3745 and 7212 controls using registry-based data on occupational history. There was no association between the risk of testicular cancer and exposure PVC plastics based on job category.

In a second Swedish study (Hardell *et al.*, 2004; Westberg *et al.*, 2005) of 791 men with germ-cell testicular cancer and 791 matched controls, exposure to PVC plastics was associated with an increased risk for testicular cancer (OR, 1.35; 95% CI: 1.06–1.71); a non-significant increased risk was reported for exposure to soft (containing plasticizer) plastics (OR, 1.48; 95% CI: 0.94–2.34; 54 cases and 37 controls), but not too rigid plastics (OR, 1.06; 95% CI: 0.55–2.01; 23 cases and 26 controls). The risk was elevated among workers with a 10-year latency (OR, 1.45; 95% CI: 1.06–1.98). However, odds ratios for exposure decreased with increasing exposure for all 4 measures of exposure (duration, maximum intensity, median intensity over the subject work history and cumulative median intensity). The questions on

exposure were focused on PVC in general and not on exposure to specific substances, which could decrease the possibility of detecting an effect due to phthalates.

The relevance of the aforementioned studies is questionable, because it is now accepted that testicular germ cell cancer (TGCC) derives from pre-cancerous abnormal germ cells in the testis, which arise in foetal life (Rajpert-De Meyts, 2006). Therefore, it is exposure of the mother in pregnancy to phthalates that would be the relevant exposure window to investigate and no such studies have been reported. Of note, effects of certain phthalates including DEHP/MEHP on foetal germ cells in human and rodent foetal testes were discussed earlier in this report.

Respiratory health

Øie *et al.* (1997) hypothesised that di(2-ethylhexyl) phthalate (DEHP) causes airways inflammation by mimicking some prostaglandins and thromboxanes with a similar chemical structure. Some monophthalates promote cytokine IL-6 and IL-8 production in the human epithelial cell line A549, indicating a potential role in inflammatory processes (Larsen *et al.*, 2001b).

Jaakkola and colleagues (1999) conducted a matched case-control study of 251 cases of bronchial obstruction and controls from a prospective Oslo Birth Cohort Study. Bronchial obstruction was defined as 2 or more episodes with symptoms and signs of bronchial obstruction. Trained experts characterised the interior surfaces and exposure assessment was based on the type of materials. The risk of bronchial obstruction was greater in the presence of PVC in the floors (adjusted OR = 1.89, 95 percent CI: 1.14, 3.14). The risk of bronchial obstruction was also related to a plasticizer exposure index (adjusted OR 2.72, 95% CI 1.50-4.91). Further analyses showed that the relation of bronchial obstruction to a plasticizer exposure index was stronger in homes with low air change than in those with high air change (Øie *et al.* 1999).

In a population-based cross-sectional study of 2568 Finnish children aged 1-7 years, the risk of wheezing, persistent phlegm, weekly nasal congestion or excretion and respiratory infections were related to the presence of plastic wall materials at home (Jaakkola *et al.*, 2000).

Bornehag and colleagues (2004) conducted a case-control study of Swedish children aged 3 to 8 years. The 198 cases included subjects with persistent allergic symptoms (106 with asthma, 79 with rhinitis and 115 with eczema) and 202 controls were free of these symptoms, both recruited from a population-based cohort of 10,852 children. The case status was related to the presence of PVC flooring in the bedroom with an adjusted OR (odds ratio) of 1.59 (95% CI (confidence interval) 1.05-2.41). The dust concentrations (mg/ g dust) of DEP, DBP, DIBP, BBzP, DEHP and DINP were determined. Median house dust concentrations of BBzP were higher in the bedrooms of cases than controls. The risk of allergic rhinitis and eczema was related to the house dust BBzP concentrations, whereas the risk of asthma was related to the concentration of DEHP (Bornehag *et al.*, 2004). Jaakkola and colleagues (2006) conducted a population-based incident case-control study to assess the relations between different types of interior surface materials and recent renovations at home and at work and the risk of asthma in adults. They systematically recruited all new cases of asthma during a 2.5-year study period (1997-2000) and randomly selected controls from a source population consisting of adults 21 to 63 years of age living in southern Finland. The clinically diagnosed cases consisted of 521 adults with new asthma and the controls of 932 adults fulfilling eligibility criteria. In logistic regression analysis adjusting for confounding, the risk of asthma was related to the presence of plastic wall materials (adjusted odds ratio (OR) = 2.43, 95% confidence interval (CI): 1.03,

5.75) and wall-to-wall carpet at work (adjusted OR = 1.73, 95% CI: 0.74, 4.09), the latter in particular in the presence of mould problems (adjusted OR = 4.64, 95% CI: 1.11, 19.4). Use of floor levelling plaster at home during the past 12 months was also a determinant of onset of asthma (adjusted OR = 1.81, 95% CI: 1.06, 3.08).

To explore the association between urinary concentrations of phthalate metabolites and asthma in children, Bertelsen *et al.*, (2013) measured 11 metabolites of 8 phthalates, including 4 metabolites of di(2-ethylhexyl) phthalate in 1 first morning void collected from 2001 through 2004 from 623 10-year-old Norwegian children. Current asthma was associated with mono(carboxyoctyl) phthalate and mono(carboxynonyl) phthalate, but not with the urinary metabolites of DEHP.

The associations between asthma diagnosed in children between 5 and 11 years of age and prenatal exposures to DEHP and other phthalates (butylbenzyl phthalate (BBzP), di-n-butyl phthalate (DnBP), and diethyl phthalate (DEP)) was studied by taking measurements from spot urine samples collected from 300 pregnant women (Whyatt *et al.*, 2014). Compared with levels in nonasthmatics, prenatal metabolites of BBzP and DnBP were associated with a history of asthma-like symptoms ($p < 0.05$) and with the diagnosis of current asthma: RR = 1.17 (95% CI: 1.01, 1.35) and RR = 1.25 (95% CI: 1.04, 1.51) per natural log-unit increase, respectively. Risk of current asthma was > 70% higher among children with maternal prenatal BBzP and DnBP metabolite concentrations in the third versus the first tertile. No association was measured with DEHP. However, results should be considered with caution, due to the limitation of measuring prenatal exposure with a single spot urine sample.

Just *et al.* (2012) measured the fractional exhaled nitric oxide, a biomarker of airway inflammation and urinary phthalate metabolites MEP, MnBP, MBzP and MEHHP in a cohort of 244 inner-city children. Independent associations were found between exposures to DEP and BBzP and fractional exhaled nitric oxide. However, such an association was not observed for DEHP exposure. Thus, DEHP exposure may not be positively associated with airway inflammation in children.

These studies suggest a correlation between PVC and/or phthalate exposure and obstructive respiratory symptoms and asthma.

Effect of DEHP metabolites on neurobehaviour

Cho *et al.* (2010) investigated the relationship between the urinary concentrations of phthalate metabolites and children's intellectual functioning. This study included 667 children at 9 elementary schools. A cross-sectional examination of urine phthalate concentrations was performed and scores on neuropsychological tests were obtained from both the children and their mothers. The geometric mean (ln) concentrations of MEHP, MEOHP and MBP were 21.3 µg/L, 18.0 µg/L and 48.9 µg/L, respectively. After adjusting for demographic and developmental covariates, the Full Scale IQ and Verbal IQ scores were negatively associated with DEHP metabolites, but not with DBP metabolites. Controlling for maternal IQ and other covariates, the results showed an inverse relationship between phthalate metabolites and IQ scores.

In a cross-sectional examination of urinary phthalate metabolites of 261 Korean children (age 8–11 years) and scores on measures of ADHD symptoms and neuropsychological dysfunction with regard to attention and impulsivity, Kim *et al.* (2009) found that teacher-rated ADHD scores were significantly associated with DEHP metabolites.

Testa *et al.*, (2012) determined DEHP metabolites MEHP, 6-OH-MEHP, 5-OH-MEHP and MEHP in spot urine samples of 48 children (36 male 12 female, mean age 11.5 yrs.) with autism spectrum disorders (ASD) and in spot urine samples of 45 healthy controls (25 male, 20 female, mean age 12.5 years). In ASD patients, significant increase in 5-OH-MEHP (52.1%, median 180 µg/L) and 5-oxo-MEHP (46.0%, median 96 µg/L) urinary concentrations were detected, with a significant positive correlation between 5-OH-MEHP and 5-oxo-MEHP. The fully oxidized form 5-oxo-MEHP showed 91.1% specificity in identifying patients with ASDs. However, the phthalate metabolite levels reported in this study (even in controls) are at least 10-fold higher than levels reported in population-based studies, which raises the possibility of contamination or measurement problems.

Recently, Stein *et al.* (2013) showed that the degree of glucuronidation of a series of DEHP metabolites in spot urine samples was lower in some children with autism spectrum disorder (ASD), indicating that alteration in glucuronidation pathway may be involved in ASD.

Kim *et al.* (2011b) explored the association between prenatal DBP and DEHP exposure and the Mental and Psychomotor Developmental Indices (MDI and PDI, respectively) of the Bayley Scales of Infant Development at 6 months in 460 mother–infant pairs from Seoul. Prenatal MEHHP, MEOHP and MBP were measured in 1 urine sample acquired from each mother during the third trimester of pregnancy. MDI was inversely associated with the natural log concentrations (mg/g creatinine) of MEHHP and MEOHP and PDI was inversely associated with MEHHP. In males, MDI was inversely associated with MEHHP, MEOHP and MBP and PDI was inversely associated with MEHHP, MEOHP and MBP. No significant linear associations were observed for females. The results suggest that prenatal exposure to phthalates may be inversely associated with the MDI and PDI of infants, particularly males, at 6 months. With regard to DEHP metabolites, it should be kept in mind that this exposure is determined by diet and therefore, confounding effects of diet is a possibility.

Whyatt *et al.* (2012) evaluated associations between phthalate metabolite concentrations in maternal prenatal urine and mental, motor and behavioural development in children at 3 years of age. Mono-n-butyl phthalate (MnBP), monobenzyl phthalate (MBzP), monoisobutyl phthalate (MiBP) and four di-2-ethylhexyl phthalate metabolites were measured in a spot urine sample collected from 319 women during the third trimester. When children were 3 years of age, the Mental Development Index (MDI) and Psychomotor Development Index (PDI) were measured using the Bayley Scales of Infant Development II and behaviour problems were assessed by maternal report on a Child Behaviour Checklist. Significant child sex differences were seen in association with MnBP and MBzP and behaviour in internalising domains ($p < 0.05$); there was no association with DEHP metabolites. The authors suggest that prenatal exposure to certain phthalates, excluding DEHP, may decrease children's mental and motor development and increase internalising behaviours.

Association with obesity, insulin resistance and type 2 diabetes

Epidemiological studies indicate that exposure to certain phthalates, commonly including DEHP and its metabolites, is associated with increased waistline, increased BMI and/or obesity. However, there is inconsistency in the reported findings between/within (e.g. male-female differences) studies. For example, Stahlhut *et al.* (2007) used data for 700-1450 men aged >18 years from NHANES and examined the relationship between waist circumference and insulin resistance (HOMA measurement) and exposure to 6 phthalate metabolites (MEP, MBP, MEHP, MEHHP, MEOHP, MBzP) measured in urine. Four metabolites (MBzP, MEHHP, MEOHP, MEP) were found to be positively associated with waist circumference whilst, MBP, MBzP and MEP were also associated with insulin resistance. Similarly, Hatch *et al.* (2008) used data for

4369 individuals from NHANES to examine the relationship of waist circumference/BMI and exposure to 6 phthalate metabolites (MEP, MEHP, MEHHP, MEOHP, MBP, MbzP). For males aged 20-59, positive associations were found with all metabolites (strongest with MBzP). In contrast, MEHP was inversely associated with waist circumference/BMI in women, although MEP was positively associated with these endpoints in adolescent girls and to a smaller extent in older women. No significant relationships were found in children and several inverse relationships were found in 60-80 year-olds. The latter contrasts a study (Lind *et al.*, 2012) that used DXA and MRI to measure fat mass/distribution in 1016 subjects aged 70 years and related this to exposure 2 years earlier to MEP, MEHP, MiBP and MMP, measured in serum. In women, MiBP was positively related to waist circumference, trunk fat mass and subcutaneous fat mass and similar, but less pronounced associations were found for MMP; no significant associations were found for MEP or MEHP and no significant associations were found at all for men.

James-Todd *et al.* (2012) examined the relationship between self-reported type 2 diabetes and exposure to 6 different phthalate metabolites (MEP, MnBP, MiBP, MBzP, MCPP and the sum of MEHP+MEHHP+MEOHP) in 2350 women from NHANES aged 20-79. Women with higher levels of MnBP, MiBP, MBzP, MCPP and the sum of DEHP metabolites had increased chance for type 2 diabetes. A relevant study in rats exposed during pregnancy and lactation to 1.25 or 6.25 mg/kg/day DEHP administered by gavage, reported abnormal β-cell ultrastructure, reduced β-cell mass and pancreatic insulin content plus changes in expression of relevant genes at weaning (Lin *et al.*, 2011). In adulthood, female, but not male rats, exhibited impaired glucose tolerance, although in both sexes there was reduced bodyweight, as had been the case at birth. The latter is similar to that reported in mice fed as young adults/adolescents with DEHP at much higher doses (100 or 1000 mg/kg/day) and maintained on a normal or a high fat diet (Feige *et al.*, 2010). DEHP exposure was found to be protective against diet-induced obesity, but this effect was abolished in PPARα-humanized mice. This highlights an important role for PPARα in mediating DEHP effects on metabolism, but also suggests important limitations in extrapolating from laboratory animal studies to humans.

As mentioned above, Hatch *et al.* (2008) found no association between phthalate exposure and waist circumference in children, which largely agrees with a recent study of 2884 children of different ethnicities aged 6-19 years from NHANES (Trasande *et al.*, 2013) that examined associations with low molecular weight, high molecular weight and DEHP metabolites. They found increased risk of overweight and obesity among non-Hispanic blacks with increased exposure to low molecular weight phthalate metabolites; no significant relationships were found for other ethnic groups and DEHP metabolites were not significantly associated with overweight and obesity in any group.

A recent meta-analysis (Goodman *et al.*, 2014) of 18 studies that investigated associations between urinary phthalate levels and obesity/waist circumference and associated diseases, concluded that there was no inter- or intra-study consistency for any phthalate metabolite for any of the indicators of overweight/obesity, diabetes mellitus or cardiovascular disease in children or adults. The associations were not statistically significant and included contradicting results. Most of these studies used cross-sectional analyses and thus could not be used to test causal hypotheses.

Association with paediatric health outcomes

Braun *et al.* (2013) reviewed the epidemiological literature examining the relationship between early life phthalate exposure and paediatric health outcomes. Five studies from Asia, Europe and the United States suggest that childhood exposure to DEHP and BBzP may increase the

risk of allergic diseases including asthma and eczema. Six studies from 4 different prospective cohorts reported that gestational BBzP, DEHP, DBP and DEP exposures were associated with alterations in infant/toddler physical development as well as parent-reported externalising, internalising and autistic-like child behaviour. However, there were inconsistencies related to the specific phthalates and behavioural domains. Two small studies reported shorter anogenital distance among male infants with higher gestational phthalate exposure, but another did not.

In a follow up of 328 inner-city mothers and their children, prenatal urinary metabolites of DEHP and di-n-butyl phthalate (DnBP), butylbenzyl phthalate, (BBzP), di-isobutyl phthalate (DiBP), diethyl phthalate were measured in late pregnancy. Phthalate exposures were within the range previously observed among general populations. The overall intelligence quotient (IQ) was evaluated in their 7 years old children. The full-scale IQ was inversely associated with prenatal urinary metabolite concentrations of DnBP and DiBP. Among children of mothers with the highest versus lowest quartile DnBP and DiBP metabolite concentrations, IQ was 6.7 (95% CI51.9, 11.4) and 7.6 (95% CI53.2, 12.1) points lower, respectively. Associations were unchanged after control for cognition at age 3 years. Significant inverse associations were also seen between maternal prenatal metabolite concentrations of DnBP and DiBP and child processing speed, perceptual reasoning and working memory; DiBP and child verbal comprehension; and BBzP and child perceptual reasoning (Factor-Litvak *et al.*, 2014)

3.4.6. Summary and conclusions on clinical and epidemiological evidence

A summary of epidemiological findings on DEHP and/or other phthalates with similar mechanisms is as follows:

Effects on testosterone production and semen quality. A number of studies have examined the association between DEHP exposure of adult men and their blood reproductive hormone levels and semen/sperm quality. There is considerable variation and inconsistency in the obtained results. Overall, an association between DEHP (or other phthalate) exposure and a decrease in testosterone/free testosterone levels has been reported. However, the described effects are small and unlikely to be of biological significance. Indeed, even in men occupationally exposed to ~100-fold higher levels of DEHP than the general population, a mean decrease in free testosterone levels of only 13% was found.
Regarding semen quality, DEHP was found to be significantly and negatively associated with sperm concentration, normal morphology and motility in one study, but these results have not been replicated in other studies investigating DEHP metabolites in other media. Therefore the association between the small adverse changes in aspects of sperm function (e.g. motility), sperm volume or DNA damage is weak.

Breast tumours- Weak association in one study with only one out of 4 DEHP urinary metabolites. Contrasting results were described with other phthalate metabolites in the same study.

Hypospadias and cryptorchidism - No association between prenatal phthalate including DEHP exposure and occurrence of hypospadias or cryptorchidism was found.

Decreased anogenital distance - Published studies so far show inconsistent evidence for an association between maternal phthalate including DEHP exposure in pregnancy and decreased anogenital distance in male offspring.

Mother/infant exposure levels - In a case-control study, higher levels of phthalates were determined in low birth weight (LBW) infants compared with the controls. In utero DEHP

exposures were associated with LBW in a dose-dependent manner. However, other studies have found no association between exposure and birth weight.

Childhood growth and pubertal development - There is no evidence of anti-androgenic effects of phthalates including DEHP in healthy boys. Current phthalate exposure in boys was not associated with pubertal timing, testosterone levels or with the presence of pubertal gynaecomastia in a cross-sectional study. In other studies with girls no association was found between total phthalate exposure and age at menarche and no association between phthalates and onset of breast development was observed. Three studies have investigated differences in urinary phthalate metabolite levels between girls with precocious puberty and controls; two found no relationship whereas the third found a positive association with total phthalate exposure.

Endometriosis - There is inconclusive evidence on this issue. Some studies indicate a correlation between the phthalate concentrations and the severity of endometriosis and some recent studies have confirmed the correlation. However, recent investigations also found no significant association between endometriosis and phatalate exposure. Further investigations are needed to resolve this disparity.

Effect of DEHP metabolites on neurobehaviour - A recent study on the relationship between the urinary concentrations of phthalate metabolites and 667 children's intellectual functioning show an inverse relationship between phthalate metabolites and IQ scores. Other results suggest that prenatal exposure to phthalates may be inversely associated with the Mental and Psychomotor Developmental Indices (MDI and PDI, respectively) of infants, particularly males, at 6 months. However there are inconsistencies related to specific phatalates and behavioural domains.

Association with obesity, insulin resistance and type 2 diabetes - Epidemiological studies indicate that exposure to certain phthalates, commonly including DEHP and its metabolites, may be associated with increased waistline, increased body mass index (BMI) and/or obesity and/or type 2 diabetes. However, results are highly inconsistent from study to study and a recent detailed meta-analysis of 18 relevant studies concluded that no association is evident.

A review of the recent epidemiological literature examining the relationship between early life phthalate exposure and paediatric health outcomes suggests that childhood exposure to DEHP and BBzP may increase the risk of allergic diseases including asthma and eczema.

Epidemiological studies on DEHP do not establish a cause-effect relationship for harmful effects on humans. The relevance of exposure metrics is often weak, being determined by means of a single urine sample (representative of short term exposure, mainly influenced by dietary habits) in which only a limited number of metabolites is measured. Indeed, an important confounding factor that applies to all of the association studies related to DEHP exposure is the role of diet. There are now several studies that demonstrate that >90% of exposure to DEHP occurs via food for the general population (Aylward *et al.*, 2011; Rudel *et al.*, 2011; Koch *et al.*, 2013). Moreover, eating a modern 'Western fast-food' diet is associated with high DEHP exposure (Rudel *et al.*, 2011). As such a diet is associated with a wide range of disorders (obesity, type 2 diabetes, liver and cardiovascular disease), it raises the possibility that dietary factors could be an important confounding factor in epidemiological studies of phthalates that have not yet been taken into account, e.g. diet is implicated in alterations in semen quality. Exposure of adults to lower molecular weight phthalates (including e.g. DBP, BBz, DEP) does not appear to be readily explained via food/diet (Koch *et al.*, 2013), an observation that also appears to apply to children (see Langer *et al.*, 2014). One implication of this difference is that

studies, which have differentially associated human disorders with exposure to DEHP or lower molecular weight phthalates, may provide additional insight.

However, analysing animal and human data along with mechanistic studies in a WoE approach, allow us to conclude that male foetuses of pregnant women and male neonates are potential groups at risk based on exposure levels above those that induce reproductive toxicity in rodent animal studies.

3.5. Alternative plasticizers in PVC medical devices

In the search for alternatives for plasticised PVC, researchers followed 3 main strategies, including: i) the development of safe plasticizer alternatives to DEHP, ii) reduction of the leaching aptitude of plasticizers and iii) the substitution of P-PVC with alternative safe polymers. Finding alternative plasticizers (van Vliet *et al.*, 2011) for DEHP is important, because it is necessary to have the appropriate mechanical and processing issues solved to significantly reduce potential health risks and the evaluation for untoward effects on blood and blood components must be carefully undertaken. An alternative to DEHP must be biocompatible and maintain mechanical properties during its entire working life. Several classes of chemicals proposed as potential alternatives to DEHP are of synthetic origin: their safety profiles have been often not fully elucidated, especially regarding long-term periods.

