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Human Health Toxicity Risk Assessment of DEHP in Medical Devices

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334-336 Illawarra Rd, MARRICKVILLE NSW 2204
GPO Box 58 SYDNEY NSW 2001
Phone: 02 8577 8800 Fax: 02 8577 8888

WEBSITE: http://www.nicnas.gov.au

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ABBREVIATIONS

2-EH	2 ethylhexanol
ACD	acid citrate dextrose
AGD	ano-genital distance
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	area under the curve
CAPD	continuous ambulatory peritoneal dialysis
CPB	cardiopulmonary bypass
DEHP	diethylhexyl phthalate
ECMO	extracorporeal membrane oxygenation
FDA	Food and Drug Administration
FPP	fresh frozen plasma
GD	gestational day
IARC	International Agency for Research on Cancer
IPCS	International Programme on Chemical Safety
LOAEL	lowest observable adverse effect level
MCL	mononuclear cell leukaemia
MEHP	mono-ethylhexyl phthalate
MOE	margin of exposure
NICU	neonatal intensive care unit
NOAEL	no observable adverse effect level
NTP CERHR	National Toxicology Program. Center for Evaluation of Risks to Human Reproduction
OR	odds ration
PND	postnatal day
PVC	polyvinyl chloride
RBC	red blood cells
TI	tolerable intake
TOTM	tris(2-ethyl hexyl)trimellitate
EVA	ethylvinyl acetate
TPN	total parenteral nutrition
WHO	World Health Organization

PREFACE

As early as 1970, studies identified and measured di(2-ethylhexyl) phthalate (DEHP), a plasticiser found in a wide variety of medical devices made from polyvinyl chloride (PVC), leached out of devices during use. These studies identified DEHP and its metabolites in the blood and other tissues of patients receiving dialysis, blood transfusions, artificial ventilation, and exchange transfusions. Numerous studies have shown that in rodents, DEHP causes cancer of the liver, developmental and reproductive toxicity and nephrotoxicity.

NICNAS was asked by the Office of Devices, Blood and Tissues to conduct a risk assessment for human health effects from DEHP exposure via medical devices. The risk assessment considers three main patient subgroups – adult males, pregnant women (prenatal development) and neonates.

The toxicity of DEHP has been reviewed by the Expert Panel on Phthalates of the Center for Evaluation of Risks to Human Reproduction, National Toxicology Program (CERHR, 2005), Health Canada (2002), FDA (2002), ATSDR (2002) and the National Chemical Inspectorate (2005). This report principally relies on these reviews. References not examined by NICNAS but quoted from other reviews as secondary citations are marked with an asterisk.

In addition, the Medline and Toxline databases were searched to find the medical and scientific reports on any new toxicology studies and human exposure to DEHP, particularly from medical procedures published since the most recent review (2005). The last literature search was performed in August 2006.

The report identifies and critically reviews the key scientific papers dealing with different aspects of exposure and toxicity to: identify the critical toxicity endpoints; estimate the no-observed-adverse-effect-levels (NOAEL) or lowest-observed-adverse-effect-levels (LOAEL); assess the relevance of the findings in animals for patients exposed to DEHP from medical procedures; and assesses whether exposure to DEHP from medical procedures may cause adverse health effects in humans. The report does not determine whether the health risks from DEHP exposure outweigh its benefits.

SUMMARY

Three critical factors influence the conclusions of this risk characterisation

(i) Choice of NOAEL from laboratory animal studies

(ii) Calculation of exposure estimate

(iii) Acceptable margin of exposure

Choice of NOAEL

The critical effects are considered to be reproductive and developmental effects in males. Testicular toxicity appears to be the most sensitive toxicity endpoint but is significantly influenced by the age at exposure. Developing and neonatal rats have been found to be much more sensitive to exposure to DEHP than adults. The younger animals responded to a much lower dose or produced a more serious lesion with a comparable dose on a mg/kg bw/day basis. For this reason, risk characterisations for three patient populations were developed: pregnant women (prenatal development), neonates and adult males. In general, the animal model best suited would utilise the same route of exposure as the human situation. However, this was not possible for these characterisations as there were no suitable long-term studies following intravenous administration of DEHP for neonatal, adult males or pregnant animals. From the available short-term studies it appears that the NOAELs and LOAELs identified for intravenous exposure to DEHP are higher than those reported following oral exposure. The NOAEL for testicular atrophy in young rats following intravenous administration was 60 mg/kg bw/day after 21 days exposure (Cammack et al, 2003) (note: the same study as AdvaMed*, 2001) while in a three generation oral study the NOAEL was 5.8 mg/kg bw/day, based on small male reproductive organ size and histopathologic changes in the testes in prenatally exposed rats (Wolfe & Layton, 2004). This may reflect a real difference in effect or differences in study design. It is biologically plausible that DEHP is less toxic by the intravenous route as conversion to MEHP is slower. However due to the inadequacy of data via the IV route, the more conservative NOAELs for oral exposure were used in the risk characterisation for parenteral exposure.

The most sensitive endpoint for adult males was effects on fertility. The NOAEL was 14 mg/kg bw/day based on a continuous breeding study in mice (Lamb et al, 1987). The most sensitive endpoint for foetal and neonatal males was effects on the testes. The NOAEL in a three generation study was 5.8 mg/kg bw/day and was used for both prenatal and neonatal development (Wolfe & Layton, 2004).

Calculation of exposure estimate

There are three variables in the calculation of the exposure estimate for each exposure scenario:

(a) clinical setting of scenario

(b) type of exposure model

(c) extraction rates of DEHP from medical device

Clinical parameters of exposure scenarios

In some respects, every clinical procedure in hospitals is a unique scenario developed with the specific requirements of the patient in mind. For the purposes of this risk characterisation, general assumptions were made for each procedure in order to arrive at an estimated exposure level. Variables that can differ include: type and volume of

solution transfused; volume, flow rate, frequency and duration of transfusion; length and internal diameter (surface area) of tubing used (if any); and patient weight. The resulting exposure estimate will also differ if any of these variables change.

Exposure model

An exposure estimate is calculated for each scenario. Ideally, this would be based on *in vivo* measurements of DEHP using area under the curve (AUC) calculations or spot serum or urine concentrations. These data are available for very few scenarios. In addition, these measurements can be influenced by differences in the procedural set-up eg. dimensions of tubing used, flow rate and bulk volume delivered leading to wide variations in the measured concentrations of DEHP. A second approach measures the amount of DEHP *"in vitro"* that leaches from common medical devices, such as blood bags or tubing, into the physiologic medium that each device contains, such as blood. The extent to which DEHP is released from PVC medical devices is a function of the lipophilicity of the fluid that comes into contact with the device, length and surface area of the tubing used, flow rate and temperature. There is also wide variability in reported rates of DEHP extraction from medical tubing for the various medical devices.

In this risk characterisation, for each scenario, a reasonable worse case estimate was made based on *in vivo* measurements (if possible) or *in vitro* leaching rates from storage devices or tubing. Critically, *in vitro* leaching rates were calculated based on surface area rather than bulk volume which has been used by other organisations (eg Health Canada). Where possible, the extraction rate to be used in a specific scenario was derived from studies of similar scenarios eg extraction rates derived from adult haemodialysis tubing were used to estimate exposure for adult haemodialysis. This was not always possible and extraction rates derived from devices used in neonatal or juvenile scenarios were used for adult scenarios. This assumed that the extraction rate from devices with smaller surface areas was the same as that of a device with a larger surface area. Not all variables could be accounted for with these extrapolations, notably flow rate and effects of temperature, as the available data was limited.

This extraction rate was then used to produce an exposure estimate based on the assumptions of the clinical scenario described previously (type and volume of solution transfused; volume, frequency and duration of transfusion; surface area of tubing used; weight of patient).

The dose of DEHP received by patients undergoing various medical procedures was estimated as a delivered dose (mg/kg/day) and time-averaged over a course of treatment to produce an average daily dose (where applicable).

This methodology had the advantage of allowing a determination of the effect of changing variables, such as tubing length or diameter, on estimated delivered dose.

Choice of DEHP extraction rates

A number of the scenarios involve delivery of blood and blood products. The concentration of DEHP varies widely in these products. For each of these scenarios, two exposure estimates were made, one using a reasonable average, the other a maximum measurement.

Acceptable margin of exposure

The margin of exposure (MOE) is calculated using the NOAEL for the critical endpoint and the estimated human dose (E_d) as:

$$MOE = \frac{NOAEL}{E_d}$$

The MOE is a measure of the likelihood that a particular adverse endpoint will occur following exposure. As the MOE increases, the risk of the adverse effect decreases. In the case of DEHP, a MOE of 100 is considered sufficient to protect against adverse health effects from DEHP in the different sub-populations (adult males, pregnant female (prenatal development) and neonates. This MOE consists of a factor of 10 applied to allow for interspecies differences and an additional factor of 10 to account for interindividual variations in the human population (WHO 1994; 2005).

With these assumptions in mind, the following procedures do not have a sufficient MOE considered to protect against the adverse health effects of DEHP.

Adult

- Drugs requiring vehicle for solubilisation, mixed and stored at room temperature. The calculated MOE in the worst-case exposure estimate, when the manufacturer's instructions are not adhered to and the drug formulation is prepared in PVC bags, stored for 24 hours and then infused using PVC tubing, is considered to be insufficient. However, when manufacturer's directions are followed, there is a sufficient MOE.
- Haemodialysis: The MOE is considered to be insufficient and there is an increased risk in this scenario particularly as these patients may have a reduced elimination capacity and so could be particularly sensitive to the effects of DEHP. The DEHP dose would be reduced if DEHP-free or heparin-coated tubing was used.
- Total Parenteral Nutrition with undiluted 20% lipid: The delivered DEHP dose is largely dependent on the lipid content of the TPN solution, the surface area of the tubing and the duration of delivery. The exposure estimate assumed that the TPN solution was stored in EVA bags (recommended practice) and delivered in PVC tubing. The MOE is considered sufficient when the dose of DEHP is derived from TPN admixtures containing 4% lipid. However the MOE is insufficient and there is an increased risk if the lipid content is 20%. The risk would be reduced if the tubing surface was reduced (shorter length), infusion time was reduced (flow rate increased) or DEHP-free tubing was used.

Neonates and Young Children

- Acute blood transfusions: ECMO, replacement transfusion, exchange transfusion: The MOE is usually based on a NOAEL/LOAEL from chronic exposure studies in laboratory animals and would not normally be considered appropriate for extrapolation to acute human scenarios. However it was considered prudent to use the NOAEL/LOAEL from chronic exposure in this scenario even though this was likely to over-estimate potential risk as infants are considered a sensitive group. Thus the MOE is considered insufficient and infants who receive acute blood transfusions in the NICU are considered at increased risk as this is a sensitive stage in development.

The exposure estimates for exchange transfusion and ECMO were based on the maximum measurements made during the procedures. The use of shorter circuits, microbore and/or heparin-coated tubing would reduce the risk. ECMO and replacement transfusions are rarely performed in Australia today.

MOE calculations for neonates were based on a body weight of 4 kg. As such a lower MOE is expected for neonates with a body weight below 4 kg. It should also be noted that a 4 kg neonate is unlikely to need long term care when compared to smaller sized babies.

- Total Parenteral Nutrition: The exposure estimate for enteral nutrition is based on the maximum extraction rate of DEHP from PVC tubing into 4% or 20% lipid over 24 hours. The risk is greatest when 20% lipid is delivered alone rather than as an admixture and would be reduced if tubing surface area was reduced (using shorter or microbore tubing), delivery time was reduced or DEHP-free tubing was used.

- Enteral nutrition: Infants of mothers undergoing medical procedures could conceivably receive doses of DEHP from breast milk directly or indirectly (if breast milk is pumped and stored). Exposure estimates were based on theoretical milk plasma partition coefficient as well as maximum DEHP serum levels in patients undergoing haemodialysis. The MOE could be increased (ie risk reduced) by the use of alternate tubing material or coated tubing circuits in the mothers undergoing medical procedures. It is assumed that the infant is not undergoing medical procedures and the the estimated DEHP dose is derived indirectly from the mother.

  In scenarios where the infant is receiving enteral nutrition as a formula or expressed breastmilk from healthy mothers, DEHP dose would be derived from the enteral feeding set. The MOE could be increased (ie risk reduced) if formulas of 20% lipid were delivered as a bolus dose or in combination in order to reduce the total lipid concentration in the solution.

Pregnancy

- Drugs requiring vehicle for solubilisation: mixed and stored at room temperature: As with the earlier adult scenario, the MOE is considered sufficient if manufacturer's instructions are adhered to and non-PVC packs and tubing are used to mix and deliver drugs that require a pharmaceutical vehicle for solubilisation.

- Acute blood transfusions (Elective surgery, Trauma, ECMO, CABG, Orthotopic heart transplant, artificial heart transplant): The MOE was based on a NOAEL that was derived from chronic studies. It was considered prudent to use the NOAEL/LOAEL from chronic exposures even though this was likely to over-estimate potential risk as fetuses are considered a sensitive group. The MOE is therefore considered insufficient to be protective. The use of heparin-coated tubing may decrease the leaching rate of DEHP and further increase the MOE.

- Haemodialysis: The MOE is considered insufficient and there is an increased risk to the developing fetus. The DEHP doses would be reduced if TOTM, heparin-coated or DEHP-free tubing was used.

- TPN with undiluted 20% lipid: The delivered DEHP dose is largely dependent on the lipid content of the TPN solution, the surface area of the tubing and the

duration of delivery. The MOE is considered sufficient when the dose of DEHP is derived from TPN admixtures containing 4% lipid. However the MOE is insufficient and there is an increased risk if the lipid content is 20%. The risk would be reduced if the tubing surface was reduced (shorter length), infusion time was reduced (flow rate increased) or DEHP-free tubing was used.

- Enteral nutrition: The MOE based on an enteral feed containing 4% lipid, prepared in PVC bags and delivered with PVC tubing is insufficient. If the enteral feeding solutions are made up in bottles (recommended practice) then the MOE is considered sufficient as DEHP is extracted from tubing only.

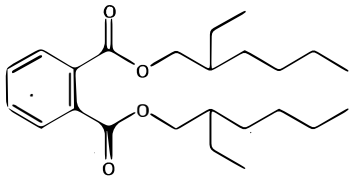
General considerations

The exposure estimates were largely based on extraction rates expressed as a function of surface area and time for specific solutions. Reducing surface area, for example by using shorter length tubing, will result in lower exposure estimates, increased MOE and reduced risk (if duration of delivery is unaltered). In particular, the use of microbore tubing has a dramatic effect on exposure estimates assuming that the same volume can be delivered over the same duration as wider bore tubing.

Scenarios involving delivery of blood and blood products can deliver a large dose of DEHP. Much of this dose is derived from DEHP present in blood bags. However, phthalates appear to contain properties that enhance the shelf-life of packed red blood cells. Exposure to DEHP in scenarios where blood is delivered can be reduced by the use of DEHP-tubing, TOTM tubing or heparin-coated tubing.

Data suggests that the extraction of DEHP into TPN solutions is greatly enhanced by increasing lipid content. The exposure estimate can be reduced if the 20% lipid solution is delivered diluted (standard formulae contain 20% lipid diluted to 4%). If it is necessary to deliver a solution containing 20% lipid then strategies such as reduced delivery time (bolus dose) and/or shorter tubing would reduce the exposure to DEHP.

1. IDENTITY

CAS Number:	117-81-7
Chemical Name:	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester
Common Name:	Diethylhexyl phthalate (DEHP)
Molecular Formula:	$C_{24}H_{38}O_4$
Structural Formula:	
Molecular Weight:	390.56
Synonyms:	DEHP; DOP; bis (2-Ethylhexyl) phthalate; Dioctyl Phthalate; 1,2-Benzenedicarboxylic acid bis (2-ethylhexyl) ester; Octoil; Ethyl hexyl phthalate; 2-Ethylhexyl phthalate; bis-(2-ethylhexyl) 1,2-benzenedicarboxylate; octyl phthalate; phthalic acid dioctyl ester; BEHP; Benzenedicarboxylic acid, bis (2-ethylhexyl) ester.

2. EXPOSURE ASSESSMENT OF DEHP IN MEDICAL DEVICES

2.1 Introduction

DEHP is the one of the most extensively used phthalates. The global production of DEHP was estimated to be between 1 and 4 million tonnes in 1994 (Klöpffer 1994 cited in Huber* et al, 1996). The worldwide consumption of plasticisers was estimated at 3.5 million tonnes/year (Cears* & Poppe, 1993). The production of DEHP in Japan was 348600 tonnes in 1993 with imports during the same period of 17400 tonnes (MITI*, 1992). The production volume of DEHP in Western Europe for 1997 was 595000 tonnes (ECPI*, 1998).

Phthalate is used as a plasticiser that is added to PVC to make it flexible. Although other materials can be used, members of the phthalate family have been commonly used for this purpose since the 1930s. Soft PVC products for building, automotive, medical and packaging applications usually contain phthalates. Typically, a flexible PVC product will contain between 20% and 50% plasticiser, of which the majority is DEHP.

According to the Australian Department of Environment and Heritage (DEH), approximately 12000 tonnes of phthalates are used annually in Australia as plasticizers in the manufacture of flexible PVC products. Australian Vinyl is Australia's only manufacturer of PVC (polyvinyl chloride or vinyl). The majority of this is used in PVC cable applications. The main suppliers of phthalates in Australia are Exxon Chemicals (mainly DEHP and DIOP) and Orica (mainly DINP) (Scheirs*, 2003).

2.2 Physico-Chemical Properties

Table 1. Summary of physico-chemical properties of DEHP

Property	Value
Physical state	liquid
Odour	Slight odour
Melting point	-47°C
Boiling point	384°C
Relative density	0.984 g/mL at 20°C
Vapour pressure at 25°C	2.19×10^{-1} Pa 1.0×10^{-7} mmHg
Water solubility	41 µg/L at 25°Ca
Partition coefficient n-octanol/water (log value) Log K_{ow}	7.50
Henry's law constant	7.9×10^{-5} kPa
Flash point	384.8 EF (196°C) (open cup)

From ATSDR (2002)

2.2.1 DEHP-Containing Medical Devices

Unplasticised PVC is hard and brittle at room temperature. As a result, plasticisers are necessary to impart flexibility to the polymer. Various compounds (e.g., adipates, citrates, phthalates) have been used as plasticisers for PVC, but di-(2-ethylhexyl) phthalate (DEHP) is most commonly used.

PVC used in medical devices contains a relatively high proportion (20-40%) of plasticiser, which imparts it with flexibility, strength and suitability at a wide range of temperatures and in a variety of sterilisation processes. PVC plastic is used to manufacture a number of medical devices, including IV and blood bags and infusion tubing, enteral and parenteral nutrition feeding bags and tubing, nasogastric tubes, peritoneal dialysis bags and tubing, and tubing used in devices for cardiopulmonary bypass (CPB), extracorporeal membrane oxygenation (ECMO), haemodialysis and infusion pumps. PVC-free or DEHP-free alternatives exist for many medical devices.

Since PVC is the most widely used plastic in medical devices, standards have been adopted for PVC formulations used in medical applications (British Pharmacopoeia, 2005). The Australian standard for blood bags states that single use, sterile, plasticised polyvinyl chloride (PVC) packs for human blood contain at least 53% by mass of PVC" and not more than 40% by mass of DEHP as outlined in Table 2.

Table 2. Typical Formulation of a PVC Blood Bag

Component	%
PVC Polymer	~ 55%
DEHP plasticiser	40%
Zinc octanoate	1%
Calcium or zinc stearate	1%
N,N' di-acyl ethylene diamine	1%
Epoxy soya or linseed oil	10%

From (Health Canada, 2002)

Since the DEHP in PVC is not chemically bound to PVC, it can leach out when a PVC-containing medical device comes in contact with fluids such as blood, plasma, and drug solutions, or it can be released when the device is heated. The rate at which DEHP migrates from a medical device depends on a number of factors: the temperature of the fluid in contact with the device; the amount of fluid in contact with the PVC; the contact time; the extent of shaking or flow rate of the fluid; and the lipophilicity of the fluid. Higher temperatures, lipophilicity of the fluid and shaking increases the migration rate.

In order to provide data on use of DEHP in Australian medical devices, the Office of Devices, Blood, and Tissues of the Therapeutic Goods Administration (TGA), Department of Health and Ageing, Canberra used the Global Medical Device Nomenclature (GMDN) number to identify the medical devices that are likely to contain DEHP and come into contact with the patients blood or mucous membranes in the following procedures in Australia: cardiopulmonary bypass, ECMO, apheresis, haemodialysis, peritoneal dialysis, blood transfusions, intravenous transfusion, total parenteral nutrition, enteral nutrition and respiratory support. These numbers were then used to identify the Australian sponsors (suppliers) of these devices. Sponsors were contacted and asked to provide information on dimensions of the device, DEHP leaching rate, DEHP content and use (Appendix C).

In total, 41 sponsor companies replied to the initial call for information. Fifteen sponsors stated that they supplied devices that did not contain DEHP or did not come in contact with the patient. Eight sponsors indicated that their products could be or were planning to be, replaced by non-DEHP alternatives. These were for enteral feeding tubes, blood lines for haemodialysis and infusion extension sets.

The information provided on DEHP-containing medical devices indicated that DEHP content ranged as follows:

Intravenous crystalloid transfusion and extension sets	20 – 45%
Intravenous drug infusion sets	30 – 45%
Blood transfusion and extension sets	20 – 32%
Blood warming coil	9.5%
Haemodialysis set	2 – 45%
ECMO/CPB	21 – 44.8 %
TPN transfusion set	28 – 45%
Peritoneal dialysis	2 – 45% (typical 30-35%)
Enteral nutrition	2 – 45% (typical 30-35%)
Breathing circuit	30%

The sponsor companies provided data on the DEHP content of each medical device, dimensions of tubing, intended use, general usage and storage recommendations. Five companies provided DEHP extraction rates. Data was supplied on extraction into blood bags, into isopropyl alcohol from PVC tubing, into ethanol solution from PVC bags and suction and irrigation tubing. No new information regarding extraction rates of DEHP into pharmacologically significant solutions were provided. The data pertaining to medical device dimensions provided by the sponsor companies were used in the calculation of exposure estimates.

2.2.2 DEHP conversion to MEHP

Patients who undergo medical procedures may also be exposed to the monoester metabolite of DEHP, mono-(2-ethylhexyl) phthalate (MEHP), which is believed to be the active metabolite responsible for many of the reported adverse reproductive and developmental effects of DEHP in rodents. Exposure to MEHP results when DEHP migrating from a PVC bag during storage or from the tubing used during infusion, is converted in the medical device to MEHP. However recent work (Ito, 2006#1414; Ito et al, 2005) suggests that MEHP was inherently present in PVC. MEHP has been measured in stored blood, blood products and peritoneal dialysate (Rock* et al, 1978; Peck* et al, 1979; Cole* et al, 1981; Labow* et al, 1986). *In vivo* conversion also occurs by enzymatic action of plasma lipases on circulating DEHP (Albro* & Thomas, 1973; Peck* et al, 1979).

Storage at 4°C significantly inhibits conversion to MEHP and storage at -30°C prevents it entirely (Rock* et al, 1978; Cole* et al, 1981). *In vitro* (such as in medical devices) conversion is enhanced by increased storage time and temperature (Cole* et al, 1981). Processes conducted at physiological temperatures will therefore increase exposure to MEHP. Heparin is reported to induce lipase activity, while DEHP itself induces extrahepatic lipase activity (Rovamo* et al, 1984a). This suggests that exposure via heparinised blood, medical devices or repeated exposure to medical procedures, may increase the rate of conversion of DEHP to MEHP.

In vivo conversion of DEHP to MEHP depends on the route of exposure. Orally administered DEHP is converted more rapidly to MEHP than intravenously

administered DEHP. After oral administration, DEHP is rapidly absorbed from the gut of rodents, mostly in the form of the monoester because of the rapid hydrolysis of DEHP by gut lipases (Albro* et al, 1982; Gaunt* & Butterworth, 1982; Pollack* et al, 1985b; Sjoberg* et al, 1985b; Eriksson* & Darnerud, 1986). In primates, including humans and marmosets, a smaller proportion of DEHP is hydrolysed and absorbed as the monoester, because of less lipase activity in primate intestine (ICI*, 1982; Shell*, 1982; Rhodes* et al, 1983; Rhodes* et al, 1984; Schmid* & Schlatter, 1985). Neonates have much lower levels of pancreatic lipase than adults (Lee* et al, 1993). However, they can convert orally administered DEHP to MEHP via lingual, gastric (Lee* et al, 1993; Armand* et al, 1996), hepatic (Terada* & Nakanuma, 1995), and plasma lipoprotein lipases (Rovamo* et al, 1984a; Rovamo* et al, 1984b) and lipase in breast milk (Hamosh*, 1996).

Most medical exposures occur via intravenous administration. Conversion of DEHP, administered intravenously, to MEHP is catalysed by plasma and hepatic lipases and is much slower than that observed when equivalent doses are administered orally (Huber* et al, 1996).

Levels of plasma and hepatic lipase enzymes in infants may be greater than levels found in adults and pre-term infants which may convert DEHP to MEHP more rapidly than at-term infants, since they have higher lipoprotein lipase levels (Rovamo* et al, 1984a; Rovamo* et al, 1984b).

There are no reliable data on the rate of *in vivo* conversion of circulating DEHP to MEHP and estimates of the total MEHP exposure from different medical procedures are unavailable.

2.3 EXPOSURE ASSESSMENT

An assessment of exposure will be made of the following scenarios in adults: intravenous administration of crystalloids or drugs, transfusion of blood and blood products, cardiopulmonary bypass, haemodialysis, total parenteral nutrition, peritoneal dialysis, enteral nutrition, inhalation and dermal. In neonates, the following scenarios will be considered: intravenous administration of crystalloids or drugs, transfusion of blood and blood products, ECMO, total parenteral nutrition, peritoneal dialysis, enteral nutrition and inhalation.

Methodology for Estimating Human Exposure

The major route of DEHP exposure from medical procedures is intravenous, through infusion of blood, blood products or lipid solutions, haemodialysis, or other bypass procedures. In these circumstances, patients are exposed to both DEHP and MEHP. DEHP migrates from the PVC bag during storage and from the tubing used during infusion and MEHP is formed by the metabolism of DEHP by blood during storage. Exposure to DEHP may also occur by inhalation (e.g., ventilators) and by ingestion (e.g., nasogastric tubes).

DEHP exposure from medical procedures is highly variable and determined by such factors as the handling of the devices during storage and use, duration of patient contact with the device and the type of fluids/gases in contact with and the extent of mass transfer through the medical device. Long storage or time of use, increased temperature, and agitation all increase the leaching of DEHP from medical devices. Leaching is also enhanced by increased lipid content or by the lipophilic nature of liquids that are in contact with DEHP - medical devices. Unanticipated exposure may result from infusing

parenteral drugs or infusates like lipid solutions, through DEHP-containing infusion sets contrary to manufacturer specifications. Variability in reported exposure measurements can result from the analytical techniques used to measure DEHP, the care with which contamination from analytic equipment is prevented, and the methods used to estimate the total dose received by the patient.

Exposures to DEHP from medical procedures can be short-term (e.g., a single blood transfusion), chronic (e.g., haemodialysis), or sub-chronic (e.g., ECMO). Chronic or recurrent treatments like haemodialysis in chronic renal failure patients or multiple long-term transfusions in cancer victims can result in cumulative exposures. Intensive procedures such as exchange transfusions in neonates can result in acutely high exposures.

Two types of studies have been conducted in order to quantify human exposure to DEHP from medical devices. The first type measures the amount of DEHP *"in vitro"* that leaches from common medical devices, such as blood bags, into the physiologic medium that each device contains, such as blood i.e. the delivered dose. The second type measures the amount of DEHP or metabolites *in vivo* found in the blood, urine, or tissues of patients following medical procedures using medical devices containing DEHP. This measurement can be influenced by both the variation in the procedural set-up, types of devices, DEHP content in devices and the metabolism of DEHP within the patient.

The most reliable studies of DEHP exposure estimate delivered dose using area under the curve (AUC) calculations based on *in vivo* measurements of DEHP or MEHP. This has been done for haemodialysis in adults and exchange transfusion in neonates. However, most exposure estimates reported in the literature are based on spot measurements of DEHP in the patient's blood or calculated from the published rates of DEHP leaching from the medical apparatus. This can result in an under-estimate of exposure if only the parent compound is measured, as DEHP is metabolised to MEHP and other metabolites. Total DEHP measured or estimated in these studies can also vary due to differences in study design and conditions, DEHP content in devices, and length of PVC tubing.

The extent to which DEHP is released from PVC medical devices is largely a function of the lipophilicity of the fluid that comes into contact with the device. Blood, plasma, red blood cell or platelet concentrates; IV lipid emulsion or total parenteral nutrition solution; and formulation aids (e.g., Polysorbate 80) used to solubilise IV medications can readily extract DEHP from PVC tubing and containers. In contrast, nonlipid-containing fluids, like crystalloid IV solutions, saline priming solution for ECMO and haemodialysis, and peritoneal dialysis solution, extract relatively small amounts of DEHP from the PVC constituents of the device.

DEHP can also be released from PVC tubing that is used in a large number of medical applications such as haemodialysis, extracorporeal membrane oxygenation, cardiopulmonary bypass procedures, IV delivery of TPN emulsions, enteral feeding, and mechanical ventilation of infants and adults. The amount of DEHP extracted depends on the differences in composition, length and surface area of the tubing used, flow rate, temperature, and the composition of the infusate. As such there is a wide variability in reported rates of DEHP extraction from medical tubing for the various medical procedures.

Exposure model

This exposure assessment will take a hierarchical approach. Estimates of DEHP exposure are based on *in vivo* measurements where possible. Where these data are not available or inadequate, then the estimates have been based on *in vitro* leaching rates from bags and tubing in a reasonable worse case estimate of exposure. Exposure calculations not based upon in vivo measurements are the least accurate, since they are subject to large errors arising from uncertainties in leaching rates of DEHP from the devices and scenario assumptions. However, estimates based on *in vitro* measurements will enable a comparison to be made between procedures.

Exposure to DEHP will be determined by scenario evaluation. For each scenario, a reasonable worse case estimate will be made based on *in vivo* measurements or *in vitro* leaching rates from storage devices (μg/ml) or tubing (μg/cm^2/day) by different solutions, dimensions and/or volume of the medical device, volume administered and duration of exposure. For adults all calculations will use the average human body weight 60 kg for females, 70 kg in males and 4 kg for neonates. The dose of DEHP received by patients undergoing various medical procedures will be estimated as a delivered dose (mg/kg/day) and time-averaged over a course of treatment to produce an average daily dose (where applicable). This methodology will allow comparisons to be made between different scenarios.

2.4 ADULT EXPOSURES

Parenteral exposure to DEHP can occur following intravenous infusion of crystalloid solutions e.g., normal saline and drugs, transfusion of blood or blood products and administration of total parenteral nutrition (TPN) solutions. In addition, patients undergoing cardiopulmonary bypass (CPB), extracorporeal membrane oxygenation (ECMO), haemodialysis or peritoneal dialysis can also be exposed to DEHP. All calculations are explained in more detail in Appendix B.

2.4.1 Intravenous infusion of crystalloid solutions

A typical IV infusion set consists of a storage bag (PVC or other) or bottle containing the solution to be delivered, PVC tubing of variable length and diameter, connectors that allow other infusion sets to be added to the same line and a catheter inserted into a vein (either peripheral or central). The flow rate can be controlled by either a drip chamber or an infusion pump. Three types of solutions can be delivered: crystalloids, drugs or colloids. Crystalloids are aqueous solutions of water-soluble molecules such as normal saline or glucose. Colloids contain larger insoluble molecules, such as blood.

There are no measurements of DEHP content in blood or serum from patients following IV infusion of crystalloid solutions. Exposure estimates in adults are based on the rate of DEHP extraction from PVC storage bags and tubing.

DEHP has very low solubility in water – less than 3 μg/L at 20°C (Staples* et al, 1997). Little or no DEHP was measured in saline solutions stored in PVC bags for more than 1 year (Dine* et al, 1991). An Australian study (Storey, 2005) found the DEHP concentration in 0.9% saline to be 11-14 μg/L. The maximum concentration reported for DEHP in crystalloid IV solutions was 172 μg/L (Corley* et al, 1977). However, when normal saline and glucose solutions are agitated, increased amounts of DEHP are extracted. Values of 285 - 360 μg/L have been reported for water after agitation and 160 μg/L for saline (Health Canada, 2002).

It is accepted practice in some medical facilities to warm IV solutions in a microwave oven. Data is unavailable on the extent to which heating a PVC bag will increase the concentration of DEHP in a crystalloid solution; however, this practice could increase the dose of DEHP delivered to a patient receiving crystalloid solutions intravenously. The use of a blood warmer is the more accepted means of warming IV fluids. During this procedure, the infused fluid is heated to about 40°C as it flows through the administration tubing into the patient. As a result, the PVC bag itself is not heated during this procedure and the heated infusate does not enter the PVC bag.

Loff et al, (2000) measured the extraction of DEHP from PVC infusion lines by an amino acid-glucose solution under conditions typical for newborn intensive care units. They found that a 140 ml solution extracted a maximum of 1.05 μg/mL DEHP from the 2.25 m of infusion tubing in 24 hours, yielding an extraction rate of 0.08 μg/cm^2/hour.

Data from non-agitated bags best represent the clinical situation in Australia. The maximum value of DEHP in crystalloid IV solutions as reported by Corley* et al, (1977) for non-agitated bags (172 μg/L) will be used to estimate exposure for this procedure. Assuming 2L of solution is delivered over 24 hours, a patient could receive 0.005 mg/kg bw/day. Using Loff's data an additional 0.008 mg/kg/day could be extracted from the tubing, giving a total DEHP dose of 0.013 mg/kg/day.

2.4.2 Intravenous infusions of drugs

A variety of drugs are administered intravenously by adding them to PVC intravenous bags. The rate at which DEHP is extracted from the bags into the drug solution depends on the lipophilicity of the drug formulation.

There are no measurements of DEHP content in blood or serum from patients following IV infusion of drugs. Exposure estimates in adults are based on the rate of DEHP extraction from PVC storage bags and tubing.

Several studies have identified a variety of drug formulations that significantly increase the extraction of DEHP from the PVC container into the solution, such as Fluconazole, Metronidazole, Teniposide and Paclitaxel (Health Canada, 2002; FDA, 2002). The highest DEHP concentrations are reached when the lipophilic drugs are pre-mixed in IV bags and agitated for 24 hr. The concentration of DEHP in low-PVC bags is assumed to not exceed 5 ppm (5 μg/ml) (FDA, 2002). These are summarised in Table 3.

Table 3. DEHP extraction into drug formulations

Drug	Handling	[DEHP] extracted	Reference
Teniposide	250 ml Bag and set RT 48 h	52 mg/bag (208 μg/ml)	(Faouzi et al, 1994)
	Bag and set RT 1h	12 μg/ml	(Faouzi et al, 1994)
Cyclosporine	Bag 0.9% NaCl after 12 hours	4.3 μg/ml (1:20 dilution) 3.37 μg/ml (1:100 dilution)	(Gotardo & Monteiro, 2005)
Etoposide	Shaking, RT 10cm tubing 1h	27.04 - 28.88 μg/ml	(Ito et al, 2005b)
Micronazole	Shaking, RT 10cm tubing 1h	53.99 - 54.64 μg/ml	(Ito et al, 2005b)
Tacrolimus	Shaking, RT 10cm tubing 1h	4.60 - 4.40 μg/ml	(Ito et al, 2005b)
Propofol	2.25m tubing, 1 hour 10 mL	1.3 mg/mL	(Loff et al, 2000)
Fentanyl	2.25m tubing, 1 hour 28.8 mL	1 mg/mL	(Loff et al, 2000)
Midazolam	2.25m tubing, 1 hour 24 mL	0.2 mg/mL	(Loff et al, 2000)
Imipenem	2.25m tubing, 1 hour 8 mL	0.8 mg/mL	(Loff et al, 2000)

The scenario is complicated further when multiple drugs are co-infused via the same IV infusion system. One such case is the co-infusion of quinine along with multivitamin preparations. Faouzi et al, (1999a) demonstrated that little DEHP is released from PVC bags containing quinine alone in solution; however, the presence of the lipophilic multivitamin cocktail dramatically increased the extent of DEHP release from the bag. Following storage of quinine/multivitamin combinations for 48 hours at 45°C, the concentration of DEHP in the bags reached 21 μg/ml.

Additional DEHP can leach from the tubing used to deliver the drug. DEHP diffused from a variety of IV tubing into etoposide infusion solution (Bagel-Boithias et al, 2005). After 6 h infusion using a 50 cm tube, the concentration of DEHP was approximately 100 μg/ml DEHP when PVC, coextruded, and triple-layered IV tubing was used while DEHP was below the limits of detection with polyethylene tubing. This experiment demonstrated that triple-layering did not prevent DEHP extraction. In addition, further variables such as flow rate, tubing length and drug concentration were also found to influence DEHP extraction rates.

There are numerous scenarios that could be generated. Several manufacturers recommend that their drugs be administered or stored in PVC bags releasing minimal DEHP. Two examples are described here using data for the drug teniposide that has the greatest potential dose as it is administered over a longer term. A patient infused with a 500 ml dose of teniposide prepared immediately before use in a low PVC bag and delivered via a PVC infusion set could receive a DEHP dose of 0.05 mg/kg bw/day. However, if the teniposide preparation was prepared and stored for 48 hours at room temperature before delivery, a patient might receive 1.51 mg/kg bw/day.

