EUROPEAN COMMISSION

Health & Consumer Protection
Directorate-General

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

Scientific Committees
on consumer products
on emerging and newly identified health risks
on health and environmental risks

Adopted after public consultation by the SCENIHR
during the 22nd Plenary of 6 February 2008

About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the sound scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems, which may pose an actual or potential threat.

They are: the Scientific Committee on Consumer Products (SCCP), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly-Identified Health Risks (SCENIHR) and are made up of external experts.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Evaluation Agency (EMEA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCENIHR

Questions concerning emerging or newly-identified risks and on broad, complex or multi-disciplinary issues requiring a comprehensive assessment of risks to consumer safety or public health and related issues not covered by other Community risk- assessment bodies.
In particular, the Committee addresses questions related to potential risks associated with interaction of risk factors, synergic effects, cumulative effects, antimicrobial resistance, new technologies such as nanotechnologies, medical devices, tissue engineering, blood products, fertility reduction, cancer of endocrine organs, physical hazards such as noise and electromagnetic fields and methodologies for assessing new risks.

Scientific Committee members

Anders Ahlbom, James Bridges, Wim De Jong, Jana Hajslová, Philippe Hartemann, Thomas Jung, Mats-Olof Mattsson, Jean-Marie Pagès, Konrad Rydzynski, Dorothea Stahl, Mogens Thomsen, David Williams

Contact:
European Commission
Health & Consumer Protection DG
Directorate C: Public Health and Risk Assessment
Unit C7 - Risk Assessment
Office: B232
B-1049 Brussels

Sanco-Sc1-Secretariat@ec.europa.eu

The opinions of the Scientific Committees reflect the views of the independent scientists who are members of the committees. They do not necessarily reflect the views of the European Commission. The opinions are published by the European Commission in their original language only.

http://ec.europa.eu/health/ph_risk/risk_en.htm

ACKNOWLEDGMENTS

Members of the working group are acknowledged for their valuable contribution to this memorandum. The members of the working group are:

The SCENIHR members:
Prof. Jim Bridges (chair)
Dr. Wim De Jong
Prof. Jana Hajslová
Prof. Dorothea Stahl

External experts:
Dr. Kristofer Almdal, Risø National Laboratory, Denmark
Prof. Jurgen Angerer, Institut für Arbeits-, Sozial- und Umweltmedizin der Universität Erlangen-Nürnberg, Germany
Prof. Gisela Degen, Scientific Committee on Consumer Products (SCCP)
Prof. Jouni Jaakkola, University of Birmingham, United Kingdom
Prof. Bo Jansson, Scientific Committee on Health and Environmental Risks (SCHER)
Dr. Ole Ladefoged, Scientific Committee on Health and Environmental Risks (SCHER), (rapporteur)
Prof. G. Latini, Division of Neonatology, Brindisi, Clinical Physiology Institute, Italy
Prof. Kimmo Peltonen, EVIRA, Finland
Prof. Elizabeta Ranucci, University of Milano, Italy
Dr. Suresh Rastogi, Scientific Committee on Consumer Products (SCCP)
Dr. Jorge Rodrigues Inacio, Karolinska University Hospital, Sweden

ABSTRACT

The Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) has evaluated the exposure to DEHP for the general population and patients during medical procedures. In some cases the exposure is significant and exceeds the toxic doses observed in animal studies. There is limited evidence suggesting a relation between DEHP exposures and some effects in humans. There is a reason for some concern for prematurely born male neonates for which the DEHP exposure may be transiently above the dose inducing reproductive toxicity in animal studies. Sofar, there is no conclusive scientific evidence that DEHP exposure via medical treatments has harmful effects in humans. But, it is recognised that especially the potentially high exposure during medical treatments may raise a concern, even in the absence of clinical or epidemiological evidence, for harmful effects in humans. Further studies are required to confirm or reject the suggestions of adverse effects of DEHP in humans. For certain uses of DEHP alternative plasticizers for PVC are available. The Committee got access to toxicity data for eight possible alternative plasticizers and compared their toxicity with that of DEHP. In respect to reproductive toxicity in animal studies DEHP induces more severe effects compared with some of the alternatives. A risk assessment of these available alternative plasticizers could not be performed due to a lack of exposure data from medical devices. Each alternative to DEHP, however, must also be evaluated with regard to their functionality in respect to medical devices. The risk and benefits of using alternative plasticizers should be evaluated case by case.

Keywords: SCENIHR, scientific opinion, DEHP, medical devices, neonates, alternative plasticizer, risk

Opinion to be cited as:

SCENIHR (Scientific Committee on Emerging and Newly-Identified Health Risks), Scientific opinion on the safety of medical devices containing DEHP-plasticized PVC or other plasticizers on neonates and other groups possibly at risk, 6 February 2008

EXECUTIVE SUMMARY

The Scientific Committee on Medicinal Products and Medical Devices (SCMPMD) published its Opinion on Medical Devices containing Di-(2-ethylhexyl)phthalate (DEHP) plasticized PVC in 2002. That Opinion stated that there were no reports concerning any adverse effects in humans following exposure to DEHP-PVC, even in neonates or other groups of relatively high exposure. In addition, there were no indications that neonates of high DEHP exposure have any altered long term fertility patterns. Since 2002, substantial new information on exposure to DEHP has become available as well as data on toxicity obtained in laboratory animal and human studies. Also for DEHP a so called tolerable daily intake (TDI) was calculated in recent risk evaluations. Therefore an overview is presented on the safety of DEHP in medical devices. In addition, the availability, suitability and safety of alternative plasticizers for DEHP have been evaluated. Alternative materials for PVC were not evaluated.

Certain medical procedures used in high risk groups result in a significant exposure to DEHP. In view of the reproductive toxicity observed in animal studies in which young immature animals were more susceptible to DEHP toxicity, newborn and pre-term born male infants are of special concern. Exchange transfusion in neonates, total parenteral nutrition in neonates, multiple procedures in sick neonates, and haemodialysis in peripuberal males are examples of procedures applied in high risk groups. Other risk groups are the male foetus and male infant of pregnant women or lactating women, respectively, in haemodialysis. Also massive infusion of blood into trauma patients is of concern due to exposure levels substantially exceeding the TDI of DEHP.

The toxicity of DEHP in laboratory animals is summarized. The reproductive effect of DEHP in developing and postnatal pups appears at low levels with a TDI of 48 µg/kg bw/d, derived from a three generation study in rats with a No Observed Adverse Effect Level (NOAEL) of 4.8 mg/kg bw and applying a uncertainty factor of 100.

Possible alternative plasticizers were evaluated for their potential toxicity and ranked according to toxicity and leaching, or leaching resulting in exposure. For reproductive toxicity the dose of DEHP is an order of magnitude lower compared with some of the alternative plasticizers. For some of the alternative plasticizers a complete evaluation could not be performed due to lack of data on either toxicity or exposure.

There are some studies published on the leaching of plasticizers from PVC materials to different fluids, but due to the very different conditions used it is difficult to compare the results between those studies. For most of the alternative plasticizers added in similar concentrations to PVC as the DEHP, the leaching in fatty medium appears to be the same order of magnitude. Although different leaching rates, both lower and higher, of some alternative PVC plasticizers in aqueous medium has been observed; the plasticizers leaching rate in aqueous medium are at least 1000 times lower than those in vegetable oils.

Some alternatives may be suitable to replace DEHP in certain medical devices, while for other devices it may be difficult to achieve the same functionality as PVC plasticized with DEHP. The risk and benefit of using alternative plasticizers should be evaluated case by case.

Compared to the previous opinion of the SCMPMD, the new information on DEHP indicates that there is still a reason for some concern for prematurely born male neonates. This concern is instigated by the potential high human exposure to DEHP especially during certain medical procedures which may be transiently above the dose inducing reproductive toxicity in animal studies.

Sofar, there is no conclusive scientific evidence that DEHP exposure via medical treatments has harmful effects in humans. However, it is recognised that especially the potentially high exposure during medical treatments may raise a concern, even in the absence of clinical or epidemiological evidence, for harmful effects in humans. Further studies are required to confirm or reject the suggestions of adverse effects of DEHP in humans.

TABLE OF CONTENTS

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 on "Medical Devices containing DEHP plasticized PVC; Neonates and Other Groups Possibly at Risk from DEHP toxicity" 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".

According to published data on reproduction toxicity, neonates and prepubertal males may suffer adverse effects from DEHP exposure in medical devices. According to a recent risk evaluation of DEHP on human health carried out in the context of the "existing" chemicals substances legal framework, a Tolerable Daily Intake (TDI) of DEHP was determined for the general exposure of humans to DEHP.

It is therefore necessary for the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) to review and possibly update the opinion adopted in 2002. Since alternative DEHP-free medical devices have been recently introduced in the market, the long-term effect of these alternative plasticizers or alternative materials, when used in medical devices, are not well known. 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 related to the safety of these alternatives for patients and in particular to high risk groups.

2. TERMS OF REFERENCE

1. Update of the scientific opinion adopted in September 2002 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 September 2002 on "Medical Devices containing DEHP Plasticized PVC; neonates and other groups possibly at risk from DEHP toxicity".

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 of DEHP leaching from soft PVC as a basis for risk assessment for high risk patient groups, taking into account the route of exposure.*

2. Medical devices containing alternative plasticizers: possible risk for certain uses or to certain patient groups. Since alternative DEHP free medical devices have been developed and are used to treat patients, the Scientific Committee is requested to evaluate the potential risks of currently available alternatives in relation to patient health, when used in medical devices.

3. SCIENTIFIC RATIONALE

3.1. Introduction

In view of the complexity of the questions addressed in the Terms of Reference. the Committee decided to concentrate on the risk assessment of plasticizers used in PVC in this opinion. Whilst recognising that there are several non-PVC based materials that could provide effective materials for use in medical devices, this opinion does not address these materials. Although the published Call for Information included both alternative plasticizers and alternative materials, only the former was submitted. The Committee recognized that there may be need for evaluation of these alternative non-PVC materials in the future.

Polyvinylchloride (PVC) is used extensively for a very wide range of purposes ranging from a lining for landfill waste disposal sites to a food wrapper for foods. 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 amounts 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 which when added to a material, usually a polymer, makes it flexible, resilient and easier to handle. There are more than 300 different types of plasticizers described of which between 50 and 100 are in commercial use. The most commonly used plasticizers are phthalates. In Western Europe about one million tonnes of phthalates are produced each year, of which approximately 900,000 tonnes are used to plasticize PVC **(**http://www.plasticisers.org). The most common are: 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. There is a reduction in the use of DEHP as plasticizer in PVC (personal communication, 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 (leak, migrate) 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 very substantial amount of research. As a consequence concerns have been raised about the implications for human health and to the environment of three particular properties of DEHP observed in experimental animals/other experimental systems namely the potential to cause:

- reproductive and developmental effects.
- endocrine disruption and testes toxicity.
- peroxisome proliferation in the liver and thereby increase the incidence of liver cancer in rodents.

Reports on these properties have resulted in calls from various organisations and individuals to replace DEHP with other plasticizers that do not show such properties.

In addition a number of bodies have called for a reduction in PVC use or even an outright ban on PVC itself because of their concerns about the environmental problems associated with PVC disposal, especially the production of dioxins as a result of the incineration of PVC. However, recently there have been improvements in the incineration technologies in Europe such that the PVC incineration minimises dioxin emission (Danish EPA 2003).

The above concerns have resulted in the SCENIHR being asked by the Commission Services to review and where appropriate update the Opinion of its predecessor committee (The Scientific Committee on Medical Products and Medical Devices Opinion (SCMPMD) of September 2002) on the risks and benefits of the use of PVC, incorporating DEHP, in medical devices. Possible alternative materials could not be evaluated in view of the lack of an analysis of the risks associated with these materials at that moment. However, it was concluded that some alternative plasticizers could replace DEHP in PVC on some conditions for which evaluation of risk and benefits should be done on a case by case basis.

In 2002 Health Canada (2002) recommended that alternative products that are already available should be utilized for all ECMO (extracorporeal membrane oxygenation) procedures in newborns and infants. Tubing and storage bags used for administration of lipophilic drugs or drugs which contain surfactants (i.e., lipophilic drug formulations) should not contain DEHP, or strategies to decrease DEHP exposure should be employed, particularly when administering these drugs to infants and children. As alternative products are already available, it was recommended that total parenteral nutrition solutions be administered to newborn and infants only via products, which do not contain DEHP. At that time the Food and Drug Administration (FDA) of USA was recommending the manufactures of medical devices to consider eliminating the use of DEHP in such devices that can result in high exposure in sensitive patients and that certain products be labelled with their DEHP content (FDA 2002).

The SCENIHR decided that in order to address this request a risk assessment needed to be carried out in which PVC containing DEHP should be the benchmark. It was also agreed that the evaluation should concentrate on new information that was not available to the SCMPMD in its deliberations in 2002.

DEHP is the main plasticizer used in PVC based medical devices. According to European Pharmacopoeia, only DEHP, ESBO and ELO should be used as plasticizers in medical devices (Medical Devices Directive 93/42/EEC). A number of other substances are used as plasticizers in medical devices (for example, butyl trihexyl citrate in blood bags), and some non-PVC based materials (for example, enteral feeding bags made of ethyl vinyl acetate) are also available as alternative to DEHP-PVC. In order to obtain the most updated information the Commission published a Call for Information in March 2006 inviting interested parties to submit:

1) Scientific peer reviewed research papers and reviews (later than 1995) on this issue.
2) Data on safety evaluation.
3) Other publicly available credible scientific information that may not be easily available and which is directly relevant to this issue.

The results of this Call for Information and information available from other sources were used as a basis for the following evaluation on DEHP and its alternatives in PVC medical devices. Consequently in this report only the risks from DEHP and possible alternative plasticizers for which sufficient suitable information has been provided are considered. Information on the following compounds was obtained from the stakeholders:

- 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)

In addition, other phthalates could be used in medical devices and SCENIHR also looked for information for these substances. A compound that is used as plasticizer in food packaging materials, DEHA, was also added to the list which thus also contains the following substances:

- Di-iso-nonyl phthalate (DINP, CAS 68515-48-0 and 28553-12-0)
- Di(2-ethylhexyl) adipate (DEHA, CAS 103-23-1)

Also polymeric plasticizers such as aliphatic polyesters can potentially be used as alternative plasticizers in PVC medical devices.

It must also be emphasised that in the following evaluation only risks and health benefits to patients who are exposed to medical devices are considered. Thus the following risk/benefit considerations are excluded from our consideration:

- Health, safety and environmental aspects of PVC manufacture and incorporation into medical devices.
- 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.

The focus of this opinion is on the possible risk for patients exposed to medical devices, but as there is a considerable exposure to plasticizers for the general public, this has been taken into account in the evaluation.

The safety assessment performed here includes currently available as well as 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. Thus, important medical devices (blood bags, catheters, dialysis equipment, enteral feed containers, gastrointestinal tubes, IV solution storage and administration sets, tubing used in neonates, tubing used for respiratory therapy and containers for total parenteral nutrition (TPN)) and potential DEHP alternatives are the focus of the evaluation.

Finally it is pertinent to point out that only the risks from the use of plasticizers in PVC medical devices have been evaluated. The 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.

In the following chapters the data on DEHP are considered first which is followed by a comparison with the biological properties of the other plasticizers.

3.2. Present use of plasticized PVC in medical devices

Quantitative information of the amount of plasticized PVC used for medical devices is not available. Medical applications account for 0.5% of the total PVC volume used in Western Europe[1]. The world PVC use was 2.94×10^7 t in 2004 with a 4.3% annual growth rate[3]. The Western European use is approximately 5.8×10^6 t. According to the EU life cycle assessment

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

report medical applications account for 0.5% of the PVC used in Europe. Thus approximately 3×10^4 t of plasticized PVC is used for medical applications annually in Europe.

It is possible to greatly reduce the use of DEHP-PVC in hospital procedures as demonstrated in several hospitals around Europe. This might be achieved by using PVC containing alternative plasticizers or using alternative materials. However, this probably can not be achieved for all medical procedures.

DEHP is used in PVC to manufacture blood bags. DEHP is leaching into the blood in which it contributes to the stability and survival by stabilising the red blood cell membrane (Labow et al. 1987). This prolongs the possibilities of blood storage up to 6-8 weeks after blood 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.

The use of plastics in medical application is increasing and the medical plastics market was anticipated to grow by more than 3% annually in 2005. There is also a considerable interest from medical plastic producers in developing alternative materials to plasticized PVC.

3.3. Physicochemical properties of plasticizers

The most important physical parameters for evaluating potential human and environmental exposures 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 physical chemical characteristic, some of which have been estimated (in Italics in the table) 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.

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

Substance	Vapor 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 100ºC (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 100ºC (4)	<20 (4)	10.0 (4)		
DINP	6 x 10^{-5} (2)	0.6 (2)	8.8 (2)	0.07	77
DOTP	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 wt % plasticizer when extracted with water at 50°C for 24 hours (ASTM D1239-55 (from Sears, 1989).
b: Loss of plasticizers from a 1 mm, PVC sheet containing 40 wt % plasticizer when extracted with kerosene at 23°C for 24 hours (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: From dossier (see Annex)

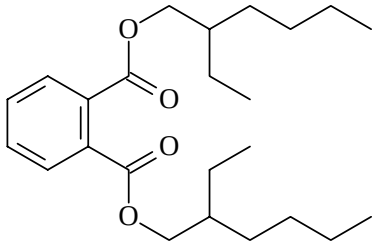
As can be seen in Table 1 the assessed plasticizers are very lipophilic, and all of them, except ATBC, have log K_{ow} values above 7 and low water solubility. In this respect the alternatives are not very different from DEHP. The leaching of these substances from PVC to body fluids/tissues can thus 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 as a basis for comparison with the different alternatives. The chemical characteristics of DEHP are presented below.

CAS Reg. No.: 117-81-7
Synonyms:
Emperical 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[2].

3.4.2. Use

The use of DEHP in Europe 1997 has been estimated to 476,000 ton and about 97% of that is used as plasticizer in polymers, mainly PVC (personal communication, ECPI 2007). About 22% of that is used for products with mainly outdoor applications, while the remaining 462,000 tons end up in products being used indoors. The use in medical devices is estimated at 0.5% of the total production of which 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 2004).

3.4.3. Metabolism of DEHP in humans

In mammals, including man, DEHP is converted into a variety of metabolites (Figure 1). The first and fast stage in the metabolism of DEHP is the hydrolytic cleavage to mono(2-ethylhexyl) phthalate (MEHP) and 2-ethylhexanol (2-EH). After oral uptake enzymatic hydrolysis occurs already in mouth (Niino et al. 2003, Niino et al. 2001) and especially in the gastrointestinal tract (Albro et al. 1982, Albro and Thomas 1973). Thus it can be assumed that the majority of DEHP is rapidly absorbed as MEHP in gut following oral administration. DEHP hydrolyzing lipases can be found in many tissues (especially in pancreas, intestinal mucosa, liver) and in blood plasma of rats (Albro and Thomas 1973, Daniel and Bratt 1974).