The alternative candidates may be classified as low molecular weight plasticizers and polymeric plasticizers. Low molecular weight PVC plasticizers such as citrates, adipates, trimellitates, azelates, sebacates, etc. are currently under investigation and are slowly accumulating market share. However, their overall use is only a small fraction of the total use of phthalates, because the main drawback is the tendency to leach from the polymer matrix.

For blood bags, in order to reduce the content of DEHP in blood components the following strategies are applied: Use cooling plates for whole blood units after blood collection to accelerate cooling to room temperature or lower temperatures; reduction, if possible, of time between blood collection and preparation of blood components; use, if possible, of additive solutions for storage as alternatives to plasma; use of fresh red blood cells for treatment of newborns; avoid storage of platelets in PVC-DEHP containers; use of frozen and thawed plasma as much as possible, although it is recognised that a certain amount of liquid-stored plasma units must be available for emergency situations.

3.5.1. Introduction

The information available for potential alternative plasticizers for DEHP in PVC medical devices is presented in Annex I. Both publicly available information (published papers) and information submitted by stakeholders for the 2008 SCENIHR Opinion on DEHP were considered. In addition, the ECHA web site was consulted and some conclusions from the Agency were reported. Although in some cases the toxicological profile is not appropriately defined, the major problem is related to the scant information about possible human exposure. Data on leaching potential in the actual conditions of use of specific medical devices are scant. In addition most of data available on toxic effects are related to oral administration: the few data available show that for some of the alternatives (e.g. COMGHA, TOMT and DEHT) the oral absorption is limited and therefore data are not relevant for defining hazards and risks following parenteral exposure, which is one of the major routes when medical devices (e.g.

tubing or bags) are concerned. The safety evaluation of medical devices and their composition including material characteristics, leaching and toxicology is described in the ISO/CEN 10993 series on Biological Evaluation of Medical Devices (ISO, Geneva, Switzerland; CEN, Brussels, Belgium).

3.5.2 Alternatives in medical devices

Modified-DEHP medical devices

Zhao *et al.* (2008) demonstrated that the blood compatibility of DEHP-PVC was improved by surface modification by end-point attachment of heparin. The influence of surface modification of DEHP-PVC tube on blood compatibility was assessed in terms of the reduction of fibrinogen and factor XII adsorption *in vitro* and the generation of thrombin-antithrombin III complex and the complement component C3a, *in vitro* and *ex vivo*.

Irradiation with 20–25 kGy is a process commonly used for sterilizing PVC medical devices. Moreover, whole blood and blood components undergo additional irradiation with 25–50 Gy to inhibit the proliferative capacity of lymphocytes and reduce the risk of transfusion-associated graft-versus-host disease (GVHD). Ferri *et al.* (2012) investigated the effects of different doses of gamma irradiation on DEHP migration from PVC blood bags using differential scanning calorimetry (DSC) analysis. The results indicated that irradiation with 25–100 Gy reduces the ability of DEHP to migrate from the blood bags and in primary containers a correlation between the doses of gamma ray irradiation was also observed. In particular, a decrease in DEHP leachability was obtained by increasing the dose of gamma ray irradiation.

Yu *et al.* (2008) prepared a plasticizer with reduced DEHP migration by incorporating 2,3,6-per-O-benzoyl-β-cyclodextrin (Bz-β-CD) into DEHP. Bz-β-CD was prepared by esterification between the hydroxyl groups of β-CD and benzoyl chloride. The presence of this cyclodextrin was expected to facilitate formation of stable complexes through π-π association with DEHP molecules. The flexible PVC was prepared with a gelation-fusion process that uses the prepared migration-resistant plasticizer and its properties (flexibility, thermal stability and clarity) were evaluated by carrying out DSC and tensile testing, TGA and haze testing, respectively. No significant changes in the physical properties of the flexible PVC were observed when Bz-β-CD was added. DEHP migration tests were carried out for the flexible PVC according to the ISO3826:1993(E) test method and the quantity of migrated DEHP was then determined with UV-vis spectroscopy. The addition of Bz-β-CD decreased the levels of DEHP migration from the flexible PVC samples by almost 40%. The authors suggested that that the reduction of DEHP migration was due to the formation of stabilised π-π attractive association and inclusion complexes of Bz-β-CD and DEHP in flexible PVC.

Gourlay *et al.* (2010) demonstrated that sulfonation of PVC surface significantly retards the migration of DEHP and is associated with the moderation of contact activation processes in blood cells. The study was carried out in 2 phases: phase 1, in which the migration rate of DEHP from DEHP plasticized PVC (PPVC) and sulfonated DEHP plasticized PVC (SPPVC) was measured; phase 2 of the study, in which the materials were incorporated into a rat recirculation biomaterial test model and blood samples taken to assess CD11b expression on neutrophils, IL-6 and Factor XIIa. The initial DEHP concentration washed from the surface after storage was 37.19 ± 1.17 mg/l in the PPVC group and 5.89 ± 0.81 mg/l in the SPPVC group ($p<0.0001$). The post-wash migration rate was 3.07 ± 0.32 mg/l/h in the PPVC group compared to 0.46 ± 0.038 mg/l/h in the SPPVC group ($p<0.0001$). In phase 2 of the study, CD11b expression increased by 228.9% ± 37% over the test period in the PPVC group

compared to 118.3% ± 46% in the SPPVC group ($p<0.01$). IL-6 levels rose from 3.1 ± 1.4 pg/ml to 263 ± 26 pg/ml in the PPVC group and 2.2 ± 1.6 pg/ml to 161 ± 29 pg/ml in the SPPVC group ($p<0.01$). Factor XIIa levels rose from 0.22 ± 0.13 g/ml to 3.7 ± 0.32 µg/ml and 0.28 ± 0.09 to 2.71 ± 0.21 µg/ml in the PPVC and SPPVC groups, respectively ($p<0.05$ at 90 minutes).

DEHP alternatives

The strategies for the use of alternatives of DEHP-PVC in some specific medical devices have been discussed in recent reviews (Van Vliet *et al.*, 2011, Sampson and Korte 2011, Simmchen *et al.*, 2012). The strategies propose the use of alternative plasticizer as well as alternative to PVC polymer.

Burgos and Jiménez (2009) investigated the surface of DINCH-PVC and ATBC-PVC after sterilisation with ethylene oxide and water vapour according to EU methods (concentration of ATBC and DINCH was not reported). The samples did not reveal significant surface alterations, with just some narrow fractures and slight increase in surface roughness, but without any evidence of serious degradation, like grooves or blisters, that could provide favourable sites for biological residue retention (bacterial colonisation). However, leaching/evaporation of plasticizers from the surfaces with narrow fractures was not studied.

Nair *et al.* (2011) studied compatibility of DINCH-PVC bags for the storage of platelets by the comparison of platelets stored in TOTM-PVC bags. The parameters studied for 6 days storage of platelets were cell count, pH, pCO_2, HCO_3, lactate, glucose, plama Na^+, K^+ and aggregation. The authors showed that DINCH-PVC bags are also suitable for the storage of platelet concentrates for more than 5 days without loss of function.

Lagerberg *et al.* (2015), after collection and overnight storage of whole blood into DEHP-containing and DEHP-free collection systems, prepared platelet (PLT) concentrates and red blood cells in DEHP-free DINCH-based systems. After addition of additive solutions, RBCs were analysed for in vitro characteristics and plasticizer levels during storage. The use of DINCH-based systems had no effect on WB composition, blood processing, and plasma quality and PLT in vitro quality variables were maintained. Hemolysis was significantly higher in DINCH-PVC (likely due to the absence of DEHP), but the use of additive solutions reduced the hemolysis to levels similar to the DEHP-bags. Leakage of DINCH into the blood product was generally less pronounced than that of DEHP (from 7 to 20 fold) depending on the matrix (plasma, RBC) and time of storage, with the exception of paediatric PLT concentrates in which the leaching rate was the same. It has to be underlined that although indicated as DEHP-free material, the release of DEHP from DINCH-based material occurs, at different extent depending on matrix and time, being most of the values 1-7 fold lower up to 20-fold.

Dumont *et al.* (2012) evaluated a candidate replacement plasticizer (DINCH) compared to DEHP in an *in vitro* feasibility study. The hypothesis was that the candidate will provide at least equivalent protection against haemolysis for RBCs stored for 42 days and periodic mixing of RBCs will add additional protection against haemolysis. Whole blood was collected into citrate-phosphate-dextrose; combined into pools of 2 ABO identical whole blood units; and divided, leuko-reduced, centrifuged and separated into plasma and RBCs. Additive solution was added and the RBCs were stored for 42 days at 1 to 6°C. In 3 parts of this study, split pools were paired as DINCH-PVC with weekly mixing vs. DINCH-PVC with no mixing, DINCH-PVC mixed vs. DEHP-PVC no mix and DINCH-PVC vs. DEHP-PVC with neither mixed. A standard panel of *in vitro* RBC characteristics was determined on Days 0 and 42. Mixing DINCH-PVC weekly improved Day 42 haemolysis (0.36 ± 0.07% vs. 0.56 ± 0.15%, $p = 0.002$) and mixed DINCH-

PVC bags were not inferior to unmixed DEHP-PVC bags (p = 0.05). DINCH-PVC bags stored without weekly mixing were inferior to unmixed DEHP-PVC bags for haemolysis on Day 42, although no individual bag exceeded 0.8% haemolysis. Periodic mixing of RBCs stored in DINCH-PVC provided additional protection against haemolysis. Unmixed DINCH-PVC bags were inferior to DEHP-PVC bags for prevention of haemolysis, but remain a candidate for replacement DEHP in RBC storage bags.

Different results were obtained in another study, showing that among different phthalate, phthalate-like, trimeliate, citrate, and adipate derivatives only DINCH, and di(2-ethylhexyl)-1,2,3,6-tetrahydro-phthalate (DOTP), exhibited a haemolysis suppression effect (9.2– 12.4%, and 5.2–7.8%, respectively) almost equal to that of DEHP (10.9%), but not other plasticizers (Haishima *et al.*, 2014). DIDP (diisodecyl phthalate) did not suppress the hemolysis because of low leachability (4.8–6.0 mg/mL).

3.5.3. Exposure to alternative plasticizers

When alternatives are used as a replacement for DEHP in medical devices, the contact of patients with these alternatives is similar to DEHP. In terms of quantitative exposure (mg/kg bw), differences may occur depending on the actual amount of plasticizer present in the medical devices used and the leaching properties of these alternatives.

The patient exposure to plasticizers in medical devices depends not only on the substance used, but also on a number of other factors. The time and area of contact between the plastic device and the biological medium/tissue is important, as well as the character of the biological medium. The plasticizer concentration in the polymer is also important and the mechanical stress of tubing in peristaltic pumps and agitation of storage samples may increase the leaching of the additives in the medium. These variables make it difficult to compare leaching measured in different studies. Thus, a comparison of plasticizers under identical conditions is the most useful approach.

A myriad of data on leaching of polymer additives from food packaging materials and some data on plasticizer leaching from PVC toys are published, but a few standardised test systems have been developed. Food simulants are used to mimic leaching of plasticizers and other additives in different types of food stored under specified temperatures and different time periods in a static system, where the concentration of the additive is analysed in the simulant. However, these data are of limited use in quantification of exposure from medical devices: the leaching rates of plasticizers from food packaging materials may be useful in the quantification of leaching of these substances during storage of biological materials in plasticized PVC container only under static conditions and depending on the composition of the food stimulant used. Artificial saliva and gastric juice simulants have been used to estimate leaching of chemicals from mouthing and ingestion of toys/toy materials. These leaching rates may have application in quantification of plasticizers under dynamic conditions, but only in aqueous medium. However, the comparison of leaching rates of various plasticizers measured by testing of food packaging and toy testing could give an indication about the relative leaching of alternative plasticizers compared to that of DEHP.

Exposure data on DEHP from PVC medical devices containing this plasticizer is available for most critical procedures obtained by using standard test methods for measuring the leaching rates of components from medical devices (ISO 10993). The best information should be obtained from studies in which the leaching of alternative plasticizers is compared to the DEHP one under identical conditions, but unfortunately these types of studies are scant or absent.

Exposure data on alternative plasticizers can be estimated/extrapolated on the basis of relative leaching rates using DEHP exposure data (see section 3.4) as a benchmark, using information coming from products other than medical devices (e.g. toys).

In a comparative study of leaching of plasticizers in different feeding solutions, Welle *et al.* (2005) compared DINCH, TOTM and ATBC with DEHP. The feeding solutions contained 4.4–10% fat and commercially available feeding sets with 29–49% plasticizer were used, except for DINCH, which was in a pilot application tube containing 30% of the plasticizer. The leaching was followed with chemical analyses for 24h. Leaching rates of various plasticizers were relatively constant over this period, except for ATBC in which leaching decreased with time. The latter may be explained by a high leaching rate for ATBC, at least 10 times higher than for DEHP. DINCH leaching was 3-10 times lower than that for DEHP, while the release of TOTM was extremely low and in 1 experiment almost 2 orders of magnitude lower than the leaching of DINCH. However, in the TOTM experiment, DEHP was significantly released, which was probably due to DEHP impurity in the TOTM-containing device.

A comparison between PVC infusion lines containing TOTM and DEHP revealed a significantly higher leaching for DEHP (about 30 times higher in one case) (Shenshu *et al.*, 2004). In another study, PVC tubes for haemodialysis plasticized with DEHP and TOTM found that leaching of DEHP was approximately 3 times higher than TOTM, but the latter also emitted DEHP (Kambia *et al.*, 2001). Leaching of DEHP from TOTM-containing products was associated with DEHP impurities.

Takahashi *et al.* (2008) measured overall extraction of DEHP during cardiopulmonary bypass when DEHP or TOTM tubing was used. Sixteen patients undergoing coronary artery bypass grafting were randomly divided into 2 groups of 8 each. Group A had tubing containing DEHP in the circuit and the tubing for group B contained TOTM. Plasma DEHP levels at the end of cardiopulmonary bypass were significantly increased compared to before anaesthesia in both groups (group A: 103 ± 60 to 2,094 ± 1,046 ng/mL; group B: 135 ± 60 to 472 ± 141 ng/mL) and were significantly higher in group A compared to group B. This study demonstrated that using tubing containing plasticisers alternative to DEHP significantly reduced the release during cardiopulmonary bypass, but confirmed that leaching of DEHP from TOTM-containing products occurs, likely associated with DEHP impurities. However, the release of TOTM from non-DEHP tubing was not measured in this study, strongly limiting the possibility to compare the two.

In a study by Subotic *et al.* (2007), 5 cm of PVC nasogastric tubes containing DEHP or polyadipate were incubated with feeding solution and gastric juice. Although leaching was at least 10 times lower compared to DEHP, no conclusion could be made from this study because the contents of the 2 plasticizers in the tubings were not described. PVC was blended with DEHP, DEHA, ATBC and BTHC plasticizers and moulded thin sheets of these materials to compare several properties. A few of the results are presented in Table 8. The higher extraction into the oil reflects the lipophilic character of these esters. The biggest difference between the compounds was seen in soapy water, with approximately a factor of 5 between the extremes.

Table 8. Extraction of some plasticizers from PVC (48 hrs at 25°C)

	Extracted fraction (%) of			
Solvent	**DEHP**	**DEHA**	**ATBC**	**BTHC**
Water	0.7	1.5	1.2	1.7
Soapy water	2.7	11.0	9.5	2.2
ASTM Oil #3	11.4	34.7	10.9	15.7

A comparison of leaching of BTHC and DEHP into blood in PVC bags revealed a slightly lower leaching of BTHC compared to DEHP (Kandler 1998). The leaching of COMGHA compared with data for DEHP and DINP (see Table 9) (Kristoffersen 2005) indicated that leaching in aqueous media is lower for COMGHA than for the phthalates tested, while in lipophilic media, leaching was similar. Data available to the EFSA in their evaluation (EFSA 2004) are included in Table 12. These results highlight the difficulties to compare results from leaching studies.

Table 9. Migration from PVC-containing COMGHA, DEHP and DINP.

Plasticizer	Reference	Leaching mg/ dm^2		
		3% acetic acid	15% ethanol	sunflower oil
COMGHA (40%)	Kristoffersen 2005	0.0058	0.0055	368
DEHP(40%)	Kristoffersen 2005	2.83	1.31	466
DINP(42%)	Kristoffersen 2005	-	-	420
COMGHA (40%)	EFSA, 2004	0.06	0.06	10.3

It is not possible to draw conclusions regarding the relative leaching of investigated plasticizers based on the studies referred to above. Identification of TOTM leaching, for instance, is much lower than DEHP, but when tested TOMT and DINCH-based materials release DEHP as well, likely due to impurities; ATBC and DOTP leaching is higher than DEHP in other studies. The general impression is that leaching of the remaining plasticizers are similar, based on their similar structures and properties generally in the range of 2-10 fold lower than DEHP. However to estimate adequate exposure scenarios, the leaching potential should be measured in the actual conditions of use of specific medical devices. Since some alternative plasticizers are already used in some medical devices present on the market (e.g. in Europe there are CE certified devices for paediatric containing DINCH-based materials), better exposure data should be available as well as appropriate follow up.

3.5.4 Toxicity of the alternative plasticizers

In general, the toxicity of alternative plasticizers is less well described than for DEHP, although for some plasticizers ECB risk assessment reports are available. Information on each alternative is presented in Annex 1. Aggregate exposure from other sources of alternative plasticizers should be taken into account for risk assessment, because they are used in many other consumer products-including toys, food can contain some residues leached from food packaging, dust and air samples may contain these plasticizers (as in the case of DEHP). Nagorka *et al.* (2011) measured concentration of DEHT and DINCH in 953 dust samples from German households. These samples were obtained in 4 studies conducted from 1997 to 2009. Maximum concentrations of 110 mg DINCH/kg dust and 440 mg DEHT/kg dust were found. Specifically, the amount of DINCH increased significantly after the market introduction of this plasticizer in 2002. Up to the beginning of 2006, DINCH was found in 44% of the dust samples and in 2009, collected dust samples contained increased concentrations for both softeners.

3.5.5. Conclusions on the risks of alternative plasticizers

To compare the toxicity a short summary of the potential genotoxicity, carcinogenicity, repeated dose toxicity and reproductive toxicity are summarised in Table 10 and 11. The lowest NOAEL measured in male or female rats has been reported (for details see Annex 1).

The critical endpoint for some plasticizers it is different from reproductive effects, which although present occurs at higher doses with respect to the critical end-point. Assuming similar leaching rates, the margin of safety associated with the critical toxicity outcome of other plasticizers will be >20 times higher for the alternatives, with a few exceptions: DINP, DINCH and DEHA (ratios with DEHP=3, 8, 8, due to effects on the liver, thyroid and kidney, respectively). It has to be noted, however, that, according to the available information, COMGHA, TOMT and DEHT are poorly absborbed after oral administration and this feature can explain at least partially the low toxicity observed in oral toxicity studies. The relevance for the parenteral route of exposure (meaning a 100% bioavailability) is therefore limited.

Table 10. Relevant NOAEL of some alternative plasticizers compared with DEHP.

Plasticizer	Relevant NOAELs mg/kg bw/d (ratio with DEHP)	Reproductive Toxicity	Critical endpoint
DEHP	4.8 (1)	Yes	Reproduction
ATBC	100 (20)	Yes: Decreased bw in F1 male rats (NOEL 100 mg/kg bw/d)	Decreased bw; haematological and biochemical changes; increased liver weight
COMGHA	1333 (278)	No (up to 1000 mg/kg bw/d)	None (highest dose tested)
BTHC	250 (52) i.v. 50 mg/kg bw/d (10)	No (up to 1.2% dietary or 500 mg/kg bw/d i.v.)	Liver weight Haematological changes
DEHA	40(8) 200 (41)	Yes	Kidney weight reduction Foetotoxicity
DINCH	107 (20) 40 (8)	No/Yes (statistically significant AGD alteration with uncertain biological meaning)	Kidney* Thyroid (follicular cell hyperplasia and adenomas)
DINP	15 (3)	Yes but NOAEL > than critical end-point	Liver
DEHT	500-700 (104) 142 (30)	Yes	Developmental Hyperplasia of the urinary bladder/adenomas in the uterus
TOTM	100 (20)	Yes	Liver, Reproduction

* Kidney effects in male rats due to alpha-2-μ macroglobulin, a specie-specific mechanism not relevant to man.

Table 11. The cancer and mutagenicity effects and maternal toxicity of plasticizers

Plasticizer	Repeated dose Toxicity, NOAEL mg/kg bw/day	Genotoxicity	Carcinogenicity	Maternal toxicity mg/kg bw/d
DEHP	29 (male rat)	Negative	LOAEL 320 (male rat)	LOAEL 750 (rat)
COMGHA	5000	Negative	No data	>1000 mg/kg bw/d
ATBC	100	Negative	Negative (limited study)	NOAEL 100 (rat)

BTHC	250	Negative	No data available	> 1.2% dietary or >500 mg/kg bw/d i.v.
DEHA	200	Negative	Negative in rats Positive in mice Hepatocellular carcinomas and adenomas°	NOAEL 400 (rat)
DINCH	40	Negative	Thyroid	NOAEL 1000 (rat)
DINP	15	Negative	Liver° and Kidney*	LOAEL 750 (rat)
DEHT	142	Negative	Uncertain	NOAEL 458 (rat)
TOTM	100	Negative	No Data	-

° Liver tumours mediated by peroxisome proliferation

* Kidney effects in male rats due to alpha-2-μ macroglobulin, a specie-specific mechanism not relevant to man

SCENIHR concludes that with the exception of BHTC and COMGHA and the uncertain results of DINCH, all the considered plasticizers can cause reproductive toxicity, although this occurs at doses several fold higher than DEHP and equal or higher than the critical end-point for their own toxicity. DINP, DINCH and DEHA have some carcinogenic potential, although the mode of action is irrelevant (as in the case of kidney tumours mediated by alpha-2-μ-macroglobulin) or not clear (as for the liver and thyroid tumours) for human health; maternal toxicity was similar or higher than DEHP for some other alternatives (such as DINP, ATBC, DEHA, DEHT). Other plasticizers such as COMGHA, BTHC and TOTM could not be evaluated for all endpoints because of few or poorly relevant data available. As already mentioned in the case of COMGHA and TOMT which are poorly absorbed after oral administration, oral studies are of limited use to assess the risk for the possible release from medical devices for which the parenteral routes are the most relevant.