2.4.3 Transfusion of Blood and Blood products

There are a number of scenarios that involve transfusion of blood and blood products, including short-term and long-term (repeated) exposures.

DEHP leaches into different blood products at different rates perhaps due to differences in the duration of storage and storage conditions (i.e. temperature) and differences in levels of lipids in blood and plasma. The mean levels of DEHP reported in blood or blood products ranges from 0 to 650 μg/mL, depending on storage conditions and blood product. Table 4 lists selected data on DEHP levels in various blood products stored in PVC bags.

Table 4. DEHP content in blood products stored in PVC bags

Blood Component	µg/mL [DEHP] [1]	Comments/Source
Whole blood	83.1 ± 9.1	2 bag types, stored.
	72.5 ± 9.0	21 d, curvilinear extraction; (Peck* et al, 1979)
Whole blood	46.4 (34.8–52.6)	21 d, 5 °C, 83–100% extraction; (Sasakawa* & Mitomi, 1978)
Whole blood	39.8 ± 2.8	21–24 d, 4 °C, CPD
	44.2 ± 10.3	21–24 d, 4 °C, ACD; (Miripol* & Stern, 1977).
Whole blood	up to 620	28-42 d, 4 °C; (Contreras* et al, 1974)
Whole blood	15.0 – 83.2	(Inoue et al, 2005)
	52.5	(Jaeger* & Rubin, 1972)
	140 to 620	
	152.5	(Peck* et al, 1979)
	123.4	(Peck* et al, 1979)
Packed RBC	14.3 ± 1.2	21–24 d, 4 °C, CPD
	13.4 ± 2.6	21–24 d, 4°C, ACD; (Miripol* & Stern, 1977)
	38.6 (32.6–46.1)	13 d, 5°C, 83–100% extraction; (Sasakawa* & Mitomi, 1978).
	44.8 (4.3 – 123)	(Plonait et al, 1993) mixed with plasma and warmed
	17.1 (7.2 - 30.4)	(Loff et al, 2000)
	54.6 (36.8 to 84.9)	(Sjoberg et al, 1985e; Sjoberg* et al, 1985d)
	152	(Peck* et al, 1979)
RBC concentrate (irradiated)	(7.4 - 36.1)	(Inoue et al, 2005)
	(6.8 - 36.5)	(Inoue et al, 2005)
Platelet-poor plasma	288 ± 33.5	3d, 20°C; (Rock* et al, 1978)
Platelet-rich plasma	181	3 d, 20°C, (Rock* et al, 1978)
Platelet-rich plasma	32.5 (23.4 - 48.8)	(Loff et al, 2000)
Platelet concentrate	267.0	(Shintani*, 1985)
Platelet concentrate	1.88 (0.41-0.32)	1d, 22°C, agitation (Buchta et al, 2005)
Platelet concentrate	6.59 (2.09 – 10.67)	5d, 22°C, agitation (Buchta et al, 2005)
Platelet concentrate	491	(Rock* et al, 1978)
Platelets	180 - 382	Pooled platelets; (Rubin* & Schiffer, 1976)
Platelets	270	3d; (Labow* et al, 1986)
Platelets	5 to 650	5 d storage, 6 different bag types; (Labow* et al, 1986)
Platelets	1.8-15.0	(Inoue et al, 2005)
Plasma	17–24	1 d, not corrected for incomplete extraction.
	71–109	30 d, not corrected; (Jensen *& Jorgen, 1977).
Plasma	261 ± 16	2 d, 22 °C
	475 ± 55	5 d, 22°C; (Shimizu* et al, 1989)
Plasma	145 (106-202)	24 d, 4 °C; (Marcel, 1973).
Plasma	100-275	30 d, 4 °C; (Vessman* & Rietz, 1974).
Plasma	44.8 (4.3 to 123.1)	(Plonait et al, 1993)
Plasma	54.6 (36.8 to 84.9)	Sjoberg et al, (1985e)
Plasma	38.0 (13.8 to 71.9)	Sjoberg et al, (1985d)
Plasma	72.5	(Shintani*, 1985)
Plasma	172.6	(Shintani*, 1985)
Plasma	363 to 545	(Dine* et al, 1991)
Fresh frozen plasma	5.6 - 10.8	12 months, -80 °C; (Sasakawa* & Mitomi, 1978).
Fresh frozen plasma	12	(Cole* et al, 1981)
Fresh frozen plasma	26.7	(Shintani*, 1985)
Fresh frozen plasma	143.4 (11.2 – 339)	(Loff et al, 2000) before circulation

Fresh frozen plasma	11.6 – 18.5	(Inoue et al, 2005) bag
Cryoprecipitate AHF	7 - 15	223 d, -20 °C; (Sasakawa* & Mitomi, 1978).
Cryoprecipitate	1.35 (0.08-1.9) mg /bag	(Marcel, 1973)
Plasma derivatives from FP:		
NSA & PPR	1 - 10	(Cole* et al, 1981)
Factor VIII & IX and serum Ig	0 - 1	(Cole* et al, 1981)

Derived from (FDA, 2002; Health Canada, 2002)
[1] Expressed as the mean (range) and/or ± SD
ACD, Acid Citrate Dextrose; CPD, Citrate Phosphate Dextrose; AHF, anti-haemophilic factor; FP, frozen plasma; NSA, normal serum albumin; PPR, plasma protein factor.

The concentration of DEHP in whole blood increases with storage time (up to 620 μg/ml at the end of its shelf life of 42 days at 4°C). However, patients requiring a transfusion generally will receive RBC, platelets, or fresh frozen plasma, rather than whole blood, which is rarely administered.

Packed Red Blood Cell

Levels of DEHP in serum from red cell concentrates prepared for exchange transfusion ranged from 4.3 to 123.1 μg/ml, with a mean of 44.8 μg/ml (Plonait et al, 1993). In this study, packed RBC had been mixed with plasma and passed through a blood warmer unit before sampling. The highest reported mean DEHP concentration was 54.6 μg/ml (Sjoberg et al, 1985e; Sjoberg* et al, 1985d). The product was also prepared as a plasma-RBC mix and passed through an infusion set before measurement. As much as 30% of the DEHP was estimated by the authors to have been derived from parts other than the bag. Neither of these studies reported the DEHP content of the packed RBC before mixing with plasma. Inoue et al, (2005) measured the levels of DEHP in bags of different blood products with a maximum measured concentration of 36.5 μg/ml. Irradiation of RBC concentrate products had no influence on the leaching of DEHP from the PVC blood bags. The amount of DEHP that leached into RBC concentrate, irradiated RBC concentrate, whole blood and blood platelet products increased with storage time.

The FDA used the maximum measurement of 123.1 μg/ml (Plonait et al, 1993), however this is only appropriate for emergency adult or infant exchange transfusion as blood warming is not routinely performed in adults in Australia (ANZCBT, 2004). It is likely that blood warming would increase the extraction of DEHP from tubing. The section of tubing that is warmed in blood warming sets available in Australia have a lower DEHP content than the extension and transfusion sets but have a greater total length. Additionally, the measurement by Plonait (1993) was taken after the packed RBC has been mixed with plasma and passed through tubing, so does not represent the DEHP content in the bag of packed RBC alone.

The maximum concentrations of DEHP as measured by Plonait et al, (1993) (123.1 μg/ml) will be used for exposure estimates, as well as the more realistic measurement of Inoue et al, (2005) (36.5 μg/ml).

Plasma

Table 4 indicates that DEHP levels of up to 545 μg/mL have been reported in stored plasma (Dine* et al, 1991). Current clinical practice uses fresh frozen plasma (FFP) instead of unfrozen, stored plasma. Levels of DEHP in FFP appear to be lower than those measured in unfrozen plasma. Reported levels of DEHP in FFP range from 5.6 to 26.7 μg/mL with only the measurements from Loff and colleagues (2000) outside this

range (11.2 – 339 μg/mL). Loff and colleagues (2000) measured levels in 14 samples. Inoue and colleagues (2005) measured 6 samples that were all in the lower range, 11.6 to 18.5 μg/ml. The maximum value of 26.7 μg/ml measured by Shintani (1985*) represents a reasonable maximum measurement and will be used to derive administered dose estimates as the DEHP levels would be expected to be lower in FFP compared with whole blood.

Platelets are commonly stored in non-PVC bags, however Inoue et al, (2005) found detectable levels of DEHP in platelets stored in polyolefin bags. The concentration of DEHP in platelets ranged from 1.8 – 15.0 μg/ml in this study. Details were not given but reading from the provided graphs indicate that the DEHP content in platelets stored in polyolefin bags was on the lower end of the range. The maximum concentrations of 15.0 μg/ml DEHP as measured by Inoue et al, (2005) will be used to derive administered dose estimates.

The FDA uses an estimated DEHP concentration of 1 μg/ml in cryoprepeciptates but it is unclear where this value arises. The maximum DEHP concentration of 15 μg/ml, as measured by Sasakawa* & Mitomi (1978), will be used for exposure estimates.

Tubing

DEHP could also be extracted from the tubing used to deliver blood and blood products to the patient. Easterling et al, (1974) found that 8.9 to 13.2 mg of DEHP was extracted from haemodialysis tubing following circulation of 500-700 ml of human plasma for 5 hours at 37°C through a haemodialysis. The level of DEHP perfusing through the plasma tubing progressively increased over the 5 hours, reaching 3.9-6.2 μg/ml in the first hour and 21.0 – 35.9 μg/ml after 5 hours. The final DEHP level would be exaggerated as the same volume of plasma was re-circulated. Karle and colleagues (1997) showed using two different infant ECMO circuits, that DEHP extraction into packed RBC was the same when corrected for surface area. In these circuits, extraction rate varied from 16-20 μg/cm^2 over the first 18 hours and ranged up to 25 μg/cm^2 by 24 hours or 1.04 μg/cm^2/hour. More recently, DEHP extraction from fresh human blood from tubing with different coatings have been measured (Hildenbrand et al, 2005). Neither ionic, non-ionic heparin nor hydrogel coating prevented the release of DEHP into fresh blood. DEHP extraction ranged from 17.4 mg/L for ionic heparin-coated tubing, 39.0 mg/L for hydrogel coating and 42.6 mg/L for uncoated tubing. The extraction rate for the uncoated tubing was 5.68 μg/cm^2 over 90 minutes or 3.79 μg/cm^2/hour. Loff et al, (2000) measured the extraction of DEHP from PVC infusion lines by packed RBC, FFP and platelet-rich plasma under conditions typical for newborn intensive care units. They found that a maximum of 5.4 μg/ml was extracted into 20 ml of packed RBC from the 2.25 m of infusion tubing over 1 hour, yielding an extraction rate of 1.39 μg/cm^2/hour. The measurements by Loff and colleagues (2000) will be used where possible to derive exposure estimates unless otherwise stated.

2.4.3.1 Short-term procedures

Acute trauma and routine surgery can require short-term blood transfusions. The highest published estimates of DEHP exposure from short-term blood transfusions assume multi-transfusions of blood or blood components that have been stored for a prolonged period of time. For example Jaeger and Rubin (1972*) estimated that a gunshot victim transfused with 63 units of whole blood could receive a DEHP dose of 8.5 mg/kg body weight.

For this scenario, it is assumed that patients undergoing routine, elective surgical procedures could receive two units of blood or blood products over 1 hour. The DEHP dose can be estimated using the maximum measurements of 36.5 μg/ml (Inoue et al, 2005) and 123.1 μg/ml (Plonait et al, 1993) for packed cells and the upper bound estimate from Loff et al, (2000) for extraction into tubing. The total dose of DEHP from bag and tubing would be 0.37 – 1.23 mg/kg/day.

Acute trauma patients can receive up to 7 units of blood. The total DEHP dose from bag and tubing estimated using the maximum measurements described above is 1.31 - 4.31 mg/kg/day.

Cardiopulmonary bypass

Cardiopulmonary bypass is used during cardiac procedures such as coronary artery bypass graft. The procedure is termed extracorporeal membrane oxygenation (ECMO) when its purpose is to supplement blood oxygenation. In ECMO, the patient's blood passes through an oxygenation device, is warmed and then returned. Additional blood transfusion may not take place. Exposure to DEHP in patients undergoing ECMO derives largely from the considerable lengths of PVC tubing typically used in this procedure (up to 600 cm of PVC tubing can be used in ECMO circuits). Patients undergoing cardiac surgical procedures (e.g., heart valve replacement, CABG surgery, heart transplantation, correction of congenital defects) would be exposed to DEHP through both ECMO and transfusions.

DEHP levels in blood taken from patients after some of these medical procedures have been measured. Estimated daily doses of DEHP for a number of cardiac bypass procedures in adults have been reported (Barry et al, 1989). The dose of DEHP received by these patients from all sources (i.e., tubing, transfusions) was estimated (Table 5).

Table 5. DEHP exposure during heart surgery

Medical Procedure	[DEHP] μg/mL	Procedure Time	Dose/Time	Dose (mg/kg bw/day	Comments/Source
Coronary artery bypass graft (n=10)	1.8–2.3	83.9 ± 15.9 min	15.4 – 72.9 mg/procedure	1.0	Calculated from post-measurements. (Barry et al, 1989)
Orthotopic Heart Transplant (n=3)	Not Stated	184 ± 54 min	2.3 – 21 mg/transfusion	0.3	Calculated from post-measurements (Barry et al, 1989)
Artificial Heart Transplant: Jarvik Bridging (n=2)	Not Stated	Several days	3.8 – 167.9 mg/day	2.4	(Barry et al, 1989)

These doses will be used in the risk estimate. Thus the DEHP dose received by a patient undergoing CABP is estimated as 1.0 mg/kg bw/day, for an orthotoptic heart transplant, 0.3 mg/kg bw/day and 2.4 mg/kg bw/day for an artificial heart transplant.

ECMO

There are no measurements of DEHP content in blood or serum from adult patients following ECMO. Patients on ECMO may receive RBCs, platelet concentrates, FFP, and cryoprecipitate. They tend to be multiply transfused and may also receive drugs

(e.g., antibiotics, vitamins). Exposure estimates in adults are based on the rate of DEHP extraction from PVC storage bags as well as the PVC tubing used in the ECMO device.

Butch* et al, (1996) reported that an adult undergoing ECMO therapy may, in addition, receive up to 46 units of blood products per day (mean = 21 units) as a combination of RBCs, platelet concentrates, FFP, and cryoprecipitate over 10 to 20 days. ECMO is very rarely used on adults and modern surgical techniques and cell saver devices mean that blood loss is usually much less than reported by Butch et al, (1996). This scenario therefore represents a rare and extreme scenario. If the mean number of units received (21 units) is used and the levels of DEHP reported earlier for RBCs, platelet concentrates, FFP, and cryoprecipitate, the patient could be exposed to 2.1 – 4.1 mg/kg bw or 0.21 – 0.41 mg DEHP/kg bw/day time-averaged over 10 days of ECMO therapy.

2.4.3.2 Long-term blood transfusions

There are no published data on DEHP levels in patients undergoing long-term transfusion of blood and blood products. All published exposure estimates are based on the levels of DEHP in stored blood and blood products together with extraction rates from tubing.

Chemotherapy, Sickle cell disease, Clotting disorders

Chronic administration of small volumes of blood or blood products is common in the treatment of patients with chemotherapy-associated anaemia, blood disorders such as leukaemia and aplastic anaemia. Patients with sickle cell disease are typically transfused with 1-2 units of packed RBC every 2-4 weeks. Using this scenario, the dose of DEHP received by a patient with sickle cell disease time-weighted over a fortnight would be approximately 0.03 - 0.09 mg/kg/day.

An average of 5.1 RBC units were infused per patient undergoing chemotherapy (Estrin* et al, 1999). Assuming one transfusion every 3 week treatment period, the maximum estimates of DEHP exposure in this scenario is about 0.009 - 0.03 mg/kg/day.

Cryoprecipitates

Cryoprecipitates containing clotting factors are administered to patients with clotting disorders. Marcel (1973) found that cryoprecipitate packs contained from 0.8 to 1.9 mg of DEHP each. Since patients with clotting disorders can receive up to 400 bags of cryoprecipitate in one year, the total DEHP dose received by these patients is in the order of 0.03 mg/kg/day.

Haemodialysis

The most reliable estimates of DEHP exposure come from studies that involve the calculation of the differences from AUC of pre- and post-infusion measurements of DEHP over the 3-5 hour haemodialysis session. In older studies, doses reported for individual patients ranged from 3.6 to 360 mg/session (Pollack* et al, 1985a). The wide range of estimates may be due to patient variation (e.g., hematocrit, triglycerides and serum cholesterol), differences in dialysis protocols, and differences in the circuits themselves. All of these studies are based upon small patient numbers, increasing the uncertainty of the exposure estimates. These data are summarised in Table 6.

Table 6. DEHP measurement in plasma following haemodialysis

Sample size	[DEHP] µg/mL	Procedure Time	Dose/procedure	Comments/Source
10		4 hours	122.95 (55 - 166.21) mg delivered 27.3 (12.50 - 42.7) mg retained	(Kambia et al, 2001a)
10		4 hours	41.8 (37.55 – 49.20) mg delivered 3.42 (2.00-6.67) retained	TOTM-DEHP tubing (Kambia et al, 2001a)
21	not given	4 hours	75 (44 - 197) mg delivered 16.6 (3.6–59.6) mg retained	AUC calculated (Faouzi et al, 1999b)
11	1.91 ± 2.1	4 hours	105 (23.8 – 360) mg/procedure	AUC calculated. (Pollack* et al, 1985a)
3	Not Stated	4 hours	60 mg/procedure	Post dialysis concentration. (Kevy* et al, 1981)
27	0.75 ± 0.4 (0.25- 1.95)	5 hours	30 mg/day	extrapolation from 1 hr result 60–70% extraction, post-dialysis concentration. (Lewis et al, 1978)
9	not given	5 hours	80 (9 – 150) mg/procedure	AUC calculated from hourly measures. (Gibson* et al, 1976)

Adapted from (Health Canada, 2002)

Faouzi et al, (1999b) reported an average of 75.2 mg DEHP was extracted during a single dialysis session, with a range of 44.3 to 197.1 mg. However, these investigators estimated that an average of only 16.6 mg DEHP (range: 3.6 - 59.6 mg) was retained by the patient.

Haishima et al, (2004) measured the amount of DEHP released using bovine blood and pump-oxygenation therapy using medical grade PVC tubing *in vitro*. The amount of DEHP released into the blood varied with the type of tubing. After 6 h in the PVC circuit, 7.5 mg (tubing covalently coated with heparin), 15.5 – 24.8 mg (uncoated) and 20.7 mg (ionic heparin coated) of DEHP had migrated into bovine blood. It should be noted that there was a wide variation in DEHP extracted from non-coated tubing from two different companies.

While the results of Kambia et al, (2001a) are more recent, the maximum measurement of Faouzi (1999b) will be used with the assumption that patients have 3 sessions each week. This would result in a delivered dose of 1.21 mg/kg bw/day and retained dose of 0.36 mg/kg bw/day.

Apheresis

Apheresis involves procedures on healthy volunteers donating blood components. The Australian Red Cross allows volunteers to donate every 2 to 3 weeks. Each donation is 650 mls and plasmapheresis lasts 40 minutes and plateletpheresis 1 h. The DEHP exposure of six plasma donors, six discontinuous-flow platelet donors and six continuous flow platelet donors was estimated by measuring DEHP and its metabolites in urine samples (Koch et al, 2005; Weisbach et al, 2006). Mean absolute DEHP estimates were 1.2 mg for discontinuous and 2.1 mg for continuous-flow plateletpheresis (maximum 2.48 mg) and 0.37 mg for plasmapheresis (similar to control value 0.41 mg) or 0.017, 0.03 and 0.005 mg/kg/day, respectively. The blood volume processed for discontinuous flow plateletpheresis was 2935 ml, 3736 ml for continuous-flow plateletpheresis and 1546 ml for plasmapheresis. These values are much higher than those estimated by Buchta et al, (2003) who found in discontinuous

plateletpheresis that the median DEHP exposure was 6.4 (range, 1.9-19.5) μg/kg bw compared to 7.2 (range, 2.0-20.3) μg/kg bw for continuous apheresis machines. The measurement by Buchta and colleagues (2003) may be an underestimate as only DEHP (not its metabolites) was measured in serum. The maximum measurement by Koch and colleagues (2005) (0.04 mg/kg bw/day for continuous-flow plateletpheresis) will be used for the estimate of exposure.

2.4.4 Total Parenteral Nutrition

Total parenteral nutrition (TPN) formulations are often administered to critically ill patients requiring nutritional supplementation. Parenteral administration involves infusion directly into the circulatory system. Typical TPN admixtures contain amino acids, dextrose, electrolytes and lipids. PVC tubing is generally required for the pump-assisted administration of TPN emulsions that contain lipids. In adults, 500 ml of a 20% lipid solution would typically be administered with an additional 2 L of electrolyte and amino acid solution over the course of one day. Alternatively the 20% lipid solution may be delivered overnight (10 hours).

There are no published data on DEHP doses in adults receiving total parenteral nutrition. Exposure estimates in adults are based on the limited data on the rate of DEHP extraction from PVC storage bags and infusion lines. Table 7 reviews the DEHP content found in TPN solutions reported in the literature. The DEHP dose is critically influenced by the lipid concentration of the TPN solution. However, storage at 4°C for one week did not increase DEHP leaching (Mazur et al, 1989; Kambia et al, 2003). Storage at 25°C for more than 2 days resulted in increased DEHP leaching as storage time increased (Mazur et al, 1989).

Mazur et al, (1989) measured the concentration of DEHP in different TPN solutions (500 ml of 0, 10, 20% lipid in 2L of amino acid/dextrose solution) over varying periods of storage after preparation. This formula is typical of an adult prescription. The maximal concentration of DEHP was 3.1 μg/ml in a 10% lipid solution stored for 48 hours. In contrast, Allwood et al, (1986) had previously reported that the concentration of DEHP in a TPN solution containing 20% Intralipid reached 40 μg/mL after 24-hr storage in a PVC set. It is possible that additional DEHP was leached, as the solution was stored in an administration set rather than bag alone.

Table 7. DEHP content on TPN solutions

Bag	% lipid	Storage conditions	[DEHP]	Reference
PVC	0%	24h stored at RT	0.1 μg/ml	(Mazur et al, 1989)
PVC	0%	48 h stored at RT	0.7 μg/ml	(Mazur et al, 1989)
PVC	1.85% of 10% lipid	24h stored at RT	0.0 μg/ml	(Mazur et al, 1989)
PVC	1.85% of 10% lipid	48 h stored at RT	3.1 μg/ml	(Mazur et al, 1989)
PVC	4 % of 20% lipid	24h stored at RT	40 μg/ml	(Allwood, 1986)
PVC	3.71% of 20% lipid	24h stored at RT	0.0 μg/ml	(Mazur et al, 1989)
PVC	3.71% of 20% lipid	48 h stored at RT	2.6 μg/ml	(Mazur et al, 1989)
EVA	3.85%	24h stored at 4°C	~0.40 μg/ml	(Kambia et al, 2001b)
	17% of 20% lipid		1.61 μg/ml	(Loff et al, 2000)
	10% lipid	24h stored at RT	160 μg/ml	(Allwood, 1986)
	20% lipid	24h stored at RT	144 μg/ml	(Allwood, 1986)

Adapted from (FDA, 2002) RT: room temperature

The maximum measurement by Mazur et al, (1989) will be used for the exposure estimate as this best describes a reasonable worst case scenario. Thus the dose of DEHP

from a 2500 ml mixture containing 20% lipid, stored for 24-48 hours in a PVC bag would be 0.11 mg/kg bw/day.

DEHP-free containers (non-DEHP plasticised PVC or polyolefin) are available to mix and deliver TPN containing lipids to adults, however these may still contain PVC-plasticised DEHP sites. The concentration of DEHP released into TPN solution containing 1.85 – 3.85% lipid, stored for 24 hours in polyethylene vinyl (EVA) bags ranged from 0.2 to 0.7 mg after 11 hours (estimated at 200 – 400 ng/ml from graphs) (Kambia et al, 2001b; Kambia et al, 2003). This would result in a DEHP dose of 0.01 mg/kg bw/day from EVA bags.

There are no data on the extraction of DEHP from PVC infusion lines during TPN procedures in adults. Allwood (1986) measured the DEHP concentration in 10% and 20% lipid as well as a TPN admixture containing 20% lipid after a 5 hour simulated infusion. After 6 hours, the DEHP concentration was greater in 10% than 20% lipid and < 1.0 μg/ml in the TPN admixture. The maximum DEHP concentration was 5.5 μg/ml. Loff et al, (2000) measured the extraction of DEHP from PVC infusion lines by lipids under conditions typical for newborn intensive care units. They found that a TPN formulation containing 20% lipid extracted a maximum of 490 μg/mL DEHP from the 2.25 m of infusion tubing in 24 hours or 6.3 $\mu g/cm^2$/hour. In this case 0.17 mg/kg bw/day would be leached from tubing over 10 hours.

This value was very different from that derived by Kambia and colleagues (2001; 2003) who measured the amount of DEHP in a TPN solution (1 – 3.85% lipid) at the PVC outlet from an infusion pump used with children and infants (Kambia et al, 2001b; Kambia et al, 2003). Estimating from a graph, the amount of DEHP released from the tubing ranged from 300 – 1200 ng/ml (varying with flow rate and lipid content). The maximum measurement extraction rate was 106 μg/hour (Kambia et al, 2001b; Kambia et al, 2003). In this scenario an additional 0.04 mg/kg bw/day would be leached from PVC tubing.

The total dose of DEHP is determined by the type of bag used and solution delivered. Doses up to 0.21 mg/kg bw/day are attained when PVC tubing is used to deliver a 20% lipid solution while doses are 0.05 mg/kg bw/day when the TPN solution contains 4% lipid.

2.4.5 Peritoneal Dialysis

DEHP content in blood or serum from patients following peritoneal dialysis has been measured and will be used for the exposure estimate. There are two types of peritoneal dialysis: continuous ambulatory peritoneal dialysis (CAPD) and cyclic peritoneal dialysis (CPD). In the first scenario, the CAPD fluid is infused via peritoneal tubing into the abdomen and the tubing then disconnected. The fluid is drained later in the evening. In CPD, the fluid is pumped in and out of the abdomen through peritoneal dialysis tubing overnight or 12 hours.

During peritoneal dialysis only aqueous solutions come in contact with PVC and, therefore, relatively small amounts of DEHP are extracted. Nassberger* et al, (1987) found levels of DEHP in peritoneal dialysis solution ranging from 0.004 to 0.011 μg/mL, while Mettang* et al, (1996) reported levels between 0.021 to 0.130 μg/mL in the dialysis solution (presumably CPD). Since the majority of an intraperitoneally injected dose of DEHP is not absorbed, the administered dose is likely to overestimate the absorbed dose for CPD (Rhodes* et al, 1983). Furthermore, a considerable amount of the infused DEHP will be returned upon drainage of the perfusate from the

peritoneum. It is interesting to note that both Nassburger* et al, (1987) and Mettang et al, (1996) observed thatthe concentration of MEHP in dialysis fluid was considerably greater than levels of DEHP.

Assuming a patient undergoing CAPD is dialyzed with 8 L of fluid/day, the maximum estimate of the daily dose of DEHP infused into the peritoneum would be in the order of 0.01 mg/kg bw/day. However, this is likely to be an overestimate for CAPD as only some of this dose will be absorbed.

2.4.6 Enteral nutrition

There are no published data on DEHP doses in adults receiving enteral nutrition. Exposure estimates in adults are based on the limited data on DEHP leaching rates from PVC bags and infusion lines.

In the context of medical procedures, oral exposure to DEHP can occur following release from enteral feeding bags and tubing or from nasogastric tubing used for aspiration of stomach contents and decompression of the stomach. Enteral feeding solutions can vary considerably depending on the patient's health, for example, patients undergoing chemotherapy have different requirements to those with renal failure. The most common methods of enteral formula administration are continuous feeding, intermittent feeding, and bolus feeding. Standard enteral feeds are isotonic, lactose-free and are designed to meet the recommended daily intakes for most nutrients in 1.5-2.0 litres (AHD, 1997). The most common general formula contains about 40 g/L of fat or 4% lipid.

There are no specific data on leaching rates from enteral nutrition storage bags. It is assumed that it is similar to that of TPN. Using a leaching rate of 3.1 μg/ml (Mazur et al, 1989) and assuming 2 L per day is delivered, then a dose of 0.09 mg/kg bw/day would be delivered.

Additional exposure would result from the PVC tubing. A variety of approaches have been used to estimate DEHP leaching from nasogastric tubing. Health Canada (2002) used a leaching rate derived from 20% lipid leached into tubing used with TPN infusion pumps based on Loff and colleagues (2000) measurements. Whereas the FDA (2002) used a leaching rate into non-biological solutions using small sections of nasogastric tubing based on data from Khaliq et al, (1992). Both have drawbacks as 20% lipid is not regularly infused into adults and the type of solution is known to influence the degree of DEHP extraction. The DEHP content of nasogastric tubes is no different than other tubing thus the leaching rates would not be expected to differ significantly from those used for TPN estimation. Using the leaching rate of 106 μg/hour (Kambia et al, 2001b; Kambia et al, 2003), the DEHP dose derived from PVC tubing would be 0.04 mg/kg bw/day.

While the nasogastric tube may stay in place for several days, it is common practice to replace the enteral giving set daily. Several studies demonstrated that less DEHP leaches out during continuous exposure (Mazur et al, 1989; Khaliq et al, 1992; Karle et al, 1997). Over 15 days extraction, the rate of extraction was approximately 25% that of the first day. Therefore, extrapolating extraction from shorter exposures over longer duration will result in an over-estimate of DEHP exposure.

2.4.7 Inhalation Exposure

PVC tubing is used in respirators and anaesthetic delivery systems and could be a theoretical source of DEHP exposure.

Some components of breathing circuits are commonly made from PVC plasticised with DEHP. There are no reliable published data on the rate of extraction of DEHP from these devices under clinical conditions of use. The rate will depend on the leaching of DEHP from the internal surface of the tubing, respiratory rate and heating of the tubing. Heated versus unheated tubes will influence the transfer of DEHP from the tube surface, because of the difference in vapour pressure. Estimates of vapour pressure using a temperature of 37°C are a better approximation of the elevated temperatures in the circuit than the use of room temperature.

Heated Respiratory Tubing (Adults)

The worst-case scenario involves breathing air saturated with DEHP continuously for 24 hours/day. For a respiratory breathing rate of 10 L/min circuits and assuming a vapour pressure of 4.8×10^{-4} Pa at 37°C then the maximum amount of DEHP extracted from heated respiratory tubing during mechanical ventilation of an adult would be 1.06 mg/day or 0.015 mg/kg bw/day.

Based on the concentration of DEHP measured in the air stream passed through PVC respiratory tubing, Hill (1997) estimated that a patient undergoing respiratory therapy would receive a daily DEHP dose ranging from 28.4 to 94.6 μg, which is equivalent to a dose of 0.0004 to 0.001 mg/kg/day for a 70 kg adult. More recently, Hill et al, (2003) measured the concentration of DEHP in air after passage through PVC medical tubing. DEHP was detectable in an unspecified proportion of samples but was not above the limit of quantification (< 16 ng/L) after 17 hours exposure at 5 ml/min.

The amount of DEHP extracted from anaesthesia breathing circuits would be similar, since these circuits are functionally equivalent to respiratory breathing circuits and similar minute flow volumes.

Oxygen Supply Tubing (Adults)

This tubing is used to deliver oxygen from a flow meter that is attached to an oxygen regulator connected to a gas cylinder, or with the flow meter directly attached to a hospital's central gas pipeline system through an outlet on the wall.

The oxygen flow rates through this tubing range from 2 L/min to 15 L/min, with typical values are < 6 L/min.

The worst-case scenario (exposure to DEHP saturated air at 37 °C and a flow rate of 15 L/min for the duration of the procedure) would be 1.6 mg DEHP/day or 0.02 mg/kg bw/day.

Tracheal or Tracheotomy Tubes (Adults)

These devices provide the interface between the patient airway and the breathing circuit or oxygen supply tubing. The flow rates through these would be the same as that for breathing circuits (i.e. at the minute volume flow rate required by the

patient), and the only exception is that flow is bi-directional. However, for the patient, it is only the inspiratory flow that is relevant, leading to the same worst-case estimate of DEHP exposure as for breathing circuits, i.e., 1.1 mg/day or 0.015 mg/kg bw/day.

2.4.8 Dermal Exposure

The potential exists for DEHP to be released from skin surface- or mucosal membrane-contacting PVC devices such as urinary catheters, drug delivery patches, occlusive dressings, oxygen masks, and endotracheal tubes. However, there are insufficient data to accurately characterize the amount of DEHP that would be released from these devices and taken up by the body. Although nasogastric tubes contact the oesophageal mucosa, it is assumed that the majority of the DEHP released from these devices is extracted from the lumenal side of the tubing and is subsequently absorbed in the gastrointestinal tract.

KEMI (2000) has estimated that a health care worker wearing gloves for 2 hours/day could receive a DEHP dose of 0.007 mg/kg/day.

2.5 PREGNANCY

Pregnant women can be exposed to DEHP from medical procedures. For example, women who experience hyperemesis gravidarum are typically rehydrated with IV fluids (Power* et al, 2001). However, further nutritional support can be provided either with TPN (Subramaniam* et al, 1998; Folk* & Leslie, 2001) or enteral feeding (Hsu* et al, 1996). DEHP exposure for pregnant women will be assumed to be the same as adult exposures already described in Section 2.3.2.

DEHP and MEHP can cross the placenta (Srivastava* et al, 1989). Latini et al, (2003a; 2003b) measured DEHP and MEHP in 24 mother-infant pairs. DEHP was detectable in 71% of maternal and 44% of cord plasma samples. The mean DEHP concentrations were 1.15 and 2.05 μg/ml respectively. There was no significant correlation between maternal and cord DEHP.

In utero exposures could be expected if the mother was exposed to DEHP from medical procedures such as haemodialysis. However, female patients with chronic renal failure on haemodialysis experience a much greater incidence of infertility than their counterparts without renal disease. Only about 1-2% of women on haemodialysis become pregnant, and only about 40-50% of those women give birth to live infants (Bagon* et al, 1998; Okundaye* et al, 1998; Toma* et al, 1999). Consequently, the number of infants that experience both prenatal exposure to DEHP (assuming maternal exposure to DEHP from haemodialysis) and postnatal exposure via procedures such as ECMO is likely to be very small.

2.5.1 Breast Milk

DEHP in human breast milk has been identified in three studies (Gruber* et al, 1998; Bruns-Weller* & Pfordt, 2000; Zhu et al, 2006). Estimates range from 71 to 398 μg/kg. Presumably none of the subjects in these studies had recently undergone medical procedures that would have exposed them to higher doses of DEHP.

Experimental data are unavailable on levels of DEHP in milk from mothers who have undergone or are undergoing medical procedures such as haemodialysis. Estimates of

exposure of infants to DEHP from milk of nursing mothers undergoing medical procedures have been made based on theoretical estimate of a human milk to human plasma partition coefficient. The coefficient is estimated by comparing reported plasma concentrations of DEHP in normal subjects (around 0.1 μg/mL) and concentrations reported in breast milk (mean 0.034 μg/kg milk) (Pfordt & Bruns-Weller, 1999). The coefficient ranges up to 0.43 in the general population.

A value of 150 ml/kg/day is assumed as typical milk consumption. Levels of DEHP in blood from patients on dialysis range from about 0.4 to 8 μg/ml, however, the latest data from Faouzi et al, (1999b) suggests that levels of about 3 μg/ml are reached after 4 hours of dialysis. The DEHP exposure to a breastfed infant of a mother on haemodialysis would be 0.2 mg/kg/day.

Although the incidence of pregnancy in women on haemodialysis is low (about 1-7%), of patients that do give birth, some would breastfeed their children. Transitory high doses might be expected in milk from patients undergoing acute medical procedures such as large blood transfusions.

Additional DEHP exposure could arise from bags used to store breast milk following the use of a breast pump. However, these bags are typically made from polyethylene or nylon coated with polyethylene. In addition, the expressed milk is not expected to come into contact with flexible PVC components of the breast pump. Consequently, it is not expected that infants will be exposed to any DEHP released from a breast pump or milk storage bags.

2.6 NEONATAL EXPOSURES

2.6.1 Intravenous infusion of crystalloid solutions

There are no measurements of DEHP content in blood or serum from neonates following IV infusion of crystalloid solutions. Neonatal patients typically receive IV fluid administration using a syringe infusion pump. Although the syringe is typically made from polypropylene, DEHP can be released from the PVC administration tubing. Exposure estimates will therefore be based on extraction rates from tubing.

Loff et al, (2000) measured the extraction of DEHP from PVC infusion lines by an amino acid-glucose solution under conditions typical for newborn intensive care units (NICUs). They found that the formulation extracted a maximum 1.8 μg/mL DEHP from the 2.25 m of infusion tubing in 24 hours or 0.08 $\mu g/cm^2$/hour. The Royal Children's Hospital, Melbourne recommends maintenance fluid requirements for neonates over 3 days old of 4 ml/kg/hour.

Using the extraction rate as measured by Loff et al, (2000), assuming 140 ml is infused, the dose received would be 0.04 mg/kg bw/day. A higher dose would be received if the infusate is warmed as typically occurs. If microbore tubing is used to deliver the solution, the delivered DEHP dose would be 0.008 mg/kg bw/day.

2.6.2 Intravenous infusions of drugs

There are no measurements of DEHP content in blood or serum from neonates following IV infusion of drugs. Drug infusions neonates are typically administered using an infusion pump. The exposure will therefore be limited to leaching as drug

passes through tubing. Exposure estimates will therefore be based on extraction rates from tubing.