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

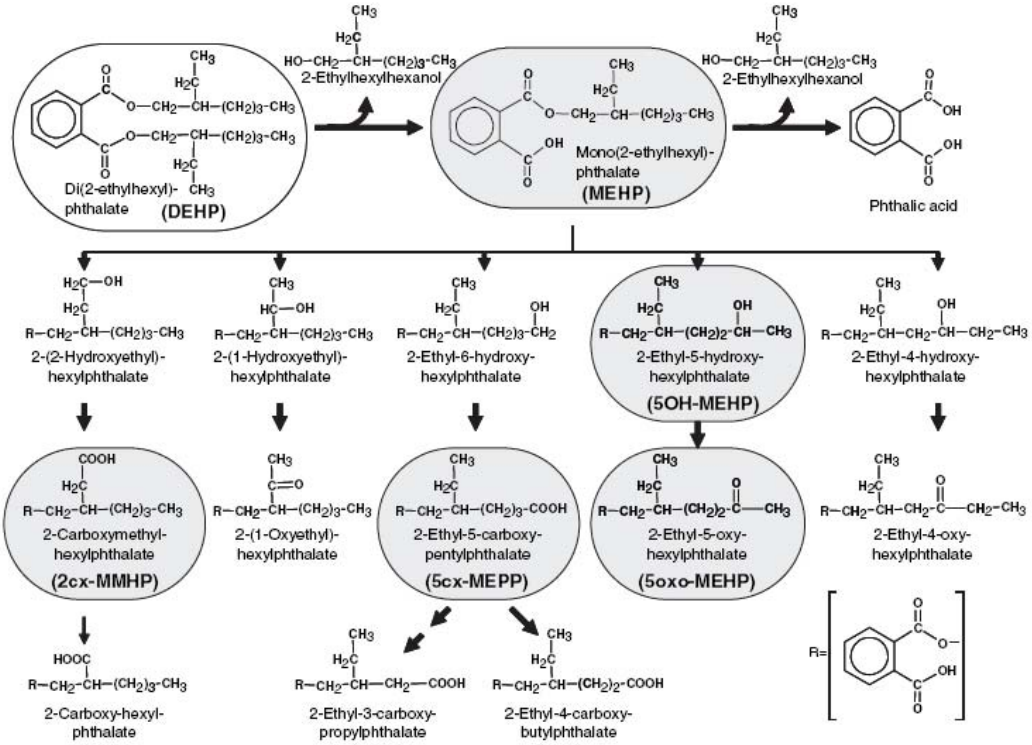


Figure 1. **DEHP metabolism**[3] (according to Albro 1982, Peck and Albro 1982, Schmid and Schlatter 1985). Major metabolites according to Koch (2005a) are highlighted.

Further metabolism takes place in the liver (Albro 1986) with 2-EH and MEHP undergoing a set of oxidative reactions. In rats the formed 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 (Albro 1975). Also in human urine several of these oxidative metabolites have been identified (Wahl et al. 2004, Wahl et al. 2001).

MEHP is metabolized to produce a large number of oxidative metabolites (Figure 1). Oxidative metabolism of MEHP starts 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).

In previous human metabolism studies urinary excretion rates between 10 and 31% after oral DEHP administration were determined determined , which indicated a maximal oral bioavailability of 50% as well (ECB 2004). However, Koch et al. (2004b, 2005a) found that the majority of orally administered DEHP is systemically absorbed in humans and excreted via urine. After two days of administration of deuterium ring-labelled DEHP (0.35 mg, 2.15 mg and 48.5 mg) to a male healthy volunteer about 75% of the dose was excreted in urine in form of the five major metabolites mono(2-ethyl-5-hydroxyhexyl) phthalate (5OH-MEHP) (24.7%), mono-(2-ethyl-5-carboxypentyl) phthalate (5cx-MEPP) (21.9%), mono(2-ethyl-5-

[3] Figure provided by Koch et al. 2005. New metabolites of di(2-ethylhexyl)phthalate (DEHP) in human urine and serum after single oral doses of deuterium-labelled DEHP. Archives Toxicology 2005; 79: 367-76 (Figure 1). With kind permission of Springer Science and Business Media and the approval of the author.

oxohexyl) phthalate (5oxo-MEHP) (14.9%), MEHP (7.3%) and mono[2-(carboxymethyl)hexyl] phthalate (2cx-MMHP) (5.4%). No dose dependency in metabolism and excretion was observed for the dose range investigated. Taking into account that further minor DEHP metabolites, such as mono(2-ethyl-3-carboxypropyl) phthalate, mono(2-ethyl-4-carboxybutyl) phthalate, and mono(2-(1-oxoethyl)hexyl) phthalate, were excreted in human urine (Figure 1) (Albro et al. 1982, Schmid and Slatter 1985, Silva et al. 2006a) and so far only periods up to 48 h after administration were observed one can assume that the majority of an orally taken DEHP dose is absorbed and excreted via urine.

In rats and non-human primates absorption rates of around 50% for doses up to about 200 mg/kg have been estimated (ECB 2004). In contrast to rodents there may be a dose-limited absorption at higher doses (2000 mg/kg per day for 14 days) in non-human primates (Rhodes et al. 1983, Rhodes et al. 1986).

Koch et al. (2004b, 2005a) found that urinary excretion in human followed at least a two-phase elimination model. The first elimination phase (after 4-8 h absorption and distribution) lasted until 14-16 h after D4-DEHP administration, with an elimination half-life of about 2 h for all five metabolites. In the second elimination phase considerably longer half-lives were estimated for the oxidized DEHP metabolites 2cx-MMHP (24 h), 5cx-MEPP (12-15 h), 5OH-MEHP (10 h), 5oxo-MEHP (10 h) than for the simple monoester MEHP (5 h). The respective half-lives in serum were estimated to be shorter than two hours except for 2cx-MMHP, for which the half-life was at least 5h. In contrast to urine MEHP was seen to be the dominant metabolite in serum.

After normalization Koch et al. (2005a) calculated a 15–100 times higher normalized area under the concentration-time curve for MEHP in human blood than previously found in rats and marmosets (Kessler et al., 2004). In the latter study the normalized AUCs of marmosets were found to be up to 16 times lower than in rats receiving the same daily oral DEHP dose per kilogram of body weight. This may indicate that a similar external exposure to DEHP results in a higher internal dose to MEHP in humans compared to rats and particularly to marmosets.

After long-term exposure, which generally may occur in the general population, the ratios among the DEHP metabolites excreted in urine seem to be shifted in favour to 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).

Apart from the first hydrolysis step to MEHP the metabolism of DEHP appears to be qualitatively unaffected by the route of administration (ECB 2004). 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 in some distance 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 rather similar to that after oral administration (Table 2), which indicates that the toxicokinetic behaviour of DEHP in humans is not different for those exposure routes.

Several studies indicate some differences in DEHP metabolism between species. In rats 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). On the other hand rats excrete much lower amounts of MEHP compared to other mammalians including primates (Peck and Albro 1982). β-oxidation may be a major metabolic pathway in rodents but not in primates and humans (Albro et al. 1982). After intravenous administration of DEHP quite similar profile of the urinary metabolites were determined in Green monkeys and humans by Albro

et al. (1981) and Peck et al. (1978). In these studies, however, 5OH-MEHP and MEHP were identified as the major metabolites, whereas the relative amounts of 5cx-MEPP were clearly lower, which is in some contrast to the recent findings from Koch et al. (2004b, 2005a).

Glucuronidation is the major conjugation pathway in mice, guinea pigs and non-human primates (Albro et al. 1982, Egestad et al. 1996). Earlier studies suggest that glucuronides are not formed in rat (Albro et al. 1982, Kluwe 1982). In a recent human study MEHP was mostly found as glucuronide conjugate in maternal urine (Calafat et al. 2006). In humans at least 65% of the MEHP derivatives in the urine seem to be excreted as glucuronides following oral or intravenous administration (Albro et al. 1982, Bronsch 1987, Schmid and Slatter 1985). Large interindividual variations in the glucuronidation were observed for some DEHP metabolites (Dirven et al. 1993, Silva et al. 2006b). 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).

Table 2. Relative distribution (in %) of the five major DEHP metabolites (sum is set as 100%) in human urine after oral administration (D4-DEHP) and intravenous exposure

Route	5OH-MEHP	5cx-MEPP	5oxo-MEHP	MEHP	2cx-MMHP	Reference
Oral	34.8	27.6	22.4	8.8	6.3	Koch 2005a
Intravenous	26.4	27.2	23.1	13.3	10.0	Koch 2005b

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>testes) (Rhodes et al. 1986). DEHP and its metabolites may be secreted into the milk of lactating rats (Dostal et al. 1987, Parmar et al. 1985) and also 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 regarding metabolism and bioavailability following inhalation and dermal exposure are limited. With respect to inhalation no reliable human or adequate animal data in a relevant animal model are available. It can be assumed that only a fraction of the amount inhaled will be available to the lungs while the majority will probably be swallowed and become orally bioavailable (ECB 2004). The dermal absorption appears to be poor in human. Wester et al. (1998) estimated that dermal absorption amounts to approximately 1.8% of a 24-hour applied dose of ^{14}C-DEHP solubilized in ethanol. In rats the bioavailability of DEHP after dermal exposure has been estimated to be around 10% (Elsisi et al. 1989, Melnick et al. 1987). However, the results of in vitro studies (Barber et al. 1992, Scott et al. 1987) indicate that the rat skin is about 4-fold more permeable for DEHP than human skin. So, approximately 2.5% of a dermal dose may be adsorbed by human skin.

There are indications 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). Also 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 by far the principal metabolite (Egestad et al. 1996, Koch et al. 2006).

3.4.4. DEHP exposure of the general population

DEHP is only physically dispersed in PVC and can therefore leach, migrate or gas out from PVC articles. Therefore DEHP can be present in air, dust, water, soils, sediments, and food and has become a ubiquitous environmental contaminant (Clark et al. 2003b). Diet has been determined as 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). DEHP contamination of food may occur due to bioaccumulation in certain foods as well as during processing, handling, transportation, packaging and storage. Further sources of DEHP exposure are indoor air, household dust, consumer products, and medical procedures.

3.4.5. DEHP exposure assessment from probabilistic calculations

Exposure estimates based on probabilistic calculations from DEHP levels in environmental media and food are given in Table 3. 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 outdated 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. The range of 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). Children are assumed to have higher exposures to DEHP than adults (Clark et al. 2003a, Meek and Chan 1994, Müller et al. 2003).

Table 3. 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 (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 (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 (2003) [d]	Adults		26
	children (7-14 years)		49
	children (1-6)		151
	infant 6-12 months		285
Wormuth (2006) [e]	Children	1.8	15.8
	Adults	2.7	15.5

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, inhalatory and dermal exposure via several pathways in Denmark
e scenario-based approach including oral, dermal and inhalation pathways for Europeans

3.4.6. DEHP exposure assessment from urinary metabolite excretion

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). Specific urinary DEHP metabolites can serve as biomarkers of DEHP exposure covering all sources and routes of exposure. So far, urinary levels of DEHP metabolites have been measured in several studies in Germany and USA, which have revealed the ubiquitous exposure of the general population to DEHP (Table 4). The data from both countries are in good accordance and lie within the same order of magnitude. While in the first studies only the simple monoester MEHP has been determined in urine, the parameter spectrum has been steadily increasing. By now the secondary metabolites have been recognized as much more reliable biomarkers for an assessment of the DEHP exposure (Koch et al. 2006, Koch et al. 2003b). They are excreted to a higher extent than MEHP and are more specific as they are not susceptible to contamination. By contrast, MEHP can be formed by hydrolysis of DEHP during sample handling and processing. Mono(2-ethyl-5-carboxypentyl) phthalate (5cx-MEPP) was found to be 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 4). This is partly in contrast to the metabolic excretion pattern found after a single dose of D4-DEHP (Koch et al. 2005a) with 5OH-MEHP as the main metabolite. However, due to the chronic exposure in the general population the ratios may be shifted to the metabolites with the longest half-lives, which are the carboxy metabolites. In general, children showed higher concentrations of DEHP metabolites than adults with higher ratios of the oxidative metabolites compared to MEHP (Becker et al. 2004, CDC 2005, Koch et al. 2004a).

Table 4. 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/day]
Blount (2000)[1]	1988-1994	298 (20-60)	n.d.	n.d.	n.d.	n.d.	2.7	>75	1.3
Koch (2003b)[2]	2002	85 (7-63)	n.d.	46.8	36.5	n.d.	10.3	100	5.8
Barr (2003)[1]	n.s.	62 (n.s.)	n.d.	35.9	28.3	n.d.	4.5	96	4.3
Silva (2004a)[1]	1999/2000	2541 (>6)	n.d.	n.d.	n.d.	n.d.	3.2	78	1.6
Becker (2004)[2]	2001/2002	254 (3-14)	n.d.	52.1	41.4	n.d.	7.2	100	(6.3)
Koch (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 (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 (2005)[1]	1999-2002	85 (>18) pregnant women	n.d.	11.4	11.1	n.d.	3.3	98	1.4
Silva (2006)[1]	2003/2004	129 (adults)	15.6	15.3	7.1	5.9	3.1	100	1.9
Wittassek (2007a)[2]	2001/2003	120 (20-29)	19.5	14.6	13.4	5.8	5.0	100	2.3

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

From the urinary concentrations measured daily DEHP exposure has been calculated by comparison with urinary excretion rates determined in human metabolism studies (Anderson et al. 2001, Koch et al. 2004b, Koch 2005a, Schmid and Slatter 1985). Since in the most metabolite excretion studies 24h urine samples were not available the amount of the DEHP metabolites excreted throughout a day has to be extrapolated from spot urine concentrations. This can be done by using reference values for the daily creatinine excretion (separately for men, women and children). For calculation of daily DEHP intake following equation has been applied:

$$\mathrm{DI}\,(\mu\mathrm{g/kg}_{\text{body weight}}/\mathrm{day}) = \frac{\mathrm{UE}_{\text{met}} \cdot \mathrm{CE}}{\mathrm{F}_{\mathrm{UE}}} \cdot MW_{DEHP}$$

UE_{met} urinary excretion of one or several DEHP metabolites in µmol/g crea
CE reference value for daily creatinine excretion [g crea/kg/day]
F_{UE} molar ratio between the urinary excreted amount of DEHP metabolite(s) and the DEHP amount taken up determined in human metabolism studies
MW_{DEHP} molecular weight of DEHP
CE: women: 18 mg/kg/day
men: 23 mg/kg/day

Calculation:
Volume related concentrations ~ Creatinine related concentrations

Alternatively, also a volume based calculation model has been applied (Wittassek et al. 2007b). Ideally, 24 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 e.g. for children not a realistic approach.

First daily DEHP intake evaluations were based on the excretion of the simple monoester MEHP only (David et al. 2000, Kohn et al. 2000). At that time, available metabolism studies indicated that urinary MEHP represented between 2.4% and 13% of the DEHP dose (Anderson et al. 2001, Schmid and Slatter 1985), which led to substantial differences in the resulting daily intake values depending on the excretion factor used. More recent daily intake calculations implement also the secondary DEHP metabolites (Koch et al. 2003a, Wittassek et al. 2007a, Wittassek et al. 2007b). Estimations based on three or five DEHP metabolites may lead to more reliable estimations of the daily DEHP intake.

In general, daily DEHP intake estimations based on urinary biomarkers give values in the same order of magnitude as those based on probabilistic calculations (Table 5). The current median DEHP exposure for the German general population has been estimated to be between 2 and 5 µg/kg bw/d (Koch et al. 2003a, Wittassek et al. 2007a). Children seemed to be higher exposed in relation to kg bw/ with a median exposure of around 4 to 8 µg/kg/d (Wittassek et al. 2007b). The results of a retrospective biomonitoring study (Wittassek et al. 2007a) indicate that the inner burden to DEHP has decreased during the last twenty years in Germany by a factor of nearly two.

Table 5. Daily DEHP intake estimations (µg/kg bw/d) deduced from urinary DEHP metabolite measurements

				DEHP intake estimate	
study	**Country**	**Sampling year**	**n (age)**	**Median**	**95th P**
David (2000)[b]	USA	1988-1994	289 (20-60)	0.6[a]	3.1
Kohn (2000)[c]	USA	1988-1994	289 (20-60)	0.7	3.6
Koch (2003a)	Germany	2002	85 (7-63)	(13.8)[d] 4.6[e]	(52.1)[d] 17.0[e]
Wittassek (2007b)[f]	Germany	2001/2002	239 (2-14)	4.3[g] 7.8[h]	15.2[g] 25.2[h]
Wittassek (2007a) [i]	Germany	2001/2003	120 (20-29)	2.7	6.4

[a] Geometric Mean
[b] Values based on MEHP; metabolic factors adopted from Anderson et al. (2001)
[c] Values based on MEHP; metabolic factors adopted from Peck and Albro (1982)
[d] Values based on 5OH-MEHP and 5oxo-MEHP; metabolic factors from Schmid and Schlatter (1985)
[e] Values based on 5OH-MEHP and 5oxo-MEHP; applying metabolic urinary factors from Koch et al. (2005)
[f] Values based on MEHP, 5OH-MEHP and 5oxo-MEHP; applying metabolic urinary factors from Koch et al. (2005a)
[g] creatinine based evaluation
[h] volume based evaluation
[i] Values based on MEHP, 5OH-MEHP, 5oxo-MEHP, 2cx-MMHP and 5cx-MEPP; applying metabolic urinary factors from Koch et al. (2005a)

3.4.7. 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 which a potential for high exposure to DEHP are identified :

- Exchange transfusion in neonates
- ECMO in neonates
- 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 infusion of blood into trauma patient
- Transfusion in adult undergoing ECMO

Depending on the medical procedure exposure to DEHP varies widely and is a function of the lipophilicity of the fluid that comes into 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). Polyethylene linings of PVC articles (e.g. tubings) do not seem to substantially prevent the release of DEHP (Bourdeaux et al. 2004, Demore et al. 2002).

3.4.8. 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 comprise haemodialysis, continuous ambulatory peritoneal dialysis (CAPD), transfusions of blood and blood products to patients with leukemia, aplastic anemia, sickle cell anemia, clotting disorders, administration of total parental nutrition

(TPN) and enteral nutrition of critically ill patients. Short-term DEHP exposures include blood transfusions e.g. in trauma patients, patients undergoing surgical procedures or extracorporeal membrane oxygenation (ECMO) procedures, and intravenous infusion of drugs.

Reported DEHP exposures estimated due to medical procedures for adults are summarized in Table 6. 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 6. 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)	Pollack (1985)
	450[a,b,c] (270-1210) – delivered dose	Faouzi (1999)
	100[a,b,c] (20-360) – retained dose	
	230[c] (50-850) – retained dose	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]	Mazur (1989) Loff (2000)
	800-2000 µg/day[d] (infants/children)	Kambia (2003)
Short-term exposures		
Transfusions of blood components		
Trauma patient	8500[f] (63 units whole blood)	Jaeger and Rubin(1972)
	1300-2600[b] (2.5l whole blood)	
During ECMO	3000-10000[f] (21-46 units combined blood products)	Sjoberg (1985b) Butch (1996)
Cardiopulmonary bypass		
During artificial heart transplant	2400[e]	Barry (1989)
IV Infusion of drugs		
Non-liphophilic drugs	< 5[f]	Health Canada (2002)
Lipophilic drugs	up to 1500[f]	Pearson (1993)

a assuming three 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 6 are based on measurements of DEHP itself. However, analytical determination of DEHP is prone to contamination during sample handling and processing. This is to be kept in mind when assessing the DEHP exposure levels estimated.

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 has been shown to increase with increasing storage time and temperature, while storage at low temperatures prevent 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).

Exposure to DEHP can also occur through voluntary medical treatments such as apheresis procedure to donate blood products (Table 7). 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 some 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. Buchta et al. (2003) estimated from serum DEHP concentrations exposures of 1.8-20.3 µg/kg/d due to apheresis procedure.

Table 7. Daily DEHP exposure of adults due to apheresis procedure using PVC medical devices calculated from measurement of urinary DEHP metabolites (Koch 2005b, Koch 2005c) or from serum DEHP concentrations (Buchta 2003)

Donation procedure (apheresis technology used)	n	Mean daily DEHP dose (range) [µg/kg/d]	Reference
Controls	5	6.2 (3.0-11.6)	Koch 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 2005c
Platelet (discontinuous)	19	6.5 (1.8-20.3)	Buchta 2003
Platelet (continuous)	17	7.2 (2.0-20.3)	

3.4.9. Newborns at risk

Developing foetus and the neonate represent the most vulnerable phases of life particularly with regard to developmental and reproductive toxicity. In particular, neonates in the Neonatal Intensive Care Unit (NICU) environment, due to their small body size, their physical condition and 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) combined with their developmental vulnerability represent a population at particularly increased risk (CERHR 2005, FDA 2002, Health Canada 2002).