With the new data available after the last Opinion, a clearer picture about the toxicological profile is available, although alternatives have not been as extensively studied as DEHP. However, a risk assessment of these alternative plasticizers in medical devices could not be performed because of a lack of adequate leaching properties and consequent poor human exposure data. This limits a proper evaluation of potential replacements for DEHP. Importantly, the risk and benefit should be carefully evaluated for each individual medical device and each medical procedure in which the alternative needs to be used.

3.6. Combined exposure to plasticizers

Combined exposure to different plasticizers of different populations and subpopulations is possible and may occur at different times or together. Due to the wide use of DEHP in the society, humans may be exposed to many different sources and exposed to other phthalates as well. Combined exposure to DEHP, DBP, BBP, DIBP and DINP and other phthalates having the same mechanism of action may potentially cause at least an additive effect, as suggested for DEHP and DINP (Burch *et al.*, 2004). In general, a common mechanism might exist if 2 compounds cause the same critical effect, act on the same molecular target at the same target tissue, and act by the same toxicological mechanism of action and share a common toxic intermediate (SCHER, SCCS, SCENIHR, 2012). This will probably be the case for combined exposure to the 5 mentioned phthalates, so that a cumulative risk assessment seems to be reasonable, also considering that the chemical structures of some alternative plasticizers

reveal that some may form the DEHP metabolite 2-ethylhexanol, which is responsible for toxicity.

ECHA in 2013 carried out a combined exposure for DINP and DIDP for liver effects and concluded no risk, but stated that a combined risk assessment of DINP and low molecular weight (LMW) phthalates could be considered in the future. However, the potency of the different phthalates has to be considered: DEHP and DBP are almost equal in potency on steoridogenesis in foetal male rats, but DIBP and BBP are less potent and DINP seems to have the smallest effect. The US CPSC did conduct a combined risk assessment, showing that DINP makes an insignificant contribution to the combined risk compared to DEHP, DBP, BBP (CPSC CHAP Report 2014). In 2012, based on a Danish restriction proposal (Danish EPA, 2011), the ECHA Committee for Risk Assessment (RAC) has adopted by consensus its opinion concluding that the proposed restriction of four classified phthalates (DEHP, DBP, BBP, and DIBP) in articles is not justified (ECHA, 2012). The RAC concluded that the available data does not indicate that there is currently a risk from combined exposure to the four phthalates. In addition, RAC was of the opinion that the existing regulatory measures and the consequential reduction in use would further reduce exposure. On the basis of these considerations, RAC concluded that the proposed restriction is not justified.

3.7. Potential alternative polymer plasticizers in PVC medical devices

In addition to the potential alternative plasticizers discussed above, another alternative to phthalates is the use of "polymeric plasticizers". These are high molecular weight solid polymers soluble in PVC in large proportions, which when blended with PVC by conventional processing, give rise to polymeric alloys that result in homogeneous blends with an almost single thermodynamically stable phase. Their macromolecular dimensions lead the molecules to undergo segment chain-chain entanglements with the PVC matrix forming strong specific interactions, thus strengthening interactions, reducing diffusion and hindering leaching outside the blend. Polymeric plasticizers of PVC are typically aliphatic polyesters. Many of these are structurally related with polyesters commonly employed as components of drug delivery systems and are biodegradable and biocompatible. Their low solubility in water further prevents extraction by aqueous media.

Extensive literature on polyester/PVC blends demonstrate that a number of homopolymeric and co-polymeric structures are, in principle, eligible as constituents of soft PVC formulations and that even a different class of polymers, as for instance polypropylene glycols, might be used to this purpose (Lindström and Hakkarainen 2006 & 2007, Hakkarainen 2005). However, a number of basic requirements must be fulfilled to fully exploit polyesters for their potential as PVC plasticizers. In addition to being miscible in nearly all proportions with PVC, their glass transition temperature must be lower than 0°C and they must show no tendency to crystallise with time within the alloy. In fact, after crystallisation, they separate into crystalline domains, which impart opacity and decrease plasticizing effect. To minimise migration, their molar mass molecular weight must be medium-sufficient high. However, in practice polymers with number average molar mass molecular weight as low as 1000 g/mol are required, because for higher molar mass phase separation occurs and the crucial homogeneous distribution of the plasticizing component is therefore, inaccessible and will no longer plasticize the PVC. Polymeric plasticizers generally make the compounds more difficult to process (Shah and Sherdukte 2003, Lindström and Hakkarainen 2007). Most of these compounds are experimental (Ferruti *et al.*, 2003) and insufficient information is available to assess the use and safety of these compounds in medical devices.

4. OPINION

4.1 Scientific Rationale

The safety of medical devices containing DEHP [di-(2-(ethylhexyl) phthalate] as plasticizer of their PVC-based components has been considered previously by EU Scientific Committees (SCMPMD, 2002 and SCENIHR, 2008). Alternative PVC softening agents were also assessed. Similarly, different scientific organisations have for many years examined the possible health risks posed by exposure to DEHP (e.g. FDA 2002; EFSA 2005; ECB, 2004, 2008; CSTEE 2004; IARC 2000, 2012; BfR 2013; ECHA, 2008, 2012, 2014).

Since the last SCENIHR Opinion on DEHP in medical devices (2008), new studies on DEHP activity have become available. Therefore, the information and literature published on this topic from 2008 up to now has been reviewed and evaluated by SCENIHR and the relevant aspects were incorporated in the present Opinion, which represents an update of the 2008 SCENIHR opinion. All the data were considered together and evaluated based on the WoE approach.

The Opinion is mainly concerned with the potential risk for patients exposed to DEHP or similar plasticising compounds leaching from medical devices. The exposure of the general population to plasticizers has been taken into account. The safety assessment includes currently available, as well as to a limited extent, proposed alternatives of DEHP in medical devices for neonates and for other patient groups, in particular in view of clinical procedures resulting in high exposure. Whilst recognising that there are several non-PVC based materials that can be effective for use in medical devices, this Opinion does not address these materials. Moreover, the Opinion does not consider PVC-related environmental aspects, nor occupational health related effects. Likewise, SCENIHR was not requested to consider the health risks from other substances that might leach out of a PVC medical device such as stabilisers, other additives and contaminants. Medical procedures using PVC medical devices can lead to DEHP exposures that are much higher than background levels, although such exposure is of limited duration (Tables 6-8). Additionally, voluntary medical treatments such as apheresis procedure to donate blood products may result in transient elevated exposure to DEHP. However voluntary donations are not provided by groups deemed to be at risk for reproductive toxicity (pregnant and nursing mothers and neonates).

4.1.1. Exposure of the general population to DEHP

As demonstrated by several metabolite excretion studies, the general population is exposed to non-negligible DEHP levels through a variety of routes, with food being the primary source. There are important differences among populations and individuals associated with various dietary habits and lifestyle, the range being 1 to 30 µg/kg bw/d estimated from all sources excluding medical and occupational exposure. In general, DEHP exposure assessments from probabilistic calculations from DEHP measurements in environmental media and dose reconstructions from urinary metabolite levels are in a comparable range. Most recent bio-monitoring studies (measuring primary and secondary excreted DEHP metabolites) suggest a current median exposure of 2 to 5 µg/kg bw/d, whereas the 95th percentile is estimated to be between 6 and 17 µg/kg bw/d. Since all DEHP metabolites have been demonstrated to have a

short half-life, it is important to record the time between exposure and urine sampling in order to characterize DEHP exposure. In adults, the ratio between primary and secondary metabolites allows short-term exposures to be distinguished from less recent ones. However, generally but not always, children show higher concentrations of DEHP metabolites than adults, with higher ratios of the secondary oxidative metabolites compared to MEHP. The median exposure of children has been estimated to be around 4 to 8 µg/kg/d.
There are indications that exposure to DEHP in the general population has decreased in recent years.

4.1.2. Exposure to DEHP following medical procedures

Medical procedures using PVC medical devices can lead to DEHP exposures much higher than the levels presented above for the general population, due to DEHP leaching from the device. The extent of exposure largely depends upon the medical treatments administered (Tables 6-8) the duration of the treatment, and in the case of plastic blood bags, by the length of storage and the storage temperature. .

The following procedures have the potential for high exposure to DEHP:

- Exchange transfusion of blood in neonates
- Extracorporeal membrane oxygenation (ECMO) treatment of neonates and of adults
- Total Parenteral Nutrition (TPN) in neonates
- Multiple procedures in preterm neonates
- Haemodialysis
- Enteral nutrition in neonates and adults
- Heart transplantation or coronary artery bypass graft surgery
- Massive blood transfusion of red blood cells and plasma
- Peritoneal dialysis

The exposure during the aforementioned procedures is caused by various types of medical devices used including blood bags, tubing, like catheters, intubation tubes and intravenous catheters and other medical devices made of PVC.

In adults, the highest short-term exposure may result from transfusions of blood components reaching DEHP doses up to approximately 8000-10000 µg/kg bw/day in trauma patients and in patients undergoing ECMO, whereas the highest chronic treatment is represented by haemodialysis, during which the maximum reported exposure is 2200 µg/kg/d. Voluntary medical treatments such as apheresis procedure to donate blood products can cause exposure to DEHP (up to 38 µg/kg/d).

The long-term total parenteral nutrition corresponds to higher exposure for infants and children, leading to a maximum exposure of 2000 µg/d, implying that the lower the body weight, the higher the exposure (i.e. for an infants of 2.5 kg bw the exposure is 800 µg/kg/d). In infants and neonates ECMO is the medical treatment which may give the highest daily exposure over repeated exposure for a short period of time (up to 35000 µg/kg over 10 days treatment in 4 kg bw infants: assuming an equal distribution over time, this would correspond approximately to 3500 µg/kg bw/d).

The FDA (2002) has estimated an upper-bound daily DEHP dose of the order of 3000 µg/kg/d for a newborn (4 kg) in the neonate intensive care unit (NICU) calculated by considering exposure from multiple devices. However, most newborns requiring medical intensive care are

prematurely born babies whose weight is significantly lower, in general between 0.5 and 2.5 kg. Therefore, the DEHP exposure in relation to bw may be even higher in premature newborns (i.e. 8000 µg/kg/d for a neonate 1.5 kg bw).

The risk of high DEHP levels during certain medical procedures needs to be assessed based on the treatment needed and the availability of suitable alternatives for each medical treatment and in some cases, DEHP-containing plasticized PVC devices are important for many treatments and their use is justified because of the benefits of these procedures. An additional benefit of DEHP is that it stabilises the membranes of red blood cells enabling blood product storage in PVC blood bags for several weeks, although some alternatives in conjunction with additive solution have given promising results in this respect.

4.1.3. Toxicokinetics and Metabolism

In human studies, a maximal and rapid oral absorption of 50% was estimated. However, since the amount recovered in the urine depends on the number of urinary metabolite measured, and the amount of excretion via bile is unknown, an almost complete absorption can be used in risk characterization (ECHA, 2013). The bioavailability following parenteral exposure is 100%. Distribution studies in rodents indicate that DEHP is widely distributed in the tissues without evidence of accumulation. In mammals, including man, DEHP is converted into a variety of metabolites (Figure 1) by lipases, ubiquitous enzymes in various tissues, and by cytochrome P450. The first and fast stage in the metabolism of DEHP is the hydrolytic cleavage catalysed by acid lipases (Carrière *et al.*, 1993) to mono(2-ethylhexyl) phthalate (MEHP) and 2-ethylhexanol (2-EH). Further metabolism takes place in the liver: 2-EH is rapidly metabolized to 2-ethylhexanoic acid, which is further oxidised by ω- and (ω-1)-oxidation and subsequent β-oxidation to acetate and CO_2; MEHP metabolism leads to a large number of secondary and tertiary oxidative metabolites, the major being OH-MEHP, COOH-MEHP, 5cx-MEPP and oxo-MEPH, which can be further metabolised. Human urine contains several of these primary, secondary and tertiary metabolites with excretion occurring mostly (more than 90%) within 24h post dose.

The comparison of the DEHP metabolic profile in humans showed a great contrast with that determined in rats. Thus, MEHP and its oxidised derivatives were excreted in rat urine mostly as unconjugated forms (87.4% in free form and only 11.2% as glucuronides in male rat at 24 hr post administration). On the contrary and similarly with humans, in the marmoset urine the metabolites were mostly glucuronide conjugates (87.7% in males at 24 hr). It is suggested that measuring the concentrations of free and conjugated forms separately may be important for estimating their toxicity, since conjugates are not biologically active. The metabolic pathway as well as the secretion pattern of DEHP in humans is qualitatively independent on the exposure routes (oral or i.v.). Neonates and infants (up to 1 year of age) can show a lower levels of conjugated metabolites, due to lower levels of the UDPGT enzymes.

4.1.4. Toxicity

DEHP shows a low oral acute toxicity (LD_{50}>25 g/kg in rats and mice). Lower LD_{50} values were obtained after parenteral exposure (200-250 mg/kg in rats). The acute toxicity of MEHP is about 5 times higher than that of DEHP.

Oral repeated toxicity in rodents indicates that DEHP induces toxicity in the kidney, liver and testis. The NOAEL derived from a two-year study in rats for kidney toxicity is 29 and 36 mg/kg/d in males and females, respectively. The lowest NOAEL for non-neoplastic effects associated with damage in the liver of mice that is 19 mg/kg bw/d, with hepatomegaly due to hepatocyte proliferation (characterised by increased replicative DNA synthesis/cell division and hypertrophy) and peroxisome proliferation.

In rhesus monkeys, sub-chronic liver toxicity has been studied after repeated transfusions through PVC tubing containing DEHP, mimicking conditions of patients undergoing repeated blood or platelet transfusion. The average cumulative amount of DEHP infused over 1 year was 69.3 mg (or 21.3 mg/kg bw) and was found to give hepatic dysfunctions and cholestasis.

DEHP has been studied extensively in a wide range of *in vitro* and *in vivo* assays for detection of gene mutations, DNA damage and chromosomal effects. The results have been negative in the majority of assays with DEHP and MEHP. Few positive results were obtained; however, it is not clear enough if they are associated with a direct interaction of DEHP or MEHP with DNA or rather to secondary oxidative stress or other events. Thus, in a WoE approach, it can be considered that DEHP and its major metabolites are non-mutagenic substances.

Several studies on the carcinogenicity (and mechanisms of carcinogenicity) of DEHP have been performed in rats and mice with oral administration. They demonstrate in rodents the induction of hepatocellular neoplasms. The NOAEL for tumour induction in male mice was 98 mg/kg bw/d, therefore higher than the NOAEL derived from the same study for non-neoplastic effects (i.e. 29 mg/kg/d). Recently, IARC (2012) has considered that there is sufficient evidence in experimental animals for the carcinogenicity of DEHP. Thus, DEHP has been classified as possibly carcinogenic to humans (Group 2B).

The mechanisms for liver carcinogenesis in rodents is mediated by PPARα-activation and consequent peroxisome proliferation, a mode of action which has a threshold and is generally considered not to be relevant in the human liver. This is because (i) human PPARα is expressed at a lower level in human liver than in rats and mice, (ii) no studies have reported any evidence that DEHP activates PPARα in human liver *in vivo,* and (iii) marked species differences with respect to hepatic response to peroxisome proliferation have been demonstrated. However, multiple molecular signals and pathways in several cell types in the liver other than the PPARα-activation have been suggested to be involved in hepatic tumour induction, so that according to the IARC evaluation, the relevance to human cancer of the molecular events that lead to cancer elicited by DEHP in several target tissues (e.g. the liver and testis) in rats and mice cannot be definitely ruled out.

The reproductive and developmental toxicity of DEHP have been studied in rats, mice, hamsters, ferrets and marmosets. On the basis of the obtained results, DEHP is classified under Regulation (EC) No 1272/2008 as toxic to reproduction category 1B. For male reproductive toxicity caused by DEHP, there is a difference in sensitivity between various animal species, rodents are more susceptible than non-human primates, with cynomolgus monkeys and marmosets showing no effect on testicular function after high prenatal DEHP exposure or mono-butyl phthalate (MBP) the active metabolite of di-n-butyl phthalate (DBP) exposure, showing similar bioactive effect and potency in rodents to DEHP. The testis toxicity of DEHP in rodents is age dependent. In marmosets, after postnatal exposure some effects similar to those in rodents were noted. These observations are of importance for extrapolation to humans because in terms of perinatal testis development and function and spermatogenesis in adulthood, the marmoset is considered to be a suitable model for studies relevant to the humans.

The NOAEL for both testicular toxicity and developmental toxicity has been derived from a multigenerational reproductive toxicity study of DEHP in rats (the most susceptible species) equal to 4.8 mg/kg bw/d.

In vitro studies using human foetal testis tissue showed no effect of the metabolites on testosterone production, whereas *in vitro* studies using testis tissue from adult men indicate that DEHP suppress testosterone production.

Altogether, these studies show that the human prenatal testis is not sensitive to anti-steroidogenic effects of DEHP. In contrast, there is an indication about the possibility of some alterations in foetal germ cells but the meaning of this has not been elucidated and it is difficult to extrapolate from in vitro results to the in vivo situation. Effects of DEHP on human germ cells need further investigation.

In the testis, peroxisome proliferator-activated receptors (PPAR) and their subtypes may explain some of the reproductive effects of phthalates. In addition, the anti-androgenic effects of some phthalates on the developing male rat foetus are a direct result of reduced androgen availability in target organs, causing malformations of male reproductive organs and low adult sperm production. In contrast, DEHP and its metabolite MEHP do not have an affinity for the human androgen receptor in an *in vitro* assay.

DEHP in experimental systems has shown the potential to interact with the immune system depending on the exposure conditions. Interestingly from a medical device perspective, immune effects were reported when parenteral routes of administration were used.

4.1.5. Epidemiological and clinical studies

Many epidemiological studies assessed in this Opinion have investigated possible associations between DEHP or similar phthalate exposures and different clinical conditions. The most important include:

Effects on testosterone production and semen quality - A number of studies have examined the association between DEHP exposure of adult men and their blood reproductive hormone levels and semen/sperm quality with considerable variation and inconsistency in the results. Overall, an association between DEHP (or other phthalate) exposure and a decrease in testosterone/free testosterone levels has been reported. However, the described effects are small and unlikely to be of biological significance. Indeed, even in men occupationally exposed to ~100-fold higher levels of DEHP than the general population, a mean decrease in free testosterone levels of only 13% was found.
Regarding semen quality, DEHP was found to be significantly and negatively associated with sperm concentration, normal morphology and motility in one study, but these results have not been replicated in other studies investigating DEHP metabolites in other media. Therefore the association between the small adverse changes in aspects of sperm function (e.g. motility), sperm volume or DNA damage is weak.

Breast tumours- A weak association was found in one study with only one out of 4 DEHP urinary metabolites. Contrasting results were described with other phatalate metabolites in the same study.

Hypospadias and cryptorchidism - No association between prenatal phthalate including DEHP exposure and occurrence of hypospadias or cryptorchidism was found.

Decreased anogenital distance - Published studies show inconsistent evidence for an association between maternal phthalates, including DEHP exposure in pregnancy, and decreased anogenital distance in male offspring.

Mother/infant exposure levels - In a case-control study, higher levels of phthalates were determined in low birth weight (LBW) infants compared with the controls. In utero DEHP exposures were associated with LBW in a dose-dependent manner. However, other studies have found no association between exposure and birth weight.

Childhood growth and pubertal development - There is no evidence of anti-androgenic effects of phthalates including DEHP in healthy boys. Current phthalate exposure was not associated with pubertal timing, testosterone levels or with the presence of pubertal gynaecomastia in a cross-sectional study of boys. In studies of girls no association was found between total phthalate exposure and age at menarche and no association between phthalates and onset of breast development was observed. Three studies have investigated if there were differences in urinary phthalate metabolite levels between girls with precocious puberty and controls; two found no relationship whereas the third found a positive association with total phthalate exposure.

Endometriosis - There is inconclusive evidence on this issue. Some studies indicate a correlation between the phthalate concentrations and the severity of endometriosis and some recent studies have confirmed the correlation. However, recent investigations also found no significant association between endometriosis and phatalate exposure. Further investigations are needed to resolve this disparity.

Effect of DEHP metabolites on neurobehaviour - A recent study on the relationship between the urinary concentrations of phthalate metabolites and intellectual functioning of a sample of children show an inverse relationship between phthalate metabolites and IQ scores. Other results suggest that prenatal exposure to phthalates may be inversely associated with the Mental and Psychomotor Developmental Indices (MDI and PDI, respectively) of infants, particularly males, at 6 months. However there are inconsistencies related to specific phatalates and behavioural domains.

Association with obesity, insulin resistance and type 2 diabetes. Epidemiological studies indicate that exposure to certain phthalates, commonly including DEHP and its metabolites, may be associated with increased waistline, increased body mass index (BMI) and/or obesity and/or type 2 diabetes. However, results are highly inconsistent from study to study and a recent detailed meta-analysis of 18 relevant studies concluded that no consistent associations are evident.

A review of the recent epidemiological literature examining the relationship between early life phthalate exposure and paediatric health outcomes suggests that childhood exposure to DEHP and BBzP may increase the risk of allergic diseases including asthma and eczema.

Epidemiological studies available on DEHP do not establish a cause-effect relationship for harmful effects on humans. The relevance of exposure metrics is often weak, being determined by means of a single urine samples (representative of short term exposure, mainly influenced by dietary habits) in which only a limited number of metabolites is measured. One of the major problems related to the association between exposure and human health effects is related to the correct identification of the level of exposure to DEHP and other phthalates. It is not often clear how the information from a snap shot urine sample affected by the short half-life of these chemicals can be associated with a causality relationship to long-term pathologies or disease requiring a long lag time before their onset. The choice of exposure metric can introduce

significant bias of varying magnitude and direction into the calculation of epidemiologic associations. This can at least partly explain the contrasting results observed in the available epidemiological studies.