Loff et al, (2000) measured the extraction of DEHP from PVC infusion lines to which syringes were connected to an infusion pump typical for newborn intensive care units. Four drugs were tested, three of which are contraindicated in young children in Australia. The DEHP concentration in a 24 mL solution of midazolam 24 hours after perfusion was 1.13 μg/ml or 0.02 μg/cm^2/hour. A scenario of a neonate receiving a sedating dose of 24 ml over 24 hours of midazolam could result in a DEHP dose of 0.007 mg/kg bw. Microbore tubing is typically used to deliver drugs in neonatal wards. If wider bore tubing is used then the DEHP dose would be greater.

2.6.3 Transfusion of Blood and Blood products

Two main scenarios arise in NICUs: replacement and exchange transfusion. Replacement transfusions are most commonly used to correct acute blood loss and hypovolaemia in preterm and term neonates, as well as the correction of anaemia associated with prematurity. Critically ill neonates also may require repeated blood sampling that may deplete their blood volume. An exchange transfusion is used to lower the serum bilirubin level; remove the infant's sensitised red blood cells and the circulating antibodies. ECMO involves the circulation of the pateint's blood for external oxygenation. Exchange transfusion and ECMO are rarely performed in Australia today.

Replacement transfusion

There are no published direct measurements of DEHP exposures from replacement blood transfusions. Exposures for replacement blood transfusions must be estimated using published data on DEHP levels in blood and blood products. If blood used for replacement transfusions is drawn up from the storage bag with a syringe and injected into the patient, there is no need to account for DEHP released from infusion sets.

It has been reported that neonates in one NICU received, on average, 33.6 ml of RBCs and 2.4 ml of FFP in the first 14 days (Ringer* et al, 1998). Infants in this study weighed about 1 kg. The Royal Prince Alfred Hospital, Camperdown recommends for stable preterm infants, infusion of 20 mls/kg (or 80 mls for a 4 kg neonate) packed red cells over 4 hours.

The time-averaged intake of DEHP leached from PVC storage bags for a premature neonate weighing 1.073 kg would be 0.09 - 0.28 mg/kg/day. The time-averaged intake of DEHP leached from PVC storage bags for a stable 4 kg neonate would be 0.05 – 0.17 mg/kg/day.

However, if the blood product was administered via an infusion pump, then the amount of DEHP received by the patient would be greater. Loff and colleagues (2000) measured the leaching rate of DEHP into packed RBC as 1.39 μg/cm^2/hour. Over 4 hours, as additional 0.11 mg/kg bw of DEHP would be leached from the tubing or 0.008 mg/kg bw/day time-averaged over 14 days.

Exchange transfusion

DEHP content in blood or serum from neonates following exchange transfusion will be used to develop exposure estimates. Data for this from the literature is shown in Table 8. In exchange transfusion, twice the infant's blood volume (2x85 mls/kg) is slowly removed and simultaneously similar aliquots are injected. Three studies have measured

the DEHP concentration in serum of infants before and after exchange transfusion (Sjoberg et al, 1985e; Sjoberg* et al, 1985d; Plonait et al, 1993). Infants receiving exchange transfusion could receive a DEHP dose up to 22.6 mg/kg according to Plonait (1993). However, the DEHP dose received by infants in the studies ranged from 0.84 to 4.22 mg/kg (Sjoberg et al, 1985e). Blood was sampled from the infusion set, accounting for DEHP leaching from the tubing and bag. Serum levels of DEHP declined rapidly after treatment.

Table 8. DEHP concentration following exchange transfusion

Medical Procedure	[DEHP] Mean (Range) µg/mL	Exposure Time	Estimated Mean Dose (range) mg/kg bw	Reference
Triple Exchange Transfusion (n=16)	12.5 (6.1 to 21.6)	1.3-3 hr	1.2 to 22.6	(Plonait et al, 1993)
Exchange Transfusion (n=4)	7.8 (3.4 - 11.1)	Not Stated	2.95 mg/kg bw (1.7 – 4.2)	(Sjoberg* et al, 1985d)
Double Volume Exchange Transfusion (n=6)	5.8 - 19.6	Not Stated	1.77 mg/kg bw (0.84-3.3)	(Sjoberg et al, 1985e)

The maximum measurement of Plonait and colleagues (1993) of 22.6 mg/kg bw/day will be used in the exposure assessment.

Extracorporeal Membrane Oxygenation

The use of ECMO has declined in Australia since the introduction of nitric oxide and High Frequency Oscillatory Ventilation and is now rarely performed. DEHP content in blood or serum from neonates undergoing ECMO will be used to develop exposure estimates. ECMO is used to treat respiratory failure in both premature infants and term infants. DEHP in patients following ECMO has been studied by several investigators as summarised in Table 9. Two groups of investigators, using different circuits, measured the DEHP concentration in serum of infants undergoing ECMO as well as after priming the circuit with blood (Shneider* et al, 1989). An estimate of the delivered dose of DEHP was then made. The reported serum levels after 14 days of treatment was 26.9 µg/mL and 33.5 µg/mL after 24 days of ECMO therapy (Shneider* et al, 1989). The *in vitro* extraction rate was 3.5 – 4.1 µg/ml/hour. They estimated that a neonate could be exposed to 42 mg/kg bw DEHP after 3 days and 140 mg/kg bw after 10 days of ECMO therapy.

More recently, much lower levels of exposure were reported from three different ECMO circuits ranging from 0 to 34.9 mg DEHP/kg bw/ treatment, depending on the circuit used (Karle et al, 1997). The circuit in which heparin-coated PVC tubing was used did not leach DEHP. The *in vitro* extraction rate for the two coated surfaces was 0.32 – 0.57 µg/ml/hour. The differences between the results reported by Karle et al, (1997) and those of Schneider* et al, (1989) may be attributed to the smaller surface areas of the newer ECMO circuits used by Karle et al, (1997) and possibly to differences in the percentage of DEHP in the tubing used leading to different extraction rates.

Table 9. DEHP concentration following ECMO

[DEHP] Mean (Range) µg/mL	Extraction rate in vitro (µg/ml/h)/session	Treatment duration	Estimated Mean Dose (range) mg/kg bw	Comment & Reference
26.9 – 33.5	3.5 – 4.1 (48, 84 h)	3, 10 d	42 – 140	(Shneider* et al, 1989)
3.5 – 8.3	0.32 (48)	3 d	4.7 – 15.5	932 cm^2 circuit (Karle et al, 1997)
	0.57 (48)	3 d	10.5 – 34.9	1563 cm^2 circuit (Karle et al, 1997)
	Negative (48)	3 d	0	932 cm^2 heparin-coated circuit (Karle et al, 1997)

The *in vitro* results obtained with the heparin-coated circuit, suggest that detectable levels of DEHP would not be released during ECMO procedures that use heparin-coated tubing.

Information is unavailable to accurately estimate the dose of DEHP received by these patients on a daily basis as the exposure period represented a range of days. However, if the maximum estimates of Shneider* and colleagues (1989) are used, then a time averaged dose of DEHP received by neonates over 10 days would 14 mg/kg/day.

To reduce circuit preparation time, many ECMO centres preprime the ECMO circuits with normal saline and hold them in a preprimed state for as long as 30 days. As might be expected, no DEHP (level of detection 120 ng/ml) was found in normal saline used to preprime ECMO circuits, even after being preprimed for as long as 4 weeks (Riley et al, 1997). Similar results were obtained by others who found no accumulation of DEHP in the circulating fluid from an ECMO circuit primed with Plasmalyte solution and stored for up to 14 days at 8°C (Han et al, 2005).

2.6.4 Total Parenteral Nutrition

There are no measurements of DEHP content in blood or serum of neonates following TPN.

TPN solutions are generally delivered using glass bottles, EVA bags or syringes depending on the volume required. DEHP exposure in this scenario would then be limited to the choice of infusion set. Although PVC-free infusion sets are available for this purpose, infusion lines are almost always made of PVC and are required for pump-assisted lipid administration, which is the procedure used for neonates and paediatric patients. Lines are changed daily.

Neonates who cannot breast feed or bottle feed generally receive their nutrition intravenously. Two estimates have been used: Loff et al, (2000) measured the extraction of DEHP from PVC infusion lines by 24 mL of TPN solution containing 20% lipid or amino acid-glucose under conditions typical for newborn intensive care units; Kambia et al, (2001a; 2003) measured the DEHP content in EVA bags and outlet attached to an infusion pump used to deliver 650 – 2200 ml of TPN solutions containing 1-4% lipid to children over the age of 2 years.

Over 24 hours infusion, a maximum of 490 µg/mL DEHP was extracted from the 2.25 m of infusion tubing or 6.3 µg/cm^2/hour or 490 µg/hour (Loff et al, 2000). These values are markedly different from Kambia and colleagues (2001, 2003) where the maximum DEHP concentrations at the outlet (ie from EVA bag and PVC tubing) was 1.6 µg/ml after 11 hours infusion or 106 µg/hour. No details regarding tubing length were given

but the set up was similar to the clinical practice of home parenteral program (Kambia et al, 2001, 2003).

Typical nutrient intake for infants is 150 ml/kg/day of a standardised electrolyte/amino acid solution. This can then be supplemented with lipid to a maximum of 18 ml/kg. Using the maximum measurements for 20% lipid from Loff et al, (2000), the total exposure for a 4 kg neonate would be 7.2 mg/kg/day. The dose of DEHP extracted would be less if a micro-volume extension set is used, as the smaller surface area would result in less DEHP leaching from the tubing (1.07 mg/kg bw/day). If microbore tubing was used to deliver 20% lipid to a 4 kg baby over 24 hours, then the estimated DEHP dose would be 1.07 mg/kg bw/day. The estimated DEHP dose will differ depending on the babies weight. In a scenario where a premature baby weighing 1 kg received a 20% lipid solution delivered over 24 hours using microbore tubing, the DEHP would be 4.3 mg/kg bw/day.

An alternative estimate can be derived using the maximum concentration of DEHP measured at the outlet of PVC tubing as reported by Kambia et al, (2001). A dose of 150 ml/kg/day of 1-4% lipid over 24 hours would yield a DEHP dose of 0.64 mg/kg/day.

2.6.5 Enteral nutrition

There are no measurements of DEHP content in blood or serum from neonates following enteral nutrition.

In the medical device context, oral exposure to DEHP can occur following release of this phthalate from enteral feeding bags and tubing or from nasogastric tubing used for aspiration of stomach contents and decompression of the stomach. For this scenario, exposure via enteral nutrition will be explored.

Neonates requiring enteral nutrition would receive 150 ml/kg via an orogastric tube delivered either as milk or prepared enteral solution. This may be delivered as a bolus dose over 30 minutes or could be delivered over 24 hours. Nasogastric tubes are available as phthalate-free as well as polyurethane. In addition, nasogastric tubes are frequently left in place for extended periods of time. However, while the nasogastric tube may stay in place for several days, it is common practice to replace the enteral giving set daily. Several studies demonstrated that less DEHP leaches out during continuous exposure (Mazur et al, 1989; Khaliq et al, 1992; Karle et al, 1997). Over 15 days extraction, the rate of extraction was approximately 25% that of the first day. However, this reasoning is not relevant where the nasogastric tubing is non-PVC. In the home setting, the giving set may be re-used resulting in an over-estimate of DEHP exposure.

There are no specific data on leaching rate of DEHP into milk from storage bags but human milk typically contains about 5% lipid. The extraction should be similar to that of formulated TPN solutions such as those used by Kambia et al, (2003). *Formula 1* (containing 3.85% lipids) was perfused at a flow rate of 177 ml/h yielding an estimated 900 ng/ml over 11 hours perfusion using PVC tubing resulting in an extraction rate of 106 μg/hour. Using this latter estimate, a 150 ml/kg feed over 24 hours would yield a DEHP dose of 0.64 mg/kg/day. This would slightly underestimate the DEHP dose as milk formulation contains more lipids and so would be expected to extract more DEHP.

If an undiluted 20% lipid solution were infused over 24 hours, then the DEHP dose would be 3.2 mg/kg bw/day.

2.6.6 Respiratory Therapy

There are no reliable published data on DEHP blood levels from respiratory therapy.

Heated Respiratory Tubing (Neonates)

Peak flow rates for an infant ventilator are in the range of 2-4 L/min compared to an adult flow rate of 10 L/min (Health Canada, 2002). The tubes are typically shorter than adult circuitry, about 1.2 meters with a resulting surface area of 0.4 to 0.7 that of the adult. Consequently, one would expect smaller quantities of DEHP to be leached from the neonate circuit per unit area of surface. Health Canada (2002) estimated the dose to children to be one-third that of adults. This estimate will be used in these scenarios.

In the worst-case scenario, the amount of DEHP extracted per day in neonatal circuitry would be one-third of the adult or 0.09 mg/kg/day.

Roth* et al, (1988) studied five pre-term infants who were ventilated using heated respiratory tubing and humidified air. The concentration of DEHP in the condensate collected from the water traps of the respirator tubing ranged from <0.001 to 4.1 mg/L. Based on these values, the authors estimated that infants could receive 1 to 4.2 mg DEHP/hour or 24 - 100.8 mg/day. However, this study has been criticised as there may have been a sampling error resulting in serious overestimates of DEHP (Health Canada, 2002). In addition, the respiratory tubing used by Roth et al, (1988*) does not appear to be the type that uses heating wires to maintain temperature throughout the inspiratory limb. Rather, the humidifier gas temperature was in the 50-60°C dropping to 30°C to 32°C at the patient end. This would result in higher concentrations of leached DEHP than would be typical with the current practice of using heated tubing with a lower mean wall temperature.

Currently respiratory tubing used with ventilators is made from polyethylene (Health Canada, 2002). DEHP exposure would be minimal in this scenario.

Tracheal or Tracheotomy Tubes (Neonates)

DEHP content of neonatal endotracheal tubes was measured before and after use (Latini & Avery, 1999). Reported loss was 0.06–0.12 mg DEHP per mg sample after use. This would represent a loss of up to 60–120 mg DEHP for a typical 1 g endotracheal tube, i.e., 11- 22 mg DEHP per day, since the average length of the procedure was 129.5 hrs. However, not all lost DEHP would result in inhalation exposure as some could be extracted into mucus that is then removed by suctioning or swallowed.

The worst-case estimate of the amount of DEHP delivered to a neonate by respiratory air stream would be one-third the amount delivered to an adult; i.e., 0.09 mg/kg/day.

2.7 Multiple Exposures

For many patients, particularly critically ill neonates, DEHP exposure may be derived from a number of different procedures eg. ECMO, multiple replacement blood, parenteral feeding, medications, and IV fluids. The total DEHP exposure will therefore vary considerably depending on the treatment protocols. Three studies have used DEHP urinary biomarkers, metabolites MEHP, 5-oxo-MEHP, and 5-OH-MEHP, to estimate DEHP exposure in the urine of infants receiving multiple treatments in the NICU (Calafat et al, 2004; Green et al, 2005; Weuve et al, 2006). Calafat and colleagues

(2004) tested six premature newborns undergoing intensive care interventions for more than 2 weeks. The geometric mean for these patients was: 1617 ng/mL 5-oxo-MEHP, 2003 ng/mL 5-OH-MEHP, and 100 ng/mL MEHP. The geometric means found in this study was several-fold higher than the MEHP geometric mean in the general US population 6 years and older (3.43 ng/mL). Green et al, (2005) measured urinary DEHP metabolites in 54 infants in a NICU. DEHP exposure was rated low, medium, or high based on the kind of medical devices used and the length of time used. Urinary MEHP levels increased with DEHP exposure. MEHP levels were 4 ng/ml, 28 ng/ml and 86 ng/ml for low, medium and high DEHP-exposure groups. This same group was re-analysed using additional DEHP metabolites to model DEHP dose (Weuve et al, 2006). Infants in the high-intensity exposure group received continuous indwelling umbilical vein catheterisation, endotracheal intubation, intravenous hyperalimentation by the central venous route (i.e., PICC line, broviac, UVC), and an indwelling gavage tube (for gastric decompression). The estimated DEHP dose in this group was 233 to 352 μg/kg bw/day.

2.8 Conclusions – Human Exposure

Medical exposures to DEHP are highly variable and although the exposure estimates have large uncertainties they are considered to be conservative.

Table 10 summarises DEHP exposure data for adults and neonates undergoing medical procedures. It shows estimates of the daily dose of DEHP received by patients undergoing a variety of medical procedures. These exposures were calculated from the data outlined in Chapter 2. The daily dose assumes a 70 kg adult and a 4 kg neonate.

Details of calculations are shown in Appendix A.

Table 10. DEHP doses and MOE in adults and neonates undergoing medical procedures

NOAEL (mg/kg bw/day) (chronic exposure):

Adult male 14 mg/kg bw/day (Lamb et al, 1987)
Developmental & Neonatal 5 mg/kg bw/day (Wolfe & Layton, 2004)

SCENARIO	ADULTS[1] (mg/kg/day)	MOE[2] adult	MOE pregnancy	NEONATES[3] (mg/kg/day)	MOE neonate
PARENTERAL					
Infusion of crystalloid solutions	0.013	1077	385	0.04 0.008 (microbore)	125 625
IV infusion of drugs	Teniposide			midazolam	
When administered according to manufacturer's instructions	0.05	280	100	0.007	714
When mixed and stored at RT for 24 hr	1.51	9	3		
Blood transfusion					
Acute					
Elective surgery[4]	0.37-1.23	38-11	14-4		
Trauma patient [4]	1.31-4.31	11-3	4-1		
ECMO	0.21-0.41	67-34	24-12	14	< 1
Replacement transfusion[4]				0.05 – 0.17	100-29
				0.09-0.28[7]	56-18[7]
Exchange transfusion				22.6	< 1
Cardiopulmonary bypass					
CABG	1	14			
Orthotopic heart transplant	0.3	47			
Artificial heart transplant	2.4	6			
Chronic					
Sickle cell transfusion [4]	0.03-0.09	467-156	167-56		
Chemotherapy[4]	0.009-0.03	1556-467	556-167		
Treatment of clotting disorders with cryoprecipitate	0.03	467	167		
Apheresis	0.04	350	125		
Haemodialysis	0.36 (retained) 1.21 (delivered)	39 12	14 4		
TPN administration					
EVA bag and PVC tubing	0.05 (4% lipid) 0.21 (20% lipid)	280 67	100 24	0.64 (4% lipid) 7.22 (20% lipid) 1.07 (microbore)	8 <1 5
Peritoneal dialysis	0.01	1400	500		
Enteral nutrition PVC tubing	0.13 (4% lipid) 0.04 (tubing)	108	38	0.64 (4% lipid or breast milk)[5] 3.2 (20% lipid) 0.2 (breast milk)[6]	8 2 25
INHALATION	0.02			0.09	
DERMAL	0.0007				

[1] Based on 70 kg body weight
[2] MOE = NOAEL/Estimated dose
[3] Based on 4 kg body weight
[4] Range refers to DEHP concentration in Packed RBC; realistic 36.5 μg/ml (Inoue et al, 2005) to maximum 123.1 μg/ml (Plonait et al, 1993)
[5] Breast milk from healthy mother
[6] Breast milk from mother undergoing hemodialysis
[7] Based on a 1 kg body weight

3. HEALTH EFFECTS

3.1 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

The toxicokinetics of DEHP has been extensively reviewed by the National Chemical Inspectorate (2005). There are a limited number of studies on the toxicokinetic of DEHP in humans. Toxicokinetic studies in experimental animals have been performed by the oral, inhalation, dermal and parenteral routes of exposure. The majority of these studies have been performed in rats by the oral route although some with non-human primates and mice have been conducted.

Oral

The first step in the metabolism of DEHP is hydrolysis by lipases to MEHP and 2-ethylhexanol (EH). Lipases are found in all tissues (intestinal mucosa, liver, kidney, lungs, skin, pancreas and adipose tissues) but especially in the pancreas, indicating that most of DEHP hydrolysis occurs in the lumen of the small intestine, and that hydrolysis of absorbed intact DEHP can occur in the liver and blood (Albro* & Thomas, 1973). The absorption of DEHP in the intestine is increased following hydrolysis to MEHP. The extent of absorption in rats, primates and humans is ~ 50% for doses up to 200 mg/kg bw. At higher doses, absorption in primates is dose-limited, in contrast to rodents (Albro* et al, 1982; Rhodes* et al, 1983). This species difference is reflected in differences in the activity of DEHP-metabolizing enzymes. The rate of MEHP formation from DEHP substrate differed by 27- to 357-fold among species being highest in CD-1 mice, next in Sprague–Dawley rats and lowest in marmosets in all organs measured (liver, lungs, kidneys, and small intestine) (Ito et al, 2005c).

Metabolic pathways for DEHP involve a number of reactions. Esteratic or hydrolytic cleavage of DEHP results in the formation of MEHP and 2-EH. MEHP is further metabolised via numerous oxidative reactions, resulting in the formation of 30 or more metabolites. Some of them can be conjugated with glucuronic acid for excretion. Oxidation of 2-EH primarily yields 2-ethylhexanoic acid and several keto acid derivatives, which are excreted in the urine. However, at lower doses, excretion is comparable between species. In the lower dose range, rats excrete 32–70% of the absorbed dose in the urine as metabolites and 20–25% with the bile in the faecal matter (Rhodes* et al, 1986).

The elimination half-life in rats was shorter after repeat exposures (2 hours) compared to a single oral dose (3-4 hours). In orally-exposed humans, approximately 65% of DEHP metabolites are excreted in the urine as glucuronide conjugates. The aglycone moieties of these conjugates as well as the non-conjugated DEHP metabolites excreted by humans are similar to those found in animals. The half-life of MEHP in humans is approximately 5 hours (Koch et al, 2006).

No studies were located regarding faecal excretion of DEHP or its metabolites in humans. However, significant amounts of DEHP were noted in the faeces of animals when given by the oral route; which presumably represents unmetabolised DEHP. MEHP and other metabolites were frequently found in faeces, in some cases associated with biliary excretion products.

There was no evidence of accumulation in rodent tissues. Limited human data from autopsies have indicated the presence of DEHP in adipose tissues and kidneys.

Kessler et al, (2004) measured the blood concentration of DEHP and MEHP in pregnant and nonpregnant rats and marmosets. Pregnant Sprague–Dawley rats were dosed by gavage with 30 or 500 mg DEHP/kg bw/day on GD 14–19. Pregnant marmosets were dosed on GD 96–124. Nonpregnant rats (2-4/dose) were gavaged with 30, 500 or 1000 (labeled and unlabelled) mg/kg bw for 7 days. Nonpregnant marmosets (8/dose) received 30 or 500 mg/kg bw DEHP for 29 days. Blood was collected up to 48 h after dosing. In rats, normalized areas under the concentration–time curves (AUCs) of MEHP were two orders of magnitude greater than DEHP. Metabolism of MEHP was saturable in rats. Repeated DEHP treatment and pregnancy had little influence on the normalized AUC of MEHP in both species. The maximum concentrations of MEHP in blood of marmosets was up to 7.5 times and the normalized AUCs up to 16 times lower than in rats receiving the same daily oral DEHP dose per kilogram of body weight, implying that DEHP would be less toxic in marmosets than rats.

Inhalation

Absorption via the respiratory tract has also been indicated, although quantitative data have not been published. The limited data regarding metabolism and excretion of DEHP in humans or animals following inhalation exposure indicate that no obvious difference should be expected when compared to oral administration. In rats exposed to an aerosol of radioactively labeled DEHP, the major route of elimination was urine (General Motors*, 1982). There are no inhalation toxicokinetic studies in humans but case studies of workers exposed to DEHP by inhalation indicate that the main metabolites in urine were MEHP (26%) and three other metabolites. There was a large human inter-individual variation in percentages of MEHP present in free form, ranging from 20 to 100%. No substantial inter-individual variation in humans in the phase I metabolism of DEHP to MEHP was found.

Parenteral

DEHP exposures through the parenteral route bypass the intestinal esterases, so the amounts of intact DEHP rather than MEHP in the organs and tissues would be expected to be higher. This is evident in data from human studies following exchange transfusions and haemodialysis. Initially there is more DEHP than MEHP in the blood (Pollack* et al, 1985a; Pollack* et al, 1985b; Sjoberg* et al, 1985a). However, DEHP levels decline rapidly with a half-life of 10 hours (Sjoberg* et al, 1985a), and the MEHP levels increase until the time-averaged concentrations are roughly equal (Pollack* et al, 1985b).

Similar results were seen in animal studies. After arterial injection DEHP was rapidly cleared from the blood of rats (half-life of 15 hours) (Pollack* et al, 1985a). In mice intravenously injected with labelled DEHP, radioactivity rapidly accumulated in the kidney and liver with high concentrations in urine, bile, and intestinal contents (Lindgren* et al, 1982). There was no evidence of retention in any tissues. The secretion by the liver, via the bile into the intestine appears to be the major route. Following intravenous administration to marmosets, approximately 40% of the dose was excreted in urine and approximately 20% in the faeces (cumulative excretion) (Elsisi* et al, 1989).

Dermal

The absorption of DEHP via the skin has been reported to be low. No human *in vivo* dermal studies were found. However, Barber* et al, (1992) and Scott* et al, (1987) compared *in vitro* absorption of DEHP through rat and human skin and found that DEHP was more rapidly absorbed through rat skin.

Dermal absorption was low in two studies conducted in rats (Melnick* et al, 1987; Elsisi* et al, 1989). In these studies, 95 or 86% of the applied dose remained at the site of application after 5 or 7 days, respectively. Several studies calculated the cumulative amount detected in excreta and tissues: excluding the dosed skin, was 6.5% (rat: Elsisi* et al, 1989), 9% (rat: Melnick* et al, 1987), 26% (guinea pig: Ng et al, 1992); and including the skin bound dose was 9.7-18.9% (guinea pig: (Chu* et al, 1996)). One study determined the percutaneous absorption rate for DEHP in PVC applied to rat skin to be 0.24 μg/cm2/hr (Deisinger* et al, 1998).

3.2 ACUTE TOXICITY

Human

Shaffer* et al, (1945) reported two adult male subjects who swallowed DEHP as single doses of 5 g and 10 g. No symptoms resulted from the 5 g dose while the ingestion of 10 g caused mild gastric disturbances and "moderate catharsis". Assuming 70 kg body weight, this equates to a dose of 0.14 g/kg.

Laboratory Animals

The acute toxicity of a single dose of DEHP has been evaluated in a number of species using oral, dermal, inhalation and intravenous routes of administration. LD_{50} values derived from these studies are shown in Table 11.

Table 11. Acute effects of DEHP

Study	Species	Results (LD50/LC50)	Ref
Oral	Rats	= 30600 mg/kg bw	(Shibko* & Blumenthal, 1973)
Oral	Rats	>20000 mg/kg bw	(NTP *, 1982)
Oral	Rats	>40000 mg/kg bw	(Nuodex*, 1981a)
Oral	Mice	>20000 mg/kg bw	(NTP *, 1982)
Oral	Mice	>9860 mg/kg bw	(Nuodex*, 1981b)
Oral	G. pig	= 26000 mg/kg bw	(Krauskopf*, 1973)
Oral	Rabbit	= 34000 mg/kg bw	(Shaffer* et al, 1945)
Dermal	Rabbits	= 24750 mg/kg bw	ATSDR, 2002
Inhalation (4 h)	Rats	>10.62 mg/l	(Hüls*, 1981)
Intravenous	Rat	250 mg/kg bw	(Schmidt* et al, 1975; Rubin* & Chang, 1978)
Intravenous	Mouse	1060 mg/kg bw	(Health Canada, 2002)
Intraperitoneal	Mouse	2800 mg/kg bw	(Lawrence* et al, 1975; Woodward* et al, 1986)
Intraperitoneal	Rat (SD)	5675 mg/kg bw	(Shaffer* et al, 1945)

Adapted from the National Chemical Inspectorate (2005)

DEHP has low acute toxicity. Intravenous and intraperitoneal DEHP has a higher acute toxicity than orally administered DEHP.

3.3 REPEATED DOSE TOXICITY

3.3.1 Oral

Human

There are no subchronic or chronic toxicity studies in humans following oral exposure to DEHP.

Laboratory Animals

The toxicity of DEHP following repeated exposures has been evaluated in a number of animal species, both over short-term (few weeks) and life-time (2 years) periods. The oral studies are summarised in Table 14, Appendix B (dietary intakes have been converted from ppm and percent into mg/kg bw/day). The studies show that rodents are the most sensitive species, followed by hamsters, guinea pigs, and primates. The most pronounced findings included effects on the liver (hepatomegaly, peroxisome proliferation, and replicative DNA synthesis), testes (tubular atrophy) and kidneys (increased kidney weights, mineralisation of renal papilla, tubule cell pigments and chronic progressive nephropathy). Other, less pronounced, effects have also been observed, such as decreased body weights/body weight gains and alterations in clinical chemistry parameters.

Liver hypertrophy, increased liver weights and peroxisomes were noted in most of the repeated dose studies. The lowest NOAEL for hepatotoxicity was 5.8 mg/kg bw, based on increased liver weights and peroxisome proliferation in a 104-week rat study (LOAEL = 28.9 mg/kg bw/day) (Moore*, 1996). In the only primate study, marmoset monkeys were given oral doses of DEHP up to 2500 mg/kg bw/day for 65 weeks with no effects on organ weights (Tomonari et al, 2006).

Decreased testicular weights, testicular atrophy, increased bilateral aspermatogenesis, immature or abnormal sperm forms, seminiferous tubular degeneration, Sertoli cell vacuolation and complete loss of spermatogenesis were evident in most of the repeated dose studies. The lowest NOAEL (3.7 mg/kg bw/day) for testicular effects was reported in a 13-week rat study, based on an increased incidence of Sertoli cell vacuolation (LOAEL = 37.6 mg/kg bw/day) (Poon et al, 1997). In the only primate study, marmoset monkeys were given oral doses of DEHP up to 2500 mg/kg bw/day for 65 weeks and no effects on the testes was observed (Tomonari et al, 2006).

Increases in kidney weights, mineralisation of renal papilla, tubule cell pigments and chronic progressive nephropathy in the kidney were consistently observed. The majority of these changes were observed in both sexes in different species following different duration. In chronic studies in rats and mice (Moore*, 1996; Moore*, 1997), there was no indication that DEHP-related changes in the kidney were reversible upon cessation of exposure. The lowest NOAEL for kidney effects consisted of a decrease in creatinine clearance in rats receiving approximately 0.9 mg/kg bw/day DEHP (Crocker* et al, 1988). This NOAEL is questioned however, on the following grounds: only few animals were used (4 rats/group), the reported data were not always clear and the effect on creatinine clearance at such low levels of DEHP has not been reported in other studies. A more reliable NOAEL for kidney effects is considered to be that reported by Moore* (1996) in a 104-week rat study of 28.9 mg/kg bw/day for males and 36.1 mg/kg bw/day in females. The LOAEL in this study was of 146.6 mg/kg bw/day, based on increased kidney weights.

3.3.2 Inhalation

Human

Three preterm infants artificially ventilated through PVC respiratory tubes, developed unusual lung disorders resembling hyaline membrane disease during the fourth week of life. One infant died two weeks after birth; the other two were healthy at follow-up 20 months later. DEHP was detected in the lung after autopsy of the infant who died. The estimated inhalation exposure in the three infants ranged between 1 - 4200 μg/h based on the concentrations of DEHP in the condensate collected from the water traps of the respirator tubing. However this is likely to be an over-estimate as infants were not exposed to the condensate. DEHP, but not MEHP, could be demonstrated in urine samples (Roth* et al, 1988).

A morbidity study was carried out on a group of workers (97 men and 4 women) employed in a German plant producing DEHP (Thiess* et al, 1978a). The average exposure period was 12 years (range 4 months to 35 years). DEHP levels measured in ambient air were generally low (0.001-0.004 ppm, ~ 0.016-0.064 mg/m^3). Higher levels, up to 0.01 ppm (~0.16 mg/m^3) were measured near the chemical reactor. Blood lipids, serum activities of liver enzymes, and routine haematological tests were normal, and no excess of any pathological condition was found. There was no referent group.

A mortality study of 221 workers exposed to DEHP in the same plant was also conducted (Thiess* et al, 1978b). Eight deaths occurred in the cohort compared with expected values of 15.9 and 17.0 for city and country workers, respectively. The cohort size was small and follow-up was short.

Three separate studies reported the incidence of polyneuropathy in workers exposed to phthlate esters (including DEHP). Milkov* et al, (1973) conducted a morbidity study in the USSR on 147 workers at a PVC-processing plant. The workers were exposed to a mixture of phthalates, including DEHP as a minor constituent. The total phthalate air concentrations recorded varied between 1.7 and 66 mg/m^3. Polyneuropathy was evident in 47 workers (32%); the incidence increased with length of employment. Vestibular abnormalities were evident in 63 of 81 workers (78%) specifically examined. No referent group was included in the study.

In a cross-sectional study symptoms and signs of polyneuropathy were reported in 12 out of 23 workers at a phthalate production plant in Italy (Gilioli* et al, 1978). The workers were exposed to a mixture of phthalates, including DEHP, but also to a lesser degree, to the corresponding alcohols and to phthalic anhydride. Total phthalate air concentrations varied between 1 and 60 mg/m^3. No referent group was included in the study.

In a study involving a Swedish PVC-processing factory 54 male workers were examined for anomalous peripheral nervous system symptoms and clinical signs (Nielsen* et al, 1985). The workers were exposed mainly to DEHP, di-isodecyl phthalate, and butylbenzyl phthalate. They were divided into three groups of approximately equal size and with mean phthalate exposures of 0.1, 0.2, or 0.7 mg/m^3. The peripheral nervous system symptoms and signs displayed were not related to the level of exposure.

Laboratory Animals

There are four inhalation studies in experimental animals. In the first study, rats were exposed up to 1000 mg/m^3, 6 hours per day, 5 days per week for 4 weeks ((BASF AG*,

1990; Klimisch* et al, 1992). In the highest dose group, there was a significant increase in relative lung weights in male rats accompanied by foam cell (macrophage) proliferation and thickening of the alveolar septi. Liver weights were slightly increased but unusually, this was not accompanied by peroxisome proliferation which had been reported in a similar study conducted earlier by BASF (Merkle* et al, 1988). No testicular toxicity was detected histologically.

A poorly described study of male mice (20 animals) exposed to air saturated with vapours of DEHP (purity not specified) for 2 hours per day, 3 days per week, for 4-16 weeks failed to reveal consistent abnormalities which could be attributed to inhalation of DEHP (Lawrence* et al, 1975). No further data were available.

Kurahashi et al, (2005) exposed 4 week old male Wistar rats to doses of 0, 5 or 25 mg/m^3, 6h per day, for 4 or 8 weeks (4 rats per treatment group). There were no differences in body or testes weights. Seminal vesicle weight was reduced after 8 weeks but not 4 weeks exposure to both doses. Histological examination showed that there were no significant pathological changes in the testes after 4 or 8 weeks exposure to either dose. The study did not show a dose-response and included a small sample size.

The only long-term inhalation study available was on hamsters (Schmezer* et al, 1988). However, only a single, very low dose (continuous inhalation of 15 $\mu g/m^3$ for 23 months) was used. No signs of any toxicological effects were reported.

3.3.3 Dermal

In the only available dermal study, DEHP was administered to mice at doses of 0.2 mL of a 10, 30, 50, or 100% solution in olive oil for one month (Watari* et al, 1978). Macroscopically, the liver was greatly enlarged. Inflammatory signs were observed in the peritoneum in the two highest dose groups. This study had several shortcomings which are described in National Chemical Inspectorate (2005).

3.3.4 Intravenous

Human

There are no subchronic or chronic toxicity studies in humans following intravenous exposure to DEHP.

Laboratory Animals

Five published reports were found on the effects of DEHP administered intravenously (IV) to animals (Jacobson* et al, 1977; Sjoberg* et al, 1985c; Greener* et al, 1987; Baxter Healthcare Corporation*, 2000; Cammack et al, 2003); two are short-term (12 days and 18 days in adult rats), two subchronic (21 days beginning in the neonatal period) and one chronic study (1 year in rhesus monkeys).

In the first study, 40-day-old Sprague Dawley rats (5-6/group) were cannulated, and 3 hour infusions of 0, 5, 50, or 500 mg/kg DEHP were performed daily every other day for a total of six infusions over 12 days (Sjoberg* et al, 1985c). This was equivalent to time-weighted average doses of 2.5, 25, and 250 mg/kg bw/day. The DEHP was emulsified with egg yolk phosphatides and administered in a glycerol solution. Animals were sacrificed 2-3 hours after the last infusion. The results showed a dose-related decrease in body weight gain, an increase in relative liver weight at the middle and highest doses but no change in the clinical chemistry parameters. Liver and kidney histology appeared unchanged except for an increase in hepatic peroxisomes. There was

no change in the relative weight of the reproductive organs but transmission electron microscopic examination revealed slight enlargements of the smooth endoplasmic reticulum in Sertoli cells at the highest dose in three of five rats. The NOAEL was 25 mg/kg bw/day for hepatic changes.

Neonatal rats (12 rats per group, 2 to 4 days old) were injected with 30.8, 91.7, or 164.8 mg/kg bw/day of DEHP (purity not specified) for 18 consecutive days (Greener* et al, 1987). Neonates were examined for signs of toxicity immediately after treatment and again 1 to 3 hours later. After sacrifice, a complete necropsy was performed and selected tissues were prepared for histopathological evaluation. Body weight gains were significantly and dose-dependently decreased for the treatment period. Liver weights were significantly increased in a dose-related manner. However, there were no conclusive histopathological changes in the liver or other organs examined. Based on these results the NOAEL for liver function impairment is 92 mg/kg bw/day.