In fact, neonates receive higher doses, in terms of body weight, of DEHP than the general population (Calafat et al. 2004b, Green et al. 2005) and their daily dose to DEHP may

increase up to 20 folds the tolerable daily intake (Jaeger et al. 2005). The combination of prenatal and postnatal exposures may exacerbate the reproductive hazard. Therefore a concern was raised about potential health effects of DEHP (CERHR 2005, ECB 2004). Accordingly research into alternatives to DEHP-containing medical devices that may come in contact with human tissues was suggested (Jaeger et al. 2005). In addition, further studies are needed to evaluate if less invasive medical treatments may reduce phthalate exposure risk (Latini et al. 2003b).

Table 8 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 premature born babies who weight significantly lighter, in general between 500 and 2500 g. Therefore, the DEHP exposure in relation to body weight may even be higher in premature newborns. The DEHP exposure estimates reach for many procedures the mg/kg 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. The highest short-term exposure may occur due to double volume exchange transfusion (up to 23 mg/kg/d) while ECMO is the medical treatment, which may give the highest daily exposure over a prolonged period of time (up to 14 mg/kg/day). 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 much higher DEHP exposure. The FDA (2002) has estimated an upper-bound daily DEHP dose on the order of 3 mg/kg/d for a newborn (4 kg) in the neonate intensive care unit (NICU) setting 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).

Table 8. Estimated dose of DEHP received by neonates undergoing medical procedures 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) of neonate (4 kg)	Reference
Infusion of pharmaceuticals		Loff (2000)
• Midazolam (24 ml)	7 a	
• Fentanyl (29 ml)	33 a	
• Propofol (1%, 10 ml, 24h)	1640 a	
TPN	30 (free of lipid) a	Loff (2000)
	2500 (lipid emulsion 20%, 27°C)	Loff (2002)
	3250 (fat infusion, 33°C) a	
Exchange transfusion – short term	1200-22600 [c]	Plonait (1993)
	840-3300 [b]	Sjoberg (1985a)
	1700-4200 [a]	Sjoberg (1985b)
Single dose Packed Red Blood Cells (20 ml)	36-152 [a]	Loff (2000)
	232 [a]	
Single dose Platelet-Rich Plasma (20 ml)	138-2020 [a]	
Single dose Fresh Frozen Plasma (20 ml)		
ECMO - sub-acute	Up to 14,000 [d] (14000 µg/kg/ 10 days)	Schneider (1989)
	0 (heparin coated PVC tubing)	Karle (1997)
	Up to 3,490 [e] (34900 µg/kg/ 10 days)	
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 and after medical procedure
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

The urinary concentrations of DEHP metabolites in neonates undergoing intensive medical interventions have been found to vary widely and reach levels that are much higher than those found in the general population (Table 9). Compared to adults the ratios among the metabolites are shifted in favour of the oxidative metabolites with 5cx-MEPP being the main metabolite (Calafat et al. 2004a, Koch et al. 2006).

Table 9. Median (95th percentile) DEHP metabolite levels in µg/l measured in urine of infants undergoing intensive medical interventions

Reference	N	Birth weight ± SD [g]	5cx-MEPP	5OH-MEHP	5oxo-MEHP	2cx-MMHP	MEHP
Calafat 2004a [a]	6	666 ± 167	n.d.	2221 (13161)	1697 (10413)	n.d.	129 (704)
Green 2005 [b], Weuve 2006	13 24 17	n.s.	n.d.	low: 27 medium: 307 high: 555	low: 29 medium: 286 high: 598	n.d.	low: 4 medium: 28 high: 86
Koch 2006 [c]	45	1976 ± 714	293 (5500)	41.6 (557)	34.8 (406)	8.3 (129)	-

[a] results of 41 urine samples of premature newborns ; intensive care interventions for more than 2 weeks
[b] DEHP exposure was rated low, medium or high based on the kind of medical devices used
[c] premature neonates treated with various medical procedures
n.d.: not determined
n.s.: not specified

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, which may reflect the variety and intensity of the medical procedures performed. The maximum estimated daily DEHP intake was 2300 µg/kg bw/d, which is separated from the NOAEL (4.8 mg/kg bw/d) for testicular and developmental toxicity in rats only by a factor of two (Wolfe and Layton 2003). Based on the data of Calafat et al. (2004a) even higher maximal DEHP exposures up to 6000 µg/kg bw/d have been estimated well above the NOAEL observed in the rat study (CERHR 2005).

3.4.10. Summary on the exposure to DEHP

The general population is exposed to DEHP through a variety of routes with food being the primary source. Several metabolite excretion studies suggest exposure to DEHP in the whole general population. In general, DEHP exposure assessments from probabilistic calculations from DEHP measurements in environmental media and dose reconstructions from urinary metabolite levels agree within an order of magnitude. Most recent studies suggest a current median exposure 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 somewhat higher body burden of DEHP than adults. There are indications that exposure to DEHP in the general population has decreased during the last years.

Medical procedures using PVC medical devices can lead to DEHP exposures much higher than the background levels. However, the extent of exposure largely depends upon the medical treatments given and the duration of the treatment. In adults, highest doses of DEHP may result by transfusions of blood components reaching up to several mg/kg bw/day. It has been shown that also voluntary medical treatments such as apheresis procedure to donate blood products can cause significant exposure to DEHP. For adults the extent of exposure varies depending on medical procedures conducted. For some treatments the mg/kg bw/day range may easily be reached. For blood transfusion procedures peak values up to 22 mg/kg bw/day have been estimated. Premature neonates in intensive care units, being dependent on multiple medical procedures, can receive even higher DEHP exposures than adults relative to their kg bw. These exposures may be in the same range as the doses inducing reproductive toxicity in animal studies.

3.4.11. Toxicity

Comprehensive reports have been issued recently which provide in depth evaluations 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 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 http://cerhr.niehs.nih.gov). SCENIHR has carefully considered these summary documents along with new pertinent original publications.

3.4.12. Animal Studies

Acute toxicity

Acute toxicity studies of good quality indicate low acute toxicity of DEHP, with an LD_{50} of >25 g/kg in rats and mice. The intravenous 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 five times higher than that of DEHP (ECB 2006, NTP-CEHRHR, 2005).

Repeated dose toxicity

Numerous studies investigated the toxicity of DEHP upon short-term and repeated administration to experimental animals, mostly rats and with application by the oral route. Many of these studies are comparable to guideline studies and conducted in conformity with GLP. Target organs for DEHP induced toxicity in rodents were kidney, liver and testis.

The effects on the kidneys included increased absolute and relative organ weights, increased incidence and severity of mineralization 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 28.9 mg/kg/day in the males and 36.1 mg/kg/day in the females) 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/day). More severe kidney lesions were observed at the highest dose level.

The most striking effects observed in the liver are hepatomegaly due to hepatocyte proliferation (characterised by increased replicative DNA synthesis/cell division and hypertrophy), peroxisome proliferation, and hepatocellular tumours. The effects on the liver (hepatomegaly) are apparently mediated by peroxisome-proliferator activated receptor (PPARα) and agonistic interaction of DEHP and its metabolite MEHP with the receptor. There are, however, marked species differences in the PPARα-mediated effects of DEHP, such that the hepatotoxic effects of DEHP in rodents are not judged to be relevant for humans (IARC, 2000).

In repeated exposure study 16 rats were pretreated with 100 mg/m^3 for 2 weeks (aerosol) 6 hours per day, 5 days per week. The study indicates that following repeated inhalation exposure long term retention does not occur. There are no other relevant studies in rodents investigating the health effects in the respiratory tract.

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 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). Positive results were obtained in assays on cell transformation, induction of aneuploidy, and cell proliferation. However, these test systems are also sensitive to several non-genotoxic substances such as tumour promoters and/or peroxisome proliferators. Thus, in conclusion, DEHP and its major metabolites are considered to be 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 summarized in the RAR report of 2006 and other summary documents (IARC, 2000).

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 two species. In the NTP studies (1982a), the LOAEL for tumour induction in mice was 3000 mg/kg DEHP in the feed (670 mg/kg bw per day 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 rat was 6000 mg/kg DEHP in the feed (320 mg/kg bw per day 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). An overall NOAEL for the tumour induction and for the effects on the liver, kidney and testis was established as 500 mg/kg DEHP in feed (29 mg/kg bw/day for male rats). 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 per day for males of the two dose groups respectively). The LOAEL and the NOAEL for non-neoplastic effects on the liver in this study were 500 and 100 ppm DEHP in the diet, respectively (98 and 19 mg/kg bw per day for males of the two dose groups respectively). Marked species differences with respect to hepatic response to peroxisome proliferation are apparent. Rat and mice seem to exhibit the highest sensitivity. Guinea pigs and monkeys are relatively insensitive. In marmosets, the liver weight was not affected and a slight increased activity of peroxonal enzymes was observed following administration of 2000 mg/kg bw for 14 days.

In conclusion: DEHP was found to induce liver tumors in rats and mice mainly by the activation of the PPARα receptor, a mechanism considered not to be relevant in the human liver.

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 ovalbumin-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 the antigen ovalbumin, When a mixture of DEHP and ovalbumin was administered intraperitoneally in PPAR-alpha knock out 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 only 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 induced allergic lung inflammation in the mouse model used (Larsen et al 2007). Similar results were obtained for the DEHP metabolite MEHP, so it was speculated whether the airway effects of DEHP are mediated by MEHP (Larsen et al 2007, Hansen et al. 2007). Although the induction of antigen (OVA) specific IgG1 antibodies is an indicator for immunogenicity and adjuvancy in mouse experimental systems, it is not clear whether this response should be considerd a protective or a risk factor for the development of IgE and thus 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 also the combined intraperitoneal 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 (monoethylhexyl phthalate) 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 exposition to DEHP.

Some monophthalates have been shown to 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).

In conclusion, DEHP was found in experimental systems to have the potential to interact with the immune system depending on the actual exposure conditions.

Reproductive toxicity

The reproductive or developmental toxicity of DEHP have 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 have been demonstrated in both male rodents and non-rodents. The testis toxicity of DEHP is age dependent (Sjoberg et al. 1985b). The lowest NOAEL is seen in the range from 3.5 to 4.8 mg/kg b.w. in rats. The females need to be exposed in the most critical period of 12-21 days during pregnancy 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 developmental toxicity of 600 and 20 mg/kg bw/day can be identified. In ferrets a LOAEL is 1200 mg/kg bw/day (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 being 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 (Rhodes et al. 1986). Also in another study, adult male marmosets treated 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. So, in studies using marmosets and cynomolgus monkeys no effect on testicular function was observed after high DEHP exposure. These observations are of importance for extrapolation to humans as for spermatogenesis the marmoset was found to have similarities to the human, and it was concluded to be a suitable model for studies relevant for human testicular function (Millar et al. 2000).

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/day 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 ECB 2006 evaluated the study in which three 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/day. 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/day.

The CSTEE agreed with the RAR to use this NOAEL rather than 3.7 mg/kg bw/day from the study of Poon et al. (1997), since the endpoints seen in the Wolfe and Layton (2003) study are more robust and the study was well performed (CSTEE 2004).

According to Council Directive 67/548/EEC, DEHP is classified Toxic, and with effects on male and female fertility Category 2, R 60 and for developmental toxicity in category 2, R61.

3.4.13. Mechanisms of Action of DEHP

In general three mechanisms have been proposed to account for liver carcinogenicity

- Hepatomegaly and peroxisome proliferation leading to oxidative stress and generation of electrophilic free radicals
- Increased hepatocyte proliferation/suppression of hepatocellular apoptosis and
- Activation of peroxisome proliferators-activated receptors (PPARs).

Still the understanding of the mechanism of action in the liver is not clarified.

The effect of DEHP on liver cells has been studied in details and the peroxisome proliferators are involved in the hepatotoxicity of DEHP. PPARs play a number of important roles in normal physiology and play a role as a modulator of signal molecules that mediate changes in gene expression to maintain lipid homeostasis (Rusyn et al. 2006).

The mechanisms of the toxic effect of DEHP on the male reproductive organ have been investigated in several animal studies. Also in the testis peroxisome proliferators-activated receptors PPAR and their subtypes are now in focus to 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 to explain the role of PPAR (Corton and Lapinski 2005, Latini et al. 2006).

The antiandrogenic effects of some phthalates have been suggested to be due to reduced androgen availability in target organs causing malformations of male reproductive organs and low adult sperm counts (Gray et al. 2000, Barlow et al. 2003). Maternal DEHP treatment from gestational day 14 to postnatal day 3 resulted in reduced testosterone synthesis to female levels (Parks et al. 2000). In addition, in contrast to the antiandrogen effect in vivo, DEHP and its metabolite MEHP did not show an affinity for the human androgen receptor in an in vitro assay. These results indicate 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). 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 seem to reduce testicular testosterone production by a similar mechanism of action (Barlow and Foster 2003, Borch et al. 2004, Borch et al. 2006). In addition, plasma LH levels in male foetuses were elevated (Borch et al. 2004). Immunohistochemistry showed a clear reduction in the nuclear receptor steroidogenic factor-1 (SF-1) and peroxisome proliferator PPAR gamma after gavage administration of 300 mg/kg bw/day DEHP (Borch et al. 2006b). Phthalates are PPAR agonists and have been found to reduce testosterone production in primary Leydig cell culture and in adult rats (Corton and Lapinski 2005).

In mice there is a study that demonstrates the same spectrum of developmental toxicity in normal mice and mice that were genetically incapable of expressing peroxisome proliferation due to lack of PPAR-alfa indicating a role for the direct toxicity (ECB 2006 in press). In laboratory animals the metabolites are less studied but one report suggests that at least in rats the antiandrogenic effect is partly caused by 2 antiandrogenic metabolites 5OXO-MEHP and 5-OH-MEHP (Stroheker et al. 2005).

In adult or prepubertal rats, other mechanisms of action 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, it has been shown that the cell structure protein vimentin and an increased caspase-3 level activity, appear to be sensitive and early markers of MEHP testis toxicity at 6 hours 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).

Little is known about the mechanism of action in humans. However, DEHP is able to induce in animals all the malformations, which are present in the so called testicular dysgenesis syndrome. The testicular dysgenesis syndrome includes the following human male reproductive disorders, cryptorchidism and hypospadias in babies or testis cancer and low sperm counts in young men. It has been proposed that maldevelopment (dysgenesis) of the foetal testis results in hormonal malfunction or other malfunctions of the testicular somatic cells eventually leading to the malformations as part of the testicular dysgenesis syndrome (Sharpe & Skakkebaek 2003).

In humans most information of DEHP exposure is obtained by measuring of the DEHP metabolites in urine (Koch et al. 2005a). However, the role of the metabolites in inducing toxic effects or possible mechanism of action is not well known. It may be assumed that the half-life of these metabolites may play a role in their ultimate toxic effects. In laboratory animals the metabolites are less studied but some studies determining DEHP metabolites suggests that at least in rats the antiandrogenic effect of DEHP is partly caused by 2 antiandrogenic metabolites, namely 5oxo-MEHP and 5OH-MEHP (Stroheker et al. 2005).

3.4.14. Evidence from epidemiological studies

Potential male developmental effects in humans include hypospadias, cryptorchism 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.

Hypospadias and cryptorchism.

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 from 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 hypospadias 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).

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 nine phthalate metabolites commonly used as biomarkers of exposure to phthalates. Anogenital distance was measured after the delivery. For the 9 urinary metabolites measured, including 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, mono-2-ethyl-5-oxohexyl phthalate. Four of these were associated with anogenital index (AGI=anogenital distance/kg bw), 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, penile volume and scrotal size, although in the study itself, no diseases or malformations were identified. However, the data were considered insufficient as solid evidence for an effect and need further elaborations with larger studies, but do add to the concern for male reproductive effects (Kaiser 2005, Sharpe 2005).

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 pthtalates concentrations were found in almost 90 % of these individuals. In this single 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). Also the mean birth weight was lower (3,150 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. This study suggests a possible effect of DEHP on pregnancy outcome.

Pubertal development

Two studies have investigated associations between pubertal development and phthalate exposure (Colon et al. 2000, Rais-Bahrami et al. 2004). The relation between serum phthalate concentrations and premature breast development was studied in a case-control study of 41 patients from the San Juan City Hospital Pediatric Endocrinology Division and 35 controls from the general pediatric care who did not have 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 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 uncertain due to laboratory and/or diagnostic procedures performed (CERHR 2005).

Rais-Bahrami et al. 2004 reported a 14-16 years 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). The results showed that 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. It was suggested that the acute and short term exposure to DEHP by the intravenous route, and a lack of conversion of DEHP to MEHP may be protective against its

long term adverse effects (Rais-Bahrami et al. 2004). 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) to very low birth weight (mean 1179 gram, n=242) individuals, assumed to have had a high DEHP exposure. The very low birth weight individuals showed educational disadvantages persisting into early adulthood. There were no differences observed concerning male fertility.

Endometriosis

Two case-control studies have investigated the relations between biomarkers of DEHP exposure and the risk of endometriosis. A case-control study of Cobellis and colleagues (2003) provided 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 two 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.

Gonadal hormones and semen quality

Phthalate monoesters including MEHP, the initial metabolite of DEHP, and MBP are known testicular toxicant in rodents. The balance of gonadotropin and gonadal hormones is an important indicator of male fertility (see 3.4.5.2).

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. MEP and MBP were positively, but weakly correlated with SHBG (Spearman correlation coefficient [r]=0.323, p=0.002 and r=0.272, p=0.01 respectively). MMP, MBBEP, and MBP were correlated with 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 suggest some phthalates may have adverse effects on human Leydig cell development and function, which may be related to incomplete virilization in infant boys exposed to phthalates.

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), luteunizing hormone (LH), follicle-stimulating hormone (FSH), and estradiol (E2). They compared blood and urine concentrations of 74 male workers in a factory producing unfoamed polyvinyl chloride 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. 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.

Duty et al. (2003a, 2003b, 2004, 2005) and Hauser et al. (2006) conducted a series of studies in male partners of subfertile 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 computing aided sperm analysis. Sperm DNA damage was measured using neutral comet assay. In an analysis of 168 males (Duty et al. 2003b), there was an exposure-response relation 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 subfertile couples (including the 168 men in the previous study) who presented semen analysis at the infertility clinic. They compared urine concentrations of phthalates esters between 76 men with compromised sperm concentrations (<20 million/mL), 221 men with compromised sperm motility (<50% motile) and 114 with compromised morphology (<4% normal) with 210 subjects whose sperm concentration, motility and morphology was normal (above the three cut points). There was a dose-response relation 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 relation 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 and the DEHP metabolites and the three 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 two 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 DNA damage. MEHP, a metabolite of DEHP, was associated with DNA damage after adjustment for the oxidative DEHP metabolites mono(2-ethyl-5-hydroxyhexyl) phthalate, and mono(2-ethyl-5-oxohexyl) phthalate. There is an indication of altered sperm motility and sperm DNA damage (as measured in chromosomal breaks) after exposure to DEHP and several other phthalates.

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 where male partner was working in a plant producing polyvinyl chloride (PVC) plastics. Exposure assessment was

based on air measurements at work place and questionnaire information on work tasks and locations. Time to pregnancy was compared between three exposure categories of no exposure, low (<0.1 mg/m3) and high (>0.1 mg/m3). There was no association between exposure and time to pregnancy.

Testicular cancer

Two epidemiologic studies of testicular cancer have used source based exposure assessment rather than measurements of specific phthalates 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 questionnaire information 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 exposures was identified. Hansen (1999) conducted a case-controls 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.

Respiratory health

Øie et al. (1997) hypothesized that di(2-ethylhexyl) phthalate (DEHP) causes airways inflammation by mimicking some prostaglandins and thromboxanes with a similar chemical structure. Some monophthalates have been shown to 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 two or more episodes with symptoms and signs of bronchial obstruction. Trained experts characterized 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 to 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 (milligram per gram dust) of six phthalates were determined: DEP, DBP, DIBP, BBzP, DEHP, and DINP. 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 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

recruited systematically 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 South 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 mold 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).