However, analysing animal and human data along with mechanistic studies in a WoE approach, allows us to conclude that male foetuses of pregnant women and male neonates are potential groups at risk based on exposure levels above those that induce reproductive toxicity in rodent animal studies.

4.1.6. Risk evaluation and characterization

The target organs of chronic toxicity are the liver, the kidney and the testis. In the liver of rodents, hepatic tumours are also induced, with a threshold mechanism involving the PPARα activation that has not been considered relevant for humans. Although the involvement of other mechanisms could not be ruled out, the tumours are induced at doses 4-5 fold higher than those eliciting non-neoplastic effects. The epidemiological evidence investigating adverse health effects in humans and DEHP exposure is generally inconsistent and needs further clarification.

The lowest relevant NOAEL is the one related to testicular and developmental toxicity equal to 4.8 mg/kg bow/d, to which an assessment factor of 100 was applied to derive the TDI of 48 rounded to 50 mg/kg bw/d (ECB, 2008; EFSA, 2005). SCENIHR supports the previously derived TDI value, considering that the new studies are in line or not sufficiently robust to justify the derivation of a new TDI.

It should be noted that TDI is a value set up for a lifelong continuous exposure, in contrast with the transient acute or subacute exposure by most of the DEHP-containing medical devices, with some exceptions (i.e. dialysis or prolonged intensive care treatment). However, if such an exposure is below the TDI, the risk can be considered extremely low, so the TDI can be a useful starting point for the risk assessment.

The key factors influencing the risks to individual patients arising from the use of DEHP used in medical devices are 1) background exposure; 2) exposure dose (leaching from each medical device used); and 3) vulnerability of patients (including the time window of the exposure). In any case the considered scenarios give rise to exposures higher than the background levels of the general populations.

DEHP exposure scenarios based on medical treatments are generally associated with acute or short term exposure. Blood transfusions to trauma adult patients or during ECMO may be the short-term procedure that gives the highest DEHP exposure in adults (up to 10000 µg/kg/d). Although the acute toxicity of DEHP is limited, also after parenteral exposure, levels are quite high and should be considered with some caution in case of short term repeated exposure.

Adult patients undergoing haemodialysis are considered to have the highest exposure, due to the chronic nature of the treatment. Long-term haemodialysis is the continuously repeated procedure, which may result in the highest cumulative dose of DEHP (up to 2200 µg/kg/d). The TDI is a reference value for chronic toxicity, therefore it can be appropriately used for the haemodialysis scenario. Median exposure levels reported in various studies exceed the TDI by 2-12 fold with peak values >40 fold higher than the TDI.

Children are potentially at higher risk, considering the DEHP toxicological profile, although humans have been indicated as less susceptible than rodents. Neonates and infants due to

their low body weight are particularly prone to a high level of exposure on a body weight basis. For blood transfusion in neonates a peak value of up to 22000 µg/kg bw/d has been estimated after transfusion of three consecutive blood units (median values are generally one order of magnitude lower). In infants and neonates ECMO is the medical treatment which may give the highest daily exposure over repeated exposure for a short period of time (up to 35000 µg/kg over 10 days treatment in 4 kg bw infants: assuming an equal distribution over time, this would correspond approximately to 3500 µg/kg bw/d).

In addition neonates in the Neonatal Intensive Care Unit (NICU) environment are very often subjected to multiple medical device-related DEHP exposure, leading to an exposure up to 6000 µg/kg bw/d, also for quite a long period of time (several weeks to months for very low-weight pre-term newborns). Due to their physical conditions, the immaturity of many systems and organs and their small size, neonates represent a particularly vulnerable population.

The exposure estimated for neonates in NICU are in the same range as the doses inducing developmental and reproductive toxicity in animal studies, without any margin of safety. Therefore, premature neonates in NICU and infants subjected to repeated medical treatment with medical devices represent the most vulnerable population, both for the levels of DEHP exposure and also due to their phases of life, particularly with regard to developmental and reproductive toxicity. Therefore, premature neonates in NICU and infants subjected to ECMO represent a high-risk population to DEHP exposure.

4.1.7. Toxicity of alternative plasticizers

The information available on leaching from alternative plasticizers is sparse, and does not allow any conclusions to be drawn. Identification of TOTM leaching, for instance, is lower than DEHP, but when tested TOMT and DINCH-based materials release DEHP as well, likely due to impurities; ATBC and DOTP leaching is higher than DEHP in other studies. It can be estimated that leaching of the different plasticizers is similar, based on their similar structures and properties and the few available data, generally in the range of 2-10 fold lower than DEHP.

Regarding toxicity, with the exception of BHTC and COMGHA and the uncertain results of DINCH, all the considered plasticizers can cause reproductive toxicity, although this occurs at doses several fold higher than DEHP and equal or higher than the critical end-point for their own toxicity. DINP, DINCH and DEHA have some carcinogenic potential, although the mode of action is irrelevant (as in the case of kidney tumours mediated by alpha-2-µ-macroglobulin) or not clear (as for the liver and thyroid tumours) for human health; maternal toxicity was similar or higher than DEHP for some other alternatives (such as DINP, ATBC, DEHA, DEHT). Other plasticizers such as COMGHA, BTHC and TOTM could not be evaluated for all endpoints because of few or poorly relevant data available. As already mentioned in the case of COMGHA and TOMT which are poorly absorbed after oral administration, oral studies are of limited use to assess the risk for the possible release from medical devices for which the parenteral routes are the most relevant.

However, paucity or the lack of data on their release from medical devices and consequent human exposure does not allow an appropriate risk assessment to be carried out. In addition aggregate exposure should be taken into account because they are used in many other consumer products (including food contact material and toys) and dust and air samples may contain these plasticizers. Castor-oil-mono-, hydrogenated, acetates (COMGHA) and TOTM could not be properly evaluated, since the only data available are from oral studies and since they are very poorly absorbed via the g.i. tract, these data are of limited use for the parenteral

route of exposure. Therefore there is a strong need to develop and collect data on exposure of alternative materials in the actual conditions of use, to refine the knowledge on their toxicological profile. The possibility of replacing DEHP with these products could then be considered taking account the efficacy in the treatment, as well as the toxicological profile and leaching properties of the alternative materials.

4.2 Responses to the questions in the Terms of Reference

Taking into consideration recent scientific developments, the SCENIHR is requested to review and update, if appropriate, the scientific Opinion adopted in February 2008 on "The safety of medical devices containing DEHP plasticized PVC or other plasticizers on neonates and other groups possibly at risk".

In particular, the Scientific Committee is requested to evaluate:

• *If DEHP in PVC plasticized medical devices is a cause for concern to neonates and children in paediatric care, in particular in relation to male fertility and tissue development.*

The degree of risk posed by exposure to DEHP in a medical procedure is determined largely by two factors. The first is the patient's sensitivity to DEHP and the second consists of the dose received which depends upon the type of procedure performed, as well as the frequency and duration of these procedures. Background levels due to DEHP exposure typical for the general population are much lower than the ones deriving from most of the treatments with PVC plasticized medical devices considered in this Opinion.

Based on the data available, the main concern derives from the animal studies showing that immature young animals are more susceptible to testicular toxicity by DEHP than older mature animals, although humans appear to be less sensitive than rodents. The developing foetus, premature neonates and neonates represent the most vulnerable phases of life particularly with regard to developmental and reproductive toxicity. As for the extent of exposure, this group is dependent in some cases on multiple medical procedures (e.g., intensive care units) and can receive doses of DEHP up to 6000 µg/kg bw/d. These exposures are in the same range as the doses inducing developmental and reproductive toxicity in animal studies without any margin of safety. Consequently, this group would appear to be a high-risk population to DEHP exposure and still a cause of concern in paediatric care.

In addition, for blood transfusion procedures in neonates peak values up to 22000 µg/kg bw/day (median values one order of magnitude lower) have been estimated. In infants and neonates, ECMO is the medical treatment which may give the highest daily exposure over repeated exposure for a short period of time (up to 35000 µg/kg over 10 days treatment in 4 kg bw infants: assuming an equal distribution over time, this would correspond approximately to 3500 µg/kg bw/d). Although these concern short-term exposures, they might represent a health concern for infants subjected to medical treatment using PVC plasticized medical devices.

There is only one follow-up study of a few highly exposed neonates (13 males) and this did not indicate an effect of DEHP on the human male reproductive system. There have been no large-scale or long-term follow-up studies of neonates highly exposed to DEHP due to medical interventions, so this is an unresolved issue; any such study will to take particular account of confounding effects of low birth weight/prematurity.

- *If there are other patient groups at risk, in particular in view of clinical procedures resulting in high exposure*

- *If it is possible to establish Tolerable Intake Values of DEHP leaching from soft PVC as a basis for risk assessment for high risk patient groups, taking into account the route of exposure.*

The scenarios consequent to medical treatments are generally associated with acute or short term exposure. The Tolerable Daily Intake (TDI) is a reference value relating to lifelong continuous exposure. Therefore, the use of TDI value for risk assessment associated with exposure via medical device represents a conservative approach. However, if such an exposure is below the TDI, the risk can be considered extremely low, so the TDI can be a useful starting point for the risk assessment. The exception is the group of dialysis patients, whose regimen of treatment can be considered chronic.

Blood transfusions to trauma adult patients or to patients undergoing ECMO may be the short-term procedure that gives the highest acute DEHP exposure in adults (up to 10000 µg/kg/d). Although the acute toxicity of DEHP is limited also after parenteral exposure, levels are quite high and should be considered with some caution in case of short-term repeated exposure.

Patients undergoing haemodialysis are considered to have the highest exposure, due to the chronic nature of the treatment. Long-term haemodialysis is the continuously repeated procedure, which may result in the highest cumulative dose of DEHP (up to 2200 µg/kg/d). Since the TDI is a reference value for chronic toxicity, it can be appropriately used for the haemodialysis scenario. The TDI value of DEHP was established at 48 µg per kg bw/d (rounded by the EFSA at 50 µg/kg bw/d), which was based on a No Observed Adverse Effect Level (NOAEL) of 4.8 mg/kg/d for reproductive toxicity in rats to which an assessment factor of 100 was applied. Median exposure levels reported in various studies exceed the TDI by 2-12 fold with peak values >40 fold higher than the TDI. The exposure values have a small Margin of Safety (lower than 100), also considering the NOAEL in rodents for induction of kidney toxicity (around 30 mg/kg/d), which is particularly relevant for that kind of patients. Therefore patients subjected to haemodialysis procedure are at risk of DEHP induced effects.

- *If it is possible, to propose possible alternative approaches that could reduce potential risks either by identifying alternative practices or by identifying alternatives to the use of DEHP in PVC plasticized in medical devices. If no clear answer can be provided on this point the SCENIHR is asked to formulate recommendations for research that could help provide scientific evidence to that end.*

The information available on leaching from alternative plasticizers is sparse, and does not allow any conclusions to be drawn. Identification of TOTM leaching, for instance, is lower than DEHP, but when tested TOMT and DINCH-based materials release DEHP as well, likely due to impurities; ATBC and DOTP leaching is higher than DEHP in other studies. It can be estimated that leaching of the different plasticizers is similar, based on their similar structures and properties and the few available data, generally in the range of 2-10 fold lower than DEHP.

Regarding toxicity, with the exception of BHTC and COMGHA and the uncertain results of DINCH, all the considered plasticizers can cause reproductive toxicity, although this occurs at doses several fold higher than DEHP and equal or higher than the critical end-point for their own toxicity. DINP, DINCH and DEHA have some carcinogenic potential, although the mode of action is irrelevant (as in the case of kidney tumours mediated by alpha-2-µ-macroglobulin) or not clear (as for the liver and thyroid tumours) for human health; maternal toxicity was similar or higher than DEHP for some other alternatives (such as DINP, ATBC, DEHA, DEHT). Other

plasticizers such as COMGHA, BTHC and TOTM could not be evaluated for all endpoints because of few or poorly relevant data available. As already mentioned in the case of COMGHA and TOMT, which are poorly absborbed after oral administration, oral studies are of limited use to assess the risk for the possible release from medical devices for which the parenteral routes are the most relevant.

However, paucity or the lack of data on their release from medical devices and consequent human exposure does not allow an appropriate risk assessment to be carried out. In addition, aggregate exposure should be taken into account because they are used in many other consumer products (including food contact material and toys) and dust and air samples may contain these plasticizers. Castor-oil-mono-, hydrogenated, acetates (COMGHA) and TOTM could not be properly evaluated, since the only data available are from oral studies and since they are very poorly absorbed via the g.i. tract, these data are of limited use for the parenteral route of exposure. Therefore there is a strong need to develop and collect data on exposure of alternative materials in the actual conditions of use, to refine the knowledge on their toxicological profile. The possibility of replacing DEHP with these products could then be considered taking into account the efficacy in the treatment, as well as the toxicological profile and leaching properties of the alternative materials. Whilst recognising that there are several non-PVC based materials that can be effective in medical devices production and use, this Opinion does not address these materials.

5. RESEARCH NEEDS

Compared to the previous Opinion there are still inconsistencies for most of the outcomes of adverse effects in humans, so these need further confirmations in well performed epidemiological studies. Studies on animals or *in vitro* studies suggest outcome other than reproductive toxicity (on which the NOAEL used as point of departure for deriving the TDI is based), but their meaning with respect to human health effects has to be elucidated. Kinetic information to better understand the route-to-route extrapolation and the identification of the UDPGT isoform active in detoxifying phthalates could give further information about susceptible populations, endowed with lower activities. This is especially needed for neonates highly exposed to DEHP within intensive care units and infants. There have been no large-scale or long-term follow-up studies of neonates/infants highly exposed to DEHP due to medical interventions, so this is an unresolved issue; any such study will need to take particular account of the confounding effects of low birth weight/prematurity.

It is important for blood establishments, suppliers and manufacturers of medical devices in general to work together to ensure that research into alternative plasticizers, or non-PVC alternatives, for use in blood establishments as well for other purposes continues to progress both in the area of defining the toxicological profile and the leaching potential in the intended conditions of use, to evaluate the human exposure. Alternatives already in use for a number of applications should be monitored in follow-up studies.

6. CONSIDERATION OF THE RESPONSES RECEIVED DURING THE CONSULTATION PROCESS

A public consultation on this opinion was opened on the website of the non-food ScientificCommittees from 22 October to 30 November 2014. Information about the public consultation was broadly communicated to national authorities, international organisations and other stakeholders.

15 organisations and individuals participated in the public consultation providing 69 comments to different chapters and sections of the Opinion. Each submission was carefully considered by the SCENIHR and the scientific Opinion has been revised to take account of relevant comments. The literature has been accordingly updated with relevant publications.

The text of the comments received and the response provided by the SCENIHR is available at:

http://ec.europa.eu/health/scientific_committees/consultations/public_consultations/scenihr_consultation_25_en.htm

7. MINORITY OPINION

None.

8. LIST OF ABBREVIATIONS

2cx-MMHP	Mono-[2-(carboxymethyl)hexyl] phthalate
2cx-MEHP	Mono-[2-(carboxyethyl)hexyl] phthalate
2-EH	2-Ethylhexanol
5-OH-MEHP	Mono-(2-ethyl-5-hydroxyhexyl) phthalate
6-OH-MEHP	Mono-(2-ethyl-6-hydroxyhexyl) phthalate
5OH-MEHP	Mono-(2-ethyl-5-hydroxyhexyl) phthalate
5cx-MEPP	Mono-(2-ethyl-5-carboxypentyl) phthalate
5oxo-MEHP	Mono-(2-ethyl-5-oxohexyl) phthalate
ABO	ABO blood group system
AGD	Anogenital distance
AGI	Anogenital distance (mm/kg bw)
COMGHA	Glycerides, Castor-oil-mono-, hydrogenated, acetates
ASTM	American Society for Testing and Materials
ATBC	Acetyl-tri-n-butyl citrate
AUC	Area under curve
BALB	Albino laboratory-inbred strain of the house mouse
BBP	Butyl benzyl phthalate
BBzP	Benzylbutylphthalate
BMI	Body Mass Index
BTHC	Buturyl-tri-n-hexyl citrate
Bz-β-CD	2,3,6-per-O-benzoyl-β-cyclodextrin
CAPD	Continuous ambulatory peritoneal dialysis
CAS	Chemical Abstracts Service
CERHR	Center for the Evaluation of Risks to Human Reproduction
CHO	Chinese Hamster Ovary
CI	Confidence interval
COMGHA	Glycerides, Castor-oil-mono-, hydrogenated, acetates
CPs	Chlorinated paraffins
CSTEE	Scientific Committee on Toxicity, Ecotoxicity and the Environment
cx-MINP	Carboxylated MINP
DBP	Di-n-butyl phthalate
DEHA	Di(2-ethylhexyl) adipate
DEHP	Di(2-ethylhexyl) phthalate
DEHT	Di(2-ethylhexyl) terephtalate

DEP	Diethyl phthalate
DFI	DNA Fragmentation Index
DG	Directorate General
DFI	DNA Fragmentation Index
DIBP	Di-iso-butyl phthalate
DIDP	Di-iso-decyl phthalate
DINCH	Di-iso-nonyl 1,2-cyclohexanedicarboxylate
DINP	Di-iso-nonyl phthalate
DMP	Dimethyl phthalate
DNA	Deoxyribonucleic acid
DOP	Di-n-octyl phthalate
DOTM	Dioctyl trimellitate
DOTP	Dioctyl terephthalate
DSC	Differential Scanning Calormetry
E2	Estradiol
EC	European Commission
ECB	European Chemical Bureau
ECDC	European Centre for Disease prevention and Control
ECHA	European Chemicals Agency
ECMO	Extracorporeal membrane oxygenation
EFSA	European Food Safety Authority
ELO	Epoxidised linseed oil
EMEA	European Medicines Evaluation Agency
EN	Enteral Nutrition
ESBO	Epoxidised soya bean oil
EU	European Union
FAI	Free Androgen Index
FSH	Follicle-stimulating hormone
FDA	US Food and Drug Administration
fT	Free testosterone
GLP	Good laboratory practice
GVHD	Graftvs-host disease
HPLC-RID/MS	High Performance Liquid Chromatography/radioisotope detection/mass spectrometry
IARC	International Agency for Research on Cancer
IgE	Immunoglobulin E

IgG_1	Subclass of Immunoglobulin G
IgG_{2a}	Subclass of Immunoglobulin G
JEM	Job-exposure matrix
LH	Luteunizing hormone
LOAEL	Lowest observed adverse effect level
MBP	Mono-n-butyl phthalate
MBzP	Monobenzyl phthalate
MBBP	Monobutylbenzylphthalate
MBzP	Monobenzyl phthalate
MDI	Mental Developmental Index
MECPP	Plastidial metabolite
MEHHP	Mono(2-ethyl-5-hydroxyhexyl) Phtalate
MEHP	Mono(-2-ethylhexyl) phthalate
MEOHP	Mono-(2-ethyl-5-oxohexyl) phthalate
MEP	Monoethyl phthalate
MIBP	Mono-iso-butyl phthalate
MINP	Mono-iso-nonyl phthalate
MMP	Monomethyl phthalate
MnBP	Mono-n-butyl phthalate
MoE	Margin of Exposure
MoS	Margin of Safety
MOTM	Monooctyl trimellitate
MOTP	Monooctyl terephthalate
NHANES	National Health and Nutrition Examination Survey
NOAEL	No observed adverse effect level
NOEL	No observed effect level
NICU	Neonate intensive care unit
NTP	US National Toxicology Programme
OECD	Organisation for Economic Co-operation and Development
OH-MEHP	Mono(2-ethyl-5-hydroxyhexyl) phthalate
OH-MINP	Hydroxylated MINP
OR	Odds ratio
OVA	Ovalbumin
oxo-MINP	Oxygenated MINP
oxo-MEHP	Oxygenated MEHP
PDI	Psychomotor Developmental Index

PPARα	Peroxisome-proliferator activated receptor
PRBC	Packed red blood cells
P-PVC	Plasticized PVC
PVC	Polyvinylchloride
RAR	Risk Assessment Report
RBC	Red blood cells
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
SANCO	Directorate General for Health and Consumer Protection
SAP	Stearic acid, 2,3-bis(acetoxy)propyl ester
SCCP	Scientific Committee on Consumer Products
SCHER	Scientific Committee on Health and Environmental Risks
SCENIHR	Scientific Committee on Emerging and Newly-Identified Health Risks
SCMPMD	Scientific Committee on Medical Products and Medical Devices Opinion
SD	Standard Deviation
SHBG	Sex-hormone binding globulin
SF-1	Steroidogenic factor-1
SPPVC	Sulfonated PPVC
TDI	Tolerable Daily Intake
Tg	Glass transition temperature
TIV	Tolerable Intake Value
Tm	Melting Temperature
TOTM	Tris(2-ethylhexyl)benzene-1,2,4-tricarboxylate
TPA	Terephthalic acid
TPN	Total parental nutrition
TOTM	Trioctyl trimellitates
TSH	Thyroid stimulating hormone
USA	United States of America
VCL	Curvilinear Velocity
VSL	Straight-line Velocity

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ANNEX I: EVALUATION OF INDIVIDUAL PLASTICIZERS

No information was submitted on the DINP and DEHA plasticizers, but they have been included in this assessment as they are already being used to substitute DEHP in a number of applications.

1. ATBC (Acetyl tri-n-butyl citrate)

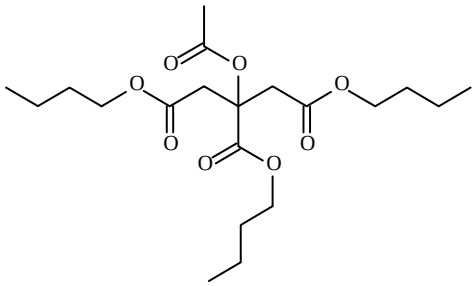
1.1. Physico-chemical properties

CAS Reg. No.:77-90-7

Synonyms: Citroflex A-4; 2-(acetyloxy)-1,2,3-propanetricarboxylic acid, tributyl ester; 1,2,3-propanetricarboxylic acid, 2(acetyloxy)-, tributyl ester; acetylcitric acid, tributyl ester; citric acid, tributyl ester, acetate; tributyl acetylcitrate; tributyl *O*-acetylcitrate; tributyl-2-(acetyloxy)-1,2,3-propanetricarboxylate; tributyl citrate
Acetate.