Neonatal male rats or rabbits were injected with either 62 mg/kg bw/day DEIIP or 4% bovine serum albumin during postnatal days 3-21 (rats) or 14-42 (rabbits) (Baxter Healthcare Corporation*, 2000). Histopathological examination of the testes and other organs of DEHP-exposed animals revealed no alterations at this dose.

Cammack et al, (2003) conducted a 21-day repeat dose study of DEHP in neonatal (3 to 5 day old) rats. Rats were injected with 0, 60, 300 or 600 mg/kg bw/day. A second group of animals was dosed for 21 days then held for a recovery period until 90 days of age. Terminal body weight was significantly less in the high dose group only. At the end of the 21-day dosing period, mean liver weight was increased and mean testes weight was decreased in the two higher dose groups. Testicular atrophy was observed in all animals in the 300 and 600 mg/kg bw/day treatment groups. The NOAEL in the study was 60 mg/kg bw/day; consistent with the results reported previously by Baxter Healthcare Corporation (2000).

Jacobson* et al, (1977) studied hepatic effects in 6-month-old rhesus monkeys receiving transfusions of plasma from DEHP-plasticised bags over a 6-month or 1 year period. The average total exposures to DEHP for the groups of monkeys transfused weekly for one year: Group 1 (plasma stored at 20°C): 3 monkeys at a mean dose of 27 mg/kg bw; Group 2 (plasma stored at 4°C): 2 monkeys at a mean dose of 8 mg/kg bw; Group 3 (transfused biweekly for 6 months, platelet poor plasma stored at 22°C): two monkeys at a mean dose of 32 mg/kg bw; Group 4 (untransfused control group): three monkeys and Group 5 (platelet-rich plasma stored for 48 hours at 22 °C in polyethylene blood bags): two monkeys. Three of the seven PVC-transfused monkeys showed some impairment of hepatic perfusion and four out of seven monkeys demonstrated abnormal sulfobromo-phthalein clearance indicative of subclinical liver disease. Six out of the seven had abnormal liver histology (aggregates of inflammatory cells, hepatocyte degeneration, and multi- and bi-nucleated giant cells) upon completion of transfusion period that persisted in three of the five surviving monkeys throughout the follow-up period of 26 months. None of the five control animals had abnormal liver histology. The results of this study are confounded by the small sample size, inconsistent responses in the two groups that received the largest (and similar) doses; use of pooled plasma to re-transfuse into the monkeys and appearance of a tuberculosis outbreak in the monkey colony, which might have contributed to the hepatic effects. The authors conceded that the observed hepatic effects were mild and could be attributed to background effects.

3.3.5 Intraperitoneal

Human

There are no human subchronic or chronic toxicity studies in humans following intraperitoneal exposure to DEHP.

Laboratory Animals

Hepatomegaly has been reported following intraperitoneal doses of DEHP in the rat and mouse but not the marmoset. The LOAEL for hepatomegaly after 56, 500 mg/kg in uremic rats over 19 weeks was 210 mg/kg bw (Leber* & Uviss, 1979). Shorter duration of exposure (7 days) also induced hepatomegaly at a dose of 3906 mg/kg bw in rats (Pollack* et al, 1989). A more recent study demonstrated that peritoneal sclerosis was produced in rats following intraperitoneal injection of a DEHP dose of 0.05 mg/kg bw/day for 7 days (Fracasso* et al, 1999). However the FDA considers this a "local" artifact of the injections.

Rhodes* et al, (1986) reported an intraperitoneal marmoset study. Five marmosets were dosed with 1□ g/kg bw/day in corn oil for 14 days. There was no indication of the length of time reported between the last dose and necroscopy. At necropsy, blood was taken for toxicokinetic studies, a gross examination was made, and "selected tissues were preserved in buffered formol saline for microscopic examination". The marmoset data was stated by CERHR (2000) to be confusing and poorly reported: a single set of bar graphs is presented, while two studies were performed. The authors state that organ weights were not changed in marmosets at 1□ g/kg bw/day IP but provide no data. Based on histology and biochemical measures, peroxisomes were not induced in marmosets. The authors presented no histological findings of testes. This is of concern as testicular pathology is the most sensitive endpoint at this exposure duration, and poor histology could well mean that lesions could go undetected.

However, hepatomegaly and other liver effects produced by DEHP in mice after oral administration (and presumably intraperitoneal) have been shown to be mediated by PPARα in this species and may not be relevant to humans.

Conclusion

While human exposure to DEHP has certainly occurred over a number of different routes of exposure, most of the studies had major limitations including lack of a referent group. The toxicity of DEHP has been evaluated in a number of animal species, both short-term (few weeks) and life-time (2 years) studies by oral, inhalation and parenteral routes of exposure. The majority of these studies have been performed in the rodent by the oral route of exposure. Reproductive endpoints have been assessed in later studies and will be described in greater detail in Section 3.8.

The studies show that rodents are the most sensitive species, followed by hamsters, guinea pigs, and primates. The most pronounced findings included effects on the liver (hepatomegaly, peroxisome proliferation, and replicative DNA synthesis), testes (tubular atrophy) and kidneys (increased kidney weights, mineralisation of renal papilla, tubule cell pigments and chronic progressive nephropathy). Other less pronounced effects have also been observed eg decreased body weights/body weight gains and alterations in clinical chemistry parameters.

DEHP is a peroxisome proliferator in rats and mice unlike hamsters, guinea pigs, and monkeys by oral and parenteral routes of exposure (inhalation and dermal route studies were inadequate). Studies in animals have shown that in general, after oral exposure to

peroxisome proliferators, rats and mice exhibit the greatest response, hamsters exhibit an intermediate response, whereas primates, guinea pigs, and dogs are either unresponsive or refractory (Huber* et al, 1996; Cattley* et al, 1998; Cattley* & Roberts, 2000). Studies conducted in patients treated with several hypolipidemic agents have provided no evidence for peroxisome proliferation or increased hepatocyte division in humans (Bentley* et al, 1993; Ashby* et al, 1994; Cattley* et al, 1998). Recent investigations have demonstrated that activation of the peroxisome proliferator-activated receptors (PPAR-α) is required for induction of the different peroxisome proliferator-induced liver effects observed in experimental animals (Peters* et al, 1997; Cattley* et al, 1998; Ward* et al, 1998). The low levels of PPAR-α found in human liver could explain the low sensitivity of the human liver to the hepatotoxic effects of peroxisome proliferators (Tugwood* et al, 1996; Palmer* et al, 1998; Woodyatt* et al, 1999).

The most sensitive endpoint is testicular toxicity. Reproductive effects are discussed in more detail in Section 3.6.1. The NOAEL (3.7 mg/kg bw/day) for testicular effects was reported in a 13-week rat study, based on an increased incidence of Sertoli cell vacuolation (LOAEL = 37.6 mg/kg bw/day).

For non-reproductive endpoints, renal effects were consistently observed. The lowest reliable oral NOAEL for kidney effects is of 28.9 mg/kg bw/day for males and 36.1 mg/kg bw/day in females reported by Moore (1996*) in a 104-week rat study. The LOAEL in this study was of 146.6 mg/kg bw/day, based on increased kidney weights.

Effects generally observed following intravenous administration of DEHP include decreased body weight, increased liver weight and testicular atrophy. The study by Sjoberg* et al, (1985c) is considered to be the most reliable. A LOAEL of 250 mg/kg bw/day was identified based on hepatic changes and the NOAEL was 25 mg/kg bw/day.

Effect of Pregnancy on Sensitivity to DEHP

It is well established in humans that changes in plasma volume, glomerular filtration rate, and body water occur during pregnancy and tend to deviate more from the nonpregnant state as the pregnancy progresses (Tracy et al, 2005). Recent studies have reported altered pharmacokinetics of various drugs in pregnancy as compared with the non-pregnant state. One of the most striking and significant physiological changes in pregnancy, from the point of view of drug disposition, is the 50% increase that occurs early in pregnancy in effective renal plasma flow, glomerular filtration rate and endogenous creatinine clearance (Morgan, 1997). Changes are accompanied by a parallel increase in the renal elimination capacity of drugs and a decrease in plasma concentrations of drugs that are eliminated from the body predominantly by the renal route. One therefore cannot necessarily extrapolate NOAELs or LOAELs derived from studies using non-pregnant females to pregnant females. The NOAEL and LOAEL for pregnancy can be ascertained from developmental toxicology studies presented in Section 3.6.2, Developmental Effects and summarised in Table 17, Appendix B.

In those experiments where exposure to DEHP was restricted to the gestational period, the LOAEL for decreased weight gain was 573 mg/kg bw/d for entire gestation in rats (20 days). By comparison in non-pregnant female rats, the lowest LOAEL for decreased body weight was 1892 mg/kg bw/d for 21 days exposure. The highest NOAELs for pregnant females was 164 mg/kg bw/d (20 days) compared to 1197 mg/kg bw/d for nonpregnant females. This analysis suggests that pregnant females may be more susceptible to DEHP than nonpregnant females.

3.4 GENETIC TOXICITY

3.4.1 Human

The frequency of chromosomal aberrations in blood lymphocytes were investigated in ten workers employed from 10-30 years in a DEHP production plant in Germany (Thiess* & Fleig, 1978). Exposure levels ranged from 0.0006 to 0.01 ppm (0.01-0.16 mg/m^3). There was no evidence of an increased frequency of chromosome aberrations in the exposed workers, however exposure levels were low and the number of workers was small.

3.4.2 Animal and Bacterial Studies

The genotoxicity of DEHP has been reviewed elsewhere (IARC, 2000; National Chemical Inspectorate, 2005). DEHP has been extensively tested in a variety of short-term genotoxicity assays with predominantly negative results. In the 15 published Ames tests, all results were negative. The maximum concentration used was 14,700 μg/plate. All but two studies in fungi were negative, failing to show any evidence of mutation or recombination events.

Results were generally negative in *in vivo* (mouse, rat, hamster and *Drosophila melanogaster*) studies testing DEHP and its main metabolites MEHP (mono-(2-ethylhexyl)phthalate) and 2-EH (2-ethylhexanol). Low levels of mutation were induced in somatic cells of *Drosophila melanogaster* but not DNA damage. Primary DNA damage, mutation, sister chromatid exchange or chromosomal aberrations was not induced in cultured mammalian cells. Cell transformation and induction of mitotic aneuploidy were induced in a number of different systems. However, these latter assays are also sensitive to non-genotoxic tumour promoters and/or peroxisome proliferators. Gene mutations were not induced *in vivo* in the liver of dosed mice and there was no evidence of chromosomal aberrations in mice or rats *in vivo*.

Taking into consideration all of the results, DEHP, and its metabolites MEHP and 2-EH are not considered to be mutagenic.

3.5 CARCINOGENICITY

Human

In a prospective cohort mortality study, eight deaths (including one carcinoma of the pancreas and one bladder papilloma) were observed (expected values of 15.9 and 17.0 from the city and county data, respectively) among 221 workers exposed to DEHP for periods of 3 months to 24 years (average 11.5 years) (Thiess* et al, 1978b). No information about exposure levels is given in the report however in two other reports by the same group, exposure levels in the plant ranged from 0.0006 to 0.01 ppm (0.01-0.16 mg/m3) (Thiess* & Fleig, 1978; Thiess* et al, 1978a).

Occupational exposure to polyvinyl chloride (PVC) and other products in the plastics industry were assessed in a case-control study on testicular cancer using self-administered questionnaires (148 cases and 315 controls) (Hardell* et al, 1997). An increased risk was observed for exposure to PVC (an increased odds ratio of 6.6; 95% confidence interval, 1.4-32), but not for other types of plastics.

Hardell's earlier study was followed up by a larger case-control study taken from the Swedish Cancer Registry during 1993-1997 (Hardell et al, 2004). A total of 791 matched pairs completed a questionnaire regarding exposure. Overall exposure to PVC plastics gave an odds ratio (OR) of 1.35 (confidence interval = 1.06-1.71). No dose-response relationships were found.

Laboratory Animals

Numerous studies on the carcinogenicity and mechanisms of carcinogenicity of DEHP have been performed *in vivo* and are reviewed in National Chemical Inspectorate (2005).

The only inhalation study available is on hamsters continuously exposed to 15 µg/m3 of DEHP by inhalation for 23 months (Schmezer* et al, 1988). There was no significant increase in tumour incidence.

In a carcinogenicity study, mice received DEHP in the diet at concentrations of 0, 100, 500, 1500, or 6000 ppm (M/F: 0/0, 19/24, 98/117, 292/354, or 1266/1458 mg/kg/day) for 104 weeks (Moore*, 1997). In an additional recovery group, mice were dosed with 6000 ppm of DEHP for 78 weeks, followed by a 26-week recovery period. Significant increased hepatocellular adenomas and carcinomas were observed at 1500 ppm and above in male mice. In the two high dose groups (1500 and 6000 ppm), induction of peroxisome proliferation but not hepatocellular proliferation was more pronounced in both sexes. The incidence of hepatocellular adenomas, but not carcinomas, was less in the 6000 ppm recovery mice, compared to the animals in 6000 ppm group. Non-tumour endpoints were described in Section 3.3.1.

In a chronic/carcinogenicity study, rats received DEHP in the diet at doses of 0, 100, 500, 2500, or 12500 ppm (M/F: 0/0, 6/7, 29/36, 147/182, or 789/936 mg/kg bw/day) for 104 weeks (Moore*, 1996). Increases in hepatocellular adenomas and mononuclear cell leukaemia (MCL) were observed in males at 2500 ppm and above and hepatocellular carcinomas in males and females at 12500 ppm. However, the incidence of hepatocellular adenomas/carcinomas was decreased in recovery animals at 12500 ppm (2-week recovery period), compared with the same dose group at the end of the dosing period. Peroxisome proliferation was induced from 2500 ppm. Effects on the liver, kidney and testis induced at 2500 ppm and above are described in Section 3.3.1.

The carcinogenicity of DEHP was tested in rats and mice in the US National Toxicology Program (NTP) in 1982-1983 (Kluwe* et al, 1982; NTP *, 1982; Kluwe* et al, 1983). Rats and mice were fed diets containing 0, 6000 or 12000 ppm (rats), and 0, 3000 or 6000 ppm (mice) of DEHP for 103 weeks. This corresponded to a daily DEHP intake of 0, 322, and 674 mg/kg bw/day for male rats; 0, 394, and 774 mg/kg bw/day for female rats; 0, 672 and 1,325 mg/kg bw/day for male mice, and 0, 799 and 1,821 mg/kg bw/day for female mice. There was a dose-dependent trend in the incidence of hepatocellular carcinomas in male and females rats with a significant increase in females at the highest dose. The combined incidence of rats with hepatocellular carcinomas or neoplastic nodules was significantly greater than controls for females at both doses and for high dose males. In mice, a dose-related trend for hepatocellular carcinomas was observed for both sexes, with a significant increase in females at both doses and in high dose males. The incidence of hepatocellular carcinomas or adenomas when combined was dose-related, with a significant increase in both sexes at both doses. Peroxisome proliferation was not reported.

DEHP was administered in the diet at 0, 600, 1897, and 6000 mg/kg to male Sprague-Dawley rats beginning at an age of 90–110 days and continuing for the remaining

lifetime of the animals (up to 159 weeks) (Berger*, 1995; Voss* et al, 2005). DEHP dose levels were 0, 30, 95, and 300 mg/kg bw/day. Significantly increased incidence of hepatocellular adenomas and carcinomas were observed at the highest dose. The percentage of benign Leydig cell tumors in the highest dose group was almost twice as high as the percentage in the control group (28.3% versus 16.4%). There was a significant dose-related trend in the incidence of hepatic neoplasms and Leydig cell tumours. Leydig cell tumours have not been reported in previous studies.

There are no long-term carcinogenicity studies following intravenous or intraperitoneal routes of exposure to DEHP.

Conclusion

DEHP, and its metabolites MEHP and 2-EH are not considered to be mutagenic. Human epidemiological studies were hampered by small sample sizes and lack of adequate exposure data and are considered inadequate.

Oral studies show that DEHP is carcinogenic in rats and mice. There was a statistically significant increase in the incidence of liver tumours with a dose-response relationship in rats and mice of both sexes, and an increase in the incidence of Leydig cell tumours and MCL in male rats. The liver effects are likely to be associated with peroxisome proliferation activity of DEHP. A Working Group of the "International Agency for Research on Cancer" (IARC) has concluded that the mechanism by which DEHP increases the incidence of liver tumours in rodents (activation of PPAR-α) is not relevant to humans. It was also noted that low doses did not cause hepatocellular tumours suggesting a threshold for this effect. Leydig cell tumours have been reported in one study. A recent review concluded LC tumours may be of relevance to humans (Cook et al, 1999). However, it was considered that the induction of LC tumours for non-genotoxic compounds (such as DEHP) may be of less relevance as humans are less sensitive than rats. An increased incidence of mononuclear cell leukaemia (MCL) was only seen in one of two rat studies and in neither of the two mouse studies. This tumour type is well known to occur spontaneously, with a high incidence in the F344 rat strain used in the study.

3.6 REPRODUCTIVE TOXICITY

There are little human reproductive data. However, there have been many studies with experimental animals following exposure to DEHP during gestation and postnatally to sexual maturation. Most of the relevant rat studies focused on effects on male offspring. Several studies of the DEHP metabolites, MEHP and 2-EH have also been performed. These are summarised in Tables 16 and 17, Appendix B.

3.6.1 EFFECTS ON FERTILITY

Human

There are limited data available on the reproductive toxicity of DEHP or its major metabolites in humans. Several studies associating adult male MEHP levels and various reproductive endpoints are described below.

Modigh* et al, (2002) evaluated time-to-pregnancy in the partners of men potentially exposed occupationally to DEHP by inhalation. Median time-to-pregnancy was 3.0 months in the unexposed group, 2.25 months in the low exposure group (< 0.1 mg/m^3), and 2.0 months in the high-exposure group (0.1-0.2 mg/m^3). The authors concluded that

there was no evidence of a DEHP-associated prolongation in time-to-pregnancy, although they recognized that there were few highly exposed men in their sample.

In a series of related studies, spot urinary MEHP and semen and sperm motion parameters and sperm DNA damage were evaluated (Duty* et al, 2003a; Duty* et al, 2003b; Duty* et al, 2004; Duty* et al, 2005). They also evaluated the relationship between serum concentrations of reproductive hormones and MEHP urine concentrations. Subjects included more than 150 men being evaluated in a clinic as part of a fertility evaluation. There were no significant associations between abnormal semen parameters, serum testosterone, sperm DNA damage and MEHP urine concentration above or below the group median.

Jonsson* et al, (2005) studied semen parameters and urinary phthalate monoester levels in 234 military recruits. There were no significant associations between highest versus lowest urinary MEHP quartile and any of the dependent variables.

Breast milk samples were analysed for a variety of phthalate monoesters, including MEHP, in a Danish–Finnish cohort study on cryptorchidism (Main et al, 2006). No association was found between phthalate monoester levels and cryptorchidism.

Cobellis* et al, (2003) measured DEHP and MEHP concentrations in the plasma and peritoneal fluid of 35 women identified by laparoscopy as having endometriosis. There was no difference in the proportion of surgical patients compared to control women with detectable DEHP or MEHP (91.4% compared to 92.6% respectively). There was a significant difference in the median concentration of DEHP in the patients compared to control women (0.57 μg/mL compared to a control value of 0.18 μg/mL) but no difference in median MEHP concentration.

The human studies are limited but are consistent in that they do not identify any significant associations between MEHP and adverse semen parameters, hormone levels, time-to-pregnancy, or infertility diagnosis.

3.6.1.1 ORAL ROUTE

Laboratory Animals

The effects of DEHP on reproductive endpoints have been tested in a variety of species including rats, mice, hamsters, ferrets and marmoset monkeys. The rat was the most sensitive followed by mice, hamsters and ferrets. Marmosets appear to be insensitive to DEHP-induced testicular toxicity. Key studies are described below.

Key studies

Poon et al, (1997) exposed groups of 4-6 week old male and female Sprague-Dawley rats to 0, 5, 50, 500 and 5000 ppm DEHP in the diet for 13 weeks. Animals were reported to reach sexual maturity approximately 50 days into the study and thus were immature for only part of the study. These dietary concentrations corresponded to average DEHP doses of 0, 0.4, 3.7, 38, and 375 mg/kg bw/day for male rats. In the testes, Sertoli cell vacuolation, described as "mild," was observed in 7/10 males in the 500 ppm group, and 9/10 males in the 5,000 ppm group. The highest group also showed bilateral, multifocal, or complete atrophy of the seminiferous tubules with complete loss of spermatogenesis and cytoplasmic vacuolation of the Sertoli cells lining the tubules. There was no measurement of reproductive function. The LOAEL, based on the testicular effects (Sertoli cell vacuolation), was 38 mg/kg bw/day and the NOAEL was 3.7 mg/kg bw/day.

Lamb et al, (1987) gave DEHP to male and female CD-1 mice (20 pairs per breeding group) at dietary levels of 0, 0.01, 0.1, or 0.3% (0, 14, 141, and 425 mg/kg bw/ day) from a 7-day premating period to 21 days after delivering litters (14 weeks in total). Decreased litters and viable pups were observed at 0.1% and above. No pairs were fertile at 0.3%. Also at this dose level (the only dose examined), increased liver weights and decreased weights of the reproductive organs in parental animals (testes, epididymes, prostate, and seminal vesicles) were evident. All but one of the high-dose males showed some degree of bilateral atrophy of the seminiferous tubules. This dose also caused decreased sperm motility and sperm concentration, and increased incidences of abnormal sperm. The LOAEL was 0.1 % (141 mg/kg bw) based on decreased fertility and the NOAEL was 0.01% (14 mg/kg bw/day).

David et al, (2000a) fed 6-week old Fischer 344 rats (50-80 males/group) diets containing 0, 100, 500, 2,500, or 12,500 ppm DEHP (0, 5.8, 29, 147, and 789 mg/kg bw/day for males) for 104 weeks. Testes weight (absolute and relative) was reduced in rats of the high-dose group. Aspermatogenesis was observed in all rats in the highest dose group at study week 78 but not in rats treated with 2500 ppm or in the control group. At study week 105, the incidence of aspermatogenesis was significantly increased in rats exposed to 100 ppm and higher. The percentage of rats with aspermatogenesis from the control to high-dose group was 58, 64, 78, 74, and 97%, respectively. The authors identified the LOAEL for aspermatogenesis as 147 mg/kg bw/d and the NOAEL as 29 mg/kg bw. However, the CERHR Expert Panel on Phthalates concluded that the findings indicate a NOAEL for testis effects of 5.8 mg/kg bw/day, because of the clear dose-response increase in the proportion of each group showing aspermatogenesis. However, this NOAEL may not be reliable because of the high frequency of aspermia in the controls. In fact the authors suggest that an increased incidence of aspermia may be a normal occurrence in the aging rat. The CERHR Expert Panel on Phthalates also suggested that suboptimal testis fixation may have obscured any early vacuolar lesions produced by DEHP.

Schilling* et al, (2001) fed Wistar rats (25 males and females in each group) DEHP in the diet at doses of 0, 1000, 3000, or 9000 ppm (0, 113, 340, or 1088 mg/kg bw/day) for two successive generations, from at least 70 days premating of the first parental generation. Increased focal tubular atrophy in the testis was observed in all treated groups (F0, F1 and F2). Decreased food consumption, body weight gain, testis weights and fertility index were seen in F0 and F1 adults at 9000 ppm. Decreased body weight gains, total number of pups, delayed vaginal opening and preputial separation, and increased the number of stillborn pups were observed in F1 and/or F2 pups at 9000 ppm. Severe effects on testicular histology, sperm morphology, fertility, and sexual development of the offspring occurred in both generations at 9000 ppm. Reduced testis weights in F2 and focal tubular atrophy were observed in male offspring in F1 and F2 pups at 3000 ppm. Focal tubular atrophy also occurred at 1000 ppm. Vacuolisation of Sertoli cells was only observed in atrophic tubuli, which were present in all exposed groups. There was no indication that Sertoli cell vacuolation preceded focal or diffuse tubular atrophy and subsequent loss of sperm production. A NOAEL was not established as Sertoli cell vacuolation was recorded in the F1 offspring generation from the lowest dose level, 1000 ppm (113 mg/kg bw).

Wolfe & Layton (2004) fed Sprague-Dawley rats (17 males and females in each group) DEHP in the diet at concentrations of 1.5, 10, 30, 100, 300, 1000, 7500, and 10000 ppm (0.1, 0.5-0.8, 1.4-2.4, 4.8-7.9, 14-23, 46-77, 359-592, and 543-775 mg/kg bw/day) for two successive generations. The F0 generation began exposure as adults. In the F0

adults, a decreased number of pups per litter were noted at 7500 ppm and above. The only reproductive effects in the F0 rats occurred at 10,000 ppm and included decreases in sperm counts and velocity, reductions in testis and epididymis weights, and increased numbers of rats with small testes in association with minimal-to-marked atrophy of seminiferous tubules characterized by loss of germ cells. The lowest dose level producing effects in F1 and F2 offspring was 7500 ppm and included decreases in number of live pups/litter and reduced male anogenital distance. Fertility was compromised in the F1 rats from the 10,000 ppm group (543 mg/kg bw), which did not produce any viable litters. Other reproductive effects observed in F1 parents were similar to those observed in F0 parents but usually occurred at lower dose levels. For example, in the non-mating F1 adult males of the 300 ppm group there was a small increase in the number of animals (3 of 45) with small testes and/or epididymides. The effects were not observed at the next higher dose (1000 ppm), but small testes were observed in 10 of 30 males of the 7500 ppm non-mating group. Small testes and epididymides were observed in all animals of the 10,000 ppm non-mating F2 group. Small testes and epididymis was also observed in 7500 ppm F3 males. Minimal to marked seminiferous tubule atrophy was noted at 10000 ppm in the F0 and F1 males, and at 7500 ppm in the F1 and F2 males. Minimal atrophy was noted 1 of 10 males in the 100 and 300 ppm F1 groups. While Sertoli cell vaculoation was observed in seminiferous tubules of the 1000 and 7500 ppm F1 males (not 10000 ppm males), the vacuolation was similar to that in the controls. It was concluded by the Pathology Working group of the Swedish Inspectorate that the vacuolation observed resulted from distortion during fixation and processing of the tissues (National Chemical Inspectorate, 2005). This distortion could also have obscured any minimal toxic effects that may have been present. Increased kidney weight was observed at 1000 ppm with accompanying histopathological changes at 1000 ppm. At doses below 1000 ppm, hepatocellular effects were the only signs of toxicity. The LOAEL was 300 ppm (14-23 mg/kg bw) based on small male reproductive organ size and histopathologic changes in the testes in the F1 generation. The isolated case of a single male showing atrophy of seminiferous tubules in testis at 100 ppm was not considered significant, as there were no other accompanying findings. The NOAEL was 100 ppm (4.8 mg/kg bw/day).

Akingbemi et al, (2001; 2004) exposed Long-Evans rats during pregnancy, lactation, and post-weaning stages to examine the effect of DEHP on Leydig cell testosterone biosynthesis. In the first series of experiments, pregnant rats were exposed to DEHP on GD 12–21, young rats were exposed on PND 21–34, 21–48, 35–48 or 62–89. DEHP was administered by gavage in corn oil at 0 or 100 mg/kg bw/day to pregnant rats or 0, 1, 10, 100, or 200 mg/kg bw/day (n = 10/dose group) to young rats. In the second series of experiments, young rats were exposed to DEHP on PND 21–48, 21–90 or 21-120 PND 62–89. DEHP was administered by gavage in corn oil at 0, 1, 10 or 100 mg/kg bw/day (n = 10/dose group). Within 24 hours of the final dose, measurements of LH and testosterone were made and the animals killed. Testicular histology was evaluated. Leydig cells were obtained from rats and incubated with LH to stimulate testosterone synthesis. Leydig cells were also incubated with testosterone biosynthesis substrates and enzyme activity measured.

There were no treatment-related effects on body weight gain or feed consumption. There was no effect of DEHP treatment in young adults at any tested dose on serum testosterone or LH or on *in vitro* Leydig cell steroidogenesis. There were no effects of any treatment on testicular histology. Treatment of prepubertal rats for 14 days (PND 21–34 or PND 35–48) did not produce alterations of serum LH or testosterone, but longer treatment (on PND 21–48) produced a dose-related increase in serum LH and

testosterone and interstitial fluid testosterone that was statistically significant at 10 mg/kg bw/day. This study suggests that younger rats are more sensitive to the effects of DEHP.

Leydig cells isolated from prepubertal rats that had been treated on PND 21–34 or 35-48 showed a decrease in basal and LH-stimulated testosterone production at and above 100 and 10 mg/kg bw/day, respectively. There were no treatment effects on cultured Leydig cells derived from 35- and 90-day-old rats that had been prenatally exposed to 100 mg/kg bw DEHP. Paradoxically, exposure of prepubertal rats for 28 days (PND 21-48) was associated with an increased Leydig cell synthesis of testosterone and LH. Exposure on PND 35-48 affected all tested enzyme activities, with the most sensitive being 17β-hydroxysteroid dehydrogenase (reduced 74% at 10 mg/kg bw/day compared to control; other enzyme activities were significantly reduced at DEHP dose levels of 100 or 200 mg/kg bw/day). There were no effects of any treatment on testicular histology in any of the groups exposed during pregnancy, lactation, and post-weaning stages. The LOAEL was 10 mg/kg bw for increased serum LH and testosterone in rats exposed from PND 21-48 with an NOAEL of 1 mg/kg bw.

In a follow-up study, Long-Evans rats were gavaged with 0, 10, or 100 mg/kg per day DEHP from PND 21 to PND 48, 90, or 120 (Akingbemi et al, 2001; Akingbemi et al, 2004). Serum LH, testosterone and estradiol were measured and RT-PCR was used to measure activity of markers of cell division. On PND 120, an increase in serum LH and testosterone was identified only in the animals exposed to DEHP at 100 mg/kg bw/day, but a decrease in basal and stimulated testosterone production in culture occurred, suggesting Leydig cell hyperplasia. mRNA levels were increased in both DEHP exposure groups for some of the markers for cell division. Leydig cell numbers were also increased in both DEHP dose groups. Serum estradiol was increased on PND 48 but not PND 90 in rats in both DEHP exposure groups.

Dostal et al, (1988) gave Sprague-Dawley rats oral doses of 0, 10, 100, 1000, or 2000 mg/kg bw of DEHP (>99% pure) by gavage in corn oil for 5 days (7-10 animals per group) at 1, 2, 3, 6, and 12 weeks of age. Absolute and relative testis weights were significantly reduced at doses of 1000 mg/kg bw/day in 1, 2, 3, and 6- week-old but not in 12-week-old rats compared to controls of the same age suggesting, differential age sensitivity. Doses of 2000 mg/kg bw/day were fatal to suckling rats and caused decreased relative testis weight but no lethality was observed in 6- and 12-week-old rats. The number of Sertoli cell nuclei per tubule was reduced by 35% at 1000 mg/kg bw in neonatal rats; two- and three-week old rats showed loss of spermatocytes but not of Sertoli cells. Loss of spermatids and spermatocytes in 6- and 12-week old rats at 1000 and 2000 mg/kg bw was also observed. These results suggest that Sertoli cells are more sensitive during their proliferative stage.

Sjoberg et al, (1986a) studied the age-dependent testis toxicity of DEHP (1000 and 1700 mg/kg bw in the diet for 14 days) in rats at 25, 40, and 60 days of age. Body weight gain was retarded in all dosed groups and testicular weight was markedly reduced in 25- and 40-day-old rats given 1700 mg/kg bw. Severe testicular damage was observed in the 25-day- and 40-day-old rats at both dose levels. No changes were found in the 60-day-old rats.

Wistar rats were dosed with 0, 0.015, 0.045, 0.135, 0.405, 1.215, 5, 15, 45, 135 or 405 mg /kg bw/day DEHP by gavage on GD6 to PND21 (approximately 10/group) (Andrade et al, 2006; Grande et al, 2006). DEHP had no effect on litter size, sex ratio or pup weight. On PND 22, testis weight was significantly increased above 5 mg/kg/day, germ cell differentiation in seminiferous tubules was reduced in exposed

animals at 135 and 405 mg DEHP/kg/day and anogenital distance (AGD) was significantly reduced in animals exposed to the highest dose (405 mg/kg/day).

A single bolus dose of DEHP (20, 100, 200, and 500 mg/kg bw) was given in corn oil to five neonatal rat (three-day old, CD Sprague-Dawley) pups per group (Li et al, 2000). MEHP (393 mg/kg bw), 2-EH (167 mg/kg), or vehicle was administered by gavage to 4 pups per group. All pups were killed 24 hours after dosing. The doses of MEHP and 2-EH were molar equivalent with 500 mg/kg DEHP. A time-course study was also conducted following a single dose of DEHP (200 mg/kg bw), where the pups were killed after 6, 9, 12, 24, or 48 h. Morphological examination revealed a dose-dependent presence of abnormally large, multi-nucleated germ cells (gonocytes) by 24 h post-treatment with DEHP (100-500 mg/kg bw). Sertoli cell proliferation was dose-dependently decreased from 100-500 mg/kg bw DEHP but not 20 mg/kg bw DEHP. There was a rebound in Sertoli cell proliferation at 48 hours following treatment with 200 mg/kg bw DEHP. MEHP (single dose group) induced similar effects as DEHP. A NOAEL for young pups of 20 mg/kg bw is derived for effects on altered gonocyte morphology and decreased Sertoli cell proliferation by a single oral dose of DEHP.

Three studies have administered oral doses of DEHP to pre- and post-pubertal marmosets for varying durations (Kurata* et al, 1998; Mitsubishi Chemical Safety Institute,*, 2003; Tomonari et al, 2006). Reproductive outcomes were assessed.

Kurata* et al, (1998) administered groups of 4 male and 4 female 12–15 month old (post-pubertal) marmosets doses of 0, 100, 500, or 2500 mg/kg bw/day DEHP in corn oil by gavage for 13 weeks. There were no treatment-related decreases in testis weight, testosterone and estradiol levels. There were no testicular histopathological changes even at the highest dose. The NOAEL is 2500 mg/kg bw/day.

The authors of the Mitsubishi-Chemical-Safety-Institute (2003*), in an unpublished report, administered DEHP by gavage in corn oil to juvenile marmosets (9 males and 6 females) beginning at 90–115 days of age until 18 months of age (young adulthood) at dose levels of 0, 100, 500, and 2500 mg/kg bw/day. Both males and females were assessed with in-life hormonal assays and with histopathology at necropsy. The results suggest little effect on testicular structure or function. Mean serum testosterone levels were highly variable, but the data suggested the possibility of a delay in the onset of puberty in male marmosets with increasing DEHP dose. Body weights and male organ weights were not affected. The NOAEL is 2,500 mg/kg bw/day.

Tomonari et al, (2006) gave 90-115 day old marmosets (5-6/sex/group) 0, 100, 500 or 2500 mg/kg bw by gavage for 65-weeks. Blood samples were taken throughout the study and analysed for DEHP, MEHP, zinc and testicular enzyme activity. At the end of the study the liver and primary and secondary sex organs were weighed and examined histologically. There were no treatment-related changes in male organ weights, no microscopic changes in male gonads, secondary organs, Leydig, Sertoli or spermatogenic cells. No increases in hepatic peroxisomal enzyme activities were noted. The NOAEL is 2,500 mg/kg bw/day.

3.6.1.2 INHALATION ROUTE

Laboratory Animals

Male Wistar rats (4 weeks old) were dosed with 5 or 25 mg/m^3 DEHP, 6 h per day for 4 or 8 weeks (Kurahashi et al, 2005). Plasma testosterone concentration and seminal

vesicle weight increased in both groups after 8 weeks. There were no significant differences in testicular histopathology.

In an earlier 4-week inhalation study conducted according to OECD guideline 412, male Wistar rats (10 rats per group) were exposed 5 days per week, 6 hours per day to 0, 0.01, 0.05, or 1 mg DEHP/L (0, 10, 50, or 1 000 mg DEHP/m^3) (99.7% pure) as liquid aerosol (Klimisch* et al, 1992). The males were mated to untreated females. No effects on male fertility were observed 2 and 6 weeks after the end of exposure and no testicular toxicity was detected histologically. However, the results were not presented and peroxisome proliferation was not observed.

3.6.1.3 DERMAL

There are no laboratory animal or human reproductive toxicology studies of DEHP delivered by the dermal route of exposure.

3.6.1.4 PARENTERAL ROUTE

Laboratory Animals

Only three (of 16) published reproductive toxicology studies with parenteral routes of exposure are useful for hazard identification. The earlier studies were considered to have significant limitations as there was either doubt over the dose administered or reproductive outcomes were not assessed.

Sjoberg* et al, (1985c) exposed 25 or 40 day old rats to 0, 5, 50 or 500 mg/kg bw intravenously every other day for 10 days (time-weighted average 0, 2.5, 25 or 250 mg/kg bw/d). There was no change in testes weight but vacuolization of the Sertoli cells and spermatocyte degeneration was observed at 250 mg/kg bw/d. The NOAEL was 25 mg/kg bw/d.

In the second study neonatal male rats or rabbits were injected with either 62 mg/kg bw/day DEHP or 4% bovine serum albumin during postnatal days 3-21 (rats) or 14-42 (rabbits) (Baxter Healthcare Corporation*, 2000). Histopathological examination of the testes and other organs of DEHP-exposed animals revealed no alterations.