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

3.4.15. Conclusion

The key factors influencing to the risks to individual patients arising from the use of DEHP used in medical devices are:

- Background exposure
- Exposure dose (leaching from each medical device used)
- Vulnerability of patients (including the time window of the exposure)

The general population is exposed to DEHP through a variety of routes with food being the primary source. Several metabolite excretion studies suggest a non-negligible exposure to DEHP in the whole general population. In general, DEHP exposure assessments from probabilistic calculations from DEHP measurements in environmental media and dose reconstructions from urinary metabolite levels agree within an order of magnitude. Most recent studies suggest a current median exposure 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 somewhat higher body burden of DEHP than adults. 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, although such exposure is of limited duration (Tables 6-8). Also during voluntary medical treatments such as apheresis procedure to donate blood products may result in significant exposure to DEHP. The extent of exposure largely depends upon the medical treatments given and the duration of the treatment (Tables 6-8). Premature neonates in intensive care can receive even higher DEHP exposures than adults relative to their body weight (up to 35 mg/kg bw over 10 day period). This exposure may be even higher than the doses observed to induce reproductive toxicity in animals. In effect, this means that there is no margin of exposure (MoE) for certain procedures. However, this is justified by the beneficial effects of these procedures.
Treatment categories involving a potential high exposure are:

- Multiple procedures in pre-term neonates
- Total Parenteral Nutrition (TPN) in neonates
- ECMO in neonates
- Exchange transfusion in neonates
- Haemodialysis patients
- Enteral nutrition in neonates and adults
- Heart transplantation or coronary artery bypass graft surgery
- Massive infusion of blood into trauma patient
- Transfusion in adult undergoing ECMO

The animal and epidemiological studies enable the likely sensitive patient groups to be identified. Animal studies have identified two lead effects liver tumours and changes in the

male reproductive system. The NOAEL for the reproductive toxicity is 4.8 mg/kg bw /day. In respect to the liver tumours there is good scientific evidence from mechanistic and other studies to indicate that DEHP is unlikely to cause this effect in man. However, for the effect in the male reproductive system both mechanistic and epidemiological findings indicate a potential hazard for man. Immature young animals are more susceptible to testicular toxicity by DEHP than older mature animals. The EU risk assessment for DEHP (ECB 2006) identified the most critical effects as on the testes, fertility, development (anogenital distance), and kidney (repeated dose). The sensitivity for such endocrine effects is highest during gestation and the first month after birth when the most sensitive organs are developing. It has to be considered that there is the potential exposure for infants to other phthalates (chapter 3.5) that are toxic to reproduction, which may have via similar mechanisms of action as DEHP.

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

- Hypospadias and cryptorchism: no evidence for potential endocrine disrupting effects
- Anogenital distance: limited indications based on one study
- Birth weight and gestational age: insufficient evidence based on one study
- Pubertal development of young females: insufficient evidence based on one study, not confirmed in another study
- Phthalate ester levels affect the severity of endometriosis: insufficient evidence
- Male fertility: no association between exposure and time to pregnancy, no effect on fertility in very low birth weight males;
- Semen quality: contradictory reports on the effects of DEHP
- Testicular cancer: no association between this cancer and exposure to PVC plastics
- Respiratory health: phthalate exposure correlates weakly with obstructive respiratory symptoms and asthma

Epidemiological studies on DEHP assessed in this report by themselves do not establish a cause-effect relationship for harmful effects on humans. However, analysing the animal and human data and mechanistics studies as a whole it can be concluded that male foetuses of pregnant women and male neonates can be considered as potential groups at risk in view of the exposure levels above those that induce reproductive toxicity in rodent animal studies. These high exposure levels during certain medical procedures have to be seen in the light of treatment needed and the availability of suitable alternatives for each medical treatment. In addition data available on non-human primate studies do not indicate effects of DEHP on the male reproductive system.

It should be noted that medical devices made from plasticized PVC provide many effective treatments and that DEHP is a particularly effective plasticizer. In addition to its beneficial effect on mechanical properties, DEHP also stabilises the membranes of red blood cells enabling blood product storage in PVC blood bags for several weeks.

3.5. Alternative plasticizers in PVC medical devices

3.5.1. Introduction

The information available for the potential alternative plasticizers for DEHP in PVC medical devices use is presented in Annex I. Both publicly available information (published papers) and information submitted by stakeholders were considered. For each individual alternative a conclusion is presented in the Annex I.

The safety evaluation of medical devices and their composing materials 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. Exposure to alternative plasticizers

When alternatives are used as replacement for DEHP, it can be expected that for the use in medical devices the contact of patients with these alternatives is similar to DEHP. In terms of quantitative exposure (mg/kg bw) obviously 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 may also be important and mechanical stress of tubing in peristaltic pumps and agitation of storage samples may increase the leaching of the additives in the medium. All these variables make it difficult to compare leaching measured in different studies, and comparisons of different plasticizers under identical conditions are therefore the most useful results.

A lot of data on leaching of polymer additives from food packaging materials and some data on plasticizer leaching from PVC toys have been published, and 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, where the concentration of the additive is analysed in the simulant. Artificial saliva and gastric juice simulants have been used to estimate leaching of chemicals from mouthing and ingestion of toys/toy materials.

These data have, however, limited use in quantification of exposure from medical devices. Thus, 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 under static conditions. The leaching rates obtained via toy testing may have application in quantification of plasticizers under dynamic conditions, but only in aqueous medium. However, the comparison of leaching rates from medical devices of various plasticizers measured by testing of food packaging packaging and toy testing will indicate the relative leaching of alternative plasticizers compared to that of DEHP. As exposure data on DEHP from PVC medical devices containing this plasticizer is available for most critical procedures, exposure data on alternative plasticizers can be generated/extrapolated on the basis of relative leaching rates using DEHP exposure data (see section 3.4) as benchmark. Standard test methods for measuring the leaching rates of components from medical devices (ISO 10993) are available, and information can be obtained from investigations where leaching of alternative plasticizers is compared under identical conditions. This kind of information for the investigated DEHP alternatives has, however, not been available to the SCENIHR.

In a comparative study of leaching of plasticizers to different feeding solutions (Welle et al. 2005) DINCH, TOTM and ATBC were compared 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 leachings were followed with chemical analyses for 24 hours. The leaching rates of various plasticizers were relatively constant over this period, except for ATBC where the leaching decreased with time. The latter may be explained by the high leaching rate for ATBC, at least ten times higher than for DEHP. The DINCH leaching were three to ten times lower than that for DEHP, while the release of TOTM was extremely low and in one experiment almost two orders of magnitude lower than the leaching of DINCH. In the TOTM

experiment the authors also measured DEHP and found 40 times more of the phthalate than of the trimellitate, which was probably due to DEHP impurity in the TOTM.

For TOTM a comparison (Senshu et al. 2004) between PVC infusion lines containing this compound and DEHP was reported. Significantly higher leaching was found for DEHP (about thirty times higher in one case). In another study (Kambia et al. 2001) PVC tubes for haemodialysis plasticized with DEHP and TOTM were compared. The leaching of DEHP was about three times higher than that of TOTM, but the latter also emitted DEHP. The leaching of DEHP from TOTM containing products is associated with the content of DEHP impurity in TOTM.

In a recently published study, 5 cm of PVC nasogastric tubes containing DEHP or polyadipate were incubated with feeding solution and gastric juice (Subotic et al. 2007). Although at least 10 times lower leaching was observed compared to that of DEHP, no conclusion can be made from this study because the contents of the two plasticizers in the tubings are not described.

PVC was blended with different plasticizers and moulded thin sheets of these materials in order to compare several properties. The plasticizers included were DEHP, DEHA, ATBC and BTHC. A few of the results are presented in Table 10. The higher extraction into the oil reflects the lipophilic character of these esters. The biggest difference between the compounds was seen in the soapy water, being approximately of a factor of five between the extremes.

Table 10. Extraction of some plasticizers from PVC (48 hours 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

In a comparison between leaching of BTHC and DEHP into blood in PVC bags containing these substances (Kandler 1998), a slightly lower leaching of BTHC could be found.

The leaching of COMGHA to some simulants have been tested (Kristoffersen 2005) and compared with the corresponding data for DEHP and DINP (see Table 11). The leachings to aqueous media seem to be much smaller for the COMGHA than for the phthalates tested, while in lipophilic media/substances the leaching was of the same order of magnitude. Different data were, however, available to EFSA in their evaluation (EFSA 2004) and are also included in Table 11. This highlights the difficulties to compare results from leaching studies.

Table 11. Leaching from PVC containing COMGHA (40%), DEHP (40%) and DINP (42%), respectively

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

It is not possible to draw any far reaching conclusions regarding the relative leachings of the investigated plasticizers based on the studies referred to above. A couple of them identify the leachings of TOTM to be several orders of magnitude lower than that of DEHP, and ATBC leaching were found to be higher than that of DEHP in a couple of investigations. The general impression is, however, that the leachings of the remaining plasticizers are

rather similar, which is not too surprising given their similar structures and properties. For some plasticizers 5 to 10 fold lower leaching rates were observed.

3.5.3. Toxicity of the alternative plasticizers

In general the toxicity of the alternative plasticizers is less well described than for DEHP, although for some plasticizers ECB risk assessment reports are available. Information on each of the alternatives considered is presented in Annex 1.

3.5.4. Conclusions on the risks of the alternative plasticizers

To compare the toxicity a short summary of the potential genotoxicity, the carcinogenicity, repeated dose toxicity and reproductive toxicity are summarised in Table 12. In the tables NOAEL is shown as the lowest effects in male or female rat.

The information of the leaching from alternative plasticizers is sparse but may be expected to be of same order of magnitude. The margin of exposure for DEHP in neonate seems to be very low. For blood transfusion peak values up to 22 mg/kg bw/day have been estimated showing a dose 4 times higher than NOAEL for DEHP.

Table 12. NOAEL of DEHP compared with some alternative plasticizers.
The critical endpoint is shown to indicate that for some of the chemicals it is different from reproductive effects.

Plasticizer	NOAEL mg/kg bw	Reproductive Toxicity	Critical endpoint	Exposure Range (neonates) µg/kg bw/day
DEHP	4.8	Yes	Reproduction	42-2300
ATBC	100	No	Decreased bw	
COMGHA	5000	No data	Decreased bw	
BTHC	250	No	Liver weight	
DEHA	200	Yes	Foetotoxicity	
DINCH	107	No	Kidney*	
DINP	15 (88)	No/Yes	Liver	
DOTP	500-700	No	Developmental	
TOTM	100	Yes	Reproduction	

bw: body weight
* Kidney effects in male rats due to alpha-2-u macroglobulin, a mechanism not relevant to man

Considering similar leaching rates, the margin of safety of other plasticizers will be least 20 times higher for most alternatives. Thus differences in leaching rates even at one order of magnitude higher than DEHP may be acceptable.

The toxicity of alternative plasticizers is shown for cancer and mutagenicity effects in Table 13.

Table 13. The cancer and mutagenicity effects and maternal toxicity of plasticizers

Plasticizer	Repeated dose Toxicity, NOAEL	Genotoxicity	Carcinogenicity	Maternal toxicity mg/kg bw/day

	mg/kg bw/day			
DEHP	29 (male rat)	Negative	LOAEL 320 (male rat)	LOAEL 750 (rat)
COMGHA	5000	Negative	No data	No data
ATBC	100	Negative	Negative	NOAEL 100 (rat)
BTHC	250	Negative	Negative	NOAEL
DEHA	200	Negative	NOAEL 1250	NOAEL 400 (rat)
DINCH	107	Negative	Negative	NOAEL 1000 (rat)
DINP	15 (88)	Negative	Kidney	LOAEL 750 (rat)
DOTP	500-700	Negative	Negative	NOAEL 458 (rat)
TOTM	100	Negative	No Data	NOAEL

It can be concluded that DEHP is causing the most severe reproductive effects in animal studies evaluating toxicity. DEHA, DINP, and TOTM are also causing reproductive toxicity, but in doses more than 20 times higher. COMGHA and TOTM could not be evaluated for all endpoint due to lack of data. Regarding the alternatives, for some compounds sufficient toxicological data is available to indicate a lower hazard compared to DEHP.

However, a risk assessment of these alternative plasticizers could not be performed due to a lack of human exposure data. For others, information on the toxicological profile is inadequate to identify the hazard. This limits the proper evaluation of the potential to replace DEHP by alternative plasticizers. 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 of different population and subpopulation is possible and may occur at different times or together. Due to the wide use of DEHP in the society humans may be exposed from many different sources and exposed to other phthalates as well. It is obvious that combined exposure to DEHP, DBP, BBP, DIBP, and DINP having the same mechanism of action may potentially cause at least an additive effect. Combined exposure to DEHP and DINP had showed an additive effect (Borch et al. 2004). In general a common mechanism might exist if two 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 may share a common toxic intermediate.

This will probably be the case for combined exposure to the five mentioned phthalates. The potency of the different phthalates should be considered. DEHP and DBP are almost equal in potency. DIBP and BBP are less potent and DINP seems to have the smallest effect considering their effect on steoridogenesis in foetal male rats.

The chemical structures of some alternative plasticizers show that some of them have a possibility to form the same metabolite 2-ethylhexanol; this is the case for DEHA, DOTP, TOTM and DEHP.

3.7. Potential alternative polymer plasticizers in PVC medical devices

In addition to the potential alternative plasticizers discussed above, another alternative to phthalates is represented by the use of "polymeric plasticizers", that is, by high molecular weight solid polymers soluble in PVC in large proportions. These polymers, when blended with PVC by conventional processing, give polymeric alloys, that is, homogeneous blends

constituted by a single thermodynamically stable phase. Their macromolecular dimensions lead to segment-segment entanglements with PVC matrix, 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 reports on polyester/PVC blends show (Lindström and Hakkarainen 2006, Hakkarainen 2005) that a number of homopolymeric and co-polymeric structures are in principle eligible as constituents of soft PVC formulations, and that even different class of polymers, as for instance polypropylene glycols, might be used to this purpose. However, a number of basic requirements must be fulfilled in order to fully exploit polyesters for their potential as PVC plasticizers. Besides being miscible in all proportions with PVC, their glass transition temperature must be lower than 0°C and, in addition, 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. In order to minimize migration their molecular weight must be medium-high. However, in practice polymers with average molecular weight as low as 1000 g/mol is used. 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 insufficent information is available to assess the use and safety of these compounds in medical devices.

3.8. Conclusion

The general population is exposed to DEHP through a variety of routes with food being the primary source. Median exposure is estimated to be 2 to 5 µg/kg bw/day. Children may have somewhat higher body burden of DEHP than adults.

Medical procedures using PVC medical devices can lead to DEHP exposures much higher than the background levels. However, the extent of exposure largely depends upon the medical treatments given and the duration of the treatment. In adults, highest doses of DEHP may result by transfusions of blood components reaching up to several mg/kg bw/day. It has been shown that also voluntary medical treatments such as apheresis procedure to donate blood products can cause significant exposure to DEHP. Premature neonates in intensive care can receive even higher DEHP exposures than adults relative to their body weight.

This is of concern in view of rodent animal studies showing that immature young animals are more susceptible to testicular toxicity by DEHP than older mature animals. Neonates may therefore be considered to be potentially at risk for the adverse reproductive and developmental effects of DEHP. As for adults the extent of exposure varies depending on medical procedures conducted, and in some cases exposure in the mg/kg bw/day range may easily be reached. For blood transfusion procedures peak values up to 22 mg/kg bw/day have been estimated. A limited number of follow-up studies of highly exposed neonates and workers did not indicate an effect of DEHP on the human male reproductive system. In addition data available of non human primate studies do not indicate effects of DEHP on the male reproductive system.

Epidemiological studies on DEHP assessed in this report do not establish a cause-effect relationship for harmful effects on humans. However, even in the absence of clinical or epidemiological evidence for harmful effects in humans, some concern may be raised in view of the exposure levels above those that induce reproductive toxicity in rodent animal

studies. The exposure levels during certain medical procedures have to be seen in the light of treatment needed and the availability of suitable alternatives for each medical treatment.

It is also noted that DEHP has beneficial properties in stabilising the membranes of red blood cells enabling blood storage for several weeks

Regarding the alternatives, for some compounds sufficient toxicological data is available to indicate a lower hazard compared to DEHP. However, a risk assessment of these alternative plasticizers could not be performed due to a lack of human exposure data. For others, information on the toxicological profile is inadequate to identify the hazard. This limits the proper evaluation of the potential to replace DEHP by alternative plasticizers. 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.

4. OPINION

In view of the complexity of the questions addressed in the Terms of Reference. the Committee decided to concentrate on the risk assessment of plasticizers used in PVC in this opinion. Whilst recognising that there are several non-PVC based materials that could provide effective materials for use in medical devices, this opinion does not address these materials. Although the published Call for Information included both alternative plasticizers and alternative materials, only the former was submitted. The Committee recognized that there may be need for evaluation of these alternative non-PVC materials in the future.

There have been concerns over possible health effect of DEHP for many years. Several times CSTE, CSTEE and SCMPMD have expressed their opinions on different aspects of the reproductive toxicity of phthalates and more specifically on DEHP. Since the last opinion on medical devices from September 2002 expressed by SCMPMD new information on the exposure and possible reproductive effects of DEHP has appeared in the literature. A better understanding of the mechanism of the antiandrogenic effects in animal models has evolved after 2002.

Recent information on the exposure of the general population and especially of the vulnerable groups raised a concern on the potential toxicity of DEHP. Vulnerable groups are male infants, male offspring of pregnant and breastfeeding women undergoing certain medical procedures that may result in general in short-term exposure to relatively high levels of DEHP.

The exposure of the general population to DEHP is already significant. The main source of DEHP for the general population is dietary, followed by inhalation of air. The exposure in adults ranges from a few µg up to 25-30 µg /kg bw/d. There are important differences among populations and individuals associated with various dietary habits and lifestyle. Infants and children are exposed to higher levels than adults, on a body weight basis.

Certain medical procedures involving plasticized PVC are already known to cause considerable exposure to phthalates. These procedures include:

- Multiple procedures in pre-term neonates
- Total Parenteral Nutrition (TPN) in neonates
- ECMO in neonates
- Exchange transfusion in neonates
- Enteral nutrition in neonates and adults
- Haemodialysis
- Heart transplantation or coronary artery bypass graft surgery
- Massive infusion of blood into trauma patients
- Transfusion in adults undergoing ECMO

However, for many of these procedures the actual extent of exposure is still unknown or spans several orders of magnitude. Research is needed to determine (i) the multiple sources and pathways of human exposure to phthalates; (ii) whether exposure to phthalates at the levels found in the general population is a cause for health concern; and (iii) to what extent human exposure to phthalates may impair human health.

Data available on the exposure to DEHP show that DEHP exposure levels of neonates during certain medical procedures are in the same order of magnitude or even higher than doses inducing reproductive toxicity in animal studies. This is of concern in view of animal studies showing that immature young animals are more susceptible to testicular toxicity by DEHP than older mature animals. Neonates may therefore be considered to be at risk for the adverse reproductive and developmental effects of DEHP. In addition, they may be exposed

to other phthalates especially DBP and DIBP, and these phthalates may act additively with DEHP.

There is limited evidence indicating a relation between DEHP exposures and some adverse effects in humans. However, the few follow-up studies after high DEHP exposures in neonates and in occupational settings, performed sofar, did not indicate that there is an effect of DEHP on fertility and/or the human male reproductive system. Regarding the effect of DEHP on semen quality and female development contradictory results were reported. It is recognised that especially the potentially high exposure during medical treatments may raise concern, even in the absence of clinical or epidemiological evidence for harmful effects in humans. Nevertheless, irrespective of the potential risk, one has to realise that especially in neonatal intensive care units, these neonates depend for their survival on a multitude of medicines and medical procedures including the use of medical devices.

Sofar, 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.