Emperical formula: $C_{20}\ H_{34}O_8$

Structure:



Molecular weight: 402.5

Melting point: -80°C

Boiling point: 173°C (1 mm Hg)
200°C (4 mm Hg)
326°C (160 mm Hg)

Vapour pressure: 0.052 mm Hg (20°C)

Solubility in water: 20 mg/L

Log Kow: 4.3 (estimated)

Purity: >99%

Impurities: Water, volatiles.

1.2. Use

ATBC is used as a plasticizer in cosmetics, in concentration of 0.7 to 7%. The substance is also used as a plasticizer in PVC, adhesives and coatings. ATBC has been approved for many food applications, including the use as a flavouring substance, in the USA. The use of ATBC in medical devices is mainly in blood bags, but also about 350 tons are used for the production of

medical tubing (Reilly Chemicals, 2006). According to latest information ATBC is mainly used in medical tubings

The substance is registered under REACH at a tonnage band > 1000 tpa. According to German/Austrian search, the following information with respect to use can be given: wide dispersive use and consumer use; Primarily used as a plasticizer in cosmetic products, toys, vinyl, adhesives, medical devices, pharmaceutical tablet coatings, food packaging, flavouring substance in foods, printing inks and plastics in concrete. Also used as a surface lubricant in the manufacture of metallic articles in contact with food.

1.3. Exposure

No information has been found describing human exposure. Higher leaching rate was found for ATBC as compared to DEHP (Welle *et al.*, 2005).

1.4. Toxicokinetics

ATBC is well absorbed after oral administration with peak blood levels being found between 2 and 4 h. It undergoes rapid and extensive metabolism to >10 polar metabolites by rat liver samples and in human serum. The first step of metabolism is hydrolysis of the ester bonds. Blood clearance of ^{14}C labelled ATBC is biphasic with corresponding half-lives of 3.9h and 39h. The slow second phase may be an artefact due to some of the radiolabel entering intermediary metabolism pathways. The main route of excretion is through the urine with monobutyl citrate being the major urinary metabolite. However some metabolites are also found in the faeces. Whether this indicates that some ATBC is biliary excreted or not absorbed is uncertain. The kinetic data indicate that ATBC has a low bioaccumulation potential in body tissues.

1.5. Toxicity

Acute toxicity

After a single oral dose of 10-30 grams per kg bw/d, administered by gavage, no systemic toxicity has been observed. ATBC can therefore be regarded as virtually nontoxic by the oral route when its administration is acute. In view of its prompt metabolism and excretion and the likelihood that it is metabolised at multiple sites to more polar metabolites it appears unlikely that ATBC will cause significant toxicity.

Irritation and sensitisation

ATBC applied dermally to rats produces moderate irritation, but it is a non-irritant following topical application to rabbits. ATBC is not a sensitiser in the guinea pig maximisation test. This finding is supported by the results of studies in which ATBC was applied to the skin of human volunteers.

Repeated dose toxicity

Three studies can be identified. The first was a four week range finding study in rats. At the highest dose (equivalent to about 2700 mg/kg bw/d) there was a small decrease in both body and organ weight. However no effects were observed in a second group of rats exposed to the lower dose of around 1000 mg/kg bw/d.

The second study was a 90 day gavage study in male and female rats. Some haematological and biochemical changes in the blood were observed at 300 mg/kg bw/d and at 1000 mg/kg bw/d there was an increase in liver weight. However no histopathological changes were seen in

either test groups. At 100 mg/kg bw/d no changes of any kind were seen and therefore, this dose may be regarded as the NOEL.

Mutagenicity and genotoxicity

A range of *in vitro* genotoxicity tests have been conducted. In bacterial tests ATBC gave consistently negative results both with and without the presence of a metabolising system. ATBC also gave negative results in 2 chromosomal aberration studies with rat lymphocytes both in the presence and absence of a metabolising system. However in mouse lymphoma cells a dose dependent increase in mutations at the HK locus was identified in 2 separate experiments.

An *in vivo* test has also been conducted using unscheduled DNA synthesis (UDS) as the endpoint. In rats treated by gavage at either 800 or 2000 mg/kg bw/d no increase in UDS could be observed. This finding indicates a low potential of ATBC to cause genotoxic effects *in vivo*. This conclusion is supported by consideration of the structure of both ATBC and its metabolites for which there are no structural alerts.

Carcinogenicity

A 2 year oral feeding study has been carried out in rats in which no significant toxic effects relating to ATBC were identified. However this study does not meet modern standards and, therefore, caution should be used in accepting this conclusion. The study does however show that ATBC is not a potent carcinogen and this is in line with the other findings discussed above.

Reproductive toxicity

Two relevant studies are available. In the first, a 2 generation study in rats, ATBC was administered in the diet at levels equivalent to 0, 100, 300 and 1000 mg/kg bw/d. The 300mg and 1000 mg doses produced a decrease in body weight in F1 male rats. In female rats a decrease in body weight was only observed at the top dose (1000 mg/kg bw/d). Thus the NOEL was identified as 100 mg/kg bw/d.

In a second study rats were exposed to ATBC in the diet at doses of 0, 100, 300 and 1000 mg/kg bw/d for four weeks before mating and then throughout the mating period. The offspring (i.e. the F1 generation) were then exposed to ATBC *in utero*, at birth and for the following 13 weeks. No effects of ATBC could be identified in any of a number of reproductive endpoints. Litter size, survival and growth rates were comparable in the control animals and all the test groups. No adverse effects were identified in any of the offspring examined and no adverse endocrine effects could be detected.

In line with the rat studies summarized above, there were some subtle liver changes (increase in weight, hypertrophy and mild peroxisome proliferation) and renal changes (some changes in urinary composition) in both sexes at the top (1000 mg/kg bw/d) dose. Minor changes were also observed in male animals at the 300 mg/kg bw/d. A NOEL of 100 mg /kg bw/d can therefore, be accepted.

1.6. Human data

No information available on toxicity in humans.

1.7. Other information

Ohta *et al.* (2003) investigated estrogenic and androgenic properties of ATBC *in vitro* and estrogenic activity *in vivo*. There were no indications of estrogenic activities in ligand-binding assays (ER, AR) *in vitro* and in *in vivo*-experiments where ovarectimised Sprague-Dawley-rats received either oral doses of 0.5 and 500 mg/kg or subcutaneous doses of 0.5 and 100 mg/kg ATBC.

1.8. Conclusion

ATBC is well absorbed following its oral administration. It is rapidly metabolised and excreted from the body. It is unlikely to accumulate in the body following frequent exposure. It has a low toxicity following acute oral administration. In repeated dose studies the oral NOEL was 100mg/kg kg bw/d, based on decreased bw; haematological and biochemical changes; increased liver weight at the higher doses.

ATBC was found to be non genotoxic; moreover in a lifetime bioassay study in rats (although not meeting modern standards for that kind of test) no dose related tumours were found.

No data are available on humans. The only indication available related to leaching potential from medical devices, suggests a higher rate than DEHP. More information are necessary on this aspect to clarify human exposure in the actual conditions of use of medical device as well as on differences among oral vs parenteral route of exposure.

References:

Johnson W (2002). Final report on the safety assessment of acetyl triethyl citrate, acetyl tributyl citrate, acetyl trihexyl citrate, and acetyl trioctyl citrate. Int J Toxicol. 21 Suppl 2:1-17.

Submission from Reilly Chemicals.

The EFSA Journal (2005) 273, 1-26.

2. BTHC (n-Butyryl-tri-n-hexyl citrate)

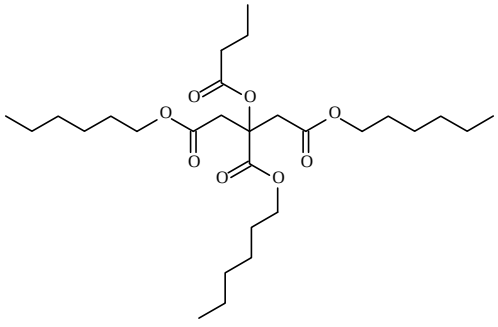
2.1. Physico-chemical properties

CAS Reg. No.: 82469-79-2

Synonyms: Citroflex B-6

Emperical Formula: $C_{28}H_{50}O_8$

Structure:



Molecular weight: 514.7

Melting point: -55°C (pour point)

Boiling point:

Vapour pressure:

Solubility in water: < 1g/L at 25°C

Log Kow: 8.2 (estimated)

Purity: >99%

Impurities: Volatiles 1.3%, water max 0.15%, heavy metals max. 10 ppm

2.2. Use

The use pattern for BTHC is similar to that of ATBC. According to latest information BTHC is mainly used in the production of blood bags.

2.3. Exposure

No information has been found describing human exposure. Slightly lower leaching rate was found as compared to DEHP, but information are very scant.

2.4. Metabolism and toxicokinetics

BTHC is well absorbed after oral administration. It is rapidly metabolised by hydrolysis of the ester bonds to a number of metabolites. The main metabolite is n-hexanol. There are no structural alerts for any of the metabolites. Radiolabelled BTHC is cleared rapidly from the body following iv administration through a combination of urinary and biliary excretion and expired air. BTHC related material does not accumulate in any of the body tissues. The clearance is biphasic with half-lives of <15 minutes and >24h. The latter half-life indicates that the radiolabel is widely incorporated into intermediary metabolism pathways. The findings indicate that BTHC is unlikely to accumulate in the body even after a prolonged period of exposure.

2.5. Toxicity

Acute toxicity

No mortality was observed by the oral route in rats for BTHC up to 5000 mg/kg bw. Acute iv injection studies with doses of up to 462 mg/kg bw did not produce any significant adverse effects. In dogs at the same iv dose level the only changes of note observed were in serum glutamate pyruvate transaminase and alkaline phosphatase. It can be concluded that BTHC has a low acute toxicity.

Irritation and sensitisation

One acute dermal study in rabbits indicates that BTHC is a very mild irritant to the skin. In a second study in rabbits undiluted BTHC (0.1ml) produced a mild and transient reaction when instilled into the eye.

Findings from the maximisation test method in guinea pigs using undiluted BTHC show a slight patchy erythema in 1 male and 1 female animal only. A further study using the Buehler

method did not show any indication of sensitisation. It can be concluded that under the conditions of these experiments BTHC has a low irritation and sensitisation potential

Repeated dose toxicity

The toxicological properties of BTHC have been investigated by both the oral and iv routes of administration. In an oral dosing study rats were given BTHC by gavage at 0, 250, 500 or 1000 mg/kg bw/d for 28 days. No clinical signs of toxicity were observed during the study. Statistically significant increases in the relative liver weight of males were noted at 500 and 1000 mg/kg bw/d but no absolute changes in liver weight were found. Statistically significant changes in urinary pH, aspartate aminotransferase, blood albumin, creatinine and blood calcium were found at the higher dose levels. These findings did not show a clear dose dependency nor were the changes consistent between the sexes. It is difficult to identify a precise NOAEL from these findings but a value of 250 mg/kg bw/d is reasonable.

In one study, BTHC was administered intravenously to adult rats at dose levels of 5, 50 and 500 mg/kg bw/d for 28 days. At 500 mg/kg bw/d no changes were observed in kg bw, but there were moderate increases in both liver and spleen weight. These changes were associated with an accumulation of pigment-laden macrophages in both organs. This dose group also showed statistically significant changes in some blood parameters. Namely, a decrease in haemoglobin, MCV and platelet levels and an increase in fibrinogen and reticulocyte levels. No other adverse histopathological changes were observed in any organs. No adverse effects were observed at the lower dose group. Thus a NOEL by the iv route of 50mg/kg bw/d can be identified.

A study was conducted in neonatal rats. BTHC was administered daily either iv or ip to male and female neonatal rats at 5, 50 and 500 mg/kg bw/d for eighteen days. At the top dose of BTHC following ip administration an increase in liver weight was noted but without evidence of adverse histopathological changes. After iv administration some histopathological changes were also observed in the lungs (macro granulomas and foreign body infiltration) at each dose. These effects following iv administration are probably due to the route of administration rather than to BTHC itself. By either administration route some tissue damage was noted around the injection sites. The study supports a NOEL by the iv and ip routes of 50 mg/kg bw/d.

A specific study was also conducted to investigate the potential of BTHC to cause peroxisome proliferation. Rats were given 3% BTHC in the diet for six weeks. No increase in hepatic peroxisome proliferation was found.

Mutagenicity and genotoxicity

No mutagenic effects were observed for BTHC in several bacterial tests either with or without the presence of a metabolic activation system. In one study the urine, from mice given oral doses of BTHC of up to 1000 mg/kg bw/d, was assessed in various Ames strains of salmonella. No mutagenic effects were observed.

In mouse lymphoma cells BTHC produced different findings in 2 experiments. In the first there was a slight, but statistically significant increase in mutations whereas in a second comparable experiment no significant changes were observed.

Using human peripheral lymphocytes no significant alteration in the incidence of either chromosomal breaks or mitotic frequency was found.

One *in vivo* study was also carried out in a bone marrow cytogenetic assay. Mice were given an oral dose of 1000 mg/kg bw/d either as an acute dose or daily for 5 days. In neither study was there any indication of BTHC genotoxicity.

It can be concluded that BTHC is not genotoxic. This conclusion is supported by the lack of structural alerts for both BTHC and its metabolites

Carcinogenicity

A lifetime bioassay test has not been conducted. However it is noted that BTHC is neither genotoxic nor is it a peroxisome proliferating agent.

Reproductive toxicity

A fertility study was carried out in albino rats at dietary levels of 0, 0.6 or 1.2% BTHC. Males were exposed to BTHC continuously to BTHC for 10 weeks prior to mating and during the mating period. Females were exposed for 2 weeks before mating, during mating, gestation and lactation. No effects on fertility and other reproductive indices, or on litter weights and pup weights were observed. The body weight of the lactating females exposed to the top dose was slightly lower. No increase in abnormalities in the F1 pups was found.

Developmental toxicity was also examined in rats following the iv administration of BTHC (0, 5, 50, 500 mg/kg bw/d)) on days 6-15 of gestation. No deaths or dose dependent changes in kg bw or uterine weight were identified. Nor were any dose related changes observed in resorptions, or embryo or foetal development or foetal toxicity. However in line with the findings from repeat dose studies changes were observed in liver, lung and spleen weight in the mothers.

An NOEL for foetal/embryo toxicity of 500 mg/kg bw/d can be estimated in this study.

2.6. Human data

No information available on toxicity in humans.

2.7. Conclusion

BTHC well absorbed following its oral administration. It is rapidly metabolised and excreted from the body. It is unlikely to accumulate in the body following frequent exposure. It has a low toxicity following acute administration by either the oral or iv routes. In repeat dose studies only non-specific effects were found. The oral NOEL was 250 mg/kg bw/d and the iv NOEL was 50 mg/kg bw/d.

BTHC was found to be non genotoxic and did not initiate hepatic peroxisome proliferation in rats. No effects of BHTC could be found in rats on reproductive efficiency nor were dose dependent foetal abnormalities or foetal deaths identified.

Slightly lower leaching rate was found as compared to DEHP, but information are very scant.

References:

Submission from Reilly Chemicals.

3. COMGHA (Glycerides, Castor-oil-mono-, hydrogenated, acetates)

3.1. Physico-chemical data

COMGHA is a mixture of 2 components: A (Ca. 84%: 12-(Acetoxy)-stearic acid, 2,3-bis(acetoxy)propyl ester) and a minor component B (Ca. 10%: Octadecanoic acid, 2,3-(bis(acetoxy)propyl ester).

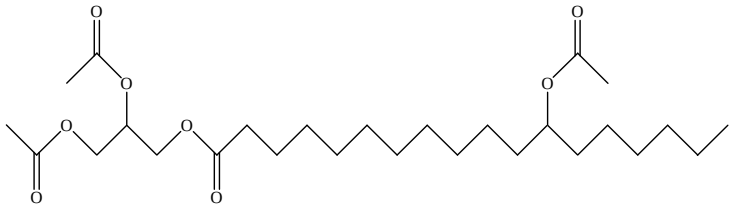
CAS Reg No : 736150-63-3 (COMGHA); Reg. No.: 330198-91-9 (component A); 33599-07-4 (component B)

Synonyms: Acetylated monoglycerides of fully hydrogenated castor oil. Acetic acid esters of monoglycerides of fully hydrogenated castor oil. Octadecanoic acid, 12-(acetoxy)-, 2, 3-bis(acetoxy)propyl ester (main component).

Emperical formula: C_{27} $H_{48}O_8$ (A) and C_{25} $H_{46}O_6$ (B) Grindsted ® Soft-n-safe

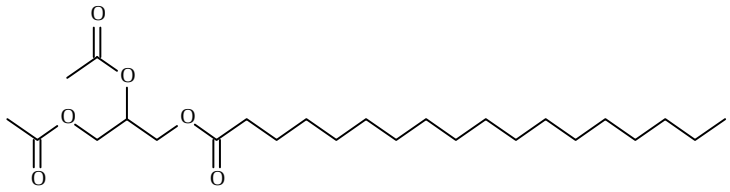
Chemical structure:

A



B

—



Molecular weight: 500.7 (A), 442.6 (B)

Melting point: -21.5°C

Boiling point: 300°C at 1 atm (decomposition)

Vapour pressure: < 2.8 x 10^{-4} Pa at 100°C

Solubility in water: 0.007 g/L

Log Kow: 6.4 (measured)

Purity: About 94% (84% and 10% of the A and B components, respectively)

Impurities: Octadecanoic acid, 12-acetoxy, 2-hydroxy, 3-acetoxypropyl ester (2%)

Octadecanoic acid, 12-oxy, 2,3-bis(acetoxy)propyl ester (1.5%)

Octadecanoic acid, 12-actyloxy, 2(acetoxy)-1,3-propanediyl ester (1.1%)

Octadecanoic acid, 3-(acetoxy)-2-hydroxypropyl ester (1.0%)

As (max 3 ppm), Pb (max 5 ppm), Hg (max 1 ppm), Cd (max 1 ppm)

3.2. Use

This mixture exhibits a performance as plasticizer similar to that of DEHP. It is approved in EU for use in food packing materials and evaluated by opinion of European Food Safety Authority (EFSA 2004) and classified in list 3. The intended primary use is in PVC (films, tubes, bottles, sealings, etc.) but product may also find use in other polymers like polyolefines, styrenics, PET.

The product is listed in the European List of Notified Substances (ELINCS) as no. 451-530-8. Under REACH it is registered at a tonnage band > 1000 tpr. German-Austrian search yielded in the following information on use: Primarily used as a plasticizer in food contact materials (approved for use in the EU, US, South America and most of Asia), medical devices, vinyl flooring, wallpaper, shrink wrap film, textile dyes, ink applications, adhesives and sealants.

3.3. Exposure

No information has been found describing human exposure. Slightly lower leaching rate to sunflower oil (368 mg/dm^2) was found as compared to DEHP (Kristofferson 2005). REACH indicates consumer use and wide dispersive use.

3.4. Toxicokinetics

Studies have been performed on absorption, distribution, biotransformation and excretion. Main conclusion suggests that hydrolysis of the compound is incomplete and that a proportion of the administered dose passes through the gastrointestinal tract and is excreted unchanged.

ECHA (2011) states:

Toxicokinetic studies on COMGHA show that there is no significant absorption of the material across gastrointestinal epithelium. COMGHA appears to be rapidly hydrolysed in the GI tract to acetic acid and fatty acids that undergoes normal fatty acid alpha- and beta-oxidation.

COMGHA does not appear to accumulate in tissues. Based on the results from a 90-days oral toxicity study, it was concluded that there were no marked effects on peroxisomal enzyme activities in liver samples at concentration levels of 0.4%, 1.2% and 3.6% in the diet (Maag *et al.*, 2010).

3.5. Toxicity

ECHA (2011) states:

Acute toxicity of COMGHA by the dermal route has been studied using OECD TG402 in rat and LD_{50} found to be > 2,000 mg/kg bw. Other acute toxicity data are not available. COMGHA was

not irritating to rabbit skin (OECD 404) and rabbit eyes (OECD 405) and also not a skin sensitizer when studied in a local lymph node assay in mice (OECD 429) (Maag *et al.*, 2010).

Repeated dose toxicity

A 13 week toxicity in SD rats fed by gavage at 3, 8.5 and 20 ml/kg bw/d. An increased incidence of thymus atrophy was recorded in the highest dosed group, but similar effects were seen in corn oil fed control group.

A second 13-week toxicity study in SD rats, where each group received diets containing 0 mg, 500 mg, 1600 mg or 5000 mg/kg bw/d. The NOAEL was 5000 mg/kg/d.

ECHA (2011) states:

In a chronic toxicity study (OECD 452) rats were administered doses of 1500, 6000 and 15000 ppm, rising to 25000 and 30000 ppm, in the diet. The concentration of the high dose group was increased during the study to ensure an average achieved dose of at least 1000 mg/kg bw/d. The mean achieved dosage for both genders was 98, 392 and 1333 mg/kg bw/d, respectively. The oral administration of COMGHA to rats for a period of up to 12 months at dietary concentrations of up to 30000 ppm did not result in effects that were considered to represent an adverse effect of treatment. The overall NOAEL for repeated dose oral toxicity (12 months) is 1333 mg/kg bw/d for both genders (information from the registration dossier).

The treatment of male rats with 8.5 ml/kg bw had no effect on palmitoyl-CoA activity whereas small, but statistically significant increases in specific and total palmitoyl-CoA were observed in male rats given 20 mg/kg bw.

Induction on peroxisome proliferation: No marked effects on peroxisomal enzyme in the livers of male and female rats were observed after 13 weeks feeding study.