Similarly, Cammack et al, (2003) conducted a 21-day repeat dose study of DEHP in neonatal (3- to 5-day old) rats. Rats were injected with 0, 60, 300 or 600 mg/kg bw/day or gavaged with 300 or 600 mg/kg bw/day. A second group of animals was dosed for 21 days then held for a recovery period until 90 days of age. At the end of the 21-day dosing period, testicular atrophy, decreased seminiferous tubules diameter and mild depletion of germinal epithelial cells were observed at 300 mg/kg bw/day. Although testicular atrophy persisted at the end of the recovery period, histopathological changes were not observed in the recovery group previously exposed to a DEHP dose of 300 mg/kg bw/day for 21 days. At equivalent doses, oral exposure induced more significant changes in testicular weight and pathology. The NOAEL for intravenous exposure in the study was 60 mg/kg bw/day and the LOAEL was 300 mg/kg bw/day.

The NOAEL for intravenous exposure to DEHP is therefore considered to be 60 mg/kg bw/d (Cammack et al, 2003), and the LOAEL is 250 mg/kg bw/d (Sjoberg* et al, 1985c) based on vacuolization of the Sertoli cells and spermatocyte degeneration.

3.6.1.5 MEHP

Sprague-Dawley rats and Swiss-Webster mice were intraperitoneally injected with 0, 50, or 100 mg/kg bw DEHP every other day for 20 days (Curto* & Thomas, 1982). In addition, rats and mice were injected subcutaneously with MEHP at 0, 1, 5, or 10 mg/kg bw/day for 5 days, 5, 10, or 20 mg/kg bw/day for 10 days or 0, 50, or 100 mg/kg bw/day IP for 5 days in mice or 0, 50, or 100 mg/kg bw every other day for 20 days in mice and rats. In rats, prostate zinc levels were reduced after 50 mg/kg bw/d MEHP or 100 mg/kg bw/day DEHP. DEHP induced a significant loss of testicular zinc whereas MEHP did not. In mice there were no effects on reproductive organ weights or zinc levels, but death occurred in 3/6 mice exposed IP to 100 mg/kg bw/day for 5 days.

The testicular toxicity of DEHP and MEHP (>98% pure) was studied in male Wistar rats (26 days old, 6 animals per group) after a single oral dose (Teirlynck et al, 1988). Rats received 2800 mg/kg bw of DEHP or 400 or 800 mg/kg bw MEHP. The doses were selected in accordance with previous data showing that oral administration of 2800 mg/kg bw DEHP and 400 mg/kg bw MEHP leads to similar MEHP plasma levels. Seven days after dosing the rats were killed. The rats showed testicular atrophy 7 days after dosing, as indicated by a significant reduction in relative testicular weight. Histological examination revealed a "dose-dependent" increase in the number of atrophic seminiferous tubules with decreased diameters of the seminiferous tubules and loss of spermatids and spermatocytes. The study suggests that MEHP is more toxic to the testes than DEHP.

Five week old male guinea pigs that received a single oral dose of 2000 mg/ml of MEHP in corn oil by gavage were sacrificed at 3, 6, and 9 h, and testicular tissues were processed for histopathological studies (Awal et al, 2005). Detachment and displacement of spermatogenic cells, thin seminiferous epithelia, vacuolisation of Sertoli cells were prominent at 6 h after MEHP treatment. The lumina of the efferent ductules were frequently occupied with sloughed seminiferous epithelia from 6 to 9 h after MEHP treatment. The incidence of apoptotic spermatogenic cells was significant from 3 to 9 h, and the maximal increase of apoptotic spermatogenic cells were observed at 9 h post MEHP treatment.

Dalgaard et al, (2001) studied the effects of MEHP on 28-day old male rats by looking at testicular morphology and apoptosis, and expression of some cellular markers (vimentin filaments, the androgen receptor, and a gene coding a Sertoli cell secretory product) 3, 6, or 12 hours (n=12) after a single oral dose of 400 mg/kg MEHP. At 3-12 hours vimentin filaments in Sertoli cells had collapsed, and the expression of the apoptosis gene Caspase-3 was increased. However, there were no other indications of apoptosis as measured by DNA ladder analyses or TUNEL staining. At 3 hours there were no histological signs of toxicity, but at 6 and 12 hours the tubuli were disorganised and germ cells detached and sloughed into the lumen of the seminiferous tubules. The results support Sertoli cells being early targets for MEHP toxicity.

Prepubertal male Fischer rats (28-day-old; number not stated) were given a single 2000 mg/kg dose of MEHP (95% pure) in corn oil by gavage (Richburg* & Boekelheide, 1996). The rats were killed at 3, 6, and 12 hours after treatment. MEHP induced collapse of Sertoli cell vimentin filament 3 hours after MEHP-administration. In control testes 44.5% of the seminiferous tubule cross-sections did not contain any apoptotic cells. However, 3 hours after MEHP treatment, the number of tubule cross sections with no incidence of apoptosis significantly increased to 63.3%. This shift was reflected by a significant decrease in the incidence of tubules containing 1-3 apoptotic cells per

cross section at 3 hours. Cross sections of the seminiferous tubules from the 6- and 12-hours groups showed a dramatic increase in the number of apoptotic events as evident by the increased incidence of seminiferous tubules which contained high categories of apoptotic germ cells and a decrease in the incidence of seminiferous tubule cross sections that contained no apoptosis.

Young male Sprague-Dawley rats (six/group 4-6 weeks old) and DSN strain Syrian hamsters (8/group) were administered 1000 mg/kg bw/day MEHP by oral intubation for 5 (rats) or 9 days (hamster) (Gray et al, 1982). Animals were also dosed with 2800 mg/kg bw/day (rats) or 4200 mg/kg bw/day (hamsters) DEHP for 9 days. In the rat, DEHP and MEHP equally resulted in reduced testes weight and considerable tubular atrophy. MEHP, but not DEHP, produced a significant reduction in testis weight in hamsters. The testes from the MEHP-exposed hamsters all showed tubular atrophy but the extent and severity of the lesion was less than that seen in rats or in hamsters exposed to DEHP. The rate of intestinal monohydrolysis of DEHP to MEHP was significantly slower in hamsters than in rats. This may explain the difference in focal seminiferous tubular atrophy induction by DEHP and MEHP in hamsters.

3.6.1.6 Mechanistic studies

The National Chemical Inspectorate (2005) reviewed current mechanisms of phthalate-mediated effects on the male reproductive system in rats. Studies have shown that DEHP and MEHP may exert a direct effect on Leydig cell structure and function as determined by testosterone output and also that DEHP and MEHP produce similar changes *in vivo* and *in vitro* in both Leydig and Sertoli cells.

Age-Differences in Vulnerability to Reproductive Toxicity

Exposure of neonates, pubertals, and adults to DEHP causes significant changes in the morphology and function of the Sertoli cells. Developing rats have been found to be much more sensitive to DEHP than adults (Sjoberg* et al, 1985a; Sjoberg et al, 1986a; Dostal et al, 1988; Arcadi* et al, 1998; Wolfe & Layton, 2004). The younger animals responded to a much lower dose or produced a more serious lesion with a comparable dose on a mg/kg bw/day basis.

Younger (developing) animals show adverse testicular effects at lower doses than older ones (Sjoberg et al, 1986a; Dostal et al, 1988; Akingbemi et al, 2001). One study also demonstrated that the number of Sertoli cell nuclei per tubule was reduced in neonatal rats; two- and three-week old rats showed loss of spermatocytes but not Sertoli cells (Dostal et al, 1988). In 6- and 12-week old rats, spermatids and spermatocytes were lost. These results demonstrated that Sertoli cells were most sensitive during their proliferative stage.

In the rat, testicular cord formation and Sertoli cell differentiation begins on gestational day (GD) 13 (reviewed in Li & Kim, 2003). The testis is distinguished morphologically on GD14, when the germinal ridge is formed and a few germ cells are seen in the gonadal cord. On GD16, the testicular cord becomes prominent, containing Sertoli cells on its margin. On GD18, the interstitium has widened in the centre of the gonad containing rich interstitial cells, while the density of germ cells in the reproductive tract increases. Sertoli cells are proliferating and gonocytes are mitotically quiescent by GD 18. On GD20, the testicular cord has developed further, although the tubular structure has not yet formed. Sertoli cell proliferation continues through PND 3 and gonocytes migrate to the seminiferous tubules and become mitotically active. Sertoli cells are the supportive cells in the seminiferous epithelium of the testes. They orchestrate

spermatogenesis by providing structural and nutritional support to germ cells. Sertoli cell proliferation stops at puberty and each Sertoli cell can only support the development of a fixed number of germ cells in an adult. The tight junctions that constitute the Sertoli barrier are established between postnatal days 10 – 20 and allow the establishment of a luminal fluid environment within the seminiferous tubules (Foley, 2001). One of the most common morphological responses of the Sertoli cell to injury is vacuolation. Subsequent to vacuolation and/or swelling of the Sertoli cells, germ cell degeneration, disorganization, or exfoliation is generally seen. Thus Sertoli cell vacuolation is seen as a precursor to germ cell loss and tubular atrophy. (Creasy, 2001).

The differences in age effects may be due to differing intestinal absorbance of DEHP. Gray* & Gangolli (1986) reported that intestinal absorption of DEHP/MEHP is greater in developing rodents than in adults, suggesting that developing rodents may be susceptible to DEHP toxicity at lower levels of exposure. Similarly, Sjoberg* et al, (1985b) found that gavage treatment with DEHP resulted in greater absorption of MEHP, and hence, a greater systemic dose to young, as compared to mature rats.

Species Differences in Sensitivity to Reproductive Toxicity

There are species differences in sensitivity to the testicular effects of DEHP. Rats, mice, and guinea pigs are the most sensitive while young adult marmosets and young adult cynomolgus monkeys exposed to high doses of DEHP show no signs of testicular toxicity.

The absence of testicular effects in marmosets can be explained, at least in part, by the poor absorption and metabolism of high doses of DEHP (see Section 3.1 Toxicokinetics).

Also, a recent review of testicular function and control in the marmoset suggests that the marmoset may be a poor model for reproductive studies as the marmoset, unlike rats, humans and other Old World primates, are relatively insensitive to steroids as they have relatively high free levels of steroids (Li et al, 2005). DEHP has been shown to reduce basal and LH-stimulated testosterone *in vitro*. If the mechanism of action of DEHP involves reduced steroid levels then marmosets may be resistant to the reproductive effects of DEHP.

Effect of Route of exposure

DEHP exposures via the intravenous route bypass the intestinal esterases, so the amount of MEHP in organs and tissues would be expected to be lower following intravenous injection of DEHP. However, studies in human and animals suggest that following intravenous injection of DEHP, the DEHP concentration initially rises but declines rapidly; thereafter MEHP levels increase. MEHP is considered to be the proximate reproductive toxicant and is a more potent toxicant than DEHP following single doses *in vivo*. *In vitro* studies also support the conclusion that MEHP is a more potent reproductive toxicant than DEHP (Gangolli*, 1982; Sjoberg* et al, 1986b; Li et al, 1998). It is therefore feasible that DEHP delivered by the intravenous route of exposure may be less toxic than by the oral route of exposure.

There are no adequate reproductive toxicity studies following inhalation or dermal exposure to DEHP. For intravenous exposure, the NOAEL for Sertoli cell damage was 25 mg/kg bw/day following exposure of adult rats to DEHP every other day for 10 days (Sjoberg* et al, 1985c). However, the NOAEL in the more sensitive, younger rats was 60 mg/kg bw/day after 21 days exposure (Cammack et al, 2003).

The NOAELs and LOAELs identified for intravenous exposure to DEHP are higher than those reported following oral exposure to DEHP. This may reflect a real difference in effect or differences in study design. The lowest NOAELs for the oral studies were derived from long-term exposure or encompassed early development. There are no similar longterm intravenous studies of the effects of DEHP in adult or neonatal animals. Oral exposure to DEHP induced more significant changes in testicular weight and pathology compared to intravenous exposure in a study using the same high equivalent doses (300 and 600 mg/kg bw/day) and short duration (21 days) (Cammack et al, 2003). The lowest NOAEL following oral exposure of neonatal rats for short durations (5 days) was 100 mg/kg bw/day (Dostal et al, 1988). It is not known if this observation would also occur at lower doses or longer durations. There is currently insufficient data to demonstrate whether intravenous exposure to DEHP is truly less toxic than oral exposure to DEHP or how relevant is the NOAEL of 60 mg/kg bw/day for longer exposure duration.

Conclusions

There is very limited human data examining the reproductive effects of DEHP. These studies largely examine the relationship between urine levels of the DEHP metabolite, MEHP, and differing measurements of male and female reproductive health. The studies were small and largely negative.

There are many experimental animal studies, primarily utilising the oral exposure route in rats and mice. The most sensitive endpoint, perturbations in testicular structure and function, have been consistently observed in several reproductive toxicity studies in rats and mice by both oral and parenteral routes of exposure (NTP *, 1982; Sjoberg* et al, 1985c; Lamb et al, 1987; Poon et al, 1997; David et al, 2000a,b; Akingbemi et al, 2001; Schilling* et al, 2001; Cammack et al, 2003; Akingbemi et al, 2004; Wolfe & Layton, 2004). *In vivo* and *in vitro* assays have demonstrated that the Sertoli cell is the most sensitive target of toxicity, causing subsequent germ cell depletion. Rats appear to be more sensitive than mice for testicular effects.

Increased LH and testosterone levels in Leydig cells were observed at 10 mg/kg bw/day with no effects at 1 mg/kg bw/day in 3 week old rats exposed for 28 days (Akingbemi et al, 2001). For testicular histopathology, the NOAEL and LOAEL were 3.7 and 38 mg/kg bw/day, respectively, in 4-6 week old rats exposed for 90 days (Poon et al, 1997). Decreases in testicular weight were reported at higher doses. For the endpoint of fertility, a NOAEL of 14 mg/kg bw/day is derived from a continuous breeding study in adult mice (Lamb et al, 1987). The LOAEL was 141 mg/kg bw/day, where the effects were reduced fertility.

The consistent finding of testicular effects in rats and mice is in contrast to studies in marmosets (Kurata* et al, 1998; Mitsubishi-Chemical-Safety-Institute*, 2003; Tomonari et al, 2006). No treatment-related changes in testicular histology or more gross parameters were observed at the highest dose used (2500 mg/kg bw).

The critical study for reproductive toxicity in neonatal males was Wolfe & Layton (2004). The NOAEL was 4.8 mg/kg bw/day and the LOAEL was 37.8 mg/kg bw/day. Other studies have similar NOAELs and LOAELs (Akingbemi et al, 2001; Poon et al, 1997; David et al, 2000a).

For the purpose of risk characterisation for adults, the oral NOAEL for fertility of 14 mg/kg bw/day is selected. For neonates and pre-pubertal males the oral NOAEL for testicular pathology of 5 mg/kg bw/day is selected. There are no long-term intravenous studies to use as a basis for selection of a parenteral NOAEL.

3.6.2 DEVELOPMENTAL EFFECTS

Human Data

There have been several studies in humans in which development of the male reproductive system has been evaluated with respect to estimates of DEHP exposure during pregnancy or early childhood.

Cord blood samples were collected from 84 consecutive newborns (including a set of twins) delivered at an Italian hospital (Latini et al, 2003b). DEHP and/or MEHP were detected in 74 of 84 cord blood samples with a mean (range) DEHP cord blood serum concentration of 1.19 (0–4.71) μg/mL and MEHP of 0.52 (0 – 2.94 μg/mL). Mean gestational age, but no other parameter, was significantly lower in MEHP-positive neonates (38.16 weeks) versus MEHP-negative neonates (39.35 weeks). However, the levels measured in blood were unusually high compared to other studies.

Association between maternal urinary phthalate monoester concentrations and genital parameters such as anogenital distance and testicular descent in children was determined in 85 mother-son pairs (Swan et al, 2005). There was no significant association between maternal urinary MEHP concentration and infant anogenital index. However, urinary concentrations of four other phthalate metabolites [monoethyl phthalate (MEP), mono-*n*-butyl phthalate (MBP), monobenzyl phthalate (MBzP), and monoisobutyl phthalate (MiBP)] (but not MEHP) were inversely related to anogenital index (AGD/weight). This study has been criticised by McEwen & Renner (2006) from the Cosmetic and Fragrance Associations of America and Europe. They suggested that anogenital distance is more likely to be proportional to height rather than weight and that maternal phthalate urinary concentrations were not normalized for urine volume. The reliability of the measurement of anogenital distance has not been verified in humans. The one study that did assess the reliability of AGD measures found the reliability of the measurement in males to be poor (0.48) (Salazar-Martinez et al, 2004).

Main et al, (2006) reported phthalate concentrations in milk collected from 1-3 months after birth by 65 Finnish and 65 Danish women as part of a study of cryptorchidism and hormone levels in male children. Phthalate monoesters (mono-methyl phthalate (mMP), MEP, MBP, MBzP, MEHP and mono-isononyl phthalate (miNP)) were measured in milk and gonadotropins, sex-hormone binding globulin (SHBG), testosterone, and inhibin B serum samples were measured in the serum of breast fed boys. Cryptorchidism was identified in 62 of the 130 children of these women; however, there was no significant association between milk phthalate concentrations and cryptorchidism.

Rais-Bahrami* et al, (2004) examined onset of puberty and sexual maturity parameters in 14–16-year-old adolescents (13 males and 6 females) who had been subjected to ECMO as neonates. Pubertal development was normal. LH, FSH, testosterone, and 17β-estradiol levels were normal.

Colon et al, (2000) compared blood phthalate levels in 41 premature thelarche patients and 35 controls. There was a statistically significant difference in average blood DEHP levels. DEHP was detected in 25 of the samples from premature thelarche patients at a mean concentration of 450 μg/L (187 - 2098 μg/L); MEHP concentration ranged from 6.3 to 38 μg/L. DEHP was detected in 5 of 35 blood samples from control patients at a mean concentration of 70 μg/L (276–719 μg/L). The reported levels in the control group are unusually high compared with the background MEHP concentration in urine

in the normal population (mean 4.27 μg/L, range 3.80–4.79 μg/L; Silva et al, 2004) and may reflect patient exposure to medical procedures within the hospital.

The data are insufficient to evaluate the prenatal effects of DEHP exposure in humans.

3.6.2.1 ORAL

Laboratory Animals

Numerous studies have shown that DEHP is embryotoxic in rats at doses close to maternally toxic levels. In mice, several studies have shown that DEHP is embryotoxic and teratogenic at dose levels below those producing observable evidence of toxicity to the dams. These studies are summarised in Table 16, Appendix B. Key studies from which a developmental NOAEL or LOAEL can be derived are described below.

Key studies

The multi-generation study of Wolfe & Layton (2004) largely complied with OECD Guideline 416. Sprague-Dawley rats were fed DEHP in the diet at concentrations of 1.5, 10, 30, 100, 300, 1000, 7500, and 10000 ppm (0.1, 0.5-0.8, 1.4-2.4, 4.8-7.9, 14-23, 46-77, 359-592, and 543-775 mg/kg bw/day) for two successive generations. The authors stated that clinical signs were generally comparable among all groups in all generations and were not treatment-related in incidence or severity. The F0 generation were exposed throughout pregnancy and allowed to litter. This F1 generation was examined at birth. In the F0 adults, a decreased number of pups per litter were noted in adult rats at 7500 ppm (592 mg/kg bw) and above. The lowest dose level producing effects in F1 offspring was 7500 ppm (391 mg/kg bw) and included decreases in number of live pups/litter, reduced male anogenital distance and delayed vaginal opening. The NOAEL for developmental effects was 1000 ppm (48 mg/kg bw/d) and the LOAEL was 7500 ppm (391 mg/kg bw/d) based on reduced anogenital distance.

Schilling* et al, (2001) fed Wistar rats (25/group) DEHP in the diet at doses of 0, 1000, 3000, or 9000 ppm (0, 113, 340, or 1088 mg/kg bw/day) for two successive generations beginning 70 days prior to mating. Increased focal tubular atrophy in the testis was observed in all treated groups (F0, F1 and F2). Decreased food consumption, body weight gain, testis weights and fertility index were observed in F0 and F1 adults at 9000 ppm. Decreased body weight gain, total number of pups, delayed vaginal opening and preputial separation, and increased numbers of stillborn pups were observed in F1 and/or F2 pups at 9000 ppm. Decreased anogenital distance was observed from 1000 ppm and was statistically significantly different from 3000 ppm. With continuing exposure, severe effects on testicular histology, sperm morphology, fertility, and sexual development of the adult offspring occurred in both generations at 9000 ppm. Reduced testis weights in F2 and focal tubular atrophy were observed in male offspring in F1 and F2 pups at 3000 ppm. Focal tubular atrophy also occurred at 1000 ppm. Vacuolisation of Sertoli cells was only observed in atrophic tubuli, which were present in all exposed groups. A developmental NOAEL was not established as Sertoli cell vacuolation was recorded in the F1 offspring generation from the lowest dose level, 1000 ppm (113 mg/kg bw).

Dietary levels of 0, 0.025, 0.05, 0.10, or 0.15% of DEHP (0, 44, 91, 190.6, or 292.5 mg/kg bw/day) were administered to mice throughout gestation (days 0-17) (NTIS*, 1984; Tyl* et al, 1988). Reduced maternal body weight gain was noted at 0.1% and above, mainly due to reduced gravid uterine weight. Increased resorptions, late foetal deaths and malformed foetuses, and decreased foetal weight and viable foetuses were

observed at 0.1% and above. Increased malformed foetuses were seen at 0.05% and above. The external malformations included unilateral and bilateral open eyes, exophthalmia, exencephaly, and short, constricted or no tail. Visceral malformations were localised predominantly in the major arteries. Skeletal defects included fused and branched ribs and misalignment and fused thoracic vertebral centra. The NOAEL for maternal toxicity was 0.05% (91 mg/kg bw/day) and for developmental toxicity was 0.025% (44 mg/kg bw/day).

The effect of DEHP on Leydig cell function in male Long-Evans rats exposed *in utero* (GD 12-21), during nursing or post-weaning stages has been evaluated (Akingbemi et al, 2001). DEHP was administered to dams by gavage in corn oil at 0 or 100 mg/kg bw/day. Males were obtained for evaluation on PND 21, 35, or 90 (n = 7 dams/group/stage). There were no effects of treatment during gestation on dam weight or weight gain or on offspring weight. Offspring testis and seminal vesicle weights were also not affected by treatment during gestation. Serum testosterone was reduced 31–33% and serum LH was reduced 50–64% in 21- and 35-day-old males exposed to DEHP during gestation. There were effects on serum testosterone or LH in 90-day-old males. Prenatal exposure to DEHP resulted in decreased testosterone production by cultured progenitor Leydig cells obtained from 21-day-old males. Basal testosterone production was reduced 47%, and LH-stimulated testosterone production was reduced 56%.

Pregnant rats received DEHP by gavage at doses of 0, 40, 200, or 1000 mg/kg bw/day from day 6 to 15 of gestation (BASF AG*, 1995; Hellwig* et al, 1997). Reduced uterus weights and increased relative kidney and liver weights were observed in dams at 1000 mg/kg bw/day. Also at this dose, decreased viable foetuses and foetal body weights, and increased implantation loss, external and skeletal malformed foetuses (predominantly of the tail, brain, urinary tract, gonads, vertebral column, and sternum) and foetuses with soft tissue, skeletal variations and retardations were seen. The NOAEL for maternal and developmental toxicity was 200 mg/kg bw/day.

DEHP at doses of 0, 40, 200, or 1000 mg /kg bw/day was administered by gavage to pregnant mice (15/group) from days 6 to 15 of gestation (Huntingdon*, 1997). At day 17 of gestation, decreased viable pups and increased resorptions and post-implantation loss were observed at 1000 mg/kg bw/day. Cardiovascular abnormalities, tri-lobed left lungs, fused ribs, fused thoracic vertebral centres and arches, immature livers, and kidney anomalies were also observed at this dose. At 200 mg/kg bw/day, there was a slight increase in foetuses with intra-muscular or nasal haemorrhage or dilated orbital sinuses. There also were a small number of foetuses with anomalous innominate or azygous blood vessels at this dose level. A NOAEL of 200 mg/kg bw/day was established for maternal toxicity and 40 mg/kg bw/day for developmental toxicity.

Female rats received DEHP in drinking water at 3.0-3.5 and 30-35 mg/kg bw/day from day 1 of pregnancy to 21 days after delivery (Arcadi* et al, 1998). Decreased pup kidney weights were observed at both doses, accompanied by histopathological findings (shrinkage of renal glomeruli with signs of glomerulonephritis, dilation of renal tubuli and light fibrosis) between weeks 0 and 4 of age. Lower testicular weights were observed, associated with severe histopathological changes which included only a few elongated spermatids in tubules showing a pervious lumen at low dose level and a generalized disorganization of the tubular epithelium with spermatogonia detached from the basal membrane, absence of elongated spermatids and spermatozoa, and with the tubular lumen filled with cellular deposits at high dose level. No NOAEL was

established. There is doubt regarding the delivered dose in this experiment as DEHP which is not soluble in water was delivered as a suspension.

Pregnant Wistar rats were gavaged from GD 7 to 21 with vehicle or 10, 30, 100 or 300 mg/kg bw/day of DEHP. Male foetuses were examined on GD 21 (Borch et al, 2006). No maternal effects were reported. Testicular testosterone production *ex vivo* and testicular testosterone levels were reduced significantly at the highest dose. Histopathological effects on gonocytes were observed at 100 and 300 mg/kg bw/day. At the highest dose level Leydig cell effects and vacuolisation of Sertoli cell were observed. There was reduced testicular mRNA expression of the steroidogenesis related factors and reduced mRNA expression of a nuclear receptor involved in regulating steroid synthesis at the two highest doses. Even at the highest dose there was no change in PPAR-a mRNA expression. The NOAEL for developmental effects was 30 mg/kg bw/day, based on testicular pathology at 300 mg/kg bw/day.

Wistar rats were dosed with 0, 0.015, 0.045, 0.135, 0.405, 1.215, 5, 15, 45, 135 or 405 mg/kg bw/day DEHP by gavage on GD6 to PND21 (approximately 10/group) (Andrade et al, 2006; Grande et al, 2006). DEHP had no effect on litter size, sex ratio or pup weight. There was a significant increase in dam liver and kidney weight at 405 mg/kg/day. There was also a significant increase in liver weight on PND1 (but not PND22) in offspring exposed to 135 and 405 mg/kg/day. Histopathological examination of the testis on PNDs 1 and 22 revealed changes at 135 and 405 mg DEHP/kg/day. The most prominent finding on PND 1 was the presence of bi- and multinucleated gonocytes. Exposure continued through lactation. There was no difference in nipple number on PND13 or anogenital distance on PND22 in female offspring. However, nipple retention and reduced anogenital distance were seen in males exposed to the highest dose (405 mg/kg/day). Delayed preputial separation was observed in males exposed to 15 mg DEHP/kg/day and higher doses. Vaginal opening, but not first estrus, was significantly delayed in females exposed to 15 mg/kg/day and above. On PND 22 signs of reduced germ cell differentiation in seminiferous tubules of exposed animals was observed. Testis weight on PND 22 was significantly increased at 5, 15, 45, and 135 mg/kg/day but not significantly increased at the two lower doses and the highest dose. The NOAEL for developmental toxicity was 5 mg/kg bw/day based on delayed pubertal onset and 135 mg/kg bw/day for maternal toxicity.

Pregnant Sprague–Dawley rats were administered 0, 11, 33, 100, or 300 mg DEHP/kg/day by oral gavage starting on gestational day (GD) 8-18 (Calafat et al, 2006). Amniotic fluid samples were collected from each pup in the litters at necropsy on GD 18. Concentrations of MEHP in amniotic fluid were strongly correlated with corresponding maternal DEHP doses.

Three studies have examined the effect of DEHP during pregnancy on male sexual differentiation following an oral dose of 750 mg/kg/day (Gray* et al, 2000; Parks* et al, 2000; Borch et al, 2005).

Pregnant rats received DEHP orally at 750 mg/kg bw/day from gestational day (GD) 14 to postnatal day (PND) 3 (Parks* et al, 2000). Maternal weight gain during gestation was significantly reduced in the DEHP-treated group. Decreased testosterone, anogenital distance and testicular weight were observed in male foetuses. Increased Leydig cell hyperplasia and multinucleated gonocytes were observed in testes. In a receptor binding study, neither DEHP nor its metabolite MEHP displayed affinity for the human androgen receptor at concentrations up to 10 μM. The results indicate that DEHP disrupted male rat sexual differentiation by reducing testosterone.

DEHP and five other phthalates were administered orally to rat dams at 750 mg/kg bw/day from GD 14 to PND 3 (Gray* et al, 2000). DEHP treatment reduced maternal weight gain and pregnancy weight gain. Decreased pup weights were observed in DEHP groups. Male, but not female pups displayed shortened anogenital distance and reduced testis weights. As infants, males had female-like areolas/nipples and a significantly increased incidence of reproductive malformations was seen, which included decreased anogenital distance, complete agenesis of the ventral prostate, the seminal vesicles and coagulating glands, permanent nipples, hypospadias, small and atrophic testes, complete unilateral testicular agenesis/atrophy or absence of both testes, and fluid-filled or undescended testes.

Borch et al, (2005) evaluated early testicular effects of prenatal exposure to DEHP with or without diethylhexyl adipate in Wistar rats. Pregnant females were treated by gavage with vehicle, 750 mg/kg bw/day DEHP or 750 mg/kg bw/day DEHP + 400 mg/kg bw/day diethylhexyl adipate beginning on GD 7. Half of the litters were evaluated on GD 21, the other half on PND 26. Evaluation of the testes on GD 21 showed vacuolisation of Sertoli cells, shedding of gonocytes, reduced interstitial cell cytoplasm, and enlarged tubules in offspring of all dams exposed to 750 mg/kg bw/day DEHP. By PND 26, tubules without spermatocytes were found in all litters exposed to DEHP compared to control litters. Malformed tubules were identified in 17–29% of DEHP exposed litters compared to none of the control litters. Leydig cell hyperplasia was identified in offspring of more dams with DEHP treatment than control dams. DNA laddering was increased by DEHP treatment, although TUNEL-positive cells and caspase-3-positive cells were not increased.

3.6.2.2 INHALATION

In rats, there was no consistent evidence of any treatment-related prenatal or postnatal developmental effects in the offspring of females (25/group) exposed to up to 300 mg/m^3 DEHP (the highest dose tested) for 6 hours/day during the period of organogenesis (gestation days 6–15) (Merkle* et al, 1988). The number of live foetuses/dam was statistically significantly decreased and the number of resorptions increased in the 50 mg/m^3 group but not at the next dose level. There was no evidence of a dose response and the systemic dose was not verified.

3.6.2.3 DERMAL

There are no laboratory animal developmental toxicology studies of DEHP delivered by the dermal route of exposure.

3.6.2.4 PARENTERAL

There are insufficient data on the developmental toxicity of DEHP administered parenterally or intraperitoneally to identify LOAELs and NOAELs. In the only published IV exposure study, no foetal toxicity was observed following intravenous administration of DEHP to pregnant rats (Lewandowski* et al, 1980). However the doses (1 - 5 mg/kg bw/day) were lower than those used in oral exposure studies. The lowest dose reported to produce foetal toxicity following IP administration was 1,970 mg/kg bw/day (Peters* & Cook, 1973). Of 10 dams dosed on GD 3, 6, and 9 only one survived to deliver. Singh et al, (1972*) administered 5 or 10 ml/kg bw (4,930 and 9,860 mg/kg bw) to groups of 5 Sprague-Dawley rats by IP injections on GD 5, 10, and

15. Maternal toxicity was not evaluated in this study. There was an increased frequency of resorptions at both doses and a decrease in foetal weights. Gross anomalies were only observed at the 9,860 mg/kg bw dose. The intraperitoneal studies are limited as only high doses were tested and group size was small.

DEHP and MEHP were orally administered to pregnant ICR mice (9-11/group) at 0, 50, 100, 200, 400 mg/kg bw/day (MEHP) or 0, 250, 500, 1000 or 2000 mg/kg bw/day DEHP on days 7, 8, and 9 of gestation (Shiota and Mima, 1985). A second group received 0, 500, 1000, 2000, 4000, 8000 mg/kg bw/day (DEHP) or 0, 50, 100, 200 mg/kg bw/day (MEHP) by IP injection on GD 7-9. In groups given DEHP orally, resorptions and malformed foetuses (anencephaly and exencephaly) increased significantly above 500 mg/kg. No teratogenic effects were revealed following IP doses of DEHP and oral or IP doses of MEHP, although high doses were abortifacient and lethal to pregnant females. Thus DEHP is highly embryotoxic and teratogenic in mice when given orally but not after IP administration. This difference may be a result of differences in metabolism, disposition, or excretion due to the route of administration. Although MEHP is a principal metabolite of DEHP and is more toxic than DEHP to adult mice, it seems that MEHP is not teratogenic in ICR mice.

3.6.2.5 MEHP and 2-EH

Several studies have focused on identifying the active developmental toxicant of DEHP. DEHP and MEHP were administered to pregnant ICR mice (9-11/group) by oral and parenteral routes of exposure (Shiota and Mima, 1985; described earlier). No teratogenic effects were revealed by oral or IP doses of MEHP, although high doses were abortifacient and lethal to pregnant females.

CD-1 mice (25–27/group) received doses of 0, 0.017, 0.035, 0.07, or 0.14% MEHP in feed on GD 0–17. Average doses were reported as 0, 35, 73, 134, or 269 mg/kg bw/day MEHP (Price* et al, 1991). MEHP-exposed females exhibited no clinical signs of maternal toxicity although there was a decrease in the adjusted body weight gain of mice in the highest dose group and increased relative liver weights in the two highest groups. The maternal NOAEL was stated to be 134 mg/kg bw/day. The percent litters with resorptions increased at all dose levels and in a dose-related manner. Foetal malformations were observed in a significantly higher percentage of litters at dose levels of 73 mg/kg bw/day and greater. A NOAEL for developmental toxicity was not observed in this study. The LOAEL (based on incidence of litters with resorptions) was 35 mg/kg bw/day MEHP.

MEHP (0, 35, 73, 134, or 269 mg/kg/bw) administered to CD-1 mice (25-27 animals per group) on gestational days 0 to 17 was shown to cause developmental toxicity and malformations at doses from 35 mg/kg/day (NTP*, 1991). A developmental LOAEL of 35 mg/kg bw/day may be derived from this study

In a gavage study in Wistar rats, on an equimolar basis, DEHP was less teratogenic than 2-EH, which in turn was less teratogenic than 2-ethylhexanoic acid (Ritter* et al, 1987). In Han:NMRI mice, the (R) enantiomer of 2-ethylhexanoic acid given intraperitoneally was highly teratogenic or embryotoxic. No such properties were seen for the (S) enantiomer (Hauck* et al, 1990). In rabbits, 2-ethylhexanoic acid did not cause developmental effects even after oral exposures (125 and 250 mg/kg/day) that were maternally toxic (Tyl*, 1988).

In vitro organ cultures of foetal and neonatal rat testes were used to assess the effect of MEHP on seminiferous cord formation in GD 13 testes and on the development of

GD18 and postnatal day 3 (PND3) testes (Li & Kim, 2003). MEHP had no effect on cord formation in the organ cultures of GD13 testes. In contrast, MEHP impaired Sertoli cell proliferation in the organ cultures of GD18 and PND3 testes. MEHP treatment did not alter the number of gonocytes in GD18 testes, whereas the number of gonocytes in PND3 testes decreased in a dose-dependent manner.

Conclusion

A number of human studies have attempted to link maternal MEHP levels with gestation length, onset of puberty and anogenital distance. However, these studies are considered inadequate for several reasons. Developmental studies in experimental animals comprise single and multiple-generation exposure largely by the oral route and predominantly in rodents. There are no dermal studies, only one single inhalation study and few studies using parenteral routes of exposure. There are no developmental studies in primates.

Data from oral multigenerational studies identified the NOAEL for developmental toxicity of 4.8 mg/kg bw/day, while 14–23 mg/kg bw/day resulted in small male reproductive organs (Wolfe & Layton, 2004). At higher levels of dietary or gavage exposure, effects on *in utero* survival, reduced anogenital distances, undescended testes, retained nipples/areolae, incomplete preputial separation, and disruption in spermatogenesis were evident in offspring. The effects of DEHP on pregnant females following repeated oral administration include body and liver weight changes (summarised in Table 15, Appendix B).

In studies where exposure was only during gestation, mice appear to be more sensitive to the developmental toxic effects of DEHP than rats. In mice, several studies have shown that DEHP is embryotoxic and teratogenic at dose levels below those producing observable evidence of toxicity to the dams. In rats, developmental studies have shown that DEHP is embryotoxic at doses close to maternally toxic dose levels when dosing encompassed early gestation exposure. DEHP induced overt structural malformations in rats exposed to 1000 mg/kg bw/d during the critical period of development (BASF AG*, 1995; Hellwig* et al, 1997). More subtle endpoints were not recorded in all studies. Reduced anogenital distance was reported in a number of studies. The LOAEL was at the non-maternotoxic dose of 113 mg/kg bw/d in rats (Schilling et al, 2001). Testes were reported to be small in male offspring of dams exposed to 250 mg/kg bw/d ie. non-maternotoxic doses during late gestation. Testicular pathology including Leydig cell hyperplasia was also noted at this dose. One other study reported decreased testicular weight and pathology at an estimated dose of 30-35 mg/kg bw/d but these results are questionable as doses administered were doubtful (Arcadi* et al, 1998). In addition, data not reported in previous evaluations showed that DEHP disrupted male rat sexual differentiation by reducing testosterone levels (Akingbemi et al, 2001).