Some alternatives may be suitable to replace DEHP in certain medical devices, while for other devices it may be difficult to obtain the same functionalities as PVC plasticized with DEHP. A risk assessment for the alternatives could not be performed due to a lack of exposure data. For other possible alternatives, adequate toxicity data is also lacking. The risk and benefit of using alternative plasticizers should be evaluated case by case. In addition, it is known that DEHP containing PVC can contribute to the stability of blood cells. However, this has not been evaluated for most alternative plasticizers.

Responses to the questions in the Terms of Reference

Question 1.

Update of the scientific opinion adopted in September 2002 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 September 2002 on "Medical Devices containing DEHP Plasticized PVC; neonates and other groups possibly at risk from DEHP toxicity".

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 of DEHP leaching from soft PVC as a basis for risk assessment for high risk patient groups, taking into account the route of exposure.*

Compared to the previous opinion of the SCMPMD, the new information indicates that there is still a reason for some concern for prematurely born male neonates. This concern is instigated by the potential high human exposure especially during certain medical procedures which may be transiently above the dose inducing reproductive toxicity in animal studies, and limited epidemiological evidence suggesting an adverse effect on the male reproductive system. However, the few follow-up studies after high DEHP exposures in neonates performed sofar, did not indicate that there is an effect of DEHP on the development of the human male reproductive system.

Sofar, 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.

Other 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 has been established and published in the EU Risk Assessment Report (RAR 2006). The TDI for DEHP is 48 µg per kg body weight per day, which was based on a No Observed Adverse Effect Level 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.

Question 2.

Medical devices containing alternative plasticizers: possible risk for certain uses or to certain patient groups. Since alternative DEHP free medical devices have been developed and are used to treat patients, the Scientific Committee is requested to evaluate the potential risks of currently available alternatives in relation to patient health, when used in medical devices.

The non-PVC alternative materials were not evaluated.

There are alternative plasticizers to PVC and also non-PVC alternative materials available. For the alternative plasticizers a generic exposure assessment could not be performed due to a lack of relevant use and human exposure data. For other possible alternatives, information on the toxicological profile was lacking. The risk and benefit should be carefully evaluated according to established protocols, for each individual medical device and each medical procedure in which the alternatives are intended to be used. For some alternative plasticizers, sufficient toxicological data is available to indicate a lower hazard compared to DEHP. The functionality of these plasticizers should be assessed before they can be used as an alternative for DEHP in PVC medical devices.

5. COMMENTS RECEIVED FROM THE PUBLIC CONSULTATION

The public consultation of the preliminary opinion took place from 15 October to 26 November 2007 and information about it was communicated to various stakeholders. During the consultation 21 contributions were received, 13 of which came from industry or industry associations, 4 from individuals, 3 from public authorities and 1 from an NGO.

In evaluating the responses from the consultation, submitted material has only been considered for revision of the opinion if

1. it is directly referring to the content of the report and relating to the issues that the report addresses,

2. it contains specific comments and suggestions on the scientific basis of the opinion,

3. it refers to peer-reviewed literature published in English, the working language of the SCENIHR and the working group,

4. it has the potential to add to the preliminary opinion of SCENIHR.

Each submission which meets these criteria has been carefully considered by the Working Group. Overall, many of the comments were of good quality and the opinion has been partly revised based on these comments. The literature has been updated with relevant publications up to early 2008.

The evaluation of the existing and additional literature on epidemiological studies on harmful effects of DEHP in man showed that there was no conclusive scientific evidence for a harmful effect of DEHP in humans. However, it is recognised that especially the potentially high exposure during medical treatments may raise a concern, even in the absence of clinical or epidemiological evidence, for harmful effects in humans. It is recommended that further studies are performed to confirm or reject the suggestions of adverse effects of DEHP in humans.

There is some concern for harmful effects of DEHP on humans. Prematurely born male infants are considered to be a high risk group as for this group the DEHP exposure may be transiently above the dose inducing reproductive toxicity in animal studies.

6. MINORITY OPINION

None.

7. LIST OF ABBREVIATIONS

2cx-MMHP	Mono-[2-(carboxymethyl)hexyl] phthalate
2-EH	2-Ethylhexanol
5OH-MEHP	Mono-(2-ethyl-5-hydroxyhexyl) phthalate
5cx-MEPP	Mono-(2-ethyl-5-carboxypentyl) phthalate
5oxo-MEHP	Mono-(2-ethyl-5-oxohexyl) phthalate
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
BBP	Butyl benzyl phthalate
BTHC	Buturyl-tri-n-hexyl citrate
CAPD	Continuous ambulatory peritoneal dialysis
CI	Confidence interval
CAS	Chemical Abstracts Service
CERHR	Center for the Evaluation of Risks to Human Reproduction
CPs	Chlorinated paraffins
cx-MINP	Carboxylated MINP
DBP	Di-n-butyl phthalate
DEHA	Di(2-ethylhexyl) adipate
DEHP	Di(2-ethylhexyl) phthalate
DEP	Diethyl phthalate
DG	Directorate General
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
DOP	Di-n-octyl phthalate
DOTM	Dioctyl trimellitate
DOTP	Dioctyl terephthalate
E2	Estradiol
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
ESBO	Epoxidised soya bean oil
FSH	Follicle-stimulating hormone
FDA	US Food and Drug Administration
fT	Free testosterone
GLP	Good laboratory practice
IARC	International Agency for Research on Cancer
JEM	Job-exposure matrix
PVC	Epoxidised linseed oil
LH	Luteunizing hormone
LOAEL	Lowest observed adverse effect level
MBP	Mono-n-butyl phthalate
MBzP	Monobenzyl phthalate
MBBP	Monobutylbenzylphthalate
MEHP	Mono(-2-ethylhexyl) phthalate
MEP	Monoethyl phthalate
MIBP	Mono-iso-butyl phthalate
MINP	Mono-iso-nonyl phthalate
MMP	Monomethyl phthalate
MOTM	Monooctyl trimellitate
MOTP	Monooctyl terephthalate
NOAEL	No observed adverse effect level
NOEL	No observed effect level
NICU	Neonate intensive care unit
NTP	US National Toxicology Programme
OH-MINP	Hydroxylated MINP
OR	Odds ratio
oxo-MINP	Oxygenated MINP
PPARα	Peroxisome-proliferator activated receptor
PVC	Polyvinylchloride
RAR	Risk Assesment Report
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
SHBG	sex-hormone binding globulin
SF-1	steroidogenic factor-1
TDI	Tolerable Daily Intake
Tg	Glass transition temperature
Tm	Melting Temperature
TOTM	Tris(2-ethylhexyl)benzene-1,2,4-tricarboxylate
TPA	Terephthalic acid
TPN	Total parental nutrition
TOTM	Trioctyl trimellitates

8. REFERENCES

Albro PW, Thomas RO, Enzymatic hydrolysis of di-(2-ethylhexyl) phthalate by lipases. Biochim Biophys Acta 1973; 306:380-90.

Albro PW. The metabolism of 2-ethylhexanol in rats. Xenobiotica 1975; 5:625-36.

Albro PW, Hass JR, Peck CC, Odam DG, Corbette JT, Bailey PW, Blatt HE, Barrett BB. Identification of the metabolites of di-(2-ethylhexyl) phthalate in urine from the African green monkey. Drug Metab Dispos 1981, 3:223-25

Albro PW, Corbett JT, Schroeder JL, Jordan S, Matthews HB. Pharmacokinetics, interactions with macromolecules and species differences in metabolism of DEHP. Environ Health Perspect 1982; 45:19-25.

Albro PW, Tondeur I, Marbury D, Jordan S, Schroeder J, Corbett JT. Polar metabolites of di-(2-ethylhexyl)phthalate in the rat. Biochim Biophys Acta 1983; 760:283-92.

Albro PW. Absorption, metabolism, and excretion of di(2-ethylhexyl) phthalate by rats and mice. Environ Health Perspect 1986; 65:293-8.

Anderson W, Castle L, Scotter M. A biomarker approach to measuring human dietary exposure to certain phthalate diesters. Food Add Contam 2001; 18:1068-1074.

Rais-Bahrami K, Nunez S, Revenis ME, Luban NLC, Short BL, Follow-up study of adolescents exposed to di-2-ethylhexyl phthalate (DEHP) as neonates on extracorporeal membrane oxygenation (ECMO) support. Environ Health Persp 2003; 112 (13):1339-1340C.

Barber ED, Genetic toxicology testing of di(2-ethylhexyl) terephthalate. Environ Mol Mutagen 1994; 23(3):228-33.

Barber ED, Topping DC. Subchronic 90-day oral toxicology of di(2-ethylhexyl) terephthalate in the rat. Food Chem Toxicol 1995; 33(11):971-8.

Barlow NJ, Foster PMD. Pathogenesis of male reproductive tract lesions from gestation through adulthood following in utero exposure to Di(n-butyl) Phthalate. Toxicologic Pathology 2003; 31:397-410.

Barlow NJ, Phillips SL, Wallace DG, Sar M, Gaido KW, Foster PM. Quantitative changes in gene expression in fetal rat testes following exposure to Di(n-butyl) Phthalate. Toxicological Sciences 2003; 73:431-451.

Barr DB, Silva MJ, Kato K, Reidy JA, Malek NA, Hurtz D, et al. Assessing human exposure to phthalates using monoesters and their oxidized metabolites as biomarkers. Environ Health Perspect 2003; 111:1148-51.

Barry YA, Labow RS, Keon WJ, Tocchi M, Rock G. Perioperative exposure to plasticizers in patients undergoing cardiopulmonary bypass. J Thorac Cardiovasc Surg 1989; 97:900-5.

Becker K, Seiwert M, Angerer J, Heger W, Koch HM, Nagorka R, et al. DEHP metabolites in urine of children and DEHP in house dust. Int J Hyg Environ Health 2004; 207:409-17.

Blount BC, Silva MJ, Caudill SP, Needham LL, Pirkle JL, Sampson EJ, et al. Levels of seven urinary phthalate metabolites in a human reference population. Environ Health Perspect 2000; 108:979-82.

Borch J, Ladefoged O, Hass U, Vinggaard AM. Steroidegenesis in fetal male rats is reduced by DEHP and DINP, but endocrine effects of DEHP are not modulated by DEHA in fetal, prepubertal and adult male rats. Reprod Toxicol 2004; 18:53-61.

Borch J, Dalgaard M, Ladefoged O. Early testicular effects in rats perinatally exposed to DEHP in combination with DEHA – apoptose assessment and immunohistochemical studies. Reprod Toxicol 2005; 19:517-525.

Borch J, Metzdorff SB, Vinggaard AM, Brokken L, Dalgaard M. Mechanisms underlying the anti-andogenic effects of diethylhexyl phthalate in fetal rat testis. Toxicology 2006; 223:144-155.

Bornehag CG, Sundell J, Weschler CJ, Sigsgaard T, Lundgren B, Hasselgren M, et al. The association between asthma and allergic symptoms in children and phtalates in house dust: a nested case-control study. Environ Health Perspect 2004; 112:1393-397.

Bourdeaux D, Sautou-Miranda V, Bagel-Boithias S, Boyer A, Chopineau J. Analysis by liquid chromatography and infrared spectrometry of di(2-ethylhexyl)phthalate released by multilayer infusion tubing. J Pharm Biomed Anal 2004; 35:57-64.

Breindahl, Petersen JH. Plasticisers in infant formulae, baby food and total diet samples. Odense Landsdelslaboratorium, Intern rapport IFE 1998; 4:46 (in Danish).

Bronsch CJT. Untersuchungen zur Exposition und zum renalen Ausscheidungsverhalten des Kunststoffweichmachers di-(2-ethylhexyl)phthalat (DEHP) beim Menschen. Dissertation No. 8459. Eidgenoessische Technische Hochschule, Zuerich, Switzerland. 1987.

Bruns-Weller E, Pfordt J. Bestimmung von Phthalsäureestern in Lebensmitteln und Frauenmilch. ERNO 2000; 1:25-28.

Buchta C, Bittner C, Hocker P, Macher M, Schmid R, Seger C, et al. Donor exposure to the plasticizer di(2-ethylhexyl)phthalate during plateletpheresis. Transfusion 2003; 43:1115-20.

Butch SH, Knafl P, Oberman HA, Bartlett RH. Blood utilization in adult patients undergoing extracorporeal membrane oxygenated therapy. Transfusion 1996; 36:61-3.

Calafat AM, Needham LL, Silva MJ, Lambert G. Exposure to di-(2-ethylhexyl) phthalate among premature neonates in a neonatal intensive care unit. Pediatrics 2004a; 113:429-34.

Calafat AM, Slakman AR, Silva MJ, Herbert AR, Needham LL. Automated solid phase extraction and quantitative analysis of human milk for 13 phthalate metabolites. J Chromatogr B Analyt Technol Biomed Life Sci 2004b; 805:49-56.

Calafat AM, Brock JW, Silva MJ, Gray LE Jr, Reidy JA, Barr DB, et al. Urinary and amniotic fluid levels of phthalate monoesters in rats after the oral administration of di(2-ethylhexyl) phthalate and di-n-butyl phthalate. Toxicology 2006; 217:22-30.

CDC (Centers for Disease Control and Prevention). Third National Report on Human Exposure to Environmental Chemicals. Department of Health and Human Services, Atlanta, GA. 2005.

CERHR (Center for the Evaluation of Risks to Human Reproduction) NTP-CERHR Expert Panel Update on the Reproductive and Developmental Toxicity of Di(2-ethylhexyl) phthalate, 2005.

Christersson A, Ljunggren L, Nilsson-Thorell A, Arge B, Diehl U, Hagstam K, et al. Int. J. Artif Organs 1991; 7:407-410.

Clark K, Cousins I, MacKay D. Assessment of critical exposure pathways. In:Staples CA, editors, The Handbook of Environmental Chemistry, 3Q: Phthalate Esters New York:Springer; 2003a

Clark K, Cousins I, MacKay D, Yamada K. Observed Concentrations in the Environment. In:Staples CA, editors, The Handbook of Environmental Chemistry, 3Q: Phthalate Esters New York:Springer; 2003b

CMA DOC ID 408565036, 1985.

Cobellis L, Latini G, De Felice C, Razzi S, Paris I, Ruggieri F, et al. High plasma concentrations of di-(2-ethylhexyl)-phthalate in women with endometriosis. Hum Reprod 2003; 18:1512-5.

Cole RS, Tocchi M, Wye E, Villeneuve DC, Rock G. Contamination of commercial blood products by di-2-ethylhexyl phthalate and mono-2-ethylhexyl phthalate. Vox Sang 1981; 40: 317-22.

Colon I, Caro D, Bourdony CJ, Rosario O. Identification of phthalate esters in the serum of young Puerto Rican girls with premature breast development. Environ Health Perspect 2000; 108:895-900.

Corton JC, Lapinski PJ. Peroxisome proliferators-activated receptors: Mediators of Phthalate ester-induced effects in male reprodsuctive tract? Toxicological Science 2005; 83:4-17.

CSTEE (Scientific Committee on Toxicity, Ecotoxicity and the Environment). 26-27 November 1998, Phthalate migration from soft PVC toys and children-care articles.

CSTEE (Scientific Committee on Toxicity, Ecotoxicity and the Environment). 8 January 2004, Opinion on the result of a second Risk Assessment of bis(2-ethylhexyl)phthalate(DEHP), Human Health Part.

Dalgaard M, Østergaard G, Lam HR, Hansen V, Ladefoged O. Toxicity study of Di(2-ethylhexyl)phthalate in combination with acetone in rats. Pharmacology & Toxicology 2000; 86:92-100.

Dalgaard M, Nellemann C, Lam HR. Sørensen IK, Ladefoged O. The acute effects of mono(2-ethylhexyl)phthalate (MEHP) on testes of pepubertal Wistar brats. Toxicology Letters 2001; 122:68-79.

Daniel JW, Bratt H. The absorption, metabolism and tissue distribution of di(2-ethylhexyl)phthalate in rats. Toxicology 1974; 2:51-65.

Danish EPA. Substance Flow Analysis for Dioxin. 2002. Environmental Project No. 811, Danish EPA, 2003, www.mst.dk/udgiv/publications/2003/87-7972-675-5/html/default_eng.htm

David MR. Exposure to phthalate esters. Environ Health Perspect 2000; 108:440.

David MR, Moore MR, Finney Dc, Guest D. Chronic toxicity of di(2-ethylhexyl)phthalate in rats. Toxicol Sci 2000a; 55:433-443.

David MR, Moore MR, Finney Dc, Guest D. Chronic toxicity of di(2-ethylhexyl)phthalate in mice. Toxicol Sci 2000b; 58:377-385.

David R, Lockhart L, Ruble K. Lack of sensitization for trimellitate, phthalate, terephthalate and isobutyrate plasticizers in a human repeated insult patch test. Food Chem Toxicol 2003; 4:589-593.

David F, Sandra P, Bart T, Vanwalleghem F, Ikonomou M. Analytical Methods Review. In: Staples CA, editors, The Handbook of Environmental Chemistry, 3Q: Phthalate Esters New York: Springer, 2003

Dearman RJ, Beresford L, Bailey L, Caddick HT, Betts CJ, Kimber I, Di-(2-ethylhexyl) phthalate is without adjuvant effect in mice on ovalbumin. Toxicology 2007; Dec 4.

Demore B, Vigneron J, Perrin A, Hoffman MA, Hoffman M. Leaching of diethylhexyl phthalate from polyvinyl chloride bags into intravenous etoposide solution. J Clin Pharm Ther 2002; 27:139-42.

Dine T, Luyckx M, Gressier B, Brunet C, Souhait J, Nogarede S, et al. A pharmacokinetic interpretation of increasing concentrations of DEHP in haemodialysed patients. Med Eng Phys 2000; 22:157-65.

Dirven HA, van den Broek PH, Jongeneelen FJ. Determination of four metabolites of the plasticizer di(2-ethylhexyl)phthalate in human urine samples. Int Arch Occup Environ Health 1993; 64:555-60.

Dostal LA, Weaver RP, Schwetz BA. Transfer of di(2-ethylhexyl) phthalate through rat milk and effects on milk composition and the mammary gland. Toxicol Appl Pharmacol 1987; 91:315-25.

Doull J, Cattley R, Elcombe C, Lake BG, Swenberg J, Wilkinson C, et al. A cancer risk assessment of di(2-ethylhexyl)phthalate: application of the new U.S. EPA Risk Assessment Guidelines. Regul Toxicol Pharmacol 1999; 29:327-57.

Duty SM, Silva MJ, Barr DB, Brock JW, Ryan L, Chen Z, et al. Phthalate exposure and human semen parameters. Epidemiology 2003a;14:269-77.

Duty SM, Singh NP, Silva MJ, Barr DB, Brock JW, Ryan L, et al. The relationship between environmental exposures to phthalates and DNA damage in human sperm using the neutral comet assay. Environ Health Perspect 2003b; 111:1164-9.

Duty SM, Calafat AM, Silva MJ, Brock JW, Ryan L, Chen Z, et al. The relationship between environmental exposure to phthalates and computer-aided sperm analysis motion parameters. J Androl 2004; 25:293-302.

Duty SM, Calafat AM, Silva MJ, Ryan L, Hauser R. Phthalate exposure and reproductive hormones in adult men. Hum Reprod 2005:20:604–610.

ECB (European Chemical Bureau) European Union Risk Assessment Report for 1,2-benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich and di-"isononyl" phthalate (DINP), 2003.

ECB (European Chemical Bureau). European Union Risk Assessment Report for Bis(2-ethylhexyl) phthalate. Consolidated Final Report 2004.

ECB (European Chemical Bureau). European Union Risk Assessment Report for Bis(2-ethylhexyl) phthalate (Consolidated Final Report: February 2004), 2006, in press.

ECPI (European Council for Plasticisers and Intermediates): Statistics 2007. personal communication.

EFSA. EFSA Journal 2004; 1-26.