Genotoxicity

Negative. Non-mutagenic in gene mutation study with or without S9 mix. *In vitro* mammalian chromosome aberration test was negative. Non-clastogenic in the chromosome aberration test.

Reproduction/developmental toxicity

ECHA (2011) states:

Developmental toxicity was examined in rats and in rabbits (OECD 414) at doses of 100, 300 and 1000 mg/kg bw/d using oral gavage administration of COMGHA. No maternal or developmental toxicity was observed at dose levels up to 1000 mg/kg bw/d and the NOEL for maternal and prenatal developmental toxicity is 1000 mg/kg bw/d (Information from the registration dossier).

Toxicity to reproduction was studied in a 2-generation study (OECD 416) in combination with a developmental neurotoxicity study (OECD 426) using rats. A dosing regimen of 0, 1500, 6000 and 15000 ppm in the diet was used. In each generation, animals allocated to the high dose group initially received an intended dietary inclusion levels of 15000 ppm, rising to 20000 ppm and then 25000 ppm during the maturation period and being sustained at the higher inclusion level until termination to ensure an average achieved dose of 1000 mg/kg bw/d.

In the 2-generation reproduction/developmental neurotoxicity study, COMGHA was found not to have adverse effects on reproduction and pre- and postnatal development in the rat when administered to 2 successive generations, including no adverse endocrine disrupting effect

using ano-genital distance and nipple count as effect parameters. Furthermore, COMGHA was found not to induce any developmental neurotoxicity in the offspring. The NOEL for adult toxicity and reproduction over the 2 generations was 25000 ppm, giving exposure of at least 1000 mg/kg bw/d throughout all of the study (lowest average exposure was 1159 mg/kg bw/d in F0 male). Other than a decrease in spleen weights for female offspring, the NOEL for offspring development was 25000 ppm and this dosage represents a clear NOAEL for offspring development. The NOEL for offspring survival and growth and, also, for developmental neurotoxicity was 25000 ppm.

3.6. Human data

No information available on toxicity in humans.

3.7. Conclusion

No original toxicity data were available. Based on the summary data submitted by industry to ECHA, it seems that the product is rather non-toxic via the oral route, most probably due to poor absorption by the g.i. mucosa. Therefore information are not relevant for exposure mediated by many medical devices use, for which the main route of exposure is parenteral, meaning a bioavailability of 100%. Slightly lower leaching rate to sunflower oil (368 mg/dm^2) was found as compared to DEHP.

References:

Submission from Danisco S/A.

ECHA (2011)

EFSA (2004). 109: 1-26.

4. DEHA (Di(2-ethylhexyl)adipate)

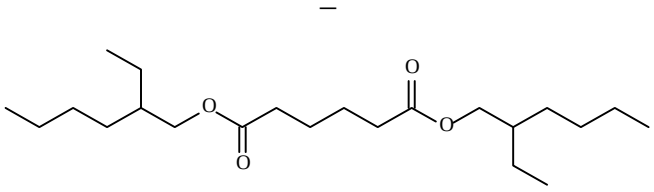
Comment: ECHA (2011) has not considered DEHA as a possible alternative to phthalates based on reprotoxic properties reported in SCENIHR (2008): "among the adipates, DEHA (or DOA) is the most used. It was however not included for further investigation in this study because it was reported by SCENIHR (2008) to have reproductive toxicity."

4.1. Physico-chemical properties

CAS Reg. No: 103-23-1

Synonymes: DEHA, di(2-ethylhexyl) adipate, DOA, dactylo adapte

Empirical Formula: $C_{22}H_{42}O_4$



Molecular weight: 370.57

Melting point: -67.8°C

Boiling point: 214°C (0.67 kappa), 417°C (SIDS)

Vapour pressure: 8.5 $x10^{-7}$ mm Hg at 25°C, 0.11 kappa (20°C), 0.32 kappa (200°C),

1.1 x 10^{-4} Pa at 20°C (SIDS)

Solubility in water: 0.78 mg/L (22°C)

Log Know: >6.11 (calculated), 8.0 (calculated)

Impurities: 0.01-0.02% atopic acid (purity >99%)

Leaching of plasticizers from food packing materials into fatty food has been studied. In a Danish survey, plastic film on the market was tested for DEHA leaching to olive oil. Of the 49 investigated samples, 42 exceeded the action limit set at 4 mg (Breidendahl and Petersen 1998), cited in CSTEE opinion (1999).

4.2. Use

DEHA is a high production volume chemical with an annual production and/or importation volumes above 1 million pounds in the U.S. DEHA is used as a plasticizer in toys, vinyl flooring, wire and cable, stationery, wood veneer, coated fabrics, gloves, tubing, artificial leather, shoes, sealants and carpet backing. Under REACH, the substance is registered at a tonnage band >1000 tpa.

4.3. Exposure

There is uncertainty about the exposure of the general population. A survey covering 112 individuals established an intake of 2.7 mg/d (medium value) related to food contact material. SCF evaluated the intake of DEHA in 2000 and concluded that the data showed DEHA intakes to be below the TDI of DEHA 0.3 mg/kg bw/ (SCF 2000, CSTEE 1999). No information has been found describing the exposure of children from PVC articles (e.g. toys).

Information available at ECHA website and from a German-Austrian search indicate the following: wide dispersive use, consumer use, high environmental release; used as a plasticizer in toys, vinyl flooring, wire and cable, stationery, wood veneer, coated fabrics, gloves, tubing, artificial leather, shoes, sealants and carpet backing. Also used in films employed in food packaging materials, fillers, paint and lacquers, adhesives, plastic in concrete and rubber products. Expected to be widely used in the near future in products for the hospital sector, printing inks and other PVC products.

4.4. Metabolism and toxicokinetics

DEHA is rapidly and completely absorbed from the gastrointestinal tract. After oral administration, DEHA is hydrolysed in the gastrointestinal tract to 2-ethylhexanol, mono(2-ethylhexyl) adipate and adipic acid. 2-ethylhexanol is also one of the metabolites of DEHP. Further details can be found in BUA, 1996.

4.5. Toxicity

Acute toxicity

DEHA has very low oral acute toxicity: LD_{50} 7.4-45.0 g/kg bw.

Irritation

DEHA has been reported to be non-irritating or slightly irritating to the skin of rabbits. It fails to produce symptoms of a sensitising potential.

Repeated dose toxicity

DEHA induces changes indicative of peroxisome proliferation in the liver, although moderate compared to those of DEHP. The metabolites appear to be the active compounds for the peroxisomal effects with 2-ethylhexanoic acid being the most active metabolite. There are no adequately performed studies, which allow a precise determination of a NOAEL for DEHA from subchronic or chronic studies. A study based on the draft protocol for the "Enhanced OECD Test guideline no 407" using oral administration of 0, 40, 200 and 1000 mg/d for 28 days showed a reduction in relative kidney weights at 200 and 1000 mg/kg/d (Miyata *et al.*, 2006).

Genotoxicity

DEHA did not induced point mutation in *Salmonella typhimurium* or mouse lymphoma cells, sister chromatide exchanges in primary hepatocytes or Chinese hamster ovary cells, nor unscheduled DNA synthesis in primary rat hepatocytes. DEHA did not cause chromosomal aberrations or micronuclei in primary rat hepatocytes. In one test on Chinese hamster ovary cells, an increase rate of chromosomal aberration was seen in the absence of a metabolic activation system; however, this study did not address cytotoxicity and have therefore some limitation. DEHA has not induced micronuclei in mouse bone marrow cells or sex-linked recessive lethals in *Drosophila melanogaster.* In a dominant-lethal test in mice using intraperitoneal administration, a slight positive effect was seen. At the same time there was a reduction in the fertility index (not seen in oral studies), suggestion cytotoxicity rather than mutagenicity being the underlying cause for the dominant lethality (BUA, 1996). In an overall assessment of the test result, the CSTEE arrived at the conclusion that DEHA does not have a genotoxic potential (CSTEE 1999).

Carcinogenicity

A 2-year cancer study of DEHA has been performed in Fischer 334 rats and B6C3F1 mice (NTP, 1982) and been discussed with chronic toxicity and carcinogenicity studies of several phthalic acid esters and compounds containing a 2-ethylhexyl moiety (Kluwe 1986). DEHA did not induce tumors in Fischer 344 rats. Hepatocellular carcinomas and adenomas occurred in mice of both sexes in a dose-related manner at incidences that were significantly higher for high–dose males and for low- and high-dose females when compared to historical controls. It is stated in the NTP report that liver tumors in male mice could not clearly be attributed to substance administration; it was concluded that DEHA was carcinogenic to female B6C3F1 mice causing increased incidences of hepatocellular carcinomas and probably carcinogenic in mice causing hepatocellular adenomas.

Reproductive toxicity

Several studies show foetotoxic effect of DEHA (CSTEE 1999). A detailed study using gavage administration of 0, 200, 400, or 800 mg/kg bw/d to pregnant rats, confirmed the foetotoxic effect. Maternal toxicity was seen at 800 mg/kg bw/d. The NOAEL for maternal toxicity was 400 mg/kg bw/d. The NOAEL was 200 mg/kg. DEHA induced a prolonged gestation period at 800 mg/kg. No anti-androgenic endpoints were affected. DEHA did not induce anti-androgenic effects similar to those of DEHP (Borch *et al.*, 2002, Dalgaard *et al.*, 2003, Borch *et al.*, 2006). Another study showed that combined perinatal exposure to a mixture of DEHA and DEHP did

not exhibit more pronounced effects in the reproductive system than those observed in males receiving DEHP alone (Jarfelt *et al.*, 2005).

In the study of Mityata *et al.* (2006) a disturbance of the oestrous cycle and increased ovarian follicle atresia were detected in the 1000 mg/kg group. In the study no maternal toxicity was observed. Developmental toxicity was observed with a decreased birth weight and an increase in postnatal death among the pups at a dose of 800 mg/kg (pre-and postnatal exposure). A NOAEL of 200 mg/kg was chosen as a precautionary approach as the 400 mg/kg dose indicated postnatal deaths that was increased and nearly reached significancy while at the 200 mg/kg dose no difference was observed between control and treated animals.

In a specific female fertility study, a significant increase in mean oestrus cycle length and post-implantation loss rate were observed in the 1,000 mg/kg and above groups, and a significant decrease in implantation rate and number of live embryos and a significant increase in pre-implantation loss rate were observed in the 2,000 mg/kg group (Wato *et al.*, 2009).

A developmental toxicity study according to OECD TG 414 has been conducted in rabbits. Summaries prepared by industry have been made available on ECHA's dissemination website. However, the original study report was not available for evaluation.

4.6. Human data

After oral administration, DEHA is first metabolised by cleavage of 2-ethylhexanol to mono-2-ethylhexyladipate (MEHA) which can be further metabolised and excreted in urine, mainly as adipic acid. In order to develop biomarkers to assess human exposure to DEHA, in vitro metabolism of DEHA was investigated by using human liver microsomes. Whereas identified adipic acid has to be considered as a non-specific biomarker, MEHA and MEHA-derived oxidative metabolites (mono-2-ethylhydroxyhexyl adipate and mono-2-ethyloxohexyl adipate) can be considered as specific DEHA metabolites (Silva *et al.*, 2015 and references cited therein). DEHA has in the meantime been taken up in biomonitoring programs (http://www.bmub.bund.de/en/press/press-releases/detailansicht-en/artikel/federal-environment-ministry-and-german-chemical-industry-association-set-new-targets-for-human-biomonitoring-1/).

4.7. Other information

In vitro dermal absorption of DEHA in human skin using a roll-on deodorant containing 1.56% DEHA was measured to be 2.2 ng/cm^2 (Zhou *et al.*, 2013).

4.8. Conclusion

In a 28 days staudy a NOAEL of 40 mg/kg bw/d can be identified based on reduced renal weight at the higher dose. From investigations described by Wato *et al.* (2009) an NOAEL of 200 mg/kg bw/d can be derived from a study of 4 week duration for developmental toxicity and foetotoxicity effects. It has to be noted that DEHA has been put on the Community rolling action plan under REACH based on concerns with respect to reproductive toxicity (http://echa.europa.eu/documents/10162/2bc79569-1f0d-4c35-ad6e-29c4c7656298).

DEHA is metabolised in humans by cleavage of 2-ethylhexanol to mono-2-ethylhexyladipate (MEHA) which can be further metabolised and excreted in urine, mainly as adipic acid. 2-ethylhexanol is likely responsible for the proxisome proliferation effects seen in the liver of animal dosed orally. NTP concluded that DEHA is carcinogenic to female B6C3F1 mice causing increased incidences of hepatocellular carcinomas and probably carcinogenic in mice causing hepatocellular adenomas.

No information is available for parenteral exposure. Regarding the leaching potential are scant, but the few indication available suggest a leachin potential higher than DEHP.

References:

Kluwe, W.M. (1986): Carcinogenic potential of phthalic acid esters and related compounds: structure-activity relationships. Env. Health. Persp. 65, 271 – 278.

NTP (1982): NTP technical report on the Carcinogenesis bioassay of Di(2-ethylhexyl)adipate (CAS No. 103-23-1) in F344 rats and B6C3F1 mice (feed study). National Cancer Institute, National toxicology program. NTP-80-29; NIH Publication No. 81-1768

Silva, M.J., Samandar, E., Ye, X. and Calafat, A. (2015): in vitro metabolites of Di-2-ethylhexyl Adipate (DEHA) as Biomarkers of Exposure in human Biomonitoring Applications. Chem. Res. Toxicol. 26, 1498 – 1502.

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5. DINCH (1,2-Cyclohexanedicarboxylic acid, diisononylester)

5.1. Physico-chemical properties

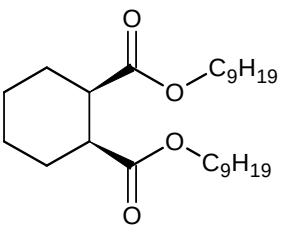
CAS Reg. No.: EU 166412-78-8, USA and Canada 474919-59-0,

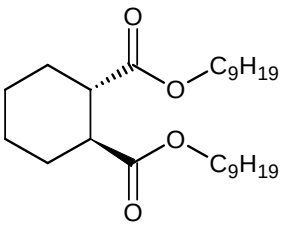
EC (ELINCS) number 431-890-2

Synonyms: Hexamoll DINCH

Emperical formula: $C_{26}\ H_{48}O_4$

Structure:





90±10% cis- isomer 10±10% trans- isomer (NICNAS, 2012)

Molecular weight: 424.6

Melting point: (liquid)

Boiling point: 240-250°C at 4 hPa

Vapour pressure: < 2.8 x 10^{-4} Pa at 100°C

Solubility in water: <0.02 mg/L at 25°C

Log Kow: 10.0 (calculated)

Purity: >99.5%

Impurities: < 0.05% 1,2-Benzenedicarboxylic acid, dinonylester, branched and linear; < 0.5% Dinonylether;

< 0.1% Nonanol, branched and linear derived from Oxo-process;< 0.5% sum of Cyclohexanecarboxylic acid, nonylester, branched and linear and 2-Methylcyclohexanecarboxylic acid, nonylester, branched and linear

5.2. Use

DINCH is suggested as an alternative to DEHP "for sensitive applications". These include medical devices, such as blood tubes and packaging for nutrient solutions. DINCH has been approved by EFSA (2006) as a food packaging material and it is used in a variety of consumer products. RIVM (2009) has concluded that – based on the migration levels tested - DINCH is not expected to pose any risk when used in toys. The European producer has extended the production capacity to 100 000 tpa by 2007, doubled in 2014. The annual production volume currently registered under REACH, however, is confidential (see ECHA website). Its physical behaviour is similar to that displayed by DEHP: the two plasticizers have similar viscosities suggesting that the relevant P-PVC formulations would not require significant changes in the plasticizer content and viscosity modifiers (Chiellini *et al.*, 2013). It has been shown to have low environmental persistence and high biodegradability compared to DEHP.

5.3. Exposure

The introduction into the commercial market and the subsequent increase in production capacity is well reflected by investigation of house dust between 2001 and 2009. Mean values of DINCH in house dust increased from < 0.31 mg/kg (detection limit: 0.29 mg/kg) in 2001/2002 and 2003/2004 up to 12 mg/kg in 2009 (Nagorka *et al.*, 2011 a and b).

In a report from Australia (NICNAS, 2012), dietary exposure in humans is estimated at 0.081 mg/kg bw/d.

In an US study which is currently in press, urinary concentrations of 3 oxidative metabolites of DINCH were measured between 2000 and 2012 in adult volunteers without known DINCH exposure. While none of the DINCH metabolites was detected in samples from the years 2000 and 2001, the detection rate for the metabolites increased from 2007 to 2012, which is in line with the commercial introduction and subsequent increase in the production of DINCH.

In a pilot study performed in Germany (Schütze *et al.*, 2012), exposure towards DINCH of the general population was investigated in 22 human volunteers (9 females, 13 males without occupational exposure to DINCH). Mean values for oxygenated metabolites of DINCH were 0.71 µg/l for OH-MINCH (Cyclohexane-1,2-dicarboxylic acid monohydroxyisononylester), 0.61 µg/l for cx-MINCH (Cyclohexane-1,2-dicarboxylic acid monocarboxyisooctylester) and 0.33 µg/l oxo-MINCH (Cyclohexane-1,2-dicarboxylic acid monooxoisononylester).

Specific DINCH metabolites (MINCH, OH-MINCH, oxo-MINCH and cx-MINCH) were investigated in 300 urine samples (24h voids) collected in the years 1999, 2003, 2006, 2009 and 2012 in

order to investigate occurrence and possible trends after introduction of DINCH into the market. No DINCH metabolites were investigated in samples from 1999 and 2003. From 2006 to 2012, the percentage of samples with DINCH metabolites above the LOQ increased significantly (7% in 2006, 43% in 2009 and 98% in 2012). OH-MINCH was the predominant metabolite. Median (and 95th percentile) concentrations (in µg/l) increased from < LOQ (0.09) in 2006 to < LOQ (1.02) in 2009 to 0.39 (2.09) in 2012. The median (95th percent) DINCH intake was calculated to be 0.14 (1.07) µg/kg bw/d (Schütze *et al.*, 2013).

Using nutrition fluids for DINCH a 8-fold lower leaching into the fluids was found as compared to DEHP. Leaching of plasticizers from food packing materials into especially fatty food has been studied, less information are available using dynamic systems and medical devices.

In a comparative study of leaching of plasticizers in different feeding solutions, Welle *et al.* (2005) compared DINCH, TOTM and ATBC with DEHP, DINCH leaching over 24 h was 3-10 times lower than that for DEHP.

By using DINCH-based bags, leakage of DINCH into the blood product was generally less pronounced than that of DEHP (from 7 to 20 fold) depending on the matrix (plasma, RBC, platelets) and time of storage, with the exception of pediatric platelet concentrates in which the leaching rate was the same. It has to be underlined that although indicated as DEHP-free material, the release of DEHP from DINCH-based material occurs, at different extent depending on matrix and time, being most of the values 1-7 fold up to 20-fold lower. (Lagerberg *et al.*, 2015).

In another study in vitro, the amount of plasticizer released from the PVC sheet in contact with blood was 53.1 26.1–36.5, and 78.4–150 mg/mL for DEHP, DINCH, and DOTP, respectively (Haishima *et al.*, 2014).

An emission rate of 0.41 µg DINCH per hr per m^2 into the gas phase was calculated for a PVC sample containing 17% DINCH under controlled conditions (Schossler *et al.*, 2011).

5.4. Metabolism and Toxicokinetics

After oral administration of radiolabelled material, DINCH showed rapid, but saturable absorption. Approximately 80% of the radioactivity is excreted 24h after dosing, and more than 90% after 48h partly via urine and mainly via faeces as unabsorbed material. Based on the amounts of radioactivity excreted in the bile and urine, the oral bioavailability of ^{14}C-1,2-cyclohexanedicarboxylic acid di(isononyl)ester is estimated to be 5-6% at the high dose of 1000 mg/kg bw and 40-49% at the low dose of 20 mg/kg bw. After oral administration, elimination of radioactivity from plasma was biphasic, highest levels in plasma were observed 1 hr after administration.

There is no indication of bioaccumulation. The characterisation of metabolites after oral and intravenous administration of DINCH indicates 2 main pathways in rodents: the partial hydrolysis of DINCH to the mono-isonyl ester followed by conjugation to glucuronic acid, which is the most abundant metabolite in bile, or the oxidation of the mono-isononylester to the corresponding hydroxy-, oxo- or carboxy-monoisononylesters and final cleavage of the second side chain which yields 1,2-cyclohexandicarboxylic acid (CHDA). The latter is regarded as an unspecific metabolite of DINCH.

The metabolism of DINCH after oral administration of approximately 50 mg DINCH has also been investigated in 3 human volunteers (Koch *et al.*, 2011, 2012, 2013) by quantification of urinary metabolites collected over 48 hr. 39.2% of the DINCH dose was excreted as

metabolites (23.7% as CHDA, 14.8% as monoesters with oxidative modifications and less than 1% as the simple, non-oxidised monoester). Over 90% of the metabolites were excreted within 24 hrs after administration. Specific DINCH metabolites (MINCH, OH-MINCH, oxo-MINCH and cx-MINCH) were detected in urine in biomonitoring studies (Schütze *et al.*, 2013).

5.5. Toxicity

All toxicity studies presented were performed under GLP conditions according to OECD guidelines.

Acute toxicity

DINCH has very low acute toxicity, the LD_{50} dose for DINCH in the rat is >5000 mg/kg bw after oral and > 2000 mg/kg bw after dermal administration.

Irritation/sensitization

DINCH was demonstrated to be a non-irritant in both the rabbit skin test and rabbit eye test and a non sensitizer in the Guinea pig maximization test.

Repeated dose toxicity

<u>28 day study</u>

The 28 day toxicity study (dosing 0-600-3000-15,000 ppm or 0-64/66-318/342-1585/1670 mg/kg bw for males/females, respectively) was followed by a 14 days recovery period. The highest dose induced gamma-glutamyltransferase serum level and degenerated epithelial cells in the urine. The NOAEL was 3000 ppm which relates to 318 mg/kg bw for males and 342 mg/kg bw for females.