Developmental effects are seen at lower doses in multigenerational studies. The NOAEL for developmental effects is therefore considered to be 100 ppm (4.8 mg/kg bw/day). This conclusion was based on the finding that testicular abnormalities in the F1 and F2 generations of a multigeneration study were much more severe than in F0, indicating the developmental phases were more sensitive to the testicular toxicity of DEHP. The LOAEL was 1000 ppm (14-23 mg/kg bw/d). It is likely however, that the lowest dose at which developmental toxicity occurs has not yet been established, as there are limited data on DEHP- induced alterations to the male reproductive tract during gestation.

There is insufficient data to determine a developmental NOAEL for parenteral, inhalation or dermal routes of exposure. In the study by Shiota and Mima (1985) it is possible to directly compare the effects of oral and IP injection of DEHP. Oral doses of DEHP at and above 1000 mg/kg bw/day induced embryolethality, decreased foetal weight and increased malformation rate. No teratogenic effects were revealed by IP doses of DEHP up to 4000 mg/kg bw/day. The next highest dose (8000 mg/kg bw/day) produced only one viable litter out of three matings.

A small number of studies have compared the relative developmental toxicity of DEHP and its metabolites. The study of Price* et al, (1991) was coordinated with the Tyl et al, study (1988) of DEHP. The maternal and developmental effects of MEHP and DEHP exposure were qualitatively similar at approximately equimolar doses administered under comparable experimental conditions. This contrasts with the smaller study of Shiota and Mima (1985) in mice, who observed that oral doses of DEHP were teratogenic above 500 mg/kg bw/day, whereas MEHP was lethal to pregnant females at 200 mg/kg bw/day and not teratogenic below that dose.

The critical study for developmental toxicity was Wolfe & Layton (2004). The NOAEL was 4.8 mg/kg bw/day and the LOAEL was 37.8 mg/kg bw/day. Another study had a similar NOAEL and LOAEL (Andrade et al, 2006).

4. RISK CHARACTERISATION

4.1 Toxicity

DEHP is readily absorbed by oral, inhalation and parenteral routes of exposure but poorly absorbed dermally. DEHP is hydrolysed by pancreatic lipases in the small intestine to form MEHP and 2-EH. Absorption of MEHP is 50% for doses up to 200 mg/kg bw but is dose-limited at higher doses in primates. Although DEHP exposure via the parenteral route bypasses the intestinal esterases, hydrolysis can occur in the liver and blood. DEHP and MEHP rapidly equalize in the blood following exchange transfusions and during haemodialysis. The elimination half-life of DEHP in rats is 2 hours. The half-life of MEHP in humans is approximately 5 hours. There was no evidence of accumulation in rodent tissues following oral or parenteral routes of exposure.

In experimental animals DEHP exhibits low acute oral, dermal and inhalation toxicity. Based on *in vitro* and *in vivo* data DEHP and its metabolites, MEHP and 2-EH, are not considered mutagenic. In carcinogenicity studies, DEHP caused an increase in the incidence of liver tumour with a dose-response relationship in rats and mice of both sexes, and an increase in the incidence of Leydig cell tumours and MCL in male rats. DEHP-induced hepatocellular carcinomas are unlikely to be of relevance to humans since the hepatotoxic effects of DEHP, including hepatocellular tumour induction, are associated with peroxisome proliferation to which humans are significantly less sensitive. The relevance of the Leydig cell tumours to humans is unknown but was only reported in one animal study. An increased incidence of mononuclear cell leukaemia (MCL) was only seen in one of two rat studies and in neither of two mouse studies. This tumour type is well known to occur spontaneously, with a high incidence in the rat strain used in the study.

Repeated dose studies show that rodents are the most sensitive species, followed by hamsters, guinea pigs, and primates. In studies in rats and mice, the most pronounced findings included effects on the liver (hepatomegaly, peroxisome proliferation, and replicative DNA synthesis), testes (tubular atrophy) and kidneys (increased kidney weights, mineralisation of renal papilla, tubule cell pigments and chronic progressive nephropathy). Other, less pronounced effects have also been observed eg decreased body weights/body weight gains and alterations in clinical chemistry parameters. Primates, however, appear to be less sensitive than rodents to the liver and testicular effects of phthalates. In a monkey study, DEHP at concentrations of up to 2500 mg/kg bw/day had no effects on liver and testicular weights, and no testicular lesions were observed.

The critical effects are considered to be reproductive and developmental effects in males. Testicular toxicity appears to be the most sensitive toxicity endpoint but is significantly influenced by the age at exposure. Developing and neonatal rats have been found to be much more sensitive to exposure to DEHP than adults. The younger animals responded to a much lower dose or produced a more serious lesion with a comparable dose on a mg/kg bw/day basis.

The most sensitive endpoint for adult males was effects on fertility. The NOAEL was 14 mg/kg bw/day based on a study in a continuous breeding study in mice (Lamb et al, 1987). The LOAEL in this study was 140 mg/kg bw/day but may not necessarily be due to male infertility as both sexes were exposed to DEHP in the diet. However, a

crossover study at the highest dose indicated both males and females had reduced fertility. This NOAEL (14 mg/kg bw/day) is selected for risk characterisation in adults.

A number of key studies exposed animals during gestation and/or early postnatal life (Wolfe & Layton, 2004; Andrade et al, 2006; Poon et al, 1987; Akingbemi et al, 2001). In a three generation study, small testes, testis seminiferous tubule atrophy and small aplastic epididymis were observed at 14 mg/kg bw/day (Wolfe & Layton, 2004). The NOAEL for this study was 5.8 mg/kg bw/day. A developmental study exposed rats from GD6 through weaning to PND 21 (Andrade et al, 2006). The NOAEL was 5 mg/kg bw/day, the LOAEL was 15 mg/kg bw/day for delayed preputial separation. In a 13 week study of 4-6 week old male rats an increased incidence of Sertoli cell vacuolation at 38 mg/kg bw/day was observed (NOAEL was 3.7 mg/kg bw/day; Poon et al, 1987). Akingbemi et al, (2001) examined the effects of short exposures (14 to 28 days) to DEHP in young male rats on hormone levels and found decreased testosterone levels at 10 mg/kg bw/day (NOAEL was 1 mg/kg bw/day). The critical study for developmental toxicity was Wolfe & Layton (2004) for testicular effects during prenatal and neonatal development. The NOAEL was 4.8 mg/kg bw/day and the LOAEL was 37.8 mg/kg bw/day. The Wolfe & Layton (2004) study covers all life stages, is well conducted and is selected as the critical study for risk characterisation for neonatal males and *in utero* exposure.

There are no suitable long-term studies following intravenous administration of DEHP for neonatal, adult or pregnant animals. The effects generally observed following short-term intravenous administration of DEHP are the same as those following oral administration: decreased body weight, increased liver weight and testicular atrophy. For reproductive toxicity, the NOAEL following intravenous exposure for Sertoli cell damage was 25 mg/kg bw/day following exposure of adult rats to DEHP every other day for 10 days (Sjoberg* et al, 1985c). However, the NOAEL in the more sensitive, younger rats was 60 mg/kg bw/day after 21 days IV exposure and the LOAEL was 300 mg/kg bw/day based on decreased testes weight and mild testicular pathology (Cammack et al, 2003). In general, the NOAELs and LOAELs identified for intravenous exposure to DEHP are higher than those reported following oral exposure to DEHP. This may reflect a real difference in effect or differences in study design. It is biologically plausible that DEHP is less toxic by the intravenous route as conversion to MEHP is slower by this route. However due to the inadequacy of data via the IV route, the NOAELs for oral exposure will be used in the risk characterisation for parenteral exposure.

Three factors are important in determining the level of risk due to exposure to DEHP:

- life stage during exposure as male foetuses and neonates appear to be a sensitive sub-population;
- dose of DEHP delivered during the medical procedure; and
- route of exposure as this influences the circulating concentration of the active metabolite, MEHP.

4.2 Risk Characterisation Methodology

The risk to different human sub-populations from exposure to DEHP leached from medical devices has been characterised using the margin of exposure methodology. The margin of exposure (MOE) is calculated using the NOAEL for the critical endpoint and the estimated human dose (E_d) as:

$$MOE = \frac{NOAEL}{E_d}$$

The MOE is a measure of the likelihood that a particular adverse endpoint will occur following exposure. As the MOE increases, the risk of the adverse effect decreases. In the case of DEHP, a MOE of 100 is considered sufficient to protect against adverse health effects from DEHP in the different sub-populations. This MOE consists of a factor of 10 applied to allow for interspecies differences and an additional factor of 10 to account for interindividual variations in the human population (WHO 1994; 2005). A greater MOE is not required for the more sensitive subpopulations, as the NOAELs selected are considered appropriate to the different subpopulations. Table 10 contains the estimated doses and MOEs for adult males, pregnant women (prenatal development) and neonates.

The NOAEL was derived from oral studies, and therefore, the risk may be over-estimated for intravenous exposure to DEHP.

4.3 Adults

The scenarios described below pertain to the potential adverse effects of DEHP to the adult. The critical endpoint was testicular toxicity. The MOE is based on the NOAEL of 14 mg/kg bw/day for reduced fertility in a continuous breeding study in mice (Lamb et al, 1987).

IV infusion of crystalloid fluids and drugs

The exposure estimate is based on the maximum concentration of DEHP in non-agitated bags of crystalloid solutions. There is a sufficient MOE for patient exposure to the amount of DEHP released from PVC IV bags following infusion of crystalloid fluids (e.g., normal saline, D5W, Ringers Lactate).

Drug formulations that require lipophilic vehicles for solubilisation can increase DEHP leaching from PVC bags and tubing. The worst-case exposure estimate assumes that the manufacturer's instructions are not adhered to and that the drug formulation is prepared in PVC bags, stored for 24 hours and then infused using PVC tubing. In this scenario, the MOE is considered not to be sufficient. When manufacturer's directions are followed ie the solution is made up in non-DEHP containing bags and delivered in non-DEHP containing tubing, there is little risk posed by exposure to the amount of DEHP released into drugs that require a pharmaceutical vehicle for solubilisation.

Chronic Blood Transfusions

DEHP exposure estimates for long-term transfusions of blood to patients with anaemia (sickle cell, chemotherapy) and treatment of clotting disorders are based on the maximum DEHP concentration measured in two different studies. When time-weighted over the duration of treatment, the MOE are considered adequate and suggest that such patients would be at minimal risk.

Exposure estimates for apheresis donors were derived from the maximum value measured during continuous-flow plateletpheresis. The estimated DEHP would be even lower if the dose is time-averaged over the donation period. Consequently, there is little concern about DEHP-associated adverse effects developing in persons donating platelets or plasma.

Haemodialysis

The exposure estimates for haemodialysis are based on the maximum values delivered and retained, time-averaged over a week. The MOE is considered insufficient and there is an increased risk in this scenario particularly as these patients may have a reduced elimination capacity, and thus could be particularly sensitive to the effects of DEHP. The DEHP doses would be reduced if TOTM-DEHP tubing were used. Heparin-coated tubing would also reduce DEHP leakage (Karle et al, 1997: Hildenbrand et al, 2005).

Acute Blood transfusion

Relatively high doses of DEHP can be received by patients who are transfused with large volumes of blood and blood products over a short period (e.g., trauma or surgical patients receiving massive transfusions). The exposure estimates for ECMO and elective surgery are based on the maximum values obtained from two studies. ECMO is very rarely used on adults and modern surgical techniques and cell saver devices mean that blood loss is usually much less than reported by Butch et al, (1996). The dose of DEHP received by adults undergoing cardiopulmonary bypass procedures and heart transplant were calculated on post-surgical measurements. No details of the volume of blood or blood products transfused were given. However, it is considered not appropriate to compare these doses with the NOAEL/LOAEL for chronic exposure. As acute toxicity of DEHP is low, the risk during acute blood transfusions is considered to be low.

Peritoneal dialysis

Since very little DEHP is released into peritoneal dialysis fluid, the risk of systemic effects developing following exposure to DEHP is low.

Total Parenteral Nutrition (TPN)

DEHP can leach both into bags used to prepare and store nutrient solutions and tubing used to deliver the solution. The delivered DEHP dose is largely dependent on the lipid content of the TPN solution, the surface area of the tubing and the duration of delivery. The highest exposure estimate is derived when TPN is stored and delivered in PVC bags and tubing. However, the use of PVC bags is no longer recommended practise. The MOE is considered sufficient when the dose of DEHP is derived from TPN admixtures containing 4% lipid. However the MOE is insufficient and there is an increased risk if the lipid content is 20%. The MOE would be increased (and the risk reduced) if the tubing surface was reduced (shorter length), infusion time was reduced (flow rate increased) or non-DEHP containing tubing was used.

Enteral nutrition

The exposure estimate is based on an enteral feed containing 4% lipid, prepared in PVC bags and delivered with PVC tubing. The MOE is considered insufficient and therefore there is an increased risk. Enteral feeding solutions are now made up in bottles and in this case (PVC tubing only), the MOE is considered sufficient.

Inhalation and Dermal

There are no reliable inhalation studies in animals and, as such, an MOE was not calculated for this route of exposure. It should also be noted that the method for estimating the exposure via inhalation was theoretical.

Although there are no dermal animal studies, the dose of DEHP received by adults through dermal routes is estimated to be small and therefore of little concern.

4.4 Neonates and Young Children

The scenarios described below pertain to the potential for adverse effects of DEHP to the neonatal and prepubertal male with the critical endpoint of testicular toxicity. The MOE is based on the NOAEL of 5 mg/kg bw/day for testicular weight and pathology and delayed puberty in a range of studies in young rats (Wolfe & Layton, 2004; Andrade et al, 2006; Poon et al, 1987; Akingbemi et al, 2001).

MOE calculations for neonates were based on a body weight of 4 kg. As such a lower MOE is expected for neonates with a body weight below 4 kg. It should also be noted that a 4 kg neonate is unlikely to need long term care when compared to smaller sized babies.

IV infusion of crystalloid fluids and drugs

The exposure estimate is based on the use of a syringe pump to deliver crystalloid solutions or drugs. The maximum concentration of DEHP leached into crystalloid solutions was used. The MOE is considered sufficient for patient exposure to the amount of DEHP released from PVC IV bags following infusion of crystalloid fluids (e.g., normal saline, D5W, Ringers Lactate). The MOE can be further increased if microbore giving sets are used.

Drug formulations that require lipophilic vehicles for solubilisation can result in greater leaching of DEHP from PVC bags and tubing. The MOE is considered sufficient for patient exposure to the amount of DEHP released from PVC tubing into midazolam. However, manufacturers advise the use of non-DEHP containing bags and tubing to be used in the preparation and delivery of certain drugs.

Blood transfusion

The exposure estimate for replacement blood transfusions is based on the maximum DEHP concentrations in packed RBC from two studies. Despite the acute exposure, the MOE is considered insufficient and there is an increased risk for infants who receive replacement transfusions in the NICU as this is a sensitive stage of development.

The exposure estimates for exchange transfusion and ECMO were based on the maximum measurements made during the procedures. These estimates are very high and the MOE for exchange transfusion is considered insufficient and the risk is increased.

The use of shorter circuits and/or heparin-coated tubing would increase the MOE.

Total Parenteral Nutrition (TPN)

The delivered DEHP dose is largely dependent on the lipid content of the TPN solution, the surface area of the tubing and the duration of delivery. The MOE for exchange transfusion is considered insufficient and the risk increased when PVC tubing only is used to deliver TPN solutions. The highest exposure estimate is derived for 20% lipid, with a 10-fold lower estimate for 4% lipids.

The MOE would be increased if the tubing surface was reduced (shorter length) or infusion time was reduced (flow rate increased) or non-DEHP containing tubing was used.

Enteral nutrition and breastfeeding

The exposure estimate for enteral nutrition is based on the maximum extraction rate of DEHP from PVC tubing into 4% or 20% lipid over 24 hours. In both scenarios the dose

estimates are high and the MOE is considered insufficient and therefore the risk is increased. The MOE would be increased if tubing surface area was reduced, delivery time was reduced or non-DEHP tubing was used.

Infants of mothers undergoing medical procedures could conceivably receive similar doses of DEHP from breast milk directly or indirectly (if breast milk is pumped and stored). There are no measurements of DEHP in the breast milk of women exposed to DEHP-containing medical devices. Exposure estimates are based on theoretical milk plasma partition coefficient as well as maximum DEHP serum levels in patients undergoing haemodialysis. Additional DEHP would not result if breast milk has been pumped and stored as the bags are typically made from polyethylene or nylon coated with polyethylene.

In this scenario, the MOE is insufficient and the risk is increased. The MOE could be increased by the strategies outlined for haemodialysis patients, namely the use of alternate tubing material or coated tubing circuits.

Inhalation

There are no reliable inhalation studies in animals and, as such, an MOE was not calculated for this route of exposure. It should also be noted that the method for estimating the exposure via inhalation was theoretical.

4.5 Pregnancy

The risk estimates described below pertain to the potential impact of DEHP on the developing embryo and foetus and not the pregnant woman. The MOE is based on the NOAEL of 5 mg/kg bw/day for testicular weight and pathology in a multi-generational study in rats (Wolfe & Layton, 2004).

The MOE is considered sufficient for IV infusion of crystalloid fluids, drugs prepared according to manufacturer's direction, chronic blood transfusions, apheresis and peritoneal dialysis.

IV drugs

Drug formulations that require lipophilic vehicles for solubilisation can extract DEHP from PVC bags and tubing. The exposure estimate assumes that the manufacturer's instructions are not strictly adhered to and that the drug formulation is prepared in PVC bags, stored for 24 hours and then infused using PVC tubing. In this scenario, the MOE is not considered sufficient and there is increased risk. If non-DEHP containing bags are used to prepare the drug solution, even if PVC tubing is used to deliver the drug, the amount of DEHP released into solution would provide a sufficient MOE.

Haemodialysis

The exposure estimates for haemodialysis are based on the maximum values delivered and retained, time-averaged over a week. The MOE is considered insufficient and there is an increased risk.

The DEHP doses would be reduced if TOTM-DEHP tubing was used and heparin-coated tubing would also reduce DEHP leakage (Karle et al, 1997: Hildenbrand et al, 2005).

Acute Blood transfusion

Relatively high doses of DEHP can be received by patients who are transfused with large volumes of blood and blood products over a short period (e.g., trauma or surgical patients receiving massive transfusions). MOE is considered insufficient and there is an increased risk despite the acute exposure as the life-stage is considered sensitive.

The use of heparin-coated tubing may decrease the leaching rate of DEHP and further increase the MOE.

Total Parenteral Nutrition (TPN)

DEHP can leach both into bags used to prepare and store nutrient solutions and tubing used to deliver the solution. The MOE is sufficient when the dose of DEHP is derived from TPN admixtures containing 4% lipid. However the MOE is insufficient if the lipid content is 20% and the risk is increased.

The MOE would be increased if the tubing surface was reduced (shorter length) or infusion time was reduced (flow rate increased). The use of non-DEHP containing tubing would also increase the MOE.

Enteral nutrition

The exposure estimate is based on an enteral feed containing 4% lipid, prepared in PVC bags and delivered with PVC tubing. Enteral feeding solutions are now made up in bottles. The MOE is considered sufficient when DEHP is extracted from tubing only.

5. OVERSEAS AGENCY DECISIONS

Several agencies have assessed the risk of DEHP exposure from medical devices. These include FDA, EU RAR, Health Canada and CERHR (FDA, 2002; Health Canada, 2002; CERHR, 2005; National Chemical Inspectorate, 2005). Although the approaches the agencies have taken differ, they fall into two general types.

The first approach, as taken in this report, is the margin of exposure (or margin of safety approach). This approach compares the estimated exposure (dose) with the NOAEL (or LOAEL) for the critical effect. The difference (or margin) is then reviewed, taking into account factors such as species differences, life stage etc, to decide whether there is a concern/increased risk. It appears that Health Canada, EU RAR and CERHR have, in general, taken this approach (Health Canada, 2002; CERHR, 2005; National Chemical Inspectorate, 2005).

The other approach, taken by the FDA, is to establish a Tolerable Daily Intake(s). This approach also identifies the critical NOAEL (or LOAEL) and then applies uncertainty factors to derive an intake of the substance which is considered to be without appreciable health risk (IPCS, 1994). Uncertainty factors are applied to take into account interspecies and interspecies differences, adequacy of the database and nature of toxicity.

In addition to differences in approach, agencies have also differed in the NOAEL (or LOAEL) they have selected and in the methods for estimating dose. There are several reasons why different NOAELs have been selected, including the data available at the time of the assessment, differences in what is considered relevant and critical effects and differences in opinion regarding adequacy of particular studies.

In calculating estimated doses, the agencies have used different methods. In some cases it is unclear how the estimate was derived. The potential dose derived from leaching from tubing was not always accounted for. In general, a maximum measurement of DEHP in the solution was used but in some scenarios a mean measurement was used. For example, the maximum DEHP in packed RBC was used to estimate DEHP dose in patients undergoing chemotherapy, whereas the mean value from the same study was used in a replacement transfusion scenario in adults (FDA, 2002). In general, short-term procedures were time-averaged. Again, discrepancies arose, for example, the DEHP dose for an adult undergoing ECMO and blood transfusions was not time-averaged.

No agency considered whether the health risks from DEHP exposure were outweighed by the benefits of the procedure. However, they all stated that when considering limiting the use of DEHP in particular medical devices and/or procedures, it is important to consider benefits, as the health benefits of the medical procedures might outweigh any risks.

5.1 Health Canada

The Medical Devices Bureau of Health Canada published a report 'DEHP in Medical Devices: An Exposure and Toxicity Assessment' in February 2002 (Health Canada, 2002).

Health Canada identified the medical procedures with the highest exposures and judged whether the exposures posed a significant risk to patients undergoing the procedure. Although not stated, it appears that Health Canada has used a margin of exposure

approach. Sub-populations of concern were identified by comparing estimated exposures with the NOAEL, taking into account factors such as species differences, toxicokinetics, and life stage of the patient.

Toxicity:

The main findings were:

- Human studies are insufficient in design and outcome to demonstrate any cause-effect relationship between human exposure to DEHP and toxicity.
- Need to extrapolate from studies in experimental animals, however, species differences in metabolism are important.
- Hepatocellular tumours in rats and mice are not relevant to humans.
- Oral exposure to DEHP can cause reproductive and developmental toxicity in rodents.
- Testicular toxicity (vacuolation of Sertoli cells) is the most sensitive toxicity end point reported to date.
- Based on toxicokinetics and possible mechanisms of action of DEHP-inducing reproductive toxicity, the data from rodent species are relevant to humans.
- The NOAEL is 3.7 mg/kg/day for testicular toxicity for oral exposure in rats (from Poon et al, 1997)
- The NOAEL is 60 mg/kg/day for testicular toxicity from intravenous exposure in rats (from AdvaMed*, 2001) (note: AdvaMed*, 2001 is the same study as Cammack et al. 2003)
- The most sensitive window for phthalate reproductive and developmental toxicity may be exposure to DEHP *in utero*, particularly during late gestation. However, there is insufficient data to identify a NOAEL for perinatal exposure.
- Route of exposure and dose are important parameters that need to be taken into account when extrapolating across species.

Exposure:

The report concluded that:

- In adults, transfusion of blood, cardiopulmonary bypass and infusion of lipophilic drugs using PVC bags and tubing (contrary to directions of use) result in very high short-term exposures relative to the general population exposure. Blood transfusions to trauma patients give the highest exposures (up to 8.5 mg/kg bw/d).
- In adults, long-term haemodialysis gives the highest chronic daily dose (0.15 – 2.20 mg/kg bw/d) and is 1 to 2 orders of magnitude above the general population exposure (0.003 – 0.030 mg/kg bw/d).
- In neonates, important sources of short-term or subacute exposures are volume exchange transfusions, ECMO, cardiac bypass procedures, TPN therapy, infusion of lipophilic drugs using PVC bags and tubing, and possibly respiratory therapy.
    - Highest acute exposure = 23 mg/kg bw/d for double exchange transfusion
    - Highest sub-acute exposure = up to 14 mg/kg bw/d during ECMO.

Conclusions:

The report concluded that there were significant data gaps, including insufficient dose-response data on adverse effects on the reproductive tract as a result of gestational exposure. They also stated that uncertainties about combinations of simultaneous exposures made it impossible to provide a quantitative risk assessment.

The report concluded that there was 'very little' concern for adults and exposure from medical procedures, based on:

- Adult marmosets exposed to high oral doses of DEHP showed no signs of reprotoxicity. Similar doses produced severe testicular toxicity in juvenile rodents;
- Adult rodents are 10 – 100 times less sensitive to reproductive toxicity of DEHP than juvenile rodents.
- There is less conversion of DEHP to MEHP (the active toxicant) by the parenteral route of exposure than by oral exposure, but extent of reduction is not known.

The report concluded that there is *'serious'* concern for critically ill infants undergoing intensive medical therapy. Greatest concern was identified for the following medical procedures: volume exchange transfusions, ECMO, cardiac bypass procedures, TPN therapy, infusion of lipophilic drugs using PVC bags and tubing and possible respiratory therapy. The conclusion was based on:

- Parenteral exposure in some procedures approach the NOAEL for IV administered DEHP;
- Parenteral exposure involving infusion of blood or blood products also exposes the infant to MEHP;
- Exposure may be increased because infants may have higher levels of plasma and hepatic lipases. Infants do not have mature glucuronidation pathways until 3 months old and this may result in prolonged exposure.
- Heparin and DEHP may induce plasma lipase activity and thus increase conversion to MEHP.
- Infants may be more susceptible, as the reproductive system is developing.
- Young Sertoli cells are more susceptible to MEHP than older cells.
- There is less conversion to MEHP by the parenteral than by oral route, which reduces the concern.

The report stated that there is inadequate information to assess the risks during pregnancy but concluded that there was *'some'* concern that pregnant women undergoing certain medical procedures may adversely affect the development of offspring. This is based on:

- The foetus is at the most vulnerable stage of life;
- Exposure to DEHP during gestation causes serious malformations in the male reproductive tract of the foetus;
- MEHP crosses the placenta and is present in breast milk;
- Short-term parenteral exposure of pregnant women to DEHP from certain medical procedures may approach, if not exceed, the NOAEL for reproductive and developmental toxicity in rodents;
- There is less conversion to MEHP via the parenteral route, which reduces the concern.

5.2 US Food and Drug Administration

The Center for Devices and Radiological Health, of the US Food and Drug Administration, published the report 'Safety Assessment of Di(2-ethylhexyl)phthalate (DEHP) Released from PVC Medical Devices' in 2002 (FDA, 2002).

The FDA stated that they did not undertake a quantitative assessment of risk but took a 'safety assessment approach' that involved comparing the doses of DEHP received by patients undergoing medical procedures to Tolerable Intake (TI) values.

Toxicity:

The main findings were:

- The critical effect is adverse effects on the testes, an organ that appears to be particularly sensitive to DEHP, at least in rodents.
- The parenteral NOAEL was based primarily on the AdvaMed* (2001) study, however similar NOAEL and LOAEL values were identified in other relevant studies, (Baxter Healthcare Corporation, 2000 and Sjoberg et al, 1985c, respectively) thus increasing the confidence of the selected NOAEL.
- For oral exposure, the Poon et al, (1997) study was selected as the most appropriate. It was also noted that the NOAEL and LOAEL in David et al, (2000a) were similar.

Derivation of Tolerable Intake:

As the potency of DEHP differs across routes of exposure, separate TIs were derived for oral and parenteral exposure.

Route	Endpoint	NOAEL or LOAEL (mg/kg/d)	Modifying factor	TI (mg/kg/d)
Parenteral	NOAEL from Baxter Healthcare Corporation, (2000), AdvaMed* (2001)	60	100	0.6
	LOAEL from Sjoberg et al, (1985c)	250	300	0.8
Oral	NOAEL from Poon et al, (1997)	3.7	100	0.04

The modifying factor was the product of uncertainty factors (UFs) for interindividual variability among humans (1 – 10); interspecies extrapolation (1 – 10); and deficiencies in the toxicological database (1 – 100).

The UF for interindividual variability was selected as 10 to account for:

- Variability in pharmacokinetic behaviour of DEHP in the general population. Individuals with high lipase activity and/or low glucuronidation activity may increase the conversion of DEHP to MEHP.
- Presumed increased sensitivity of neonates and in critically ill patients.

The UF for interspecies differences was selected as 3 to account for:

- The testicular effects in rodents are assumed to be relevant to humans;
- Spermatogenesis in the marmoset is functionally similar to that in humans and thus marmosets are assumed to be an appropriate model; and
- Nonhuman primates are less sensitive to DEHP than rodents following oral exposure.

The UF for deficiencies in the toxicological database is 3 to account for:

- Some differences between the AdvaMed* (2001) study and conditions under which patients are exposed in a clinical setting (eg length of exposure).

- Note: an additional UF of 3 was applied to Sjoberg et al, (1985c) as a NOAEL was not identified in the study.

Conclusions:

The report noted that when assessing the significance of TI/dose ratios, the comparison should not be viewed as a bright-line (ie greater or less than 1), but rather as a general index of safety. The values should be used in a relative sense to assess the likelihood that exposure will cause adverse effects. It should also be kept in mind that when interpreting the significance of the values: 1) the TI has an uncertainty that spans perhaps an order of magnitude; and 2) the TI/dose ratios based on a comparison between short-term or one-time exposures (such as acute transfusion) and a TI based on repeat-dose toxicity study are likely to be conservative.

In drawing conclusions about risk:

- Estimates for dose received via IV medical procedures were compared against the TI for parenteral exposure (0.6 mg/kg/d).
- Estimates for dose received for enteral nutrition were compared against the TI for oral exposure (0.04 mg/kg/d).

Comparisons of the TIs and estimated doses during medical procedures are summarised in Table 12.

The report concluded that there may be an increased risk of DEHP-mediated adverse effects for:

- Adult patients undergoing blood transfusions over a short period (however this will be an overestimate); cardiopulmonary bypass or enteral nutrition.
- Neonatal patients undergoing TPN therapy with admixtures containing lipids; exchange transfusion; ECMO; or enteral nutrition.
- Neonates exposed to DEHP from multiple medical devices, such as infants undergoing IV administration of sedatives, administration of TPN and replacement transfusion.

The report concluded that there was little or no risk (concern) for:

- Adult patients undergoing infusion of crystalloid fluids; IV infusion of drugs requiring pharmaceutical vehicles for solubilisation; TPN treatment; replacement blood transfusions; treatment with cryoprecipitate; apheresis; haemodialysis or peritoneal dialysis.
- Neonate patients undergoing infusion of crystalloid fluids; IV infusion of drugs requiring pharmaceutical vehicles for solubilisation; TPN treatment without lipids; or replacement transfusion.

Table 12. Comparison of Tolerable Intake (TI) values to the dose of DEHP received by adult and neonatal patients undergoing various medical procedures

	ADULTS[1]		NEONATES[2]	
	DEHP dose (mg/kg/d)	**TI/dose ratio[3]**	**DEHP dose (mg/kg/d)**	**TI/dose ratio[3]**
Infusion of crystalloid IV solutions	0.005	120	0.03	20
IV infusion of drugs requiring pharmaceutical vehicles for solubilisation	0.15	4	0.03	20
				4
TPN administration				
Without added lipid	0.03	20	0.03	20
With added lipid	0.13	5	2.5	0.2
EVA bag with PVC tubing	0.06	10		
Blood transfusion				
Trauma patient	8.5	0.1		
Transfusion/ECMO pts	3.0	0.2		
Exchange transfusion			22.6	0.02
Replacement transfusion Neonate in NICU			0.3	2
Replacement transfusion Correction of anemia in pts receiving chemotherapy & pts with sickle cell disease	0.09	7		
Replacement transfusion surgical pts undergoing CABG	0.28	2		
Treatment of clotting disorders with cryoprecipitate	0.03	20		
Cardiopulmonary Bypass				
CABG	1	0.6		
Orthotopic heart transplant	0.3	2		
Artificial heart transplant	2.4	0.3		
ECMO			14	0.04
Apheresis	0.03	20		
Haemodialysis	0.36	2		
Peritoneal dialysis	< 0.01	> 60		
Enteral nutrition	0.14	0.3	0.14	0.3

[1] 70 kg body weight
[2] 4 kg body weight
[3] Based on TI of 0.6 mg/kg/day for parenteral exposures and 0.04 mg/kg/day for enteral nutrition

5.3 European Commission Scientific Committee on Medicinal Products and Medical Devices

The European Commission's Scientific Committee on Medicinal Products and Medical Devices adopted an 'Opinion on Medical Devices Containing DEHP Plasticised PVC: Neonates and Other Groups Possibly at Risk from DEHP Toxicity' on 26 September 2002 (SCMPMD (Scientific Committee on Medicinal Products and Medical Devices), 2002).

The methodology for deriving their conclusions was not described.

Toxicity:

The report concluded that:

One study suggested that the NOAEL could be as low as 3.7 mg/kg/d, but the criteria for toxicity in the study was Sertoli cell vacuolation believed to be a precursor for tubular atrophy in the context of testicular toxicity.

- Most other studies with different endpoints produce NOAEL values ranging from 30 to 300 mg/kg/d.
- The majority of data is from rodent studies and there are significant differences within the species and between rodents and other species (eg. no toxicity observed in marmosets).
- Extrapolation of the rodent results to humans has not been demonstrated.
- Young immature animals appear to be more sensitive compared to mature animals.
- There are no concerns over carcinogenicity in humans on the basis of animal studies.

Conclusions:

The report made the following conclusions:

- The mechanisms for adverse effects do exist in rodents, but these do not appear to be of great significance in non-human primates and the evidence that such mechanisms could be operative in humans is lacking.
- The levels of DEHP that induce toxic effects in rodents are of the same order as the exposure experienced by some neonates in clinical practice;
- There are no reports concerning adverse effects in humans following exposure to DEHP-PVC, even in neonates or other groups of relatively high exposure, however a lack of evidence of causation does not mean that there are no risks;
- 'On the basis of the evidence presented in this report, no Tolerable Intake Value for DEHP in medical devices can be recommended.'
- The Scientific Committee (now the Scientific Committee on Emerging and Newly Identified Health Risks, SCENIHR) has been asked to review and update, if appropriate, the scientific opinion of September 2002 (SCENIHR, 2005). The Committee has been asked to report back by February 2007.

5.4 EU Risk Assessment (draft 2006)

As part of the EU Risk Assessment of Bis(2-ethylhexyl)phthalate, risks during some medical procedures were assessed (National Chemical Inspectorate, 2005).

The EU used a margin of safety (MOS) approach.

Toxicity:

The report concluded that:

- the most critical effects are effects on the testis, fertility, development and kidney (repeated dose toxicity);
- severe and irreversible testicular injury was induced in rats exposed to low oral dose levels in 3 different studies (Wolfe & Layton, 2004; Poon et al, 1997; Arcadi et al, 1999, 1998);
- severe developmental effects were observed in rats and mice in the absence of maternal toxicity (Wolfe & Layton, 2004; Arcadi et al, 1998, Lamb et al, 1987);
- endocrine effects (eg underlying the testicular toxicity) are very serious effects, and the sensitivity to this effect is highest during gestation and the first few months after birth when the most sensitive systems are still developing;
- the choice of NOAEL in the Wolfe & Layton (2004) study may be considered a conservative choice.

The report identified critical effects as kidney, testicular, fertility and developmental, with the following NOAELs:

- Effects on the kidney: oral NOAEL 14.5 mg/kg bw/d (Moore, 1996)
- Testicular effects: oral NOAEL 4.8 mg/kg bw/d (Wolfe & Layton, 2004)
- Fertility effects: oral NOAEL 10 mg/kg bw/d (Lamb et al, 1987)
- Developmental effects: oral NOAEL 4.8 mg/kg bw/d (Wolfe & Layton, 2004)

Exposure:

The report estimated worst-case exposures for long-term haemodialysis for adults; long-term transfusion for adult haemophiliacs; long-term blood transfusion for children; and transfusion for neonates. See Table 13 for a summary of the MOS data in these populations.

Conclusions:

The minimum MOS for all endpoints is considered to be 100 for adults and older children. Based on the severity of the effect (testicular toxicity) and the risk for combined exposure (egg via breast milk), the minimum MOS is increased to 250 for neonates (< 3 months old).

Table 13. MOS for medically treated persons exposed to DEHP.

Effect	Exposure (mg/kg bw/d)	NOAEL (mg/kg bw/d)	$NOAEL_{Systemic}$ [1] (mg/kg bw/d)	MOS ($MOS_{NOAEL, Systemic}$) [2]
1. Long-term haemodialysis (adults)				
Intravenous				
RDT	3.1	29.0	14.5	4.6
Testicular	3.1	4.8	4.8	2.0
Fertility	3.1	20.0	10.0	3.2
Developmental	3.1	4.8	4.8	2.0
2. Long-term blood transfusion (adults) Haemophiliacs				
Intravenous				
RDT	0.03	29.0	14.5	483
Testicular	0.03	4.8	4.8	160
Fertility	0.03	20.0	10.0	333
Developmental	0.03	4.8	4.8	160
3. Long term blood transfusion (children)				
Intravenous::				
RDT	0.075	29.0	14.5	193
Testicular	0.075	4.8	4.8	64
Fertility	0.075	20.0	10.0	133
4. Transfusions (neonates)				
Intravenous::				
Testicular	1.7	4.8	4.8	3.0
Fertility	1.7	20.0	10.0	5.9

[1] Correction of NOAEL for 50% oral absorption in the rat (no correction of the NOAELs for testicular and developmental toxicity is needed as the Wolfe study is considered to directly give a systemic NOAEL because of a major part of the exposure is to young animals with 100% absorption)
[2] MOS derived based on the systemic oral NOAEL for rats

Based on a quantitative risk assessment, the report concluded that there is concern for:

- Testicular, fertility and developmental effects for adults undergoing long-term haemodialysis;
- Testicular effects for long-term blood transfusion in children;
- Testicular and fertility effects for transfusions in neonates.