Egestad B, Green G, Sjoberg P, Klasson-Wehler E, Gustafsson J. Chromatographic fractionation and analysis by mass spectrometry of conjugated metabolites of bis(2-ethylhexyl)phthalate in urine. J Chromatogr B Biomed Appl 1996; 677:99-109.

Elsisi AE, Carter DE, Sipes IG. Dermal absorption of phthalate diesters in rats. Fundam Appl Toxicol 1989; 12:70-7.

Fabjan E, Hulzebos E, Mennes W, Piersma AH. A category approach for reproductive effects of phthalates. Crit Rev Toxicol 2006; 36:695-726.

Faouzi MA, Dine T, Gressier B, Kambia K, Luyckx M, Pagniez D, et al. Exposure of hemodialysis patients to di-2-ethylhexyl phthalate. Int J Pharm 1999; 25(180):113-121.

FDA (US Food and Drug Administration) Safety Assessment of di(2-ethylhexyl)phthalate (DEHP) Released from Medical Devices. Center for Devices and Radiological Health, 2002.

Ferruti P, Mancin I, Ranucci E, De Felice C, Latini G, Laus M. Polycaprolactone-Poly(ethylene glycol) Multiblock Copolymers as Potential Substitutes for Di(ethylhexyl) Phthalate in Flexible Poly(vinyl chloride) Formulations. Biomacromolecules 2003; 4:181-188.

Gaunt IF, Butterworth KR. Autoradiographic study of orally administered di-(2-ethylhexyl) phthalate in the mouse. Food Chem Toxicol 1982; 20:215-7.

Gray LE Jr, Ostby J, Furr J, Price M, Veeramachaneni DN, Parks L. Perinatal exposure to the phthalates DEHP, BBP, and DINP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male rat. Toxicol Sci. 2000; 58(2):350-65

Green R, Hauser R, Calafat AM, Weuve J, Schettler T, et al. Use of di(2-ethylhexyl) phthalate-containing medical products and urinary levels of mono(2-ethylhexyl) phthalate in neonatal intensive care unit infants. Environ Health Perspect 2005; 113:1222-5.

Gruber L, Wolz GOP. Untersuchung von Phthalaten in Baby-Nahrung. Deutsche Lebensmittel-Rundschau 1998;9.

Hack M, Flannery DJ, Schluchter M, Cartar L, Borawski E, Klein N. Outcomes in young adulthood for very low birth weight infants. N Engl J Med 2002; 346: 149-57.

Hakkarainen M. New PVC materials for medical applications - the release profile of PVC/polycaprolactone-polycarbonate aged in aqueous environments. Polym. Degrad. Stab. 2003; 80:451-458

Haishima Y, Seshimo F, Higuchi T, Yamazaki H, Hasegawa C, et al. Development of a simple method for predicting the levels of di(2-ethylhexyl) phthalate migrated from PVC medical devices into pharmaceutical solutions. Int J Pharm 2005; 298:126-42.

Hanawa T, Muramatsu E, Asakawa K, Suzuki M, Tanaka M, et al. Investigation of the release behavior of diethylhexyl phthalate from the polyvinyl-chloride tubing for intravenous administration. Int J Pharm 2000; 210:109-15.

Hanawa T, Endoh N, Kazuno F, Suzuki M, Kobayashi D, et al. Investigation of the release behavior of diethylhexyl phthalate from polyvinyl chloride tubing for intravenous administration based on HCO60. Int J Pharm 2003; 267:141-9.

Hansen J. Risk for testicular cancer after occupational exposure to plastics. Int J Cancer 1999; 82:911-2.

Hansen JS, Larsen ST, Poulsen LK, Nielsen GD. Adjuvant effect of inhaled mono-ethylhexyl phthalate in BALB/cJ mice. Toxicology 2007; 232: 79-88.

Hardell L, Ohlson CG, Fredrikson M. Occupational exposure to polyvinyl chloride as a risk factor for testicular cancer evaluated in a case-control study. Int J Cancer 1997; 73:828-30.

Hauser R, Meeker JD, Singh NP, Silva MJ, Ryan L, Duty S, et al. DNA damage in human sperm is related to urinary levels of phthalate monoester and oxidative metabolites, Hum Reprod 2007; 22:688-95.

Health Canada. An exposure and toxicity assessment. Medical Devices Bureau, Therapeutic Products Directorate, Health Products and Food Branch, Ottawa, Canada. 2002.

Huber WW, Grasl-Kraupp B, Schulte-Hermann R. Hepatocarcinogenic potential of di(2-ethylhexyl)phthalate in rodents and its implications on human risk. Crit Rev Toxicol 1996, 26, 365-481.

IARC (International Agency for Research on Cancer), Monograph on the evaluation of carcinogenic risk to humans, Some industrial chemicals, Di(2-ethylhexyl) phthalate, 2000: 77, 41-148.

ISO/CEN 10993 series on Biological Evaluation of Medical Devices (ISO, Geneva, Switzerland, CEN, Brussels, Belgium).

Jaakkola JJK, Øie L, Nafstad P, Botten G, Samuelsen SO, Magnus P. Surface materials in the home and development of bronchial obstruction in young children in Oslo, Norway. Am J Publ Health 1999; 84:188-92.

Jaakkola JJK, Verkasalo PA, Jaakkola N. Plastic interior materials and respiratory health in preschool children. Am J Public Health 2000; 90:797-99.

Jaakkola JJK, Ieromnimon A, Jaakkola MS. Interior surface materials and asthma in adults: a population-based incident case-control study. Am J Epidemiol 2006; 15(164):742-9.

Jacobson MS, Kevy SV, Grand RJ. Effects of a plasticizer leached from polyvinyl chloride on the subhuman primate: a consequence of chronic transfusion therapy. J Lab Clin Med 1977; 89:1066-79.

Jaeger RJ, Rubin RJ: Leakage of a phthalate ester plasticizer from polyvinyl chloride blood bags into stored human blood and its localization in human tissues. N Engl J Med 1972; 287:1114-1118.

Jaeger RJ, Weiss AL, Brown K. Infusion of di-2-ethylhexylphthalate for neonates: a review of potential health risk. J Infus Nurs 2005; 28:54-60.

Kaiser J. Toxicology - Panel finds no proof that phthalates harm infant reproductive systems. Science 2005; 310(5747): 422-422

Kambia K, Dine T, Azar R, Gressier B, Luyckx M, BrunC, Comparative study of leachability of di(2-ethylhexyl)phthalate and tri(2-ethylhexyl)trimellitate for hemodialysis. Int J Pharm 2001; 22:139-146.

Kambia K, Dine T, Gressier B, Bah S, Germe AF, Luyckx M, et al. Evaluation of childhood exposure to di(2-ethylhexyl) phthalate from perfusion kits during long-term parenteral nutrition. Int J Pharm 2003; 262:83-91.

Kandler RL, Levels of Citroflex B-6 plasticiser leaching into platelet concentrates from PL 2209. Fenwal Division, Project No. FE272, Final Report (FPS #M011-87). Round Lake IL. USA, 1998.

Karle VA, Short BL, Martin GR, Bulas DI, Getson PR, Luban NL, et al. Extracorporeal membrane oxygenation exposes infants to the plasticizer, di(2-ethylhexyl)phthalate. Crit Care Med 1997; 25:696-703.

Kato K, Silva MJ, Reidy JA, Hurtz D, 3rd, Malek NA, Needham LL, et al. Mono(2-ethyl-5-hydroxyhexyl) phthalate and mono-(2-ethyl-5-oxohexyl) phthalate as biomarkers for human exposure assessment to di-(2-ethylhexyl) phthalate. Environ Health Perspect 2004; 112:327-30.

Kessler W, Numtip W, Grote K, Csanady GA, et al. Toxicol Appl Pharmacol 2004; 195: 142-53.

Kluwe WM. Overview of phthalate ester pharmacokinetics in mammalian species. Environ Health Perspect 1982; 45:3-9.

Koch HM, Drexler H, Angerer J. An estimation of the daily intake of di(2-ethylhexyl)phthalate (DEHP) and other phthalates in the general population. Int J Hyg Environ Health 2003a; 206:77-83.

Koch HM, Rossbach B, Drexler H, Angerer J. Internal exposure of the general population to DEHP and other phthalates--determination of secondary and primary phthalate monoester metabolites in urine. Environ Res 2003b, 93, 177-85.

Koch HM, Drexler H, Angerer J. Internal exposure of nursery-school children and their parents and teachers to di(2-ethylhexyl)phthalate (DEHP). Int J Hyg Environ Health 2004a; 207: 15-22.

Koch HM, Bolt HM, Angerer J. Di(2-ethylhexyl)phthalate (DEHP) metabolites in human urine and serum after a single oral dose of deuterium-labelled DEHP. Arch Toxicol 2004b; 78: 123-30.

Koch HM, Bolt HM, Preuss R, Angerer J. New metabolites of di(2-ethylhexyl)phthalate (DEHP) in human urine and serum after single oral doses of deuterium-labelled DEHP. Arch Toxicol 2005a; 79: 367-76.

Koch HM, Angerer J, Drexler H, Eckstein R, Weisbach V. Di(2-ethylhexyl)phthalate (DEHP) exposure of voluntary plasma and platelet donors. Int J Hyg Environ Health 2005b; 208: 489-98.

Koch HM, Bolt HM, Preuss R, Eckstein R, Weisbach V, Angerer J. Intravenous exposure to di(2-ethylhexyl)phthalate (DEHP): metabolites of DEHP in urine after a voluntary platelet donation. Arch Toxicol 2005c; 79: 689-693.

Koch HM, Preuss R, Angerer J. Di(2-ethylhexyl)phthalate (DEHP): human metabolism and internal exposure - an update and latest results. Int J Androl 2006; 29: 155-65.

Kohn MC, Parham F, Masten SA, Portier CJ, Shelby MD, Brock JW, et al. Human exposure estimates for phthalates. Environ Health Perspect 2000; 108:440-2.

Kristoffersen BL. Ud med phthalaterne? Dansk Kemi, 2005; 86 (3): 22-23.

Kurata Y, Kidachi F, Yokoyama M, Toyota N, Tsuchitani M, Katoh M, Subchronic toxicity of di(2-ethylhexyl)phthalate (DEHP) in common marmosets: Lack of hepatic peroxisomal proliferation, testicular atrophy, or pancreatic acinar cell hyperplasia. Toxicological Sciences 1998; 42: 49-56.

Labow RS, Tocchi M, Rock G. Contamination of platelet storage bags by phthalate esters. J Toxicol Environ Health 1986; 19:591-8.

Larsen ST, Hansen JS, Thygesen P, Begtrup M, Poulsen OM, Nielsen GD. Adjuvant and immunosuppressive effect of six monophtalates in a subcutaneous injection model with BALB/c mice. Toxicology 2001a; 169:37-51.

Larsen ST, Lund RM, Nielsen GD, et al. Di-(ethylhexyl) phthalate possesses an adjuvant effect in subcutaneous injection model with BALB/c mice. Toxicol Lett 2001b; 125:11-18.

Larsen ST, Hansen JS, Hansen EW, Clausen PA, Nielsen GD. Airway inflammation and adjuvant effect after repeated airborne exposures to di-(2-ethylhexyl)phthalate and ovalbumin in BALB/c mice. Toxicology 2007; 235: 119-129.

Larsen ST, Nielsen GD. The adjuvant effect of di-(2-ethylhexyl)phthalate is mediated through a PPARalpha-independent mechanism. Toxicol Lett 2007; 170: 223-228.

Latini G, Avery GB. Materials degradation in endotracheal tubes: a potential contributor to bronchopulmonary dysplasia. Acta Paediatr 1999; 88:1174-5.

Latini G, De Felice C, Presta G, Del Vecchio A, Paris I, Ruggieri F, et al. In Utero exposure to Di-(2-Ethylhexyl)-Phthalate and Duration of Human Pregnancy. Environ Health Perspect 2003a; 111:1783-1785.

Latini G, De Felice C, Presta G, Rosati E, Vacca P. Minimal Handling and Bronchopulmonary Dysplasia in Extremely Low Birth Weight Infants Eur J Pediatr 2003b;162:227-229.

Latini G. Monitoring phthalate exposure in humans. Clin Chim Acta 2005; 361(1-2):20-29.

Latini G, Del Vecchio A, Massaro M, Verrotti A, De Felice C. Phthalate exposure and male infertility. Toxicology 2006; 226:90-8.

Lindgren A, Lindquist NG, Lyden A, Olsson T, Ullberg S. A whole body autoradiographic study on the distribution of 14C-labelled di-(2-ethylhexyl)phthalate in mice. Toxicology 1982; 23:149-58.

Lindström A, Hakkarainen M. Environmentally Friendly Plasticizers for Poly(vinyl chloride)-Improved Mechanical Properties and Compatibility by Using Branched Poly(butylene adipate) as a Polymeric Plasticizer. J Appl Polym Sci 2006; 100:2180-2188.

Lindström A, Hakkarainen M. Migration Resistant Polymeric Plasticizer for Poly(vinyl chloride). J Appl Polym Sci 2007; 104:2458-2467.

Loff S, Kabs F, Witt K, Sartoris J, Mandl B, Niessen KH, Waag KL. Polyvinylchloride infusion lines expose infants to large amounts of toxic plasticizers. J Pediatr Surg 2000; 35:1775-81.

Loff S, Kabs F, Subotic U, Schaible T, Reinecke F, Langbein M. Kinetics of diethylhexyl-phthalate extraction From polyvinylchloride-infusion lines. JPEN J Parenter Enteral Nutr 2002; 26:305-9.

Loff S, Subotic U, Reinicke F, Wischmann H, Brade J. Extraction of Di-ethylhexyl-phthalate from Perfusion Lines of Various Material, Length and Brand by Lipid Emulsions. J Pediatr Gastroenterol Nutr 2004; 39:341-345.

MAFF. Survey of plasticiser levels in food contact materials and in foods. Food Surveillance Papers No 21. Ministry of Agriculture, Fisheries and Food, 1996.

Main KM, Mortensen GK, Kaleva MM, Boisen KA, Damgaard IN, Chellakooty M, et al. Human breast milk contamination with phthalates and alterations of endogenous reproductive hormones in infants three months of age. Environ Health Perspect 2006; 114:270-6.

Mazur HI, Stennett DJ, Egging PK. Extraction of diethylhexylphthalate from total nutrient solution-containing polyvinyl chloride bags. JPEN J Parenter Enteral Nutr 1989; 13:59-62.

Meek ME, Chan PK. Bis(2-ethylhexyl)phthalate: evaluation of risks to health from environmental exposure in Canada. Journal of Environmental Science and Health. Part C, Environmental Carcinogenesis & Ecotoxicology Reviews 1994; 12:179-194.

Melnick RL, Morrissey RE, Tomaszewski KE. Studies by the National Toxicology Program on di(2-ethylhexyl)phthalate. Toxicol Ind Health 1987; 3:99-118.

Mettang T, Thomas S, Kiefer T, Fischer FP, Kuhlmann U, Wodarz R, et al. The fate of leached di(2-ethylhexyl)phthalate in patients undergoing CAPD treatment. Perit Dial Int 1996; 16:58-62.

MR Millar, RM Sharpe, GF Weinbauer, HM Fraser, PT Saunders, Marmoset Spermatogenesis: organizational similarities to the human. Int J Androl, 2000; 23 (5): 266-277.

Ministry of Health and Welfare, Japan 1996, Toxicity testing Report Vol 4, 695-720.

Miyata K, Shiraishi K, Houshuyama S, Imatanaka N, Umano T, Minobe Y, et al. Subacute oral toxicity study of di(2-ethylhexyl)adipate based on the draft protocol for the "Enhanced OECD Test Guideline no. 407". Arch Toxicol 2006; 80: 181-186.

Modigh CM, Bodin SL, Lillienberg L, Dahlman-Hoglund A, Akesson B, Axelsson G. Time to pregnancy among partners of men exposed to di(2-ethylhexyl)phthalate. Scand J Work Environ Health 2002; 28:418-28.

Moore, MR: Oncogenicity study in mice with Di(2-ethylhexyl)phthalate including ancillary hepatocellular proliferation and biochemical analyses, Corning Hazleton Incorporate (CHV), 9200 Leesburg Pike, Vienna, Virginia 22182-1699. Laboratory study identification: CHV 663-134, 1996; Sponsor: Eastman Chemical Compny, First America Center, P.O. Box 1994, Kingsport, Tennessee 37662-5394.

Moore, MR: Oncogenicity study in rat with Di(2-ethylhexyl)phthalate including ancillary hepatocellular proliferation and biochemical analyses, Corning Hazleton Incorporate (CHV), 9200 Leesburg Pike, Vienna, Virginia 22182-1699. Laboratory study identification: CHV , 663-135, 1997; Sponsor: Eastman Chemical Compny, First America Center, P.O. Box 1994, Kingsport, Tennessee 37662-5394.

Mortensen GK, Main KM, Andersson AM, Leffers H, Skakkebaek NE. Determination of phthalate monoesters in human milk, consumer milk, and infant formula by tandem mass spectrometry (LC-MS-MS). Anal Bioanal Chem 2005; 382:1084-92.

Müller AK, Nielsen E, Ladefoget O. Human exposure to selected phthalates in Denmark. Institute of Food Safety and Nutrition, Søborg, Denmark. 2003.

Niino T, Ishibashi T, Itho T, Sakai S, Ishiwata H, Yamada T, et al. Monoester formation by hydrolysis of dialkyl phthalate migrating from polyvinyl chloride products in human saliva. Journal of health science 2001; 47:318-322.

Niino T, Ishibashi T, Ishiwata H, Takeda K, Onodera S. Characterization of human salivary esterase in enzymatic hydrolysis of phthalate esters. Journal of health science 2003; 49:76-81.

NTP (National Toxicology Program). (1982a). Carcinogenesis bioassay of di(2-ethylhexyl) phthalate in F344 rats and B6C3F1 mice (feed study). NTP Technical Report No. 217, 01-82. NTP-CEHRHR, 2005

Øie L, Hersoug LG, Madsen JØ. Residential exposure to plasticizers and its possible role in the pathogenesis of asthma. Environ Health Perspect 1997; 105:972-978.

Øie L, Nafstad P, Botten G, Jaakkola JJK. Ventilation in the homes and bronchial obstruction in young children. Epidemiology 1999; 110:294-99.

Pan G, Hanaoka T, Yoshimura M, Zhang S, Wang P, Tsukino H, et al. Decreased Serum Free Testosterone in Workers Exposed to High Levels of Di-n-butyl Phthalate (DBP) and Di-2-ethylhexyl Phthalate (DEHP): A Cross-Sectional Study in China. Environ Health Perspect. 2006;114:1643-1648.

Parks LG, Ostby JS, Lambright CR, Abbott BD, Klinefelder GR, Barlow NJ, Gray LE. The plasticizer diethylhexyl phthalate induces malformations by decreasing fetal testosterone synthesis during sexual differentiation in the male rat. Toxicolo Sci 2000; 58: 339-49.

Parmar D, Srivastava SP, Seth PK. Hepatic mixed function oxidases and cytochrome P-450 contents in rat pups exposed to di-(2-ethylhexyl)phthalate through mother's milk. Drug Metab Dispos 1985; 13: 368-70.

Pearson SD, Trissel LA. Leaching of diethylhexyl phthalate from polyvinyl chloride containers by selected drugs and formulation components. Am J Hosp Pharm 1993; 50:1405-9.

Peck, C. C., Albro, P. W., Hass, J. R., Odom, D. G., et al., Clinical Research 1978, 25, 101A.

Peck CC, Odom DG, Friedman HI, Albro PW, Hass JR, Brady JT, Jess DA. Di-2-ethylhexyl phthalate (DEHP) and mono-2-ethylexyl phthalate (MEHP) accumulation in whole blood and red cell concentrates. Transfusion 1979, 19, 137-46.

Peck CC, Albro PW. Toxic potential of the plasticizer Di(2-ethylhexyl) phthalate in the context of its disposition and metabolism in primates and man. Environ Health Perspect 1982; 45:11-7.