Single dose and repeated dose i.v. toxicity studies (continous infusion for up to 29 days) indicated that there was no specific systemic toxicity at nominal concentrations up to 300 mg/kg bw/day, indicating no significant differences among route of exposure.

<u>90-day study</u>

The 90 repeated dose toxicity study was performed with the doses 1500-4500-15000 ppm which relates to 107/128, 325/389 and 1102/1311 mg/kg bw for male/female animals, respectively.

There was no effect on mortality, clinical signs or haematology. Alterations were observed for clinical pathology including an increase in serum gamma-glutamyl transferase and TSH increase, in addition in urine blood and transitional epithelium cells were observed. The following pathological effects were present: an increase in liver weight, an increase thyroid weight, which was in line with the histology of showing hyperplasia/hypertrophy of the thyroid follicles. In the kidney alpha 2-microglobulin accumulation in the tubules was observed. This mechanism is considered specific for the male rat and not relevant for man. In the liver in the mid and high dosed group, enzyme induction of phase I and phase II enzymes was observed. The increased gamma-glutamyltransferase and TSH value, increases in liver and thyroid gland, as well as the thyroid hypertrophy/hyperplasia suggest a common pathogenesis of enzyme induction process. This is not considered an adverse effect.

In the testes there was a significant increased in the mean relative weights in all 3 dose groups with no dose-response relationship. Histopathologically there was no obstructive process present in the male rete testis or other areas of the male reproductive system.

Based on kidney effects the NOAEL was 1,500 ppm (107.1 /mg/kg/d) in male and 4,500 ppm (389.4 mg/kg/d) in females. Also in the 2 generation study thyroid hyperplasia/hypertrophy was observed with a NOAEL of 100 mg/kg/d.

Genotoxicity

DINCH has been evaluated for mutagenicity, both in bacterial (*Salmonella typhymurium/ Escherichia coli* reverse mutation assay) and mammalian cell tests (*In vitro* mutation test in CHO cells), with negative results. It was non-clastogenic in tests conducted *in vitro* (chromosome aberration assay in Chinese hamster V79 cells) and *in vivo* (Micronucleus assay bone marrow cells mouse). DINCH is considered as non-genotoxic.

Carcinogenicity

In a 2 year combined chronic toxicity/carcinogenicity study (doses 40, 200, 1,000 mg/kg bw/d) the thyroid was identified as target organ. Thyroid weight was increased in both sexes with follicular cell hyperplasia and the presence of follicular adenomas. The effect was considered due to secondary mechanisms via liver enzyme induction, a mechanisms which is considered not relevant for humans. The NOAEL was 40 mg/kg in males and 200 mg/kg in females. Similar to the short term study transitional epithelial cells of the urinary tract were present in the urine. These were temporarily present and considered as adaptive as no histopathological lesions were observed in the kidneys at 12 and 24 moths.

Reproductive toxicity

Prenatal development studies

In prenatal toxicity study in rabbits, DINCH was orally administered from day 6 to day 29 of gestation with doses of 100, 300 and 1,000 mg/kg bw/d. There were no signs for maternal toxicity, no influence on gestation parameters, no signs for developmental effects in pups or teratogenic effects. Soft tissue malformations were equal to control values. The NOAEL was determined at the highest dose investigated, 1,000 mg/kg bw/d.

In the prenatal development study in rats, no effects were observed. The dosing of the mothers was form day 6-19 post coitum. The NOAEL was equal to the highest dose administered by gavage being 1,200 mg/kg bw/d.

In a pre- and postnatal developmental study DINCH was administered orally to the mother animals from day 3 post coitum to day 20 post-partum (750 and 1,000 mg/kg bw/d). Exposure of the offspring was via the mother animals during gestation and the lactation period until day 20 post-partum. The offspring (all males and 3 females) was raised to days 100-105 post-partum and then evaluated. Anogenital distance (AGD) and anogenital index (AGI, AGD divided by kg bw/) was measured at day 1 after birth and sexual maturation was determined (testes descendance, balanopreputional separation, penis evaluation/inspection, sperm evaluation and vaginal opening for females). Gross pathology was performed and testes and epididymus were collected for histology. The results indicated that there was no toxicity in F1 progeny with a NOAEL of 1,000 mg/kg/d. The AGD ($p<0.05$) and AGI ($p<0.01$) were significantly decreased in the male high dose group (1,000 mg/kg bw/day): AGD 7% and AGI 8% below the control group. Also in females of the high dose group the AGI was significantly reduced by 8%. The AGI was significantly ($p<0.05$) decreased also in females.

Although statistically significant, the limited alterations (7-8% change compared to controls), in the AGD and AGI are not considered of biological significance by the authors as other corresponding parameters like testes descendance, preputial separation, vaginal opening,

testes weight and histology and sperm parameters were not affected. Also in females the AGI was decreased to the same extent, contradicting the AGI to be an effect of impaired androgen dependent development. In the 2 generation study no effects were reported (but AGD and AGI not determined).

Two generation study

The 2 generation study was performed with continuous dietary administration (doses 0-100-300-1000 mg/kg bw/d). The animals remained in the same dosing group as their parents. Evaluated were sexual maturation of the F1 generation and sperm parameters of the F0 and F1 generation. There were no effects on fertility and reproduction performance and no substance related effects on the evaluated F1 and F2 generation. In the F0 parents an increase in gamma glutamyltransferase in females, decreased total bilirubin in females and increased liver, kidney and thyroid weight in both males and females was observed. At the highest dose investigated (1000 mg/kg bw): in the F1 parents similar effects were noted including thyroid weight increase with thyroid hypertrophy/hyperplasia. The NOAEL for fertility and reproductive performance was 1000 mg/kg bw for both F0 and F1 parents and 1000 mg/kg bw for developmental toxicity in F1 and F2 pups.

Other information

In an *in vivo* screening assay foetal testes testosterone production after administration of several phthalate esters and DINCH has been investigated. Pregnant Sprague-Dawley rats received 750 mg/kg/d of the tested substances by oral gavage from gestational day (GD) 14-18. Foetal testes from 3 fetuses were collected on GD 18, cultured for 3h and investigated for testosterone production. While fetal testes production was significantly reduced compared to control in most of the phthalate esters investigated, no effect was seen with DINCH (Lambright *et al.*, 2011).

5.6. Human data

No information available on toxicity in humans.

5.7 Conclusion

After oral administration of radiolabelled material, DINCH showed rapid, but saturable absorption: the oral bioavailability of DINCH is estimated to be 5-6% at 1000 mg/kg bw and 40-49% at 20 mg/kg bw. Apart from a species-specific effects on the kidneys (not relevant to humans) repeated toxicity studies including a 2 generation study showed induction of thyroid hyperplasia/hypertrophy with a NOAEL of 40 mg/kg/d: it has to be determined whether or not the mode of action is relavant to humans. Anogenital distance (AGD) and anogenital index were altered: although statistically significant, the alterations were limited (7-8% change compared to controls), therefore their meaning should be clarified.

Single dose and repeated dose i.v. toxicity studies (continous infusion for up to 29 days) indicated that there was no specific systemic toxicity at nominal concentrations up to 300 mg/kg bw/day, indicating no significant differences among route of exposure.

It should be taken into account that DINCH based medical devices are on the market worldwide. In Europe there are CE certified devices for pediatric used in use since 2 years, Oxygen masks etc. This should made it possible to have in the future both a better exposure assessment as well as a follow up related to these applications.

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6. DINP(di-iso-nonyl phthalate)

A recent report prepared by ECHA has summarized all recent findings on DINP (ECHA 2013), conducting a risk assessment to understand the possibility to chance the restriction of DINP use in toys toys and childcare articles that can be mouthed (Regulation (EU) No 1907/2006, Annex XVII, 52).

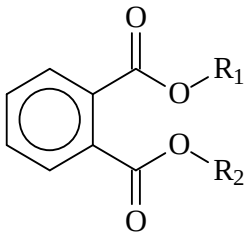
6.1. Physico-chemical properties

CAS Reg. No: 68515-48-0 and 28553-12-0 (different alcohol chains depending on production method)

Synonyms: 1,2-Benzenedicarboxylic acid, di-C8-10 branched alkylesters, C-9 rich (DINP-1); Jayflex® for CAS 68515-48-0; Di-"isononyl"phthalate (DINP-2) for CAS 26761-40-0; Palatinol N® and Palatinol DN®

Empirical Formula: $C_{26}H_{42}O_4$ (average)

Structure:



—

DINP-1 (68515-48-0) contains alcohol groups made from octane, by the "polygas" process At least 95 percent of these alcohol groups comprise roughly equal amounts of 3,4-, 3,5-, 3,6-, 4,5-, 4,6-, and 5,6-dimethyl heptan-1-ol.

DINP-2 (28553-12-0) contains alcohol groups made from n-butene, which results mainly in methyl octanols and dimethyl heptanols.

Molecular weight: 420.6 (average)

Melting point: -40 to -54°C

Boiling point: 424°C

Vapour pressure: 6×10^{-5} Pa at 20°C

Solubility in water: 0.6 µg/L

Log Kow: 8.8

Purity: DINP is not a pure substance, but a complex mixture containing mainly C9-branched isomers, with mean formula $C_{26}H_{42}O_4$. Phthalates are produced with a high degree of purity (> 99.5%), in terms of ester content (ECB, 2003).

Impurities: In ECB (2003) the following impurities according to the manufacturer are listed: iso-Nonanol ca. 0.04%, iso-nonylbenzoate ca. 0.03%, n-butyl-iso-nonyl phthalate ca. 0.1%, water 0.02-0.03%.

6.2. Use

There are currently four producers of DINP in EU. Approximately 95% of DINP are used in PVC as a plasticizer (ECB 2003). According to old data it has limited use in food packing material and is not used in medical products (CSTEE 2001); however so far it has not been used in medical devices. DINP is used as a plasticizer in toys (with restriction, vinyl flooring, wire and cable, stationery, wood veneer, coated fabrics, gloves, tubing, artificial leather, shoes, sealants and carpet backing. The substance is registered under REACH at a tonnage band > 1000 tpa (100000 – 1000000 tpa). DINP is restricted for use in toys according to Regulation (EU) No 1907/2006, Annex XVII, 52 i.e. not to be used for toys and childcare articles that can be mouthed. Further, DINP is listed in Regulation (EU) No 10/2011.

6.3. Exposure

The estimated maximum combined total daily intake for an occupationally exposed adult is 1.12 mg/kg bw/d. For non-occupational exposed adults and children a maximum exposure of 20 µg/kg bw/d is estimated. Based on probabilistic estimation the maximum total daily intake from consumer sources is 0.25 mg/kg bw/d and via the environment 0.16 mg/kg bw/d. (combined exposure 0.41 mg/kg bw/d). These estimates are based on DINP measurements in several environmental media and consumer products (ECB 2003). A more recent, detailed overview on exposure to DINP from various sources is given in ECHA (2013). From urinary DINP metabolite concentrations median daily intakes of approx. 0.2 µg/kg bw/d have been calculated for the general population with maximal values of 20 µg/kg/d (David 2000; Kohn 2000; Wittassek *et al.*, 2011). When considering results from biomonitoring studies performed between 2007 and 2012, substantial variability was observed between studies. However, most studies have reported median adult exposure to DINP of around 1 µg/kg bw/day with 95th percentile intakes being generally less than 10 µg/kg/day (ECHA, 2012).

6.4. Toxicokinetics

In rats DINP is readily absorbed and approximately 50% of an oral DINP dose is excreted in urine, mainly as oxidised metabolites of the monoester mono-iso-nonyl phthalate (MINP) (ECB 2003; McKee 2002; Silva 2006a). These oxidised metabolites have also been identified in humans (Koch and Angerer, 2007; Silva *et al.*, 2006a, 2006b). More than 40% of an applied DINP dose to a male volunteer was recovered in urine in form of oxidised MINP-isomers with hydroxy (20%), oxo (11%) and carboxy (11%) functional groups (Koch and Angerer, 2007). The simple monester MINP urinary excreted accounted only for 2% of the dose. Elimination was at least bi-phasic and elimination half-lives in the second phase (beginning 24h post dose) were 12h for OH-MINP and oxo-MINP and 18h for carboxy-MINP. Further metabolites may be breakdown products through α- and β-oxidation of the alkyl side chain and those with more than one functional groups through oxidation (Koch and Angerer, 2007; Silva 2006a).

Conclusions of ECHA (2013) on Toxicokinetics of DINP are:

The conclusions from the EU Risk Assessment (ECB 2003) concerning the toxicokinetics of DINP are generally still valid. Studies in animals and humans demonstrate that DINP is rapidly absorbed orally and quickly metabolized. Absorption of DINP is saturable at high dose levels: astudy with DINP indicates absorption of roughly 40-55% at dose levels as high as 500 mg/kg/day. As biliary excretion occurs, an unknown percentage of the radioactivity excreted in feces is to be added to the radioactivity excreted in urine to estimate the absorption. The absorption of DINP and DIDP can therefore be assumed to be in the range of 50-70% in the rat. Human volunteer studies with DEHP clearly demonstrate that the amount recovered in urine is dependent on the type and amount of metabolites that are measured in those studies. Measuring all metabolites most likely would result in near to 100% recovery of radioactivity in urine. An unknown amount of excretion via bile contributes further to the absorption estimate. However, it is acknowledged that the studies in humans have not been designed to determine absorption. RAC concludes that adult rats can be assumed to absorb 50-70%, whereas humans absorb 100% also based on read-across from DEHP.
For inhalation a bioavailability factor of 75% can be assumed for adults and 100% for newborns and infants as a vulnerable sub-population. Dermal internal exposure for consumers can be derived using a maximum dermal absorption rate of 0.024 µg/cm^2/h.

6.5. Toxicity

Acute toxicity

Upon single exposure, DINP has a low acute toxicity by all routes of administration.

Irritation

DINP may be considered as a very slight skin and eyes irritant, with effects reversible in short time (ECB 2003).

Sensitisation

Conclusions of ECHA (2013) on Sensitization of DINP are:

In general, phthalates (including DINP) lack intrinsic sensitising potential. It has however been suggested that phthalates could be one possible contributor to the increasing prevalence of atopic (IgE-mediated) allergic diseases and asthma in Europe and other Western countries as they have adjuvant potential (Kimber and Daerman, 2010). Many phthalates, including DINP, can affect serum levels of IgG1 and IgE if mice are exposed via the subcutaneous or intraperitoneal routes. For DINP data also indicate a potential to aggravate atopic inflammatory processes. It can be concluded that DINP shares at least some of the adjuvant properties demonstrated for phthalates and an effect on atopic responses in humans cannot be excluded.

Repeated dose toxicity

The liver is a target for chronic toxicity and a NOAEL of 88 mg/kg bw/d can be assumed on hepatic biochemical and histopathological findings. In 2001 CSTEE expressed an opinion on DINP-RAR and disagreed with a use of a NOAEL of 88 mg/kg/d. CSTEE support the use of spongiosis hepatis in rat as the critical effect for DINP, applying a benchmark dose of 12 mg/kg/d. Two studies show spongiosis hepatica with a benchmark dose 12-15 mg/kg/d (Aristech, 1994; Moor, 1998 cited from CSTEE 2001).

For kidney effects, a NOAEL of 88 mg/kg bw/d based on increase kidney weights can be assumed.

ECHA (2013) comes to the following conclusion with respect to repeated dose toxicity of DINP:

A NOAEL of 15 mg/kg bw/day with a LOAEL of 152 mg/kg bw/day (Exxon 1986) and a NOAEL of 88 mg/kg/day with a LOAEL of 359 mg/kg bw/day (Aristech 1994) were identified in the two key repeated dose toxicity studies based on statistically significant increases of incidence of spongiosis hepatis together with other signs of hepatotoxicity. Considering the dose spacing in those studies, in particular the Exxon study with 152 mg/kg as the next higher dose, the true NAEL (No Adverse Effect Level) could be argued to be somewhere between 88 and 152 mg/kg/day. However, there were differences in methodology between both studies: the Exxon (1986) study evaluated 4-5 liver sections, whereas the Aristech (1994) study examined 1-2 sections. Also, the 88 mg/kg bw/day dose group in the Aristech (1994) study could be seen as an outlier.

As a result of the methodological difference, the Exxon (1986) study was considered the most appropriate to use. Thus a NOAEL of 15 mg/kg bw/day was selected for repeated dose toxicity of DINP. This conclusion was supported by RAC (ECHA 2013a). RAC however noted that the NOAEL could be higher given the large dose spacing in the Exxon study.

Genotoxicity

DINP is not mutagenic *in vitro* in bacterial mutation assays or mammalian gene mutation assays (with or without metabolic activation) and is not clastogenic in one cytogenic assay on CHO cells and in one *in vivo* assay on bone marrow cell of Fisher rats. This suggests that DINP is not genotoxic.

Carcinogenicity

In chronic/carcinogenicity studies, DINP was found to induce significant excess of liver neoplasia in rats and mice. This is explained by peroxisome proliferation mode of action. DINP induce kidney tumours in male rats mediated by the alpha 2μ-globulin, a mechanism which is not considered as relevant to humans.

DINP in 2 studies increased the mononuclear cell leukaemia in Fisher rat. IARC has classified this leukaemia of no relevance for humans. However, increased incidences of MNCL in rats remain difficult to interpret in the light of the high and variable background incidences and the unclear relevance to humans. As a reasonable approach it would be possible to conclude that the carcinogenicity findings further strengthen the selected NOAELs for repeated dose toxicity.

With respect to carcinogenicity, ECHA (2013) concludes:

The renal tumors seen in rats are assumed to stem from an alpha-2μ-globulin mode of action which is not considered to be relevant for humans.
Liver neoplasia were seen in rats and mice with a NOAEL of 112 mg/kg bw/day. It is believed that peroxisome proliferation is the underlying mode of action for development of liver tumors with DINP, and that PPARα is involved in hepatic tumour formation. However, the more recent literature indicates that the mechanisms of liver carcinogenicity in rodents with peroxisome proliferators have not entirely been elucidated and that multiple pathways seem to exist. Some of those pathways seem to be PPARα-independent, which might indicate a need for some caution when interpreting the relevance of rodent carcinogenicity with DINP to humans.

The increased incidences in MNCL seen in rats with a NOAEL of 15 mg/kg bw/day might have a human counterpart. The available information does not allow to draw definite conclusions on the relevance of the findings. As MNCL is likely to follow a threshold mode of action with a NOAEL equal to that for repeated dose toxicity, the finding would not be a driver for the risk assessment. Therefore, the endpoint is not taken further to the risk characterisation step.

Reproductive/developmental toxicity

In mice, a very high dose (>5g/kg bw/d) lead to a decrease in testicular weight with abnormal/immature sperm forms and uterus/ovaries atrophy in a 13-week study. A NOAEL of 276 mg/kg bw/d for testicular effects can be assumed in a 104-week chronic rat study based on a reduced testicular weight at 742 mg/kg.

In the developmental studies, visceral and skeletal variations increased on litter basis at 1,000 mg/kg/d, leading to a NOAEL of 500 mg/kg bw/d. A decrease of mean offspring kg bw/ was observed following parenteral administration of DINP in the one and 2-generation study from the lowest dose tested (LOAEL of 159/mg/kg bw/d).

DINP is not estrogenic *in vitro* but recent studies after perinatal exposure indicated that male displayed female like areolas/nipple retention and that incidence of reproductive malformation was slightly, but significantly increased (7.7% vs. 91% observed with DEHP) (Gray *et al.*, 2000). The reproductive effect of DINP is similar to the profile for DEHP, but DINP appears to be about half potent as DEHP.

Conclusions of ECHA (2013) on reproductive toxicity of DINP are:

A NOAEL of 50 mg/kg bw/d based on decreased T production/level and histopathological changes in foetal/pup testis at a LOAEL of 250 mg/kg bw/d is proposed (based on Clewell *et al.*, 2011a and supported by Clewell *et al.*, 2011b; Hannas *et al.*, 2011a and b and 2012; Boberg *et al.*, 2011). The histopathological changes include increased multinuclear gonocytes (MNGs) and Leydig cell aggregates in foetal/pup testis. In a two-generation reproductive toxicity study the offspring bodyweight was decreased with a LOAEL of 159 mg/kg bw/day (no NOAEL) and increased skeletal variations were observed in a prenatal developmental toxicity study with a NOAEL of 100 mg/kg bw/day. The *in vivo* findings suggest that DINP has anti-androgenic potential, but may also exhibit its effects through other modes of action. The decrease in testicular T levels is transient and permanent changes were not generally seen in all studies with DINP.

Many of the changes were mostly reversible, e.g., increase in multinucleated germ cells in 2 studies (Boberg *et al.*, 2011; Clewell *et al.*, 2011b). The permanent effects seem to appear at high dose levels, e.g., reduction in motile sperm and low incidence of permanent nipples/areolae and small testes and epididymides were observed at and above a LOAEL of 128 mg/kg bw/d (Boberg *et al.*, 2011; Gray *et al.*, 2000). Detection of low incidence of malformations/effects requires enough statistical power to be detected. DINP causes low incidences of similar permanent effects observed in with other phthalates likely via same modes of action including androgen deficiency.

Effects on fertility occur at higher dose levels, with a NOAEL for decreased live birth and survival indices of 622 mg/kg bw/day and a NOAEL of 276 mg/kg bw/day for decreased testicular weights.

6.6. Human data

No information available on toxicity in humans.

6.7. Conclusion

In rats an oral DINP dose is approximately 50-70% absorbed and is excreted in urine, mainly as oxidised metabolites of the monoester mono-iso-nonyl phthalate (MINP) which have also been identified in humans. The most sensitive endpoints are repeated dose toxicity with a NOAEL of 15 mg/kg bw/day, using the spongiosis hepatis as the critical endpoint and reproductive toxicity with a NOAEL of 50 mg/kg bw/day.
The reproductive effect of DINP indicates a similar hazard profile as shown for DEHP, with DINP showing lower potency. The mechanism of action is related to an effect on steroidogenesis of testosterone in the foetal male rat like DEHP. DINP can induce liver and kidney tumors after chronic oral administration to rats: the former via peroxisome proliferation whereas the latter with a mechanism not considered relevant for humans.