Based on a qualitative risk assessment, there is concern for testicular, fertility and kidney effects for ECMO in children.

5.5 NTP CERHR

The NTP CERHR (Centre for the Evaluation of Risks To Human Reproduction Expert) Expert Panel published a Report on the Reproductive and Developmental Toxicity of Di(2-ethylhexyl) phthalate in October 2000 (CERHR, 2000). The report was updated in November 2005 (CERHR, 2005) and a draft NTP Brief published in May 2006 (CERHR, 2006).

Toxicity:

NTP CERHR concluded that:

- Human data are insufficient to evaluate developmental and reproductive effects;
- DEHP is a developmental toxicant in rats by dietary, oral and IV routes and the data are assumed to be relevant to humans;

- Oral NOAEL based on malformations in rodents was approx. 40 mg/kg bw/d (Tyl 1988, Ema, 1997; Huntingdon, 1996; Price 1988) and for testicular effects was 3.7 – 14 mg/kg bw/d in rodents. A recent NTP study (Wolfe & Layton, 2004) also indicates DEHP causes developmental effects (small or absent male reproductive organs) at 14-23 mg/kg bw/d during gestation and/or early postnatal life in rats;
- Reproductive toxicity has been observed in rats, mice, guinea pigs and ferrets and the data are assumed to be relevant to humans;
- Marmosets may be less susceptible to hormonal disruption, a key feature of DEHP toxicity, than most other species, including rats and humans;
- Oral LOAEL for reproductive effects in rodents is ~38 mg/kg bw/d (Poon et al, 1997) and NOAEL is ~3.7 – 14 mg/kg bw/d (Poon et al, 1997, Lamb et al, 1987).
- Non-oral studies are too limited to derive NOAEL/LOAELs.

Exposure:
The majority of the exposure estimates in this report are those of the (FDA, 2002).

Conclusions:

NTP CERHR concluded that:

- The level of concern is '*minimal*' for adults medically exposed to DEHP. The oral LOAEL of 425 mg/kg bw/d (Lamb et al, 1987) remains informative. Humans have 2-3 fold lower levels of intestinal lipases than rats.
- For critically ill infants undergoing medical treatments, there is '*serious*' concern that exposure may adversely affect male reproductive tract development. Intensively medically treated infants are exposed to doses that are toxic in rodents, however there is less conversion of DEHP to MEHP by the parenteral route although the exact degree of reduction is not known.
- There is '*concern*' for adverse effects on male offspring of pregnant and breast-feeding women undergoing certain medical procedures that may result in high levels of exposure to DEHP. The lowest LOAEL for testicular effects was 14-23 mg/kg bw/d, with a NOAEL of 5 – 8 mg/kg bw/d (NTP 2004).

APPENDIX A: EXPOSURE CALCULATIONS

ADULT

1. Intravenous infusion of crystalloid solutions

The maximum value of DEHP reported for crystalloid IV solution was 0.172 μg/mL (Corley* et al, 1977). More recent Australian figures are much lower (0.011-0.014 μg/mL) (Storey, 2005).

The daily exposure to DEHP leached from PVC storage bags is calculated as follows:

$$E_d = \frac{Conc_{crystal} \times Vol \times 0.001}{BW}$$

Where:

E_d	=	Daily exposure (mg/kg/day)
$Conc_{crystal}$	=	DEHP concentration in storage bag containing crystalloid solution (0.172 μg/mL)
Vol	=	volume delivered daily (2000 mL/day)
BW	=	body weight (70 kg)

Using the maximum value of 0.172 μg/mL, the daily intake of DEHP leached from PVC storage bags containing a crystalloid solution would be 0.005 mg/kg/day.

If DEHP-containing tubing is used to deliver the solution then an additional load of DEHP could be leached from the tubing. There is no data for adults but Loff and colleagues (2000) found a maximum concentration of 1.05 μg/ml DEHP was leached from 2.25 m of PVC tubing after 140 ml of an amino acid-glucose solution was infused over 24 hours. With an internal surface area of 77.8 cm^2, this yields an extraction rate of 0.08 $\mu g/cm^2$/hour.

Data supplied by Australian sponsors suggest that in a typical adult scenario, tubing dimensions are 280 cm in length with an internal diameter of 3.2mm (SA = 281 cm^2). The daily exposure to DEHP leached from PVC tubing could be calculated as follows:

$$E_d = \frac{TL_{crystal} \times SA \times D \times 0.001}{BW}$$

Where:

E_d	=	Daily exposure (mg/kg/day)
$TL_{crystal}$	=	DEHP leaching rate into crystalloid solution (0.08 $\mu g/cm^2$/hr)
SA	=	Surface area (281 cm^2)
D	=	Duration (24 hours)
BW	=	body weight (70 kg)

In this case, an additional 0.38 mg DEHP would be released if the extraction rate is linear, equivalent to 0.008 mg/kg/day.

If both DEHP-containing bag and tubing were used during the procedure then the estimated dose would be 0.013 mg/kg/day. Infusate may be heated to 40°C. This will not go to bag but will pass through tubing. It would be expected that additional DEHP would be extracted in this scenario.

2. Intravenous infusions of drugs

The degree to which DEHP is extracted depends on hydrophobicity of the drug formulation and whether the drug is mixed then stored or mixed and administered just before use.

(a) Teniposide, mixed and stored at room temp for 48h in PVC bag

Faouzi et al, (1994) conducted a simulated infusion of a teniposide solution (400 mg/ml) prepared in 250 ml of 5% dextrose solution in PVC bags and PVC tubing for 1 hour. The solution was either prepared immediately before infusion or stored in PVC bags at room temperature for 48 hours. The data was presented in a graph.

After storage, DEHP concentration in the PVC bag was an estimated 210 μg/ml.

Teniposide stored before use and delivered in a low PVC bag with a prescription of 250 ml twice daily, would deliver the following DEHP dose:

$$E_d = \frac{Conc_{drug} \times Vol \times F \times 0.001}{BW}$$

Where:

E_d	=	Daily exposure (mg/kg/day)
$Conc_{drug}$	=	DEHP concentration in storage bag containing drug (210 μg/ml)
Vol	=	volume delivered daily (250 mL/day)
F	=	frequency per day (twice)
BW	=	body weight (70 kg)

This would result in a DEHP dose of 1.5 mg/kg/day from the bag.

In the leaching experiment described above, it can be extrapolated form the graph that the PVC tubing alone contributed 2 μg/ml DEHP (Faouzi et al, 1994).

The daily exposure to DEHP leached from PVC tubing into teniposide solution is calculated as follows:

$$E_d = \frac{TL_{drug} \times Vol \times F \times 0.001}{BW}$$

Where:

E_d	=	Daily exposure (mg/kg/day)
TL_{drug}	=	DEHP leaching rate into drug solution (2 μg/ml)
Vol	=	volume delivered daily (250 mL/day)
F	=	frequency per day (twice)
BW	=	body weight (70 kg)

Thus, an additional 0.01 mg/kg bw/day from the tubing, giving a total DEHP dose of 1.51 mg/kg bw/day.

(a) Teniposide prepared immediately before use

It is not recommended that teniposide (Vumon) be prepared in DEHP-containing containers (MIMS). Rather glass or polyolefin containers are recommended along with non-DEHP containing administration sets. DEHP was not detected in teniposide formulations stored in glass bottles or polyolefin containers (Faouzi et al, 1994).

The concentration of DEHP in low PVC bags is assumed to be 5 μg/ml (FDA, 2002). Using the prescription and formula as described above, the daily intake of DEHP

leached from low PVC storage bags containing teniposide would be 0.04 mg/kg/day with an additional 0.01 mg/kg bw/day leached if PVC tubing is used to deliver the drug.

If manufacturer's instructions are adhered to and teniposide is prepared immediately before use in non-DEHP containing bags and delivered using non-PVC tubing, the dose of DEHP would be negligible.

3. Transfusion of Blood and Blood products

The following values for DEHP concentration will be used in the exposure estimates.

Packed RBC	36.5 μg/ml (Inoue et al, 2005) - 123.1 μg/ml (Plonait et al, 1993)
Fresh Frozen Plasma	26.7 μg/ml (Shintani*, 1985)
Platelets	15.0 μg/ml (Inoue et al, 2005)
Cryoprecipitate	15.0 μg/ml (Sasakawa* & Mitomi, 1978)

3.1 Short term blood transfusions

Routine elective surgery

A patient requiring routine elective surgery would receive 700mL of blood (2 units of 350 ml) over 1 hour. If this patient received only packed RBC, the DEHP dose from the blood bag would be as follows:

$$E_d = \frac{Conc_{RBC} \times Vol \times 0.001}{BW}$$

Where:

E_d	=	Daily exposure (mg/kg/day)
$Conc_{RBC}$	=	DEHP concentration in storage bag containing packed RBC (36.5 – 123.1 μg/ml)
Vol	=	volume delivered daily (700 ml/day)
BW	=	body weight (70 kg)

The daily intake of DEHP leached from PVC storage bags containing packed RBC would be 0.37 – 1.23 mg/kg/day. The upper value using the maximum measurement from Plonait and colleagues (1993) was made after the RBC had been mixed with plasma, passed through an administration set and then a blood warmer. This value is likely to represent an over-estimate as blood is not generally warmed for adult administration.

If DEHP-containing tubing is used to deliver the packed RBC then an additional load of DEHP could result from leaching from tubing. This adjustment is not necessary for the estimate derived from Plonait and colleagues (1993) as the DEHP concentration was measured after the packed RBC had passed through tubing, however, it does assume that the tubing surface areas are the same. The complete dimensions of the tubing used by Plonait and colleagues (1993) are unknown.

DEHP leaching rate has been estimated for infant ECMO circuits using whole blood (Karle et al, 1997), heparinised fresh human blood in a Chandler loop (Hildenbrand et al, 2005), whole blood during a haemodialysis sessions in adult patients (Easterling et al, 1974; Pollack* et al, 1985a) and packed RBC, platelet-rich plasma or fresh frozen plasma through infant infusion pumps (Loff et al, 2000). The data provided by Loff et

al, (2000) will be used as the extraction rates are for packed RBC. It is known that the extraction rate of DEHP into whole blood from bags is greater than packed RBC.

There are no data for adults, but Loff and colleagues (2000) found a maximum concentration of 5.4 μg/ml DEHP was leached from 2.25m of PVC tubing after 20 ml of packed RBC was infused over 1 hour. With an internal surface area of 77.8 cm^2, this yields an extraction rate of 1.39 $\mu g/cm^2$/hour.

Data supplied by Australian sponsors suggest that in a typical adult scenario, tubing dimensions are 190 cm length with an internal diameter of 3.2mm (SA = 191 cm^2). The daily exposure to DEHP leached from PVC tubing could be calculated as follows:

$$E_d = \frac{TL_{RBCl} \times SA \times D \times 0.001}{BW}$$

Where:

E_d = Time-weighted daily exposure (mg/kg/day)
TL_{RBC} = DEHP leaching rate into packed RBC (1.39 $\mu g/cm^2$/hr)
SA = Surface area (191 cm^2)
D = duration (1 hour)
BW = body weight (70 kg)

Assuming a linear extraction rate, an additional 0.26 mg DEHP would be extracted over 1 hour or 0.003 mg/kg/day. The length of tubing can range up to 455 cm and the dose would then be greater.

If both DEHP-containing bag and tubing were used then the estimated dose would be 0.37 – 1.23 mg/kg/day.

Adult Trauma patient

An acute trauma patient could receive 2.5L of blood (7 units of 350ml) over 3.5 hours. If this patient received only packed RBC (an unlikely scenario) the DEHP dose from the blood bag could be calculated as follows:

$$E_d = \frac{Conc_{RBC} \times Vol \times 0.001}{BW}$$

Where:

E_d = Daily exposure (mg/kg/day)
$Conc_{RBC}$ = DEHP concentration in storage bag containing packed RBC (36.5 – 123.1 μg/ml)
Vol = volume delivered daily (2450 ml/day)
BW = body weight (70 kg)

The daily intake of DEHP leached from PVC storage bags containing packed RBC would be 1.3 – 4.3 mg/kg/day. The upper value is likely to represent an over-estimate as blood is not generally warmed for adult administration.

If DEHP-containing tubing is used to deliver the packed RBC, then an additional amount of DEHP could be leached from the tubing. This adjustment is not necessary for the estimate derived from Plonait and colleagues (1993), however, it does assume that the tubing lengths are the same.

Data supplied by Australian sponsors suggest that in a typical adult scenario, tubing dimensions are 190 cm length with an internal diameter of 3.2mm (SA = 191 cm^2). The daily exposure to DEHP leached from PVC tubing is calculated as follows:

$$E_d = \frac{TL_{RBCl} \times SA \times D \times 0.001}{BW}$$

Where:

E_d = Time-weighted daily exposure (mg/kg/day)
TL_{RBC} = DEHP leaching rate into packed RBC (1.39 $\mu g/cm^2/hr$)
SA = Surface area (191 cm^2)
D = duration (3.5 hours)
BW = body weight (70 kg)

Assuming a linear extraction rate, an additional 0.93 mg DEHP would be extracted over 3.5 hours or 0.013 mg/kg/day.

If both DEHP-containing bag and tubing were used then the estimated dose would be 1.31 – 4.31 mg/kg/day.

ECMO

Patients undergoing ECMO receive DEHP from PVC tubing as well as DEHP in stored blood products. In the scenario of Butch et al, (1996), 21 units of packed RBC, FFP, platelets and cryoprecipitate were infused over 10 to 20 days as follows:

	[DEHP] µg/ml	**#units**	**Vol/unit (ml)**	**DEHP dose (mg)**
RBC	36.5 – 123.1	4.6	350	58.77 – 198.19
Platelet	15.0	15	100	22.5
FFP	26.7	0.5	200	2.67
Cryoprecipitate	15.0	1	50	0.75
TOTAL		21.1		84.69 – 224.11

The DEHP dose from the blood bag is calculated as follows:

$$E_d = \frac{Conc \times Vol_{unit} \times Units \times 0.001}{BW}$$

Where:

E_d = Time-weighted daily exposure (mg/kg/day)
Conc = DEHP concentration in storage bag containing packed RBC/platelet/FFP/Cryoprecipitate (µg/ml)
$Volume_{unit}$ = volume/unit (ml)
Units = number of units each procedure
BW = body weight (70 kg)

The total intake of DEHP leached from PVC storage bags in the above scenario would be 1.2-3.2 mg/kg bw or 0.12 – 0.32 mg/kg/day if time-averaged over 10 days.

In a typical adult scenario, ECMO circuitry can be 280 cm length with an internal diameter of 8 mm (SA = 704 cm^2). Assuming infusion takes 5 hours (an over-estimate as infusion takes place over several days) the bulk of the fluid perfused is packed RBC.

Using the leaching rate for this component, exposure to DEHP leached from PVC tubing is calculated as follows:

$$E_d = \frac{TL_{RBC} \times SA \times D \times 0.001}{BW}$$

Where:

E_d = Daily exposure (mg/kg/day)
TL_{RBC} = DEHP leaching rate into packed RBC (1.39 μg/cm^2/hr)
SA = Surface area (704 cm^2)
D = duration (5 hours)
BW = body weight (70 kg)

In this case an additional 0.07 mg/kg bw/day DEHP would be extracted (0.003 mg/kg bw/day time-averaged). Alternatively, if the higher leaching rate for FFP is used (17.05 ug/cm^2/hour) then an additional 0.86 mg/kg bw DEHP would be infused. This would amount to 0.086 mg/kg bw/day if time-averaged over 10 days.

If both DEHP-containing bag and tubing were used then the estimated dose would be 0.21 – 0.41 mg/kg bw/day.

3.2 Long term blood transfusions

Sickle-cell disease

Patients with sickle cell disease are typically transfused with 1-2 units of packed RBC every 2-4 weeks. Each unit is 350 ml. The time-weighted DEHP dose from the blood bag is calculated as follows:

$$E_d = \frac{Conc_{RBC} \times Vol_{unit} \times Units \times 0.001}{BW \times F}$$

Where:

E_d = Daily exposure (mg/kg/day)
$Conc_{RBC}$ = DEHP concentration in storage bag containing packed RBC (36.5 – 123.1 μg/ml)
$Volume_{unit}$ = volume/unit (350 ml)
Units = number of units each fortnight (2)
BW = body weight (70 kg)
F = frequency of procedure (14 days)

The daily intake of DEHP leached from PVC storage bags containing packed RBC would be 0.03 – 0.09 mg/kg/day.

Data supplied by Australian sponsors suggest that in a typical adult scenario, tubing dimensions are 200 cm length with an internal diameter of 3.2mm (SA = 201 cm^2). Assuming infusion takes one hour, the daily exposure to DEHP leached from PVC tubing is calculated as follows:

$$E_d = \frac{TL_{RBC} \times SA \times D \times S \times 0.001}{BW \times F}$$

Where:

E_d = Daily exposure (mg/kg/day)
TL_{RBC} = DEHP leaching rate into packed RBC (1.39 μg/cm^2/hr)

SA = Surface area (201 cm^2)
D = duration (1 hours)
S = number of sessions/fortnight (2)
BW = body weight (70 kg)
F = frequency of procedure (14 days)

In this case an additional 0.04 mg DEHP would be extracted or 0.0006 mg/kg/day.

If both DEHP-containing bag and tubing were used then the estimated dose would be 0.03 – 0.09 mg/kg/day.

Chemotherapy

In a study of Taxol chemotherapy, patients received about 3 transfusions during the course of therapy, or about 1 transfusion (presumably packed red cells) every 3 weeks (Veach* et al, (1998) reported in FDA, (2002). Assuming one transfusion every 3 week treatment period, then the time-weighted DEHP dose from the blood bag is calculated as follows:

$$E_d = \frac{Conc_{RBC} \times Vol_{unit} \times Units \times 0.001}{BW \times F}$$

Where:

E_d = Time-weighted daily exposure (mg/kg/day)
$Conc_{RBC}$ = DEHP concentration in storage bag containing packed RBC (36.5 – 123.1 μg/ml)
$Volume_{unit}$ = volume/unit (350 ml)
Units = number of units each three weeks (1)
BW = body weight (70 kg)
F = frequency of procedure (21 days)

The daily intake of DEHP leached from PVC storage bags containing packed RBC would be 0.009 – 0.03 mg/kg/day.

Data supplied by Australian sponsors suggest that in a typical adult scenario, tubing dimensions are 200 cm length with an internal diameter of 3.2mm (SA = 201 cm^2). Assuming infusion takes 30 minutes, the daily exposure to DEHP leached from PVC tubing could be calculated as follows:

$$E_d = \frac{TL_{RBC} \times SA \times D \times S \times 0.001}{BW \times F}$$

Where:

E_d = Daily exposure (mg/kg/day)
TL_{RBC} = DEHP leaching rate into packed RBC (1.39 $\mu g/cm^2/hr$)
SA = Surface area (201 cm^2)
D = duration (1 hour)
S = number of sessions/three weeks (1)
BW = body weight (70 kg)
F = frequency of procedure (21 days)

In this case a minor amount of DEHP would be extracted.

If both DEHP-containing bag and tubing were used then the estimated dose would be 0.009 – 0.03 mg/kg/day.

Clotting disorders

Cryoprecipitates containing clotting factors are administered to patients with clotting disorders. Marcel (1973*) found that cryoprecipitate packs contained from 0.8 to 1.9 mg of DEHP. Since patients with clotting disorders can receive up to 400 bags of cryoprecipitate in one year, using the upper bound measurement, the time-weighted DEHP dose received by these patients could be calculated as follows:

$$E_d = \frac{Conc_{cryo} \times Units}{BW \times F}$$

Where:

E_d = Daily exposure (mg/kg/day)
$Conc_{cryo}$ = DEHP concentration in storage bag containing cryoprecipitate (1.9 mg/pack)
Units = number of units each year (400)
BW = body weight (70 kg)
F = frequency of procedure (365 days)

The maximum daily intake of DEHP leached from PVC storage bags containing cryoprecipitate would be 0.03 mg/kg/day. There is no data available on the extraction rate of DEHP from tubing into cryoprecipitate.

Haemodialysis

Not all DEHP is retained by the patient during dialysis. For example, Faouzi et al, (1999b) estimate that while 75 (44-197) mg DEHP is delivered in each session only 3.6-59.6 mg is retained in a single session. Assuming the average patient has three sessions each week, then the time-weighted DEHP dose from haemodialysis can be calculated as follows:

$$E_d = \frac{Conc_{AUC} \times S}{BW \times F}$$

Where:

E_d = time-weighted daily exposure (mg/kg/day)
$Conc_{AUC}$ = DEHP dose estimated from AUC ($59.6^{retained}$, 197 $mg^{delivered}$)
S = number of session/week (3)
BW = body weight (70 kg)
F = frequency of procedure (7 days)

The maximum daily delivered dose of DEHP leached from PVC storage bags in the above scenario would be 1.21 mg/kg/day with an estimated maximum of 0.36 mg/kg bw/day retained.

Kambia and colleagues (2001a) measured DEHP during haemodialysis using DEHP and TOTM-DEHP tubing. For DEHP tubing, the mean delivered dose was 122.95 mg while the mean retained dose was 27.3 mg. For TOTM-DEHP tubing, the maximum delivered and retained doses were 49.20 mg and 6.7 mg, respectively. Using the formula above, this would equate to a time-averaged daily doses of 0.30 mg/kg bw/day (delivered) and 0.04 mg/kg bw/day (retained).

4. Total Parenteral nutrition

In adults, 500 ml of a 20% lipid solution would typically be administered with an additional 2 L of electrolyte and amino acid solution over the course of one day. The DEHP dose from the lipid solution will be estimated from the maximum measurements of Mazur et al, (1989) for 10% lipid in PVC bags (3.1 μg/ml) and Kambia et al, (2003) for 4% lipid in EVA bags (0.4 μg/ml).

The DEHP dose from the nutrient bag was calculated as follows:

$$E_d = \frac{\text{Conc x Vol x } 0.001}{\text{BW}}$$

Where:

E_d = Daily exposure (mg/kg/day)
Conc = DEHP concentration in storage bag containing lipid (0.4 or 3.1 μg/ml)
Volume = volume (2500 mL)
BW = body weight (70 kg)

The daily intake of DEHP leached from PVC storage bags in the above scenario would be 0.11 mg/kg/day (PVC bag) or 0.01 (EVA bag). If the solution does not contain lipids, then the DEHP dose would be considerably less (see Crystalloid solution scenario).

The maximum concentration of DEHP in a TPN solution containing 1 – 3.85% lipid measured in PVC tubing was 600 ng/ml with a flow rate of 177 ml/hour or 106 μg/hour (Kambia et al, 2001b; Kambia et al, 2003).

Assuming a linear extraction rate, infusion over 24 hours, DEHP leached from PVC tubing could be calculated as follows:

$$E_d = \frac{TL_{lipid} \text{ x Dx } 0.001}{\text{BW}}$$

Where:

E_d = Daily exposure (mg/kg/day)
D = duration (hours)
TL_{lipid} = DEHP leaching rate (106 μg/h)
BW = body weight (70 kg)

In this case an additional 0.04 mg/kg bw/day DEHP would be extracted into PVC tubing.

However, if the 20% lipid admixture is delivered undiluted, the concentration of DEHP leached from the tubing will be greater. Loff and colleagues (2000) found a maximum

concentration of 490 μg/ml DEHP was leached from 2.25 m of PVC tubing after 24 ml of a 20% lipid emulsion was infused over 24 hours. With an internal surface area of 77.8 cm^2, this yields an extraction rate of 6.3 $\mu g/cm^2$/hour.

Assuming the above prescription and tubing of the dimensions 200 cm x 3.0 mm (SA = 189 cm^2), the daily exposure to DEHP leached from PVC tubing into a 20% lipid solution overnight (10 hours) would total:

$$E_d = \frac{TL \times SA \times D \times 0.001}{BW}$$

Where:

E_d	=	Daily exposure (mg/kg/day)			
$TL_{crystal}$	=	DEHP leaching rate (6.3 $\mu g/cm^2$/hr)	SA	=	Surface area (189 cm^2)
D	=	Duration (10 hours)	BW	=	body weight (70 kg)

In this case an additional 0.17 mg/kg bw DEHP would be extracted into PVC tubing. There is no data on the amount of DEHP released into non-PVC tubing.

If a 20% lipid solution is delivered without dilution using EVA bags and PVC tubing then the maximum daily delivered dose of DEHP would be 0.21 mg/kg/day. If non-PVC bags were used with PVC tubing to deliver a 5% lipid solution (typical TPN solution) the delivered DEHP dose would be 0.05 mg/kg/day.

5. Peritoneal dialysis

Using the maximum measurement of 0.021-0.13 μg/ml and assuming 8 L is intraperitoneally injected (Mettang* et al, 1996): The DEHP dose from dialysate is calculated as follows:

$$E_d = \frac{Conc_{dial} \times Vol \times 0.001}{BW}$$

Where:

E_d	=	Daily exposure (mg/kg/day)
$Conc_{dial}$	=	DEHP concentration in dialysate (0.13 μg/ml)
Volume	=	volume (8000 mL)
BW	=	body weight (70 kg)

Assuming the tubing length in Australian scenarios is similar to that measured by Mettang et al, (1996), the daily intake of DEHP leached from tubing used in peritoneal dialysis would be 0.01 mg/kg/day. The retained dose would be less.

6. Enteral nutrition

The maximal concentration of DEHP was 3.1 μg/ml in a 10% lipid solution stored for 48 hours (Mazur et al, 1989).

Assuming 2 L per day is delivered, the DEHP dose from the enteral feeding bag can calculated as follows:

$$E_d = \frac{Conc \times Vol \times 0.001}{BW}$$

Where:

E_d = Daily exposure (mg/kg/day)
Conc = DEHP concentration in storage bag containing 10% lipid (3.1 μg/ml)
Volume = volume (2000 mL)
BW = body weight (70kg)

The daily intake of DEHP leached from PVC storage bags in this scenario would be 0.09 mg/kg/day. If the solution does not contain lipids or the feeding solution is prepared and stored in bottles, then the DEHP dose would be considerably less.

Additional exposure would come from the PVC nasogastric tubing and extension set. Again, there is no specific data for adult exposure from leaching of DEHP into enteral delivery devices. The maximum concentration of DEHP in a TPN solution containing 1 – 3.85% lipid measured in PVC tubing was 600 ng/ml with a flow rate of 177 ml/hour or 106 μg/hour (Kambia et al, 2001b; Kambia et al, 2003).

Assuming a linear extraction rate, infusion of 2000 ml of a 4% lipid solution over 24 hours, DEHP leached from PVC tubing is calculated as follows:

$$E_d = \frac{TL_{lipid} \times D \times 0.001}{BW}$$

Where:

E_d = Daily exposure (mg/kg/day)
TL_{lipid} = DEHP leaching rate (106 μg/h) D = duration (hours)
BW = body weight (70 kg)

In this case an additional 0.04 mg/kg bw/day DEHP would be extracted into PVC tubing.

The maximum daily delivered dose of DEHP if both PVC storage bags and tubing were used would be 0.13 mg/kg/day. In the more typical scenario, non-PVC bags would be used with PVC tubing. In this case the daily delivered dose would be 0.04 mg/kg/day. The daily delivered dose would be greatly increased if the feed comprised 20% lipid without dilution.

7. Inhalation Exposure

Heated Respiratory Tubing (Adults)
The worst-case scenario involves breathing air saturated with DEHP continuously for 24 hours/day. Assuming a vapour pressure of 4.8×10^{-4} Pa at 37°C (4.8×10^{-9} atm) and a mean flow rate of 10 L/min, the daily volume of DEHP would be 6.9×10^{-5} L/day (Health Canada, 2002).

The standard volume is (gas constant) x (1 + temp/degree Kelvin) or
22.4 x (1+37/273) = 25.4 L.

Therefore the DEHP daily mass would be:

$$\text{DEHP mass} = \frac{\text{Daily volume x MW x 0.01}}{\text{Standard volume}}$$

Where:

Daily mass = DEHP (mg/day)

Daily volume = (6.9×10^{-5}) L/day MW = 390.57 g/L

Standard volume = 25.4 L

Assuming the patient is breathing a saturated volume of air, DEHP leached from PVC tubing would be 1.06 mg/day or 0.015 mg/kg bw/day.

Oxygen Supply Tubing (Adults)

The worst-case scenario (exposure to DEHP saturated air at 37 °C and a flow rate of 15 L/min for the duration of the procedure) would result in a DEHP exposure of 1.6 mg DEHP/day or 0.02 mg/kg bw/day using the same formaulae as above.

8. Breast Milk

Dostal and colleagues (1987b) report that the milk:plasma partition coefficient in rats for DEHP was < 200 due to the lipophilic nature of this compound. However in humans, plasma concentrations of DEHP in normal subjects (around 0.1 μg/mL) and concentrations reported in breast milk by Pfordt & Bruns-Weller, (1999) (median 43 μg/kg milk), the milk:plasma partition coefficient in the general population was calculated to be 0.43 (Health Canada, 2002).

Typical milk consumption is 150 ml/kg/day. Faouzi et al, (1999b) measured DEHP plasma levels of 3 μg/ml after 4 hours of haemodialysis. The DEHP exposure to a breastfed infant of a mother on haemodialysis is estimated as follows.

$$E_d = Conc_{milk} \times PC \times Vol \times 0.001$$

Where:

E_d = Daily exposure (mg/kg/day)
PC = Partition co-efficient milk:plasma (0.43)
$Conc_{milk}$ = DEHP concentration in milk (3 μg/ml)
Volume = volume (150 mL/kg)

The maximum daily delivered dose of DEHP from breast milk would be 0.2 mg/kg/day. The dose of DEHP received by infants would be higher (90 mg/kg/day) if milk:plasma partition coefficient for DEHP in rats (200) was used.

NEONATES

Some information regarding possible exposure scenarios was obtained by visiting a NICU at Westmead Hospital, Sydney. These data were used in the development of scenarios for intravenous infusion of drugs and TPN. Procedures in neonatal wards at other hospitals may differ.

1. Intravenous Infusion of crystalloid solutions

As crystalloid solutions are delivered using a syringe pump, the only source of DEHP is tubing. Loff et al, (2000) took measurements before and after perfusion. The content after perfusion will be used in the exposure estimate as it represents both the residual DEHP content in the stored solution and the DEHP extracted after running the solution through PVC tubing. Loff and colleagues (2000) found a maximum concentration of 1.8 μg/ml DEHP was leached from 2.25m of PVC tubing after 140 ml of an amino acid-glucose solution was infused over 24 hours. Flow rate was therefore 1ml/hour, internal surface area was 77.8 cm^2 and extraction rate was 0.08 $\mu g/cm^2$/hour.

Data supplied by Australian sponsors suggest that syringe pump tubing can be 225 cm in length with an internal diameter of 1.1 mm (SA = 77.8 cm^2). The daily exposure to DEHP leached from PVC tubing could be calculated as follows:

$$E_d = \frac{TL_{crystal} \times SA \times D \times 0.001}{BW}$$

Where:

E_d = Daily exposure (mg/kg/day)
$TL_{crystal}$ = DEHP leaching rate into crystalloid solution (0.08 $\mu g/cm^2$/hr)
SA = Surface area (77.8 cm^2)
D = Duration (24 hours)
BW = body weight (4 kg)

The daily DEHP dose would be 0.04 mg/kg bw/day.

Syringe pumps may also use a microbore giving set. For example, a micro-volume giving set of 91.5 cm may have an internal diameter of 0.6 mm (Surface area = 17 cm^2). Using the formula above the amount of DEHP extracted would be 0.008 mg/kg bw/day.

2. Intravenous infusion of drugs

Drug infusions to neonates are typically administered using an infusion pump.

Extraction rates for infusion pumps and tubing in typical NICUs were reported as follows: Imipenem, 0.78 μg/ml; midazolam, 1.13 μg/ml; fentanyl, 4.89 μg/ml and propofol, 656 μg/ml (Loff et al, 2000). Given the volumes infused and length of tubing, the extraction rates were: Imipenem, 0.003 $\mu g/cm^2$/h, midazolam, 0.02 $\mu g/cm^2$/h, 0.077; fentanyl, 0.02 $\mu g/cm^2$/h and propofol, 3.95 $\mu g/cm^2$/h.

Of these drugs, only midazolam is not contra-indicated in young children. A typical dose of DEHP following infusion of midazolam, using syringe pump microbore tubing of 200 cm x 0.9 mm dimensions is calculated as follows:

$$E_d = \frac{TL_{midazolam} \times SA \times D \times 0.001}{BW}$$

Where:

E_d = Daily exposure (mg/kg/day)
$TL_{midazolam}$ = DEHP leaching rate into midazolam solution (0.02 $\mu g/cm^2$/hr)
SA = Surface area (56.6 cm^2)
D = Duration (24 hours)
BW = body weight (4 kg)

The daily intake of DEHP leached from PVC tubing delivering midazolam would be 0.007 mg/kg/day. Microbore tubing is typically used to deliver drugs in neonatal wards.

3. Transfusion of Blood and Blood Products

Replacement transfusion

Two scenarios are modelled. In the first scenario, Ringer* et al, (1998) reported that neonates in one neonatal intensive care unit (NICU) received, on average, 33.6 ml of RBCs and 2.4 ml of FFP in the first 14 days. Infants in this study weighed about 1 kg. In the second scenario, The Royal Prince Alfred Hospital, Camperdown recommends for stable preterm infants, infusion of 20 mls/kg packed red cells over 4 hours. The DEHP load is estimated as follows:

$$E_d = \frac{Conc_{RBC} \times Vol \times 0.001/14}{BW}$$

Where:

E_d = Daily exposure (mg/kg/day)
$Conc_{RBC}$ = DEHP concentration in storage bag containing packed RBC (36.5-123.1μg/ml)
Vol = volume delivered per fortnight (ml)
BW = body weight (kg)

In the first scenario, a premature neonate weighing 1.073 kg is infused with 33.6 ml of packed RBC over 14 days. The DEHP dose would be 1.2 – 4.1 mg or 0.08 - 0.28 mg/kg/day if time-averaged over 14 days.

In the second scenario, for a stable 4 kg neonate infused with 20 mls/kg packed RBC, the DEHP dose would be 0.73 – 2.46 mg/kg bw or 0.05 – 0.18 mg/kg/day time-averaged over 14 days.

However, if the blood product was administered via an infusion pump, then the amount of DEHP received by the patient would be greater. Loff et al, (2000) infused packed red blood cells through an infusion pump over 1 hour. DEHP was measured before infusion (present in blood product bags) and after infusion (contribution from tubing). They found a maximum concentration of 5.4 μg/ml DEHP was leached from 2.25 m of PVC tubing after 20 ml of packed RBC was infused over 1 hour. With an internal surface area of 77.8 cm^2, this yields an extraction rate of 1.39 $\mu g/cm^2$/hour.

Tubing dimensions can vary but a typical pump infusion set had dimension of 225 cm by 1.1 mm (SA = 77.8 cm^2). If 80 ml of packed RBC was infused into a 4 kg neonate then the additional exposure to DEHP leached from PVC tubing is calculated as follows:

$$E_d = \frac{TL_{RBCl} \times SA \times D \times 0.001}{BW}$$

Where:

E_d = Daily exposure (mg/kg/day)
TL_{RBC} = DEHP leaching rate into packed RBC (1.39 $\mu g/cm^2$/hr)
SA = Surface area (77.8 cm^2)
D = duration (4 hours)
BW = body weight (4 kg)

In this case an additional 0.11 mg/kg/day DEHP would be extracted. This would equate to 0.008 mg/kg/day if time-averaged over 14 days.

The total DEHP dose from replacement transfusion of a premature neonate would be 0.09 - 0.28 mg/kg/day if time-averaged over 14 days and 0.06 – 0.18 mg/kg/day for a stable 4 kg neonate.

ECMO

Up to 140 mg/kg bw was estimated as the DEHP dose following ECMO treatment over 10 days (Shneider* et al, 1989). If 80 ml of packed RBC was infused into a 4 kg neonate then the additional exposure to DEHP leached from PVC tubing is calculated as follows:

$$E_d = \text{Dose} / 10$$

Where:

E_d = Daily exposure (mg/kg/day)
Dose = DEHP dose over 10 days (140 mg/kg)

Therefore the estimated daily dose would be 14 mg/kg bw/day.

4. Total Parenteral nutrition

Small neonates will typically receive TPN using a syringe infuser with a microbore extension set. Larger babies will have the TPN made up into EVA bags or bottles with a normal extension set.

Typical nutrient intake for infants is 150 ml/kg/day of a standardised electrolyte/amino acid solution. This can then be supplemented with lipid to a maximum of 18 ml/kg (containing 12 ml of 20% lipid). The lipid and electrolyte solutions are generally administered separately. For the purposes of this exposure estimate, the maximum concentration measured by Loff and colleagues (2000) will be used. They found a maximum concentration of 490 μg/ml DEHP was leached from 2.25 m of PVC tubing after 24 ml of a 20% lipid emulsion was infused over 24 hours and 1.05 μg/ml into 140 ml of an amino acid/glucose/electrolyte solution. With an internal surface area of 77.8 cm^2, this yields an extraction rate of 6.3 $\mu g/cm^2$/hour and 0.08 $\mu g/cm^2$/hour respectively.