Peterson JH, Breindahl T. Plasticizers in total diet samples, baby food and infant formulae. Food Additives and Contaminants 2000; 17:133-141.

Pierik FH, Burdorf A, Deddens JA, et al. Maternal and paternal risk factors for cryptorchidism and hypospadias: A case-control study in newborn boys. Environmental Health Perspectives 2004; 112 (15):1570-1576.

Plonait SL, Nau H, Maier RF, Wittfoht W, Obladen M. Exposure of newborn infants to di-(2-ethylhexyl)-phthalate and 2-ethylhexanoic acid following exchange transfusion with polyvinylchloride catheters. Transfusion 1993; 33:598-605.

Pollack GM, Buchanan JF, Slaughter RL, Kohli RK, Shen DD. Circulating concentrations of di(2-ethylhexyl) phthalate and its de-esterified phthalic acid products following plasticizer exposure in patients receiving hemodialysis. Toxicol Appl Pharmacol 1985a; 79:257-67.

Pollack GM, Li RC, Ermer JC, Shen DD. Effects of route of administration and repetitive dosing on the disposition kinetics of di(2-ethylhexyl) phthalate and its mono-de-esterified metabolite in rats. Toxicol Appl Pharmacol 1985b; 79:246-56.

Poon R, Lecavalier P, Mueller R, Valli VE, Procter BG, Chu I. Subchronic oral toxicity of Di-n-Octyl Phthalate and Di(2-Ethylhexyl)phthalate in the rat. Food Chem Toxicol 1997; 35:225-239.

Preuss R, Koch HM, Angerer J. Biological monitoring of the five major metabolites of di-(2-ethylhexyl)phthalate (DEHP) in human urine using column-switching liquid chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2005; 816:269-80.

Pugh G, Isenberg JS, Kamendulis LM, Ackley DC, Clare LJ, Brown R, Lington AW, Smith JH, Klaunig JE. Effects of di-isononyl phthalate, di-2ethylhexyl phthalate and clofibrate in cynomolgus monkeys. Toxicol Sci 2000; 56: 181-8.

Rais-Bahrami K, Nunez S, Revenis ME, Luban NLC, Short BL. Follow-up study of adolescents exposed to di-2-ethylhexyl phthalate (DEHP) as neonates on extracorporeal membrane oxygenation (ECMO) support. Environ Health Perspect 2004; 112(13):1339-1340.

Reddy BS, Rozati R, Reddy S, Kodampur S, Reddy P, Reddy R. High plasma concentrations of polychlorinated biphenyls and phthalate esters in women with endometriosis: a prospective case control study Fertil Steril. 2006a; 85:775-9.

Reddy BS, Rozati R, Reddy BV, Raman NV. Association of phthalate esters with endometriosis in Indian women. BJOG 2006b; 113:515-20.

Rhodes C, Elcombe CR, Batten PL, Bratt H, Jackson SJ, Pratt IS, Orton TC. The disposition of 14C-di-2-ethylhexylphthalate (DEHP) in the marmoset. Dev Toxicol Environ Sci 1983; 11:579-81.

Rhodes C, Orton TC, Pratt IS, Batten PL, Bratt H, Jackson SJ, Elcombe CR. Comparative pharmacokinetics and subacute toxicity of di(2-ethylhexyl) phthalate (DEHP) in rats and marmosets: extrapolation of effects in rodents to man. Environ Health Perspect 1986; 65:299-307.

Rock G, Secours VE, Franklin CA, Chu I, Villeneuve DC. The accumulation of mono-2-ethylhexylphthalate (MEHP) during storage of whole blood and plasma. Transfusion 1978; 18:553-8.

Rosenberg EM, Chambers LA, Gunter JM, Good JA. A program to limit donor exposures to neonates undergoing extracorporeal membrane oxygenation. Pediatrics 1994; 94:341-346.

Rusyn I, Peters JM, Cunningham ML. Mode of action and species-specific effects of di-(2ethylhexyl)phthalates in the liver. Cri Rev Toxicol 2006; 36:459-79.

Schmid P, Schlatter C. Excretion and metabolism of di(2-ethylhexyl)phthalate in man. Xenobiotica 1985; 15:251-6.

Schneider B, Schena J, Truog R, Jacobson M, Kevy S. Exposure to di(2-ethylhexyl)phthalate in infants receiving extracorporeal membrane oxygenation. N Engl J Med 1989; 320:1563.

SCMPMD (Scientific Committee on Medicinal Products and Medical Devices), 26 September 2002 Medical devices containing DEHP Plasticised PVC; Neonates and other groups possibly at risk from DEHP toxicity.

Scott RC, Dugard PH, Ramsey JD, Rhodes C. In vitro absorption of some o-phthalate diesters through human and rat skin. Environ Health Perspect 1987; 74:223-7.

Seidl S, Gosda W, Reppucci AJ, The in vitro and in vivo evaluation of whole blood and red blood cell concentrates drawn on CPDA-1and stored in a non-DEHP plasticized PVC container. Vox Sang 1991; 61: 8-13

Sharpe RM, Skakkebaek N E. Male reproductive disorders and the role of endocrine disruption: advances in understanding and identification of areas for future research. Pure and Applied Chemistry 2003; 75:2023-2038.

Shah BL, Shertukde VV. Effect of Plasticizers on Mechanical, Electrical, Permanence, and Thermal Properties of Poly(vinyl chloride). J Appl. Polym. Sci. 2003; 90:3278-3284.

Sharpe RM Phthalate exposure during pregnancy and lower anogenital index in boys: wider implications for the general population. Environmental Health Perspectives 2005; 113(8): 504-A505.

Shenshu K, Takenaka, M, Miyahara Y, Ichishi M, Koyama S. Study on drug absorption of polyvinyl chloride-infusion line containing tris(2-ethylhexyl)trimellitate as the plasticiser. – Japanese Journal of Pharmaceutical Health Care and Sciences, 2004; 30(2):136-142.

Silva MJ, Barr DB, Reidy JA, Malek NA, Hodge CC, Caudill SP, et al. Urinary levels of seven phthalate metabolites in the U.S. population from the National Health and Nutrition Examination Survey (NHANES) 1999-2000. Environ Health Perspect 2004a; 112:331-8.

Silva MJ, Reidy JA, Herbert AR, Preau JL, Jr., Needham LL, Calafat AM. Detection of phthalate metabolites in human amniotic fluid. Bull Environ Contam Toxicol 2004b, 72, 1226-31.

Silva MJ, Kato K, Wolf C, Samandar E, Silva SS, Gray EL, Needham LL, Calafat AM. Urinary biomarkers of di-isononyl phthalate in rats. Toxicology 2006a; 223:101-12.

Silva MJ, Reidy JA, Preau JL Jr, Needham LL, Calafat AM. Oxidative metabolites of diisononyl phthalate as biomarkers for human exposure assessment. Environ Health Perspect 2006b; 114:1158-61.

Singh AR, Lawrence WH, Autian J. Maternal-fetal transfer of 14C-di-2-ethylhexyl phthalate and 14C-diethyl phthalate in rats. J Pharm Sci 1975; 64:1347-50.

Sjoberg P, Bondesson U, Kjellen L, Lindquist N.G, Montin G. Ploen,L, Kinetics of di-(2-ethylhexyl) phthalate in immature and mature rats and effect on testis. Acta Pharmacol.Toxicol.(Copenh), 1985a; 56:30-7.

Sjoberg P, Bondesson U, Sedin G, Gustafsson J. Dispositions of di- and mono-(2-ethylhexyl) phthalate in newborn infants subjected to exchange transfusions. Eur J Clin Invest 1985b; 15:430-6.

Srivastava S, Awasthi VK, Srivastava SP, Seth PK. Biochemical alterations in rat fetal liver following in utero exposure to di(2-ethylhexyl)phthalate (DEHP). Indian J Exp Biol 1989; 27:885-8.

Stroheker T, Cabaton N, Nourdin G, Régnier J-F, Lhuguenot J-C, Chagnon M-C. Evaluation of anti-androgenic activity of di-(2-ethylhexyl)phthalate. Toxicology 2005; 208:115-121.

Subotic U., Hannmann, Kiss M, Brade J, Breitkopf K and Loff S. Extraction of the plasticizers diethylhexylphthalate and polyadipate from polyvinylchloride nasogastric tubes through gastric juice and feeding solution. J Ped Gastroenterol Nutr 2007, 44: 71-76.

Sullivan FM, Watkins WJ, van der Venne M. Reproductive Toxicity. Volume 1, C EC 1993.

Swan SH, Main KM, Liu F, Stewart SL, Kruse RL, Calafat AM, et al. Ternand CL, Sullivan S, Teague JL. Decrease in anogenital distance among male infants with prenatal phthalate exposure. Environ Health Perspect 2005; 113:1056-61.

Takano H, Yanagisawa R, Inoue K, Sadakane K, Yoshikawa T. Di-(2-ethylhexyl) phthalate enhances atopic dermatitis like skin lesions in mice. Environ Health Perspect 2006;114: 1266-1269.

Tomonari Y, Kurata Y, Kawasuso T, David R, GansG, Tsuchitani M, et al. Effect of Di(2-Ethylhexyl) Phthalate (DEHP) on Genital Organs from Juvenile Common Marmosets: I. Morphological and Biochemical Investigation in 65-Week Toxicity Study, J Toxicol Environ Health 2006; 69 (17): 1651-1672.

Van Tongeren M, Nieuwenhuijsen MJ, Gardiner K, Armstrong B, Vrijheid M, Dolk H, et al. A job-exposure matrix for potential endocrine-disrupting chemicals developed for a study into the association between maternal occupational exposure and hypospadias, Ann. Occup. Hyg. 2002: 46(5):465-477.

Vrijheid M, Armstrong B, Dolk H, van Tongeren M, Botting B. Risk of hypospadias in relation to maternal occupational exposure to potential endocrine disrupting chemicals. Occup Environ Med 2003; 60:543-50.

Wahl HG, Hong Q, Stube D, Maier ME, Haring HU, Liebich HM. Simultaneous analysis of the di(2-ethylhexyl)phthalate metabolites 2-ethylhexanoic acid, 2-ethyl-3-hydroxyhexanoic acid and 2-ethyl-3-oxohexanoic acid in urine by gas chromatography-mass spectrometry. J Chromatogr B Biomed Sci Appl 2001; 758:213-9.

Wahl HG, Hong Q, Hildenbrand S, Risler T, Luft D, Liebich H. 4-Heptanone is a metabolite of the plasticizer di(2-ethylhexyl) phthalate (DEHP) in haemodialysis patients. Nephrol Dial Transplant 2004; 19:2576-83.

Wester RC, Melendres J, Sedik L, Maibach H, Riviere JE. Percutaneous absorption of salicylic acid, theophylline, 2, 4-dimethylamine, diethyl hexyl phthalic acid, and p-aminobenzoic acid in the isolated perfused porcine skin flap compared to man in vivo. Toxicol Appl Pharmacol 1998, 151:159-65.

Weuve J, Sanchez BN, Calafat AM, Schettler T, Green RA, Hu H, et al. Exposure to phthalates in neonatal intensive care unit infants: urinary concentrations of monoesters and oxidative metabolites. Environ Health Perspect 2006; 114:1424-31.

Wittassek M, Wiesmüllerc GA, Koch HM, Eckard R, Dobler L, et al. Inner burden to phthalate plasticizers over the last two decades – a retrospective human biomonitoring study. Int J Hyg Environ Health 2007a; 210(3-4):319-333

Wittassek M, Heger W, Koch HM, Becker K, Angerer J, Kolossa-Gehring M. Daily intake of di(2-ethylhexyl)phthalate (DEHP) by German children - a comparison of two estimation models based on urinary DEHP metabolite levels. Int J Hyg Environ Health 2007b; 210:35–42

Wolfe GW, Layton KA. Multigeneration reproduction toxicity study in rats (unaudited draft): Diethylhexylphthalate: Multigenerational reproductive assessment when administered to Sprague-Dawley rats in the diet. TherImmune Research Corporation (Gaithersburg, Maryland), TRC Study n° 7244-200, 2003

Wormuth M, Scheringer M, Vollenweider M, Hungerbuhler K. What are the sources of exposure to eight frequently used phthalic acid esters in Europeans? Risk Anal 2006; 26:803-24.

Zhu J, Phillips SP, Feng YL, Yang X. Phthalate esters in human milk: concentration variations over a 6-month postpartum time. Environ Sci Technol 2006; 40:5276-81.

ANNEX I evaluation of individual plasticizers

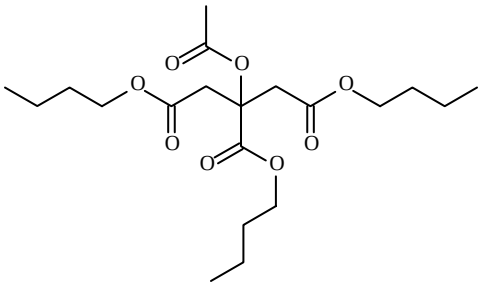
No information was submitted on the DINP and DEHA plasticizers, but they have been included in this assessment as they are already being used to substitute DEHP in a number of applications.

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. For medical devices Johnson (2002) says that the major compound being used is acetyltrihexyl citrate. 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,

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. Metabolism

ATBC is well absorbed after oral administration with peak blood levels being found between 2 and 4 hours. It undergoes rapid and extensive metabolism to 10 or more polar metabolites. The principal mode of metabolism is hydrolysis of the ester bonds. Blood clearance of C14 labelled ATBC has been shown to be biphasic with corresponding half lives of 3.9hours and 39 hours.

The slow second phase may be an artefact due to some of the radiolabel entering intermediary metabolism pathways. The main route of clearance is through the urine with monobutyl citrate being the principle metabolite found. However some metabolites are also found in the faeces. Whether this indicates that some ATBC is biliary excreted or not absorbed is uncertain. ATBC is also extensively metabolised in human serum and by rat liver samples. The kinetic data indicate that ATBC is very unlikely to accumulate in body tissues even if frequent exposure occurs.

1.5. Toxicity

Acute toxicity
After a single oral dose of 10-30grams per kg kg bw/ per day, 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 at other sites of exposure.

Irritation and sensitisation
ATBC applied dermally to rats produces moderate irritation but has been shown to be 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

Repeat dose toxicity
Three relevant 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/day) 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/day.

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 kg bw/day and at 1000 mg/kg kg bw/day there was an increase in liver weight. However no histopathological changes were seen in either test group. At 100 mg/kg bw/day no changes of any kind were seen and therefore this dose may be regarded as the NOEL

A third study involving the in utero exposure of rats is discussed below (in the section on reproductive effects).

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 two 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 two separate experiments.

An in vivo test has also been conducted using unscheduled DNA synthesis as the endpoint. In rats treated by gavage at either 800 mg or 2000 mg /kg bw/day no increase in UDS could be observed. This finding indicates a low or zero 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 two year oral feeding study has been carried out in rats in which no significant toxic effects relating to ATBC were identified. However this study was not to 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 studies.
Two relevant studies are available. In the first, a two generation study in rats, ATBC was administered in the diet at levels equivalent to 0, 100, 300 and 1000 mg/kg bw/day. The 300mg and 1000 mg doses produced a decrease in kg bw/ in F1 male rats. In the female rats a decrease in kg bw/ was only observed at the top dose (1000 mg/kg w/day). Thus the NOEL was identified as 100 mg//kg bw/day.

In a second study rats were exposed to ATBC in the diet at doses of 0, 100, 300 and 1000 mg/kg bw/day 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/day) dose. Minor changes were also observed in male animals at the 300 mg/kg bw/ day. A NOEL of 100 mg /kg bw/day can therefore be accepted.

1.6. Human data

No information available on toxicity in humans.

1.7. Conclusion

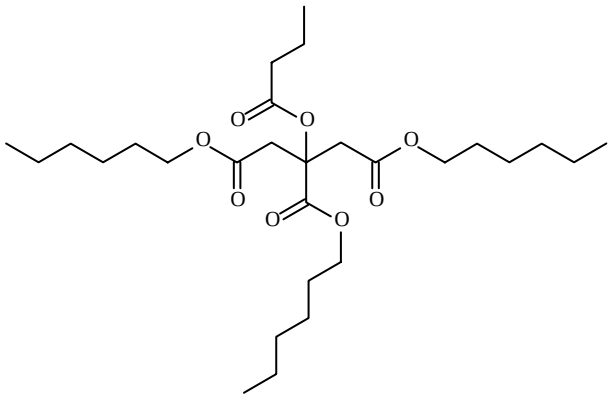
ATBC is well absorbed following its oral administration. It is rapidly metabolised and excreted from the body. It is unlikely to accumulate in the body following frequent exposure. It has a low toxicity following acute oral administration. In repeat dose studies only non specific effects were found. The oral NOEL was 100mg/kg kg bw//day.
ATBC was found to be non genotoxic and was a very mild hepatic peroxisome proliferator in rats. Moreover in a lifetime bioassay study in rats no dose related tumours were found.
.

References:
Submission from Reilly Chemicals.

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.

2.4. Metabolism

BTHC is well absorbed after oral administration. It is rapidly metabolised by hydrolysis of the ester bonds to a number of metabolites. The principal 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 >24hours. 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 kg bw. Acute iv injection studies with doses of up to 462 mg/kg 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 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 one male and one 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 kg bw/day 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 1000mg/kg kg bw/ per day 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 NOEL from these findings but a value of 250mg/kg kg bw/ per day is reasonable.

In one study BTHC was administered intravenously to adult rats at dose levels of 5, 50, and 500 mg/kg bw/day for 28 days. At 500 mg/kg bw/day no changes were observed in kg body weight, 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 two lower dose groups.Thus an NOEL by the iv route of 50mg/kg bw//day 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 kg bw/ per day 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 50mg/kg bw/day.

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/day, was assessed in various Ames strains of salmonella. No mutagenic effects were observed.

In mouse lymphoma cells BTHC produced different findings in two 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/day either as an acute dose or daily for five 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 studies.
A fertility study was carried out in albino rats at dietary levels of 0,0.6 0r 1.2% BTHC. Males were exposed to BTHC continuously to BTHC for ten weeks prior to mating and during the mating period. Females were exposed for two 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 kg bw/ 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 kg bw/ /day) 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 kg bw/day 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 po NOEL was 250 mg/kg kg bw/day and the iv NOEL was 50 mg/kg kg bw/day.

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.

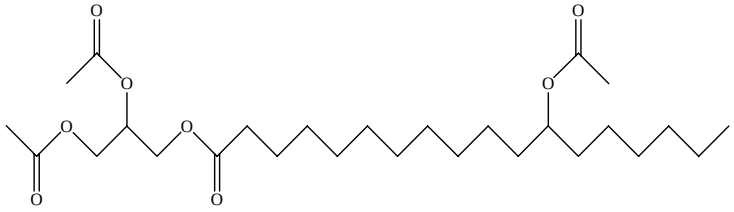
References:
Submission from Reilly Chemicals.

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

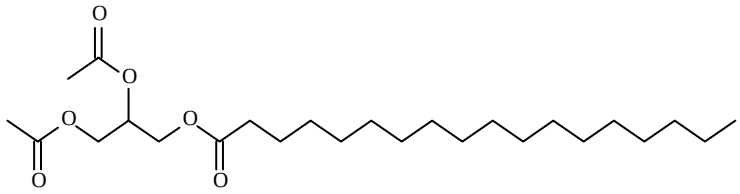
3.1. Physico-chemical data

COMGHA is a mixture of two 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)
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 plasticizer exhibits a performance similar to that of DEHP. It is approved in EU for use in food contact material. The intended primary use is in PVC (films, tubes, bottles, sealings, etc.), and the product may also find use in other polymers like polyolefines, styrenics, PET, etc. The product is recognised as a food packing material and evaluated by opinion of European Food Safety Authority (EFSA) 2004, 109, 1-26. Classified list 3. This product is notified as “new substances” in the context of 6th Amendment of Directive 67/548/EEC and listed in the European List of Notified Substances (ELINCS) as no. 451-530-8.