It has to be noted that DINP is restricted for use in toys (i.e. not to be used for toys and childcare articles that can be mouthed), but the recent evaluation by ECHA (2013) gave indication that no further action are needed, since for the actual exposure scenarios it appears no further risks occur. This conclusion was endorsed by the European Commission in a decision published in January 2014.

References:

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Wittassek M, Koch HM, Angerer J, Brüning T. (2011) Assessing exposure to phthalates - the human biomonitoring approach. Mol Nutr Food Res. 55(1):7-31

7. DEHT (Di(2-ethylhexyl) terephtalate)

7.1. Physico-chemical properties

CAS Reg. No: 6422-86-2

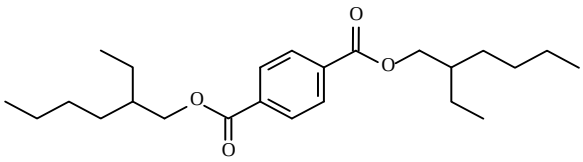
Synonyms: 1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester;

dioctylterephthalate (DOTP); Eastman Plasticizer 168.

Emperical formula: $C_{24}H_{38}O_4$

Molecular weight: 390.56

Structure:



Melting point: -48°C

Boiling point: 363°C (383 enl IUCLID)

Vapour pressure: 28.5 hPa at 25°C, 1013 hPa at 398°C

Solubility in water: 0.4 µg/L (well water), 0.35-1.5 mg/L (sea water)

Log Kow: 5.72 (well water), 5.26 (sea water)

Purity: 98.5%

Impurities: <2% w/w 2-ethylhexyl methyl terephthalate

Information on stability in water is given (in section 3.2.1 of IUCLID set) and the calculated rate constants for hydrolysis. GC-ECD method for parent compound determination (e.g. page 50 of IUCLID)

7.2. Use

DEHT is a high production volume chemical and is annually produced in volumes above 50 million pounds in the U.S.

DEHT is used as a general purpose, low-volatility plasticizer for polyvinyl chloride and other polymeric materials. It is used in a wide range of applications including toys, childcare articles and other consumer products, transportation and beverage closures. According to IUCLID Data Set, the production volume in 1998 was 25000 – 50000 tonnes in the US.

7.3. Exposure

DEHT production uses a closed system. Occupational exposure could occur when the chemical is put into drums or during quality control. It is said that minimal consumer exposure is expected based on limited use in consumer products and low leaching of the compound out of the polymer matrix in its major use as plasticizer.

Information from ECHA gives a different picture: wide dispersive use, consumer use; Primarily used as a plasticizer for PVC toys, childcare articles, consumer products, beverage closures and other polymer materials including cellulose acetate-butyrate, cellulose nitrate and chloroprene rubbers. No information about the possible uses in medical devices.

7.4. Toxicokinetics

In vitro: The metabolic hydrolysis rate of DEHT determined by the formation of free 2-ethylhexanol (2-EH) was studied with rat intestinal homogenate ($t_{1/2}$ was 53 min; and stoichiometry at termination showed about 2 mol of 2-EH per mol DEHT. Results indicated complete hydrolysis to terephthalic acid (TPA). This was in contrast to DEHP (with $t_{1/2}$ of 13 min and a yield of 1.2 mol of 2-EH per mol DEHP) indicating it forms a stable monoester.

Oral study: Absorption and metabolism were studied for DEHT (^{14}C labelled) mixed with corn oil and administered by gavage in a single dose of 100 mg/kg bw to 10 adult male SD rats. About 93% of the total radioactivity was recovered, most of it in the faeces (56.5%) and urine (31.9%) and 3.6% was expired as CO_2. The mean amount of unchanged radioactive DEHT recovered in the faeces was 36.6% representing unabsorbed material; around 50.5% of the total DEHT dose recovered in the urine was detected as unlabelled TPA. After 24h more than 95% of the administered dose was excreted. The amount of mono(2-ethylhexyl) terephthalate (MEHT) and its metabolites was limited to a maximum of 9.3% of orally administered dose (Barber *et al.* 1994).

A study to assess dermal absorption rate indicated that DEHT has a very low potential to penetrate the skin (0.103 µg/cm2/hr), which further limits systemic exposure potential (ECHA, 2011).

7.5. Toxicity

Acute toxicity

Acute oral toxicity data are reported for rats and mice. LD_{50} was >5000 mg/kg and 3200 mg/kg bw in oral studies and >20 ml/kg for dermal toxicity in guinea pigs

Irritation:

DEHT was concluded to be a slight dermal irritant in male guinea pigs with no evidence of percutaneous absorption following a single-dose occlusive dermal application and 24h exposure. DEHT produces slight irritation to rabbit eyes in a study using a procedure similar to OECD 405. (ECHA, 2011).

Sensitisation:

In studies with some limitations, no skin sensitisation was observed in humans or guinea pigs (ECHA, 2011).

Repeated dose toxicity

4-5 studies are available, some conducted according to GLP. Groups of male and female rats were fed diets containing DEHT at 0.1 up to 1% and 2.5% w/w for up to 90 days:

SD rats 90 day (GLP) study: NOEL was 0.5% or 277 and 309 mg/kg bw for males and females, respectively; the NOAEL was 1% or 584 and 617 mg/kg bw for males and females, respectively. Slight increases in relative liver weight (max about 11%) were seen at the 1% dose level. No adverse effects on the testes were found at any dose (Barber & Topping 1995).

Fisher 344 rats 21 day (GLP) study: NOEL was 0.5% or 487 and 505 mg/kg bw for females and males respectively; the NOAEL was 1.2% or approx: 1000 and 1100 mg/kg bw for males and females, respectively. DEHT caused only slight peroxisome proliferation at 2.5%, whilst DEHP caused a moderate increase at 1.2% and a marked increase at 2.5% in this study (Topping *et al.* 1987). The effect seen at the 2.5% exposure level was believed to be secondary to significant decreases in food intake and body weight reduction.

Two other repeated dose studies, one in SD rats with oral feeding at levels of 0.1 and 1% for 2 weeks, the other with inhalation (6h per d for 10 days) of 46.3 mg/m^3 revealed no signs of toxicity; the NOEL for these studies were the highest tested doses.

Genotoxicity

No evidence for genotoxicity was found in assays assessing mutagenicity, *i.e.* gene mutation in bacterial (Ames test) or mammalian (CHO / hgprt) system. DEHT did not induce chromosomal aberrations in mammalian cultured cells with or without an exogenous metabolic activation system. The results for mono(ethylhexyl)terephthalate (MEHT) in the Ames assay were also negative (Barber 1994).

Carcinogenicity

Data from a chronic 104 weeks oral study in rats indicate a NOEL for carcinogenicity of 12000 ppm (highest dose tested), equivalent to 666 mg/kg/d in males and 901 mg/kg/d in females. The NOEL for chronic toxicity in the study was 1500 ppm equivalent to 79 mg/kg/d in males and 102 mg/kg/d in females.

A further chronic study is described in ECHA (2011):

DEHT was evaluated for combined chronic toxicity and carcinogenicity. The test substance was administered in the diets of male and female Fischer-344 inbred rats at concentrations of 20, 142 and 1,000 mg/kg/d. Clinical evaluations revealed no treatment-related signs, however, eye opacities (cataracts) occurred frequently in all groups. At 1,000 mg/kg/d, body weights and female liver weights were reduced. There were no consistent reductions in food consumption. There were no treatment-related effects evident from the gross and histopathologic examinations conducted at 6 and 12 months. At 18 months, 2 basic lesions of the females in the 1,000 mg/kg/d level appear to be associated with treatment. These were hyperplasia and/or transitional cell adenomas of the urinary bladder and adenomas or adenocarcinomas of the uterus.

Reproduction/ developmental toxicity

In a 2 generation reproductive toxicity study following OECD guideline 416, DEHT was given to 30 male and 30 female SD rats at doses of 0, 0.3, 0.6 and 1% in the diet (approx. 0, 150-200; 300-400; 500-700 mg/kg/d for males and 0, 250-300, 500-600, 800-1000 mg/kg/d for females). The F0 animals received DEHT for at least 70 days before mating and until termination; the F1 generation received diets following weaning (following PND 22) and for at least 70 days before mating. Reproductive parameters were unaffected by DEHT. Mean maternal kg bw/s was reduced in the 1% group throughout gestation and lactation and throughout the F1 generation. No critical histopathological changes observed: the NOAEL for reproductive toxicity was concluded to be 1% in the diet (500-700 mg/kg/d in males).

Oral developmental toxicity

Study 1 following OECD guideline 414: Groups of 25 pregnant SD rats received DEHT doses of 0, 0.3, 0.6 and 1% in the diet (approx. 0, 226, 458, or 747 mg/kg/d) from GD 0 to GD 20. Uteri and contents were excised by caeserean section and examined (foetuses, implantation sites): No evidence of embryotoxicity, fetotoxicity and no effect of treatment on the number of viable foetuses. No visceral or skeletal anomalies attributed to treatment. Changes in maternal kg bw/ were seen at the highest exposure level and the NOAEL for maternal toxicity was 0.6% (458 mg/kg/d); the NOAEL for developmental tox was 1% (747 mg/kg/d).

Study 2: 10 Controls and 8 pregnant SD rats received DEHT from GD14 to PND3 by gavage at 0 and 750 mg/kg bw (dose adjusted based on individual maternal weight changes throughout dosing period) and their male offspring were examined for several parameters of demasculinisation: No changes in AGD, testes weight, testes descent, testes lesions, presence of areolas/nipples or vaginal pouches, reproductive organs weights, reproductive malformations or mating behaviour were noted. In contrast, DEHP also assessed in the same study, yielded adverse effects at this dose (750 mg/kg bw) (Gray *et al.*, 2000).

Study 3 following OECD guideline 414: Groups of pregnant CD mice received DEHT doses of 0, 0.1, 0.3 and 0.7% in the diet (approx. 0, 197, 592, or 1,382 mg/kg/d) from GD0 to GD18. Changes in maternal weights were seen in the mid and high exposure animals and the NOEL for maternal toxicity was 0.1% (197 mg/kg bw); the NOEL for developmental toxicity was 0.7% (1,382 mg/kg).

ECHA (2011) states: Results of an uterotrophic assay in which immature females were given up to 2,000 mg/kg/day DEHT by gavage on PND 19-21 also indicate that DEHT does not possess estrogenic activity.

7.6. Human data

There are 2 small human studies reported, both with dermal application of DEHT, one to test primary dermal irritation, the other on skin sensitisation. Under the conditions of the study DEHT was found to be non-irritating and did not elicit evidence of sensitisation. No other human studies

7.8. Conclusion Da rivedere

DEHT is not genotoxic. DEHT is less active in the induction of peroxisome-proliferation in rats than DEHP and this is explained by the smaller amounts of monoester produced during DEHT metabolism. At doses where DEHP, BBP and DINP all altered sexual differentiation, DEHT was inactive.

References:

Submission from Eastman Chemical Company.

ECHA web site

8. TOTM (Trioctyltrimellitate)

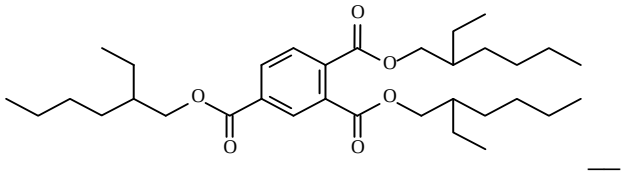
8.1. Physico-chemical properties

CAS Reg. No: 3319-31-1

Synonyms: Tris(2-ethylhexyl)benzene-1,2,4-tricarboxylate, trioctyl trimellitate; tri(2-ethylhexyl) trimellitate (TEHTM); trioctyl benzene-1,2,4-tricarboxylate; 1,2,4-bezenetricarboxylic acid, trioctyl ester.

Emperical Formula: C_{33} $H_{54}O_6$

Structure:



Molecular weight: 546.8

Melting point: -50°C (-35 in IUCLID)

Boiling point: 283°C at 4 hPa

Vapour pressure: 5.6 Pa at 20°C

Solubility in water: 0.13 (0.00039) mg/L at 25°C / 1.08 µg/L at 25°C (measured)

Log Kow: 5.94 (4.35) at 25°C Log Pow = 8.00 (T= 25°C) (measured)

Purity: ≥ 98.4 % (w/w)

Impurities: 7 unknown impurities with typical concentrations ranging between 0.01 and 0.42 % have been reported.

In the dossiers no method of determination of substance and metabolites were presented. The open literature gave 2 papers in which HPLC methodology were applied for TOTM analysis (Christensson et.al. 1991, Kambia et.al. 2001). No methods for the metabolites are available, however most probably DEHP methods are applicable.

8.2. Use

The production volume in Japan is about 20.000 tonnes/year and there are 5 manufacturers in Japan. Estimated global production is 40,000-100,000 tonnes/year. In Europe, the substance has been registered under REACH at a tonnage band of > 1000 tpa (10000 – 100000 tpa). TOTM is mainly used as a plasticizer for PVC electrical cables and wire. In medical devices TOTM is used as a PVC plasticizer in various infusion equipments.Trimellitate plasticizers are the alternative for phthalate plasticizers when high temperature applications and low volatility are of importance. The end products include oil resistance products, gasoline hoses, rain shoes, gasketing and vehicle engine wires. TOTM has low leaching properties and extraction resistance properties.

8.3. Exposure

TOTM is produced and used in closed systems and therefore, the occupational exposure is limited in the case of sampling and maintenance at the production facilities. Moreover, the exposure time is very short. The major route of occupational exposure is inhalation and dermal. TOTM is relatively difficult to extract from the polymeric matrix which lowers the consumer (patient) exposure.

German/Austrian search mentions the following with respect to use: wide dispersive use, consumer use; primarily used for heat-resistant PVC articles, PVC-products used in the hospital sector (blood platelet bags), packing, cables, profiles and floor/wall coverings

8.4. Metabolism and toxicokinetics

Absorption and metabolism were studied for TOTM (^{14}C labelled) mixed with corn oil and administered by gavage in a single dose of 100 mg/kg bw in 4 male SD rats. About 75% of the dose was excreted in the faeces, 16% in the urine as metabolites and 1.9% was expired as CO_2. Radioactivity excreted in the faeces was unchanged TOTM (85% of the faecal radioactivity) mono and di(2-ethylhexyl)trimellitate (MOTM and DOTM) and as unidentified polar metabolites. Metabolites in the urine were identified as MOTM nabd metabolites of 2-ethylhexanol. Less than 0.6% of the dose remained in the tissues (SIDS Initial Assessment Report for 13th SIAM, 2001).

8.5. Toxicity

Acute toxicity

Acute toxicity data are mainly reported for rat, mice and rabbits. LD_{50} was >2000 mg/kg and 3200 mg/kg bw in oral or ip administration in rats (Ministry of Health and Welfare, Japan 1996).

Repeated dose toxicity

Oral administration of TOTM in the diet to groups of 5 male and 5 female Fisher 344 rats at the level of 0, 184, 650, 1826 mg/kg bw/d for 28 days. There were no statistical significant differences in body weight between control and exposed groups, but a significant difference was reported in the absolute and relative liver weights, serum albumin, cholesterol levels, palmitoyl CoA oxidation and catalase activity at the mid and high dose.. The NOAEL was 184 mg/kg /day(CMA 1985).

In the second study the NOAEL was 1000 mg/kg/d, but few the information is available (Ministry of Health and Welfare, Japan 1996).

The third study was the OECD preliminary reproduction study. Administration was by gavage at the doses of 100, 300 and 1000 mg/kg/d. The decrease of spermatocytes and spermatides in males was observed at 300 and 1000 mg/kg/d doses by histopatohological examinations. The NOAEL was 100 mg/kg for males and 1000 mg/kg/d in females (Ministry of Health and Welfare, Japan 1998).

In a non GLP compliant study rats were exposed to TOTM and DEHP (28 days, 0.2%; 0.67%; 2.00%). The data demonstrated the same spectrum of morphological and biochemical changes in the livers of rats exposed to TOTM as did DEHP. TOTM, however, was much less potent in its action, with a dietary level of 2%, causing less peroxisome proliferation and enzyme induction than 0.67% DEHP (Hodgson, 1987).

Adult male rats receiving TOTM intraperitoneally for 7 days exhibited no significant changes in the P450-mediated activities of hepatic aminopyrine-N-demethylase, aryl hydrocarbon hydroxylase or glutathione-S-transferase or in glutathione contents. However, except for the glutathione level, the DEHP shoved significant increases in the activities of these particular enzymes (Rathinam et.al., 1990).

TOTM (50, 225 and 1000 mg/kg bw day) was tested in a sub-chronic feeding toxicity test in rodents (OECD Guideline 408) (Longobardi, 2012 robust summary available on the ECHA web site). The test included additionally an analysis of spermatogenic cycling (histology of testis) and oestrous cycle (last 2 weeks of treatment, vaginal smear). No adverse effects were observed on these parameters and on the histology of the reproductive organs. A NOEL/NOAEL of 50/225 mg/kg bw day was proposed. Moreover a mechanistic transcriptional profiling study further supports the evidence that TOMT differs with regard to reproductive toxicity to the phthalates DEHP and MEHP.

Genotoxicity

GLP compliant or non complaint studies for Ames test were carried out indicating that TOTM did not induce gene mutation in bacterial system and chromosomal aberration in mammalian cultured cells with or without an exogenous metabolic activation system (Ministry of Health and Welfare, Japan 1996, ECHA web site).

Reverse gene mutation assay was conducted according to OECD TG 471,and 472 using preincubation method TOTM was not mutagenic in Salmonella TA100, TA1535, TA 98, TA1537 and E.coli WP2 uvrA at concentration of up to 5000 µg/plate, with or without tan exogenous metabolic activation (Ministry of Health and Welfare, Japan 1996).

Chromosomal aberration test by OECD TG 473 was conducted in cultured Chinese hamster lung cells. Structural chromosomal aberrations and polyploidy were not induced to a max conc. of 5,0 mg/ml on continuous treatment (Ministry of Health and Welfare, Japan 1996).

TOMT was also negative when tested in mammalian cell gene mutation assay (mouse lymphoma L5178Y cells) test with and without metabolic activation. The substance was not mutagenic in a dominant lethal assay conducted in the mouse and does not induce unscheduled DNA synthesis in primary rat hepatocytes (ECHA web site).

Carcinogenicity

No data available.

Reproduction/developmental toxicity

Gavage study in SD rats according to OECD guideline 421 (Reproduction/Developmental toxicity screening test) conducted at doses of 100, 300 and 1000 mg/kg/day (male 46 days, females from 14 days before mating to day 3 of lactation) of TOTM. Histopathological examination of testes revealed decreased spermatocytes and spermatids in males of the 300 and 1000 mg/kg/d groups. No effects of TOTM were detected on general appearance, body weight, food consumption, autopsy findings and weight of reproductive organs of both sexes or on histopathological examination of the ovary. On the basis of this observation the NOAEL for males is 100 mg/kg/d and 1000 mg/kg/d in females (Ministry of Health and Welfare, Japan 1998).

No influence of TOTM was detected regarding reproduction ability, organ weights or histopathological appearance of the ovaries, delivery or maternal behaviours of dams. No effects were seen on viability, general appearance, of weight or autopsy findings of offspring. The NOAEL for repro/developmental toxicity is considered to be 100 mg/kg/d for males, 1000 mg/kg/d for females and 1000 mg/kg/d for offspring (Ministry of Health and Welfare, Japan 1996).

8.7. Human data

The leaching of plasticizers from blood line was studied in 11 patients. During the treatment the plasma level of DEHP rose from 0.1 microg/ml (<0.05-0.17, n=11) to 0.7 microg/ml (0.30-1.6, n=11). When patients were changed to tubing containing TOTM, the concentration of DEHP was below or close to the detection limit (LOD 0.5 microg/ml) and TOTM could not be detected (LOD 0.5 microg/ml) (Christersson et.al. 1991).

The circulating concentrations of DEHP and TOTM resulting from the release from dialyzer tubes were estimated by HPLC. A DEHP quantity of 122.95 ± 33.98 mg (n=10) was extracted from tubing during a single dialysis session (4h). By using TOTM-DEHP 1:1 mixture, 41.80± 4.47 mg of DEHP and 75.11± 25.72 mg of TOTM were extracted (Kambia et.K. *et al.*, 2001).

203 human volunteers were tested for sensitisation to several plasticizers following 3 weeks of dermal application 3 times a week. Slight erythema was observed in four individuals exposed to TOTM, 2 of which resolved within 96h and one that occurred only after 96h (David *et al.*, 2003).

8.8. Conclusion

TOTM has a low acute toxic potential. Based on the data available TOTM seem to be poorly absorbed and metabolised. This may partially explain the low liver toxicity of the compound. No clear toxicological mode of action can be identified. TOMT was not genotoxic. The spectrum of some morphological and biochemical changes in rat liver were the same in TOTM and DEHP, but the degree of damage was by far lower in TOTM exposed animals than in DEHP. The overall NOAEL can be set to 100 mg/kg in male based on the damage reported in testes in animals.

Comment: in Europe, the substance is not approved for food contact material. The substance was put into the community rolling action plan under REACH to clarify inter alia PBT concern. The process has been finalized in the meantime and the outcome with respect to T (toxicity) criterion was that this criterion is not fulfilled (http://echa.europa.eu/documents/10162/d8cb11fc-9996-4112-81d3-666e91f799bf). With respect to reproductive toxicity the following conclusion was drawn: "Due to the overall negative results for reproductive toxicity: 1/in a 90 day study extended with an analysis of spermatogenic cycling and oestrous cycling, 2/in a developmental study extended with an

analysis of post-natal development till 15 weeks (male offspring) and 6 weeks (female offspring) including endocrine and developmental parameters as well as histology of reproductive organs, and 3/in developmental/reproductive tests according to OECD TG 421 and 422 with full histology, no further testing appears necessary."

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