Assuming the above scenario for a larger baby, tubing of the dimensions 200 cm x 3.0 mm (SA = 189 cm^2) could be used. The daily exposure to DEHP leached from PVC tubing into lipid and electrolyte solutions delivered in separate tubing over 24 hours would total:

$$E_d = \frac{TL_{crystal} \times SA \times D \times 0.001}{BW}$$

Where:

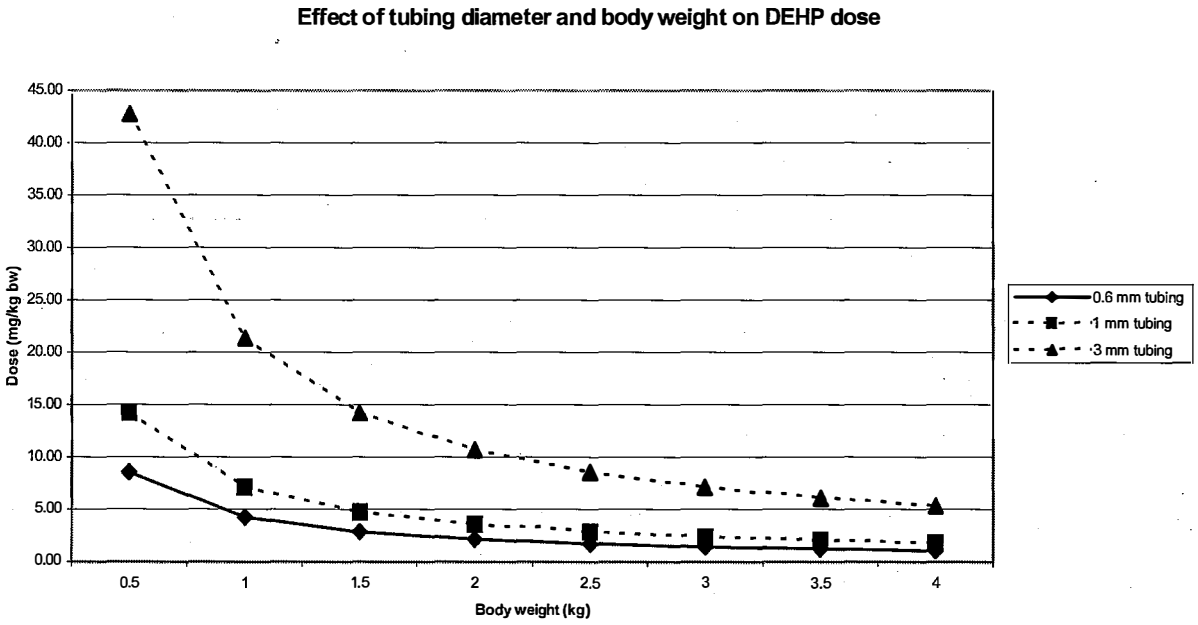
E_d = Daily exposure (mg/kg/day)
$TL_{crystal}$ = DEHP leaching rate ($0.08^{crystalloid}$, 6.3^{lipid} $\mu g/cm^2$/hr)
SA = Surface area (189 cm^2)
D = Duration (24 hours)
BW = body weight (4 kg)

Solution	Extraction rate ($\mu g/cm^2$/hr)	DEHP extracted (mg)	DEHP (mg/kg bw/day)
Amino acid/glucose	0.08	0.36	0.09
20% lipid	6.3	28.58	7.13

Total exposure for a 4 kg neonate would be 7.22 mg/kg/day.

Syringe pumps may also use a microbore giving set. For example, a micro-volume giving set of 150 cm may have an internal diameter of 0.6 mm (Surface area = 28 cm^2). Using the formula above the amount of DEHP extracted from 20% lipid solution would be 1.07 mg/kg bw/day.

The estimated DEHP dose will differ depending on the babies weight. In the graph below, the estimated DEHP dose from different diameter tubing of the same length (150 cm) has been calculated assuming an extraction rate of 6.3 $\mu g/cm^2/hr$ for 20% lipid over 24 hours for babies of different weights. The relative DEHP dose will be much greater on a mg/kg bw basis when a 20% lipid solution is delivered over 24 hours to smaller babies.



An alternative estimate can be derived using the maximum concentration of DEHP measured at outlet of PVC tubing as reported by Kambia et al, (2003). The maximum concentration of DEHP in a TPN solution containing 1 – 3.85% lipid measured in PVC tubing was 600 ng/ml with a flow rate of 177 ml/hour or 106 µg/hour (Kambia et al, 2001b; Kambia et al, 2003).

Assuming a linear extraction rate, infusion of 150 ml/kg of lipid solution over 24 hours, DEHP leached from PVC tubing could be calculated as follows:

$$E_d = \frac{TL_{lipid} \text{ x } Dx\ 0.001}{BW}$$

Where:

E_d = Daily exposure (mg/kg/day)
TL_{lipid} = DEHP leaching rate (106 µg/h)
D = duration (24 hours)
BW = body weight (4 kg)

The DEHP dose leached would be 0.64 mg/kg bw/day. This assumes that the giving set used by Kambia et al, (2001, 2003) is the same dimension as those used in an Australian setting.

If the solution does not contain lipids, then the DEHP dose would be considerably less (see Crystalloid scenario).

5. Enteral nutrition

Assuming that the nasogastric tube is non-PVC then the estimated DEHP dose would be derived from the giving set (if used). The maximum concentration of DEHP in a TPN solution containing 1 – 3.85% lipid measured in PVC tubing was 600 ng/ml with a flow rate of 177 ml/hour or 106 μg/hour (Kambia et al, 2001b; Kambia et al, 2003). Assuming the nasogastric tubing is PVC-free then the DEHP dose would derive from the giving set.

Assuming a linear extraction rate, infusion of 150 ml/kg of lipid solution over 24 hours, DEHP leached from PVC tubing is calculated as follows:

$$E_d = \frac{TL_{lipid} \text{ x } Dx\ 0.001}{BW}$$

Where:

E_d = Daily exposure (mg/kg/day)
TL_{lipid} = DEHP leaching rate (106 μg/h)
D = duration (24 hours)
BW = body weight (4 kg)

The DEHP dose leached would be 0.64 mg/kg bw/day. If the solution does not contain lipids, then the DEHP dose would be considerably less (see Crystalloid scenario).

However, if a 20% lipid admixture is delivered, the concentration of DEHP leached from the tubing will be greater. Loff and colleagues (2000) found a maximum concentration of 490 μg/ml DEHP was leached from 2.25 m of PVC tubing after 24 ml of a 20% lipid emulsion was infused over 24 hours. With an internal surface area of 77.8 cm^2, this yields an extraction rate of 6.3 $\mu g/cm^2$/hour. Assuming the above scenario and tubing of the dimensions 150 cm x 1.8 mm (SA = 84.9 cm^2), the daily exposure to DEHP leached from PVC tubing into a 20% lipid solution for 24 hours would total:

$$E_d = \frac{TL \text{ x } SA \text{ x } D \text{ x } 0.001}{BW}$$

Where:

E_d = Daily exposure (mg/kg/day)
$TL_{crystal}$ = DEHP leaching rate (6.3 $\mu g/cm^2$/hr)
SA = Surface area (84.9 cm^2)
D = Duration (24 hours)
BW = body weight (4 kg)

In this case an additional 3.2 mg/kg bw DEHP would be extracted into PVC tubing.

6. Inhalation exposure

Conversion factor (adult to neonate)
The surface area of an adult breathing circuit is typically five times that of the infant circuit.

$$SA = 2\pi rh$$

Adult = 2π x1.0 x 1.8 m = 1131 cm^2
Child = 2π x 0.3 x 1.2 m = 226 cm^2

Other considerations include differences in respiratory flow rates between adults and neonates.

Heated Respiratory Tubing (neonate)
(1/3) x 6.9 x 10^{-5} L/day x 390.57/25.4 gm/L = 0.35 mg/day.

APPENDIX B

Table 14. Effects of DEHP following repeat administration

Species, Study Duration, route	Doses (mg/kg bw/day)	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	RESULTS	REFERENCES
Short-term studies (up to 28-days exposure)					
Rat, Sprague-Dawley Females 5 days (2-6, 6-10, or 14-18 of lactation) gavage, corn oil	0, 2000	N/A	2000	↓bw liver: ↑relw all groups, ↑p.enz.act. ↓TG, ↓CHO	(Dostal* et al, 1987b)
Rat, Sprague-Dawley Males 5 days (from day 6, 14-16, 21, 42, 86 of age) gavage, corn oil	0, 10, 100, 1000, 2000	10	100	mortality: all pups in three youngest age groups at 2000 mg/kg bw/day groups ↓bw in two highest dose groups liver: ↑absw and relw from 100 mg/kg bw/day, ↑pp, ↑p.enz.act. ↓TG, ↓CHO	(Dostal* et al, 1987a)
Rat, F344 5 males/group 1 week, diet	0, 1.2% (0, 670)	NA	670	liver: ↑absw and relw	(Takagi* et al, 1992)
Rat, F344 8 males 1 week, diet	0, 2% (0, 1600)	NA	1600	↓CHO, ↓TG ↑ absw and relw for liver and kidney no histological findings in liver, kidney, or testes	(Exxon*, 1982)
Rat, Wistar 4 males/ dose group 6 male controls 3, 10, or 21 days, diet	0 or 2% (1650-1830)	NA	1650-1830	↓bw after 10-21 days liver: ↑relw in all dosed males, ↑pp, ↑pSER, ↑p.enz.act., mitoch changed	(Mann* et al, 1984)
Rat, Alderley Park	0, 50, 200, 1000	NA	50	↓bw at 1000 mg/kg bw/day for 9 months	(CEFIC*, 1982)

Species, Study Duration, route	Doses (mg/kg bw/day)	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	RESULTS	REFERENCES
20 rats/sex/group 30 rats/sex in control group 3, 7, 14, 28 days, or 9 months, diet				liver: ↑w from 50 mg/kg bw/day, ↑pp, ↑pSER, ↑p.enz.act., mitoch changed (males)	(Mitchell* et al, 1985)
Rat, Wistar 6 rats/sex 7 or 21 days, gavage	0, 2500	NA	2500	↓bwg (males) liver: ↑relw, no histological findings, ↑nb peroxisomes, ↑pSER	(Mangham* et al, 1981)
Rat, F344 4-5 rats/sex/group 1 or 3 weeks, diet	0, 0.1, 0.6, 1.2% (0, 80, 480, 960)	NA	80	liver: ↑w from 0.1%, hepatocellular hypertrophy from 0.6% (males) and at 1.2% (females), ↑nb peroxisomes at 1.2%, ↑p.enz.act. from 0.1% ↓TG from 0.1% ↓CHO from 0.6%	(CMA*, 1982)
Rat, F344 5 rats/sex/group 14 days, diet	0, 6300, 12500, 25000, 50000, 100000 ppm (0, 630, 1250, 2500, 5000, 10000)	630	1250	mortality: 2/5 males and 4/5 females at 100000 ppm ↓bwg from 25000 ppm (males), from 50000 ppm (females) testes: atrophy from 12500 ppm	(NTP *, 1982)
Rat, Alderley Park 10 rats/sex/group 14 days, gavage	0, 2000	NA	2000	↓bwg (males) liver: ↑absw, ↑relw, ↑pp, ↑pSER, mitoch changed kidney: ↑weight (females), ↑pp testes: ↓weight, atrophy ↓CHO (males), ↓TG (males)	(ICI*, 1982) (Rhodes* et al, 1986)
Rat, Sprague-Dawley 6 males per group 14 day gavage	0, 1000	NA	1000	liver: ↑relw, ↑p.enz.act.	(Lake* et al, 1984b)
Rat, Sprague-Dawley 5 males per group 14 days, gavage	0, 25, 100, 250, 1000	NA	25	liver: ↑relw from 100 mg/kg bw/day, ↑p.enz.act. from 25 mg/kg bw/day	(Lake* et al, 1984a)
Rat, Wistar 6 males per group 2 or 4 weeks, diet	0, 5, 18, 52, 182, 549	5	18	liver: dose-related ↑absw from 182 mg/kg bw/d following 2 or 4 weeks of treatment, ↑nb peroxisomes from 18 mg/kg bw/d, ↑p.enz.act. from 5 mg/kg bw/d	(RIVM*, 1992)

Species, Study Duration, route	Doses (mg/kg bw/day)	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	RESULTS	REFERENCES
Rat, Wistar 5 males per group 14 days, gavage	0, 250, 500, 1000, 2000	250	500	↓bw from 1000 mg/kg bw/day liver: ↑absw from 1000 mg/kg bw/day, ↑relw from 500 mg/kg bw/day	(Khaliq* & Srivastava, 1993)
Rat, Wistar 5-6 males/group 16 days, diet	0, 0.01, 0.025, 0.05, 0.1, 0.5, 1.0% (0, 8, 22, 42, 88, 500, 900	42	88	liver: ↑relw at 88 mg/kg bw/day	(Fukuhara* & Takabatake, 1977)
Rat, F344 4-5 males per group 3 weeks, diet	0, 2%	NA		liver: ↑relw, ↑p.enz.act. hypolipidemia	(Moody* & Reddy, 1978)
Rat, Sprague-Dawley 4 males 3 weeks, diet	0, 2% (0, 900)	NA	900	↓bw and bwg liver: ↑absw and relw, ↑nb peroxisomes, ↑pSER, ↑p.enz.act. kidney: ↑absw and relw ↑CHO and TG (trend only)	(General Motors*, 1982)
Rat, F344 5 rats/sex/group 21 days, diet	0, 0.01, 0.1, 0.6, 1.2, 2.5% (0, 11, 105, 667, 1224, 2101 [M]; 0, 12, 109, 643, 1197, or 1892 [F])	NA	11	↓bw at 2.5%; liver: ↑absw and relw from 0.6%, histological findings from 0.6%, ↑nb peroxisomes from 0.1% (males) and from 0.6% (females), ↑p.enz.act.; kidney: ↑relw at 2.5% testes: ↓w at 2.5%; ↓TG from 0.6% (males) ↑TG at 0.01%(males), 1.2% (females)	(CMA*, 1984) (Barber* et al, 1987)
Rat, F344, 5 males per group 21 days, diet	0, 100, 1000, 6000, 12000, 25000 ppm (0, 11, 105, 667, 1223, 2100)	11	105	liver: ↑relw from 6000 ppm, ↑nb peroxisomes from 6000 ppm, ↑p.enz.act. from 1000 ppm	(Short* et al, 1987)
Rat, Wistar 3 males/group 2-4 weeks, diet	0, 2% (4 w) or 2 w, 2% +2 w control diet	NA		liver: ↑ weight during treatment period, ↓ during withdrawal period; ↑ p.enz.act. during treatment, ↓ during withdrawal	(Miyazawa* et al, 1980)
Rat, F344 5 rats/sex/group 28 days, diet	0, 0.2, 0.67, 2.0% (0, 150, 504, 1563 [M]; 0, 147, 490, 1416 [F])	NA	150	↓bw at 0.67% liver: ↑absw and relw from 0.2%, ↑p.enz.act. ↓total lipid from 0.2%	(Nuodex*, 1981c)
Rat, F344	0, 1000	NA	1000	liver: ↑absw and relw	(Tenneco*, 1981)

Species, Study Duration, route	Doses (mg/kg bw/day)	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	RESULTS	REFERENCES
5 males 28 days, gavage					
Rat, F344 5 rats/sex/group 28 days, diet	0, 0.67% (0, 350)	NA	350	liver: ↑relw, ↑nb peroxisomes, and ↑p.enz.act. at 0.67%	(Hodgson*, 1987)
Rat, F344 5 rats/sex/group 21 days, gavage	0 700	NA	700	liver: ↑relw, ↑nb peroxisomes, and ↑p.enz.act. at 700 mg/kg bw/day	(Hodgson*, 1987)
Rat, F344 5 males per dose group 10 male controls 28 days, diet	0, 0.02, 0.05, 0.1, 0.5, 1.0, 2.5% (0, 24, 52, 115, 559, 1093, 2496)	NA	24	↓bw at 2.5% liver: ↑absw from 0.5%, ↑relw from 0.02%, ↑p.enz.act. from 0.1% testes: ↓absw and relw and atrophy at 2.5%	(BIBRA*, 1990)
Mouse, B6C3F1 10 males per group 5 days, gavage	0, 1879, 2844, 4304, 6514, 9860	NA	1879	liver: enlarged with a slight dose-response trend from 1879 mg/kg bw/day	(Nuodex*, 1981b)
Mouse, CD-1 10 females per group 8 days, gavage, corn oil	0, 6000, 7690, 9860	NA	6000	clinical signs of toxicity from 6000 mg/kg bw/day	(Hazleton*, 1983)
Mouse, B6C3F1 5 mice/sex/group 14 days, diet	0, 6300, 12500, 25000, 50000, 100000 ppm (0, 630, 1250, 2500, 5000, 10000)	1250	2500	mortality: 5/5 (males) and 5/5 (females) at 100,000 ppm, 1/5 (males) and 4/5 (females) at 50,000 ppm ↓bw: from 25000 ppm (males) and from 50000 ppm (females) ↓bwg : dose-related	(NTP *, 1982)
Mouse, B6C3F1 10 mice/sex/group 4 weeks, diet	0, 1000, 5000, 10000, 25000 ppm (0, 250, 1210, 2580, or 6990 [M]) 0, 270, 1430, 2890, 7900 [F])	250	1210	mortality: 4/10 (males) and 3/10 (females) at 25000 ppm ↓bw and bwg from 5000 ppm (males) and at 25000 ppm (females) liver: ↑absw and relw from 5000 ppm, hepatocellular hypertrophy at 25000 ppm kidney: ↓absw from 5000 ppm (males), inflammation from 5000 ppm; testes: ↓absw and relw from 10000 ppm, atrophy at 25000 ppm thymus: atrophy at 25000 ppm	(Eastman Kodak*, 1992a)

Species, Study Duration, route	Doses (mg/kg bw/day)	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	RESULTS	REFERENCES
				ovaries: absence of corpora lutea at 25000 ppm)	
F344 rats, 5 males/gp. and B6C3F1 mice, 5 females/gp 14 days, gavage	0, 2000 mg/kg bw	NA	2000	Enzymes responsible for production of H_2O_2 peroxisomal palmitoyl CoA oxidase): 9-fold and 21-fold ↑ in rats and mice, respectively. Enzymes responsible for degradation of H_2O_2 Catalase: 2-fold and 3-fold ↑ in rats and mice, respectively. Glutathione peroxidase: ↓ to 50% and 35% of the control in rats and mice respectively.	(Tomaszewski* et al, 1986)
Subchronic Studies (>28-days exposure <chronic exposure)					
Rat, Sprague-Dawley 10 rats/sex/group 13 weeks, diet	0, 5, 50, 500, 5000 ppm (0, 0.4, 3.7, 37.6, 375.2 [M]) 0, 0.4, 4.2, 42.2, 419.3 [F])	3.7	37.6	liver: enlarged (both sexes), ↑absw and relw, hypertrophy, ↑nb peroxisomes at 5000 ppm kidney: ↑relw at 5000 ppm (both sexes) thyroid: histological changes at 5000 ppm testes: mild to moderate Sertoli cell vacuolation from 500 ppm (7/10); ↓ absw and relw testicular weight, atrophy, and complete loss of spermatogenesis at 5000 ppm (9/10)	(Poon et al, 1997)
Rat, F344 10 rats/sex/group 13 weeks, diet	0, 1600, 3100, 6300, 12500, 25000 ppm (0, 80, 160, 320, 630, 1250)	320	630	↓bwg at 25000 ppm testes: atrophy from 12500 ppm	(NTP *, 1982)
Rat, F344 10 rats/sex/group 13 weeks, diet	0, 1000, 4000, 12500, 25000 ppm (0, 63, 261, 859, 1724 [M]; 0, 37, 302, 918, 1858 [F])	NA	63-73 (females	↓bwg from 12500 ppm (females) and at 25000 ppm (males) liver: ↑absw and relw from 1000 ppm, hepatocellular hypertrophy from 4000 ppm (males) and from 12500 ppm (females) kidney: ↑relw from 4000 ppm (males) and from 12500 ppm (females) testes: ↓w from 12500 ppm	(Eastman Kodak*, 1992b)
Mouse, B6C3F1	0, 800, 1600, 3100,	NA	100	↓bwg from 3100 ppm (males) and from 800 ppm (females)	(NTP *, 1982)

Species, Study Duration, route	Doses (mg/kg bw/day)	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	RESULTS	REFERENCES
10 mice/sex/group 13 weeks, diet	6300, 12500 ppm (0, 100, 200, 400, 800, 1600)				
Chronic Toxicity studies (>10% of the test animals lifespan					
Rat, Sprague-Dawley 15 rats/sex/group 17 weeks, diet;	0, 0.2, 1.0, or 2.0% (0, 143, 737, 1440 [M]; 0, 154, 797, 1414 [F])	NA	143	↓bw from 1.0% liver: ↑absw and relw from 0.2%, no histological findings testes: ↓absw and ↑relw from 1.0%, atrophy	(Gray* et al, 1977)
Rat, F344 5-10 males per group 1, 2, 4, 8, 18, 39, 77, 151, or 365 days, diet	0 or 1.2% (0, 600)	NA	600	↑p.enz.act.	(Conway* et al, 1989)
Rat, Sprague-Dawley 520 males in total1 102 weeks, diet	0, 0.02, 0.2, 2.0% (0, 7, 70, or 700)	NA	7	↓bw from 0.2% liver: ↑pp and nb mitoch from 0.2%, ↑p.enz.act. from 0.02%, no tumours testes: atrophy and inhibition of spermatogenesis from 0.02%	(Ganning* et al, 1987; Ganning* et al, 1990)
Rat, F344 50 rats/sex/group 103 weeks, diet	0, 6000, 12000 ppm (0, 322, 674 [M]; 0, 394, 774 [F])	NA	322	↓bw at 12000 ppm hypertrophy anterior pituitary at 12000 ppm (males); liver: neoplastic lesions from 6000 ppm; testes: seminiferous tubular degeneration at 6000 (5%) and at 12000 ppm (90%)	(NTP *, 1982)
Rat, F344 70-85/sex/group recovery group: 55/sex 104 weeks, diet	0, 100, 500, 2500, 12500 ppm (0, 5.8, 28.9, 146.6, 789.0 [M]; 0, 7.3, 36.1, 181.7, 938.5 [F]) or 12500 ppm for 78 weeks, followed by a recovery period of 26 weeks	28.9 [M] 36.1 [F]	146.6-181.7	liver: ↑ weight (males) and peroxisome proliferation at 500 ppm; kidney: ↑ weight from 2500 ppm; ↑ materialisation of the renal papilla (males), tubule cell pigment (both sexes), and chronic progressive nephropathy (males) at 12500 ppm; pituitary: ↑ castration cells (30/60 males) at 12500 ppm testes: ↓ weight, ↑ incidence and severity of bilateral aspermatogenesis; ↓ incidence of interstitial cell neoplasms; epididymis: ↑ immature or abnormal sperm forms and	(Moore*, 1996)

Species, Study Duration, route	Doses (mg/kg bw/day)	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	RESULTS	REFERENCES
				hypospermia from 12500 ppm; changes in the kidneys, testes, and pituitary were not reversible upon cessation of exposure	
Rat, Sprague-Dawley 5 dosed males, 8 control males 2 years, diet	0, 2% (0, 1000)	NA	1000	liver: ↑relw, ↑ nb mitoch ↑nb peroxisome ↑p.enz.act. and lipid peroxidation	(Lake* et al, 1987)
Mouse, B6C3F1 50 mice/sex/group 103 weeks, diet	0, 3000, 6000 ppm (0, 672, 1325 [M] 0, 799, 1821 [F])	672-799	1325-1821	↓bw at 6000 ppm (females) liver: hepatocellular neoplastic lesions kidney: inflammation at 6000 ppm (males) testes: seminiferous tubular degeneration and testicular atrophy at 6000 ppm	(NTP *, 1982)
Mouse, B6C3F1 70-85/sex/group; recovery group: 55/sex 104 weeks, diet	0, 100, 500, 1500, 6000 ppm (0, 19.2, 98.5, 292.2, 1266.1 [M]; (0, 23.8, 116.8, 354.2, 1458.2 [F]) or 6000 ppm followed by a recovery period of 26 weeks	19.2 [M] 23.8 [F])	98.5-116.8	liver: peroxisome proliferation and ↑ weight (males) from 500 ppm; ↑ weight, adenomas and carcinomas (both sexes) from 1500 ppm; kidney: ↓ weight (especially males) and chronic progressive nephropathy (both sexes) from 1500 ppm; testes: ↓ weight, ↑ incidence and severity of bilateral hypospermia from 1500 ppm; epididymis: ↑ immature or abnormal sperm forms and hypospermia from 1500 ppm; ↓ survival (males) changes in liver, kidneys, and testes were at least partially reversible following recovery period;	(Moore*, 1997)
Male rats, One year, by gavage, three times weekly	0, 0.9, 0.9 leachate from toluene extraction	NA	0.9	statistically significant increase (p= 0.04) in the incidence of focal cystic changes was observed in kidneys of rats received DEHP or leachate and killed at 12-months interval. Creatinine clearance was significantly decreased (p= <0.01) only in rats received pure DEHP and killed at the 12-months interval.	(Crocker* et al, 1988)
Marmoset monkeys (4/sex) 13 weeks, gavage	0, 100, 500 2500	2500	NA	No effects	(Kurata* et al, 1998)

Species, Study Duration, route	Doses (mg/kg bw/day)	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	RESULTS	REFERENCES
Marmoset monkeys (9M, 6 F) 65 weeks, daily gavage	0, 100, 500 2500	2500	NA	no effects on liver and testes weights, accessory male reproductive organs no testicular lesions, no differences in sperm counts.	(Mitsubishi-Chemical-Safety-Institute*, 2003) (McKee et al, 2004)
Marmoset monkeys (5-6/sex/group) 65 weeks, gavage	0, 100, 500 2500	2500	NA	No effects	(Tomonari et al, 2006)
INHALATION					
Rats, Wistar (10/group) 4 weeks	0, 0, 10, 50, 1000 mg/m^3	50	1000	↑ lung wt	(BASF AG*, 1990; Klimisch* et al, 1992)
Rats, Wistar (4/group) 4-8 weeks	0, 5, 25 mg/m^3	5	25	↓ seminal vesicle wt	(Kurahashi et al, 2005)
Hamster, Syrian golden 23 months	15 ug/m^3	15	NA	No effects	(Schmezer* et al, 1988)
PARENTERAL					
Rats, Sprague-Dawley (5-6/gp) 12 days, IV	0, 2.5, 25, 250	25	250	Testes: Sertoli cell change at 250	(Sjoberg* et al, 1985c)
Rat, strain not specified (12/group) 18 days, IV	0, 30.8, 91.7, 164.8	92	165	↓ body wt, ↑ liver wt at 165	(Greener* et al, 1987)
Rat, strain not specified (7/group) 18 days, IV	0, 62	62	-	No effect	(Baxter Healthcare Corporation*, 2000)
Rabbit, strain not specified (5/group) 28 days, IV	0, 62	62	-	No effect	(Baxter Healthcare Corporation*, 2000)
Rat, Sprague-Dawley (7/group) 21 days, IV	0, 60, 300, 600	60	300	Testicular atrophy at 300	(Cammack et al, 2003)
Mice, Swiss Webster (6 male/group) 5 days, IP or every other day for 20	0, 50, 100	100	-	No effects	(Curto* & Thomas, 1982)

Species, Study Duration, route	Doses (mg/kg bw/day)	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	RESULTS	REFERENCES
days					
Rat, Sprague-Dawley Mice, Swiss Webster (6 male/group) every other day for 20 days, IP	0, 50, 100	50	100	↓ gonadal zinc at 100	(Curto* & Thomas, 1982)
Monkey, rhesus (3 male/group) 6-12 month, IV	0, 8 (plasma stored at 4C), 27 (plasma stored at 20C), 32 (platelet poor plasma stored at 22C)	32	-	Probable non-treatment related effects due to disease in colony	(Jacobson* et al, 1977)
Rat (uremic), 14 days, IP	2000	2000		No adverse effects	(Leber* & Uviss, 1979)
Rat 56 doses over 19 weeks, IP	500		210.5	Hepatomegaly, ↑ MFO activity, ↓ GST activity	(Leber* & Uviss, 1979)
Rat 7 days, IP	3906		3906	Hepatomegaly	(Pollack* et al, 1989)
Rat Every other day for 12 days, IP	Up to 7.5	3.8		No adverse effects, ↓ liver vitamin A at 0.75 and above	(Nair* et al, 1998)
Dog. 6 doses/week for 6 weeks, IV		1		No adverse effects	(Rutter*, 1975)

*This Table has been adapted from (FDA, 2002; EURAR, 2003; National Chemical Inspectorate, 2005)

[M]: male; [F]: female
NA:
↓ / ↑ = decreased and increased, respectively
bw / bwg = body weight and body weight gain, respectively
absw / relw = absolute and relative weight, respectively
mitoch = mitochondria
nb = number
p.enz.act. = peroxisomal enzyme activity or activities; pp = peroxisome proliferation
pSER = proliferation of smooth endoplasmic reticulum
CHO / TG = cholesterol and triglyceride, respectively = weight; ** the conversion of daily intakes into mg/kg bw/day were from IUCLID

Table 15. Effects of DEHP on body and liver weight in pregnant females following repeated oral administration

SPECIES	PROTOCOL	RESULTS	REFERENCES
Lifetime including Gestational exposure			
Rat, Wistar 25/group	Diet, 2 generations 0, 1000, 3000, or 9000 ppm (0, 113, 340, or 1088 mg/kg bw/day)	↓ weight gain in F0 at 1088 mg/kg bw/day	(Schilling* et al, 2001)
Rat, Sprague-Dawley 17/group	Diet, 3 generations 1.5, 10, 30, 100, 300, 1000, 7500, and 10000 ppm (F0/F1/F2: 0.1, 0.5-0.8, 1.4-2.4, 4.8-7.9, 14-23, 46-77, 359-592, and 543-775 mg/kg bw/day)	↓ weight gain in F0 at 775 mg/kg bw/day ↑ rel liver weight in F0 at 77 mg/kg bw/day	(Wolfe & Layton, 2004)
mice CD-1 20 pairs of mice	Diet, 98 days 0, 14, 141, and 420 mg/kg bw/day	↑ liver weight at 420 mg/kg bw/day	(Lamb et al, 1987)
Gestational exposure			
Rat, Sprague-Dawley 5-8 /group	gavage 0, 375, 750, 1500 mg/kg bw GD3-PND21	↓ weight gain at 750 mg/kg bw/day	(Moore* et al, 2001)
rat, F344/CrlBr 34-25 females/group	*Diet* 0, 0.5, 1.0, 1.5, or 2% 0, 357,666,856,1055 mg/kg bw/d GD 0-20	↓ maternal bw gain at 666 mg/kg bw/day	(NTIS*, 1984) (Tyl* et al, 1988)
Rats, Fischer 344	diet 0, 0.25, 0.5, or 1.0% 0, 164, 313, 573 mg/kg bw/d GD 0 to 20	↓ maternal weight gain at 573 mg/day.	(Price* et al, 1988)

SPECIES	PROTOCOL	RESULTS	REFERENCES
	evaluated: postnatal		
rat, Wistar 9-10 females/group	gavage, oil 0, 40, 200, or 1000 mg/kg bw/d GD 6-15	↓ maternal bw at 1000 mg/kg bw/day ↑ liver weights at 1000 mg/kg bw/day	(BASF AG*, 1995; Hellwig* et al, 1997)
Rat, Sprague-Dawley 3-9/group	Gavage, oil 0, 125, 250, 500 mg/kg bw/d GD 7–18 Examined: GD 20, 5 or 10 weeks (Exp 2)	No effect on mat body wt at GD20	(Shirota* et al, 2005)
Rat, Sprague-Dawley 6 dams/group	Gavage, oil 0, 500, or 1000 mg/kg bw/d GD 7–18 Evaluated: GD 12, 14, 16, 18, or 20, 7 weeks (Exp 1)	↓ Dam weight at 1000 mg/kg bw/day	(Shirota* et al, 2005)
Rat, Long-Evans 7/group	Gavage, oil 0 or 100 mg/kg bw GD 12–21 Evaluated: PND 21, 35, or 90	no effects on dam weight or weight gain	(Akingbemi et al, 2001)
Rat, Sprague-Dawley 11 rats/dose	Gavage 0, 750 mg/kg bw/d GD 14-PND2	↓ mat wt gain at 750 mg/kg bw/day	(Parks* et al, 2000)
Rat, Sprague-Dawley 5-19/dose	Gavage 0, 750 mg/kg bw/d GD 14-PND 3	↓ maternal weight gain at 750 mg/kg bw/day	(Gray* et al, 2000)
Rat, Long Evans 12 rats/group	Oral, drinking water 0, 3.0-3.5, 30-35 mg/kg bw/d GD 1-21	no effects on dam weight or weight gain	(Arcadi* et al, 1998)

Table 16. Important reproductive studies with DEHP in laboratory animals

Species	Protocol	Most sensitive outcome	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Reference
Two-generation studies					
Rat, Sprague-Dawley 17/males/group	Diet, 3 generations 1.5, 10, 30, 100, 300, 1000, 7500, and 10000 ppm (F0/F1/F2: 0.1, 0.5-0.8, 1.4-2.4, 4.8-7.9, 14-23, 46-77, 359-592, and 543-775 mg/kg bw/day)	dose-dependent effects male reproductive organ weights, seminiferous tubule atrophy ↓ litter size in F0 at 7500ppm ↓ pup weights in F1 at 10000ppm dose-dependent ↓ in male reproductive organ weights in F2,3 at 100ppm ↓ anogenital distance in F1 at 75ppm00	4.8 for test.tox and dev. tox. 46 for fertility	14-23	(NTP, 1994) (Wolfe & Layton, 2004)
Rat, Wistar; 25/gp	Diet, 2 generations 0, 1000, 3000, or 9000 ppm (0, 113, 340, or 1088 mg/kg bw/day)	Increased abnormal sperm, decreased male fertility, decreased testis size, decreased litter size and viability, deficit in growing follicles ↓ anogenital distance in F1 at 1000 ppm	*Study authors:* **~340** *Expert Panel:* Not applicable	*Study authors:* **~1088** *Expert Panel:* **~113,** based on testicular histopathology	(Schilling* et al, 2001)

Postnatal studies					
Continuous breeding studies					
Species	**Protocol**	**Most sensitive outcome**	**NOAEL (mg/kg bw/day)**	**LOAEL (mg/kg bw/day)**	**Reference**
11-week-old CD-1 mice. 20 pairs of mice	Diet, 98 days 0, 14, 141, 425 mg/kg bw/day	Reduced fertility ↓ Live pups per litter	14	141	(Lamb et al, 1987)
Rat, Long-Evans; 10/group	0, 10, 100 mg/kg bw/day by gavage × 70 or 100 days	↑ serum LH and testosterone, ↓ ex vivo Leydig cell	1	10	(Akingbemi et al, 2004)
Rat, F344 24 males/group	60 days, diet 0, 320, 1250, 5000, 20000 ppm (0, 18, 69, 284, or 1156 mg/kg bw/day)	Dose-dependent ↓in total body, testis, epididymis, and prostate weights from 5000 ppm ↓mean litter size at 20000 ppm correlated with degenerative testicular changes, ↓ testicular zinc content, epididymal sperm density and motility, ↑number abnormal sperm cells	69	284	(Agarwal* et al, 1986a; Agarwal* et al, 1986b)
Fischer-344 rats 50-80/group	104 weeks, diet 0, 100, 500, 2500, 12500 ppm DEHP (0, 5.8, 29, 147, and 789 mg/kg bw/day).	↓ Testes weight, spermatogenesis in 12,500 ppm ↑ aspermatogenesis in 500 ppm and higher (age related ?)	5.8	28.9 (aspermatogenesis).	(David et al, 2000a)
marmosets - 4 male and 4 female 12–15 months (post pubertal)	13 weeks, gavage 0, 100, 500, 2,500 mg/kg bw/day.	no treatment-related effects on testis weight, testosterone and estradiol levels, testicular zinc concentration or testicular histology	2500 mg/kg bw	NA	(Kurata* et al, 1998)
marmosets – 5/sex/group 90-115 d (pre pubertal)	65 weeks, gavage 0, 100, 500, 2,500	no treatment-related effects on organ weight, testosterone and estradiol levels, testicular zinc concentration, testicular or hepatic histology	2500 mg/kg bw	NA	(Tomonari et al, 2006)

Neonatal and Prepubertal (Male exposed)					
Species	**Protocol**	**Most sensitive outcome**	**NOAEL (mg/kg bw/day)**	**LOAEL (mg/kg bw/day)**	**Reference**
Rat, Sprague-Dawley (4–6-week-old 10 rats/sex/group	90-Day repeat-dose, diet received doses of: M: 0, 0.4, 3.7, 38, 375;F: 0.2 429	Mild Sertoli cell vacuolation	3.7 (M)	38 (M)	(Poon et al, 1997)
Rat, Long-Evans PND 21–34, 35–48, or 21–48, PND 62–89. 10/group	14 or 28 days, gavage 0, 1, 10, 100, or 200 mg/kg bw/day	↓ 17α-hydroxylase in testis, altered ex vivo Leydig cell testosterone synthesis	1	10	(Akingbemi et al, 2001)
Rat, Wistar GD6-PND21 10/gp	Gavage 0, 0.015, 0.045, 0.135, 0.405, 1.215, 5, 15, 45, 135, 405 mg/kg bw/day	↑ dam liver wt ↑ nipple retention at 405 mg/kg bw/d ↓ AGD at 405 mg/kg bw/d ↓ testes weight above 5 mg/kg bw/d ↑ test pathology above 135 mg/