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).

3.4. Metabolism

Quite detailed 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.

3.5. Toxicity

Repeated dose toxicity
Similar effect as administering corn oil. The NOAEL is 3 ml/kg bw/day. 90 day oral toxicity. A 13 week toxicity in SD rats fed by gavage at 3, 8.5, and 20 ml/kg bw/day. The NOAEL was less than 3 ml//kg bw/day. 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 o, 500 mg, 1600 mg or 5000 mg/mg/kg bw/day. The NOAEL was 5000/mg/kg/day. A chronic toxicity/ carcinogenicity study was not submitted.

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.

Mutagenity and 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
No studies submitted. A review of the toxicity of 12-hydroxy-octadecanoic acid, 12-acetoxyoctadecanoic acid and the systemic toxicity of acetic acid concluded no adverse effect have been reported of the two compounds but no data was available on the toxicity of 12-acetoxy-octadecanoic acid.

3.6. Human data

No information available on toxicity in humans.

3.7. Conclusion

Good information on fully functional replacement of DEHP is available. The compound migrates less than DEHP. Replaced 1:1 with DEHP.

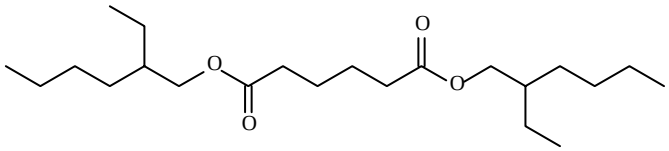
No original toxicity data were available. Based on the summary data it seems that the product is rather non-toxic. However basal toxicity on reproduction and immunotoxicity, sensitisation and chronic toxicity and cancer studies are missing.

References:
Submission from Danisco S/A.

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

4.1. Physico-chemical properties

CAS Reg. No: 103-23-1
Synonyms: DEHA, di(2-ethylhexyl) adipate, DOA, dioctyl adipate
Empirical Formula: $C_{22}H_{42}O_4$



Molecular weight: 370.57
Melting point: -67.8°C
Boiling point: 214°C (0.67 kPa), 417°C (SIDS)
Vapour pressure: 8.5 $x10^{-7}$ mm Hg at 25°C, 0.11 kPa (20°C), 0.32 kPa (200°C), 1.1 x 10^{-4} Pa at 20°C (SIDS)
Solubility in water: 0.78 mg/L (22°C)
Log Kow: >6.11 (calculated), 8.0 (calculated)
Purity:
Impurities: 0.01-0.02% adipic acid (purity >99%)

Leaching of plasticizers from food packing materials into especially fatty food has been studied a lot. 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 that have an annual production and/or importation volumes above 1 million pounds in the U.S. DINP 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.

4.3. Exposure

There has been uncertainty about the exposure of the general population. A survey covering 112 individuals established an intake of 2.7 mg/day (medium value). 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 kg bw/ (SCF 2000, CSTEE 1999). No information has been found describing the exposure of children from PVC articles

4.4. Metabolism

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 (Short term effects).
DEHA has very low 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

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

Genotoxicity
DEHA has not induced point mutation in *Salmonella typhimurium* or mouse lymphoma cells, sister chromatide exchanges in primary hepatocytes or Chinise 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. 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 orally 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
Chronic toxicity and carcinogenicity study of several phthalic acid esters and compounds containing a 2-ethylhexyl moiety was conducted in Fischer 334 rats and $B6C3F_1$ mice (Kluwe 1986). In general, the toxic manifestation of the phthalic acid ester was closely correlated with their ester substituents. Although many of the phthalic esters possessed some carcinogenic activity, target sites for such effects were dissimilar, suggesting the absence of a common mode of action. In contrast, all of the 2-ethylhexyl-containing compounds studied possessed some hepatocarcinogenic activity, indicating that this moiety may have a propensity for causing hepatocarcinogenesis in mice. The 2-ethylhexyl compound that caused the greatest hepatocarcinogenic response in mice (DEHP), was also hepatocarcinogenic in rats.

Reproductive toxicity
Several studies show foetotoxic effect of DEHA (CSTEE 1999). A new detailed study using gavage administration of 0, 200, 400, or 800 mg/kg/day to pregnant rats, confirmed the foetotoxic effect. Maternal toxicity was seen at 800 mg/kg bw/day. The NOAEL for maternal toxicity was 400 mg/kg bw/day. The NOAEL was 200 mg/kg. DEHA induced a prolonged gestation period at 800 mg/kg. No antiandrogenic endpoints were affected. DEHA did not induce antiandrogenic effects similar to those of DEHP (Borch et al. 2002, Dalgaard et al. 2003, Borch et al. 2006). A recent 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 estrous cycle and increased ovarian follicle atresia were detected in the 1000 mg/kg group.

4.6. Human data

No information available on toxicity in humans.

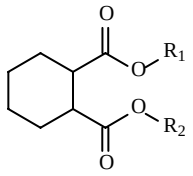
4.7. Conclusion.

DEHA does not show the specific toxicity on reproductive organs like DEHP on male pups after *in utero* exposure. A NOAEL of 200 mg/kg/bw for developmental toxicity and foetotoxicity can be established.

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:



R_1 and R_2 (not necessary identical) either mainly C_8H_{17} to $C_{10}H_{21}$ or C_9H_{19} isomers. In the case where R_1 and R_2 is C_9H_{19} isomersisomers it isis 10 % n-nonyl, 35-40 % methyloctyl, 40-45 % dimethylheptyl, 5-10 % methylethylhexyl

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 was introduced recently and it is suggested as an alternative to DEHP “for sensitive applications”. These include medical devices, such as blood tubes and packaging for nutrient solutions. The European producer has a capacity of 25,000 tpa but that is now going to be extended to 100,000 tpa.

5.3. Exposure

No information has been found describing human exposure. 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 a lot.

5.4. Metabolism

After oral administration DINCH showed rapid but saturable absorption and extensive elimination 24 hours after dosing approximately 80% of the radioactivity is excreted, after 48 hours more than 90 % is excreted via urine and mainly via feces. Based on the amounts of radioactivity excreted in the bile and urine, the bioavailability of ^{14}C-1,2-Cyclohexanedicarboxylic acid di(isononyl)ester is estimated to be 5-6% at the high dose and 40-49 % at the low dose.

There is no indication of bioaccumulation. The characterisation of metabolites after oral and intravenous administration of DINCH indicates two main pathways: the partial hydrolysis of DINCH to the mono-isonyl ester followed by conjugation to glucuronic acid, which is the most ab Undant metabolite in bile, or the hydrolysis of the remaining ester bond to yield free cyclohexane dicarboxylic acid, the predominant urinary metabolite.

5.5. Toxicity

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

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.

Acute toxicity
DINCH has very low acute toxicity, the LD50 dose for DINCH in the rat is >5000 mg/kg bw after oral, and > 2000 mg/kg bw after dermal administration.

Repeated dose toxicity

28 day study
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.

90 day study
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.

(NOTE the alpha 2-macroglobulin is considered specific for the rat and the mechanism thought not relevant for man). In the liver 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 mean relative weights in all 3 dose groups with no dose-response relationsip. Histopathologically there was no obstructive proces 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/day) in male and 4,500 ppm (389.4 mg/kg/day) in females. Also in the two generation study thyroid hyperplasia/trophy was observed with a NOAEL of 100 mg/kg/day.

Mutagenity and 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 two year combined chronic toxicity/carcinogenicity study (doses 40, 200, 1,000 mg/kg bw/day) also 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 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/day. 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/day.

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 being 1,200 mg/kg bw/day

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/day). 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/day. 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), respectively 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 also in females significantly ($p<0.05$) decreased.

The limited (7-8% change compared to controls), although significant alterations in the AGD and AGI are not considered of biological significance as other corresponding parameters were not affected like testes descendance, preputial separation, vaginal opening, testes weight and histology, and sperm parameters. Also in females the AGI was decreased to the same extent, contradicting the AGI to be an effect of impaired androgen dependent development. In addition, in the two generation study no effects were noted (but AGD and AGI not determined).

Two generation study

The two generation study was performed with continuous dietary administration (doses 0-100-300-1000 mg/kg bw/day). 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) For 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

5.6. Human data

No information available on toxicity in humans.

5.7. Conclusion

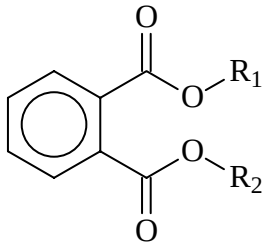
The toxicity of DINCH is lower than that of DEHP. DINCH also shows a different "hazard profile" from DEHP for reproductive toxicity and peroxisome proliferation. The magnitude of exposure resulting from differences in leaching of DEHP and DINCH from the plastics of interest, is less for DINCH. In addition, effects of DINCH are observed at higher exposure doses than DEHP.

References:
Submission from BASF.

6. DINP (di-iso-nonyl phthalate)

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



R_1 and R_2 (not necessary identical) either mainly C_8H_{17} to $C_{10}H_{21}$ or C_9H_{19} isomers. In the case where R_1 and R_2 is C_9H_{19} isomers it is 10 % n-nonyl, 35-40 % methyloctyl, 40-45 % dimethylheptyl, 5-10 % methylethylhexyl

Molecular weight:	420.6 (average)
Melting point:	-40 to -54°C
Boiling point:	424°C
Vapour pressure:	6 x 10^{-5} Pa at 20°C
Solubility in water:	0.6 µg/L
Log Kow:	8.8
Purity:	These products are mixtures of different composition and can contain up to at least 40 different substances
Impurities:	DINP is not a pure substance, but a complex mixture containing mainly C9-branched isomers: 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. (RAR EU 2003). It has limited use in food packing material and is not used in medical products (CSTEE 2001). DINP 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.

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. These estimates are based on DINP measurements in several environmental media and consumer products (ECB 2003). 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 submitted).

Infants (0.5-3 years old)
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) (ECB 2003).

6.4. Metabolism

In rats DINP is readily absorbed and approximately 50% of an oral DINP dose is excreted renally, 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 in press b; 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 in press a). 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 12 hours for OH-MINP and oxo-MINP and 18 hours 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 in press a; Silva 2006a).

6.5. Toxicity

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

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.

Mutagenity and 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 in two studies increased the mononuclear cell leukaemia in Fisher rat. IARC has classified this leukaemia of no relevance for human.

DINP induce kidney tumours in male rats but this 2u globulin induced tumours is not considered as relevant to humans.

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 two-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 that male displayed female like areolas/nipple retention and that incidence of reproductive malformation was slightly but significantly increased (7.7% versus 91% with DEHP) Gray et al. (2000). (The reproductive effect of DINP is similar to the profile shown for DEHP but DINP is only half or less potent as DEHP. There is an increasing use of DINP but the reproductive toxicity of all the isomers is not well investigated (CSTEE 2001).

6.6. Human data

No information available on toxicity in humans.

6.7. Conclusion

The reproductive seffect of DINP indicate a similar hazard profile (except age) as shown for DEHP, but DINP is only half or less potent as DEHP. The mechanism of action is an effect on steroidogenesis of testosterone in the fetal male rat like shown by DEHP. CSTEE (2001) has previously recommended that the NOAEL effect is lower than the one reported in the RAR if using the spongiosis hepatis as the critical endpoint. This is seen in doses of 12-15 mg/kg/d.

References:

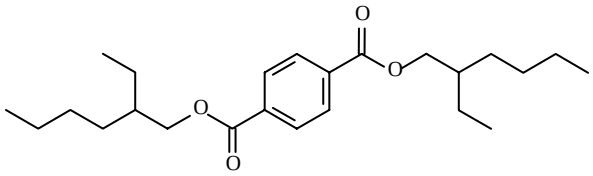
ECB. European Union Risk Assessment Report for 1,2-benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich and di-"isononyl" phthalate (DINP) (Final Report 2003). Doc. No. European Chemical Bureau, 2003.
Koch HM and Angerer J. Di-iso-nonylphthalate (DINP) metabolites in human urine after a single dose of deuterium-labelled DINP. *Int J Hyg Environ Health* in press a
Koch HM, Muller J and Angerer J. Determination of secondary, oxidised di-iso-nonylphthalate (DINP) metabolites in human urine representative for the exposure to commercial DINP plasticizers. *J Chromatogr B Analyt Technol Biomed Life Sci* in press b, doi:10.1016/j.jchromb.2006.09.044
McKee RH, El-Hawari M, Stoltz M, Pallas F and Lington AW. Absorption, disposition and metabolism of di-isononyl phthalate (DINP) in F-344 rats. *J Appl Toxicol* 2002, 22, 293-302.
Silva MJ, Kato K, Wolf C, Samandar E, Silva SS, Gray EL, Needham LL and Calafat AM. Urinary biomarkers of di-isononyl phthalate in rats. *Toxicology* 2006a, 223, 101-12.

Silva MJ, Reidy JA, Preau JL, Jr., Needham LL and Calafat AM. Oxidative metabolites of diisononyl phthalate as biomarkers for human exposure assessment. *Environ Health Perspect* 2006b, 114, 1158-61.

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.

7.4. Metabolism

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, indicating 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 of 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% and the percentage of the total DEHP dose recovered in the urine, as unlabeled TPA, was 50.5%. In total 91.7 % of the dose can be accounted for as either unchanged DEHT (in faeces), unlabeled TPA (in urine) or exhaled CO_2. This balance sheet thus limits the amount of mono(2-ethylhexyl) terephthalate (MEHT), and its metabolites to a maximum of 9.3 % of orally administered dose: After 24 hours more than 95%of the radioactivity was excreted [Barber et al. 1994].

Apparently; DEHT is not readily absorbed from the GI tract upon oral exposure; and extensively hydrolyzed to TPA and 2-EH (before and after absorption) and it is rapidly excreted. This contrasts to the metabolite profile of the ortho-phthalate DEHP which primarily undergoes hydrolysis to form the monoester (MEHP).

7.5. Toxicity

Acute toxicity

Acute toxicity data are mainly reported for rats and, mice. LD50 was >5000 mg/kg and 3200 mg/kg bw in oral studies and >20 ml/kg for dermal toxicity in guinea pigs

Repeated dose toxicity
4-5 studies conducted; some 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:
[a] 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].

[b] 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.

Mutagenity and 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 indicate a NOEL for carcinogenicity of 12000 ppm (highest dose tested), equivalent to 666 mg/kg/day in males and 901 mg/kg/day in females.

The NOEL for chronic toxicity in the study was 1500 ppm equivalent to 79 mg/kg/day in males and 102 mg/kg/day in females.

Reproduction/ developmental toxicity
In a two 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/day for males, and 0, 250-300, 500-600, 800-1000 mg/kg/day 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 were 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.

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/day) from GD 0 to GD 20. Uteri and contents were excised by caeserean section and examined (fetuses, 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/day); the NOAEL for developmental tox was 1% (747 mg/kg/day).

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 demasculinization: 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/day) 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).

7.6. Human data

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

7.7. Conclusion

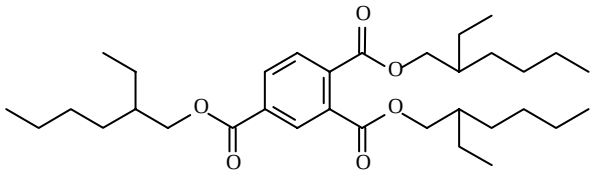
DEHT is not genotoxic (like its isomeric relative DEHP). DEHT is less active in the induction of peroxisome-proliferation in rats than DEHP, and this is explained by the smaller amounts of monoester produced during DEHT metabolism. At doses where DEHP, BBP and DINP all altered sexual differentiation, DEHT was inactive. (DEHP, BBP were of equivalent potency, DINP was about an order of magnitude less active).

References:
Submission from Eastman Chemical Company.

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
Log Kow: 5.94 (4.35) at 25°C
Purity:
Impurities:

In the dossiers no method of determination of substance and metabolites were presented. The open literature gave two 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. 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 unique low leaching properties and extraction resistance properties that are required for dishwasher gaskets, medical tubing and photograph storage.

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.

8.4. Metabolism

Absorption and metabolism were studied for TOTM (14C labelled) mixed with corn oil and administered by gavage in a single dose of 100 mg/kg of kg bw/ in 4 male SD rats. About 75% of the dose was excreted unchanged in the faeces, 16% in the urine as metabolites and 1.9% was expired as CO2. Radioactivity was excreted in the faeces as 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. LD50 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/day for 28 days. There were no statistical significant differences in kg bw/ between the control and the exposed groups. There was a significant difference in between the control and exposed groups in the following absolute and relative liver weights, serum albumin and cholesterol levels. Liver biochemistry (palmitoyl CoA oxidation and catalase activity were induced) revealed statistically significant differences between treated and control groups. The NOAEL was 184 mg/kg (CMA 1985)/day.
In the second study the NOAEL was 1000 mg/kg/day; not all the informations are 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/day. The decrease of spermatocytes and spermatides in males was observed at 300 and 1000 mg/kg/day doses by histopatohological examinations. The NOAEL was 100 mg/kg for males and 1000 mg/kg/day in females (Ministry of Health and Welfare, Japan 1998).

In a non GLP compliance 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 J. Toxicology and Industrial Health 1987).

Adult male rats receiving TOTM intraperitoneally for seven days exhibited no significant changes in the 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 K. et.al.1990).

Mutagenity and genotoxicity
One GLP level study for Ames test was carried out and several (4 to5) non GLP compliant studies exist. In the GLP compliant study 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).

Reverse gene mutation assay was conducted by 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 5,0mg/ml on continuous treatment (Ministry of Health and Welfare, Japan 1996).

Carcinogenicity
No data available.

Reproduction/developmental toxicity
Gavage study in SD rats 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/day groups. No effects of TOTM were detected general appearance, kg bw/, food consumption autopsy findings and weight of repro 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/day and 1000 mg/kg/day 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/day for males, 1000 mg/kg/day for females and 1000 mg/kg/day for offspring (Ministry of Health and Welfare, Japan 1996).

8.6. 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 using an 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). (1-2) 139-146.)

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

8.7. Conclusion

TOTM has a low acute toxic potential. Based on the data available TOTM seem to have low metabolic transformation capacity and no major single water soluble metabolite can be identified. This may partially explain the low liver toxicity of the compound. No clear toxicological mode of action can be identified. However, 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.

References:

IARC, 2000.
IARC (1995). International Agency for Research on Cancer. Peroxisome Proliferation and its role in Carcinogenesis, views and expert opinions of an IARC Working Group Lyon, 7-11 Dec 1994, IARC Technical Report No.24 Lyon.]Christersson A., Ljunggren L., Nilsson-Thorell., Arge B., Diehl U., Hagstam K., Lundberg M. Int. J. Artif Organs 1991(7) 407-410
CMA DOC ID 408565036, 1985.
David R., Lockhart L., Ruble K. Lack of sensitization for trimellitate, phthalate, terephthalate and isobutyrate plasticizers in a human repeated insult patch test. Food Chem Toxicol 2003 (4) 589-593)
Hodgson J. Results of peroxisome induction studies on tri(2-ethylhexyl)trimellitate and 2-ethylhexanol. Toxicology and Industrial Health 1987 (1) 49-61
Kambia K., Dine T., Azar R., Gressier B., Luyckx M., Brunet C. Comparative study of the leachability of di(2-ethylhexyl)phthalate and tri(2-ethylhexyl)trimellitate from haemodialysis tubing. Int J. Pharm. 2001 (1-2) 139-146.
Ministry of Health and Welfare, Japan 1996, Toxicity testing Report Vol 4, 695-720)
Ministry of Health and Welfare, Japan 1998, Toxicity testing Report Vol 6, 564-578)
Rathinam K., Srivastava S., Seth P. Hepatic studies of intraperitoneally administered tris(2-ethylhexyl)trimellitate (TOTM) and di(2-ethylhexyl)phthalate in rats. J. App. Toxicology 1990 (10) 39-41.
SIDS Initial Assessment Report for 13th SIAM, 2001, pages 1-8.
Submission from Polynt S.p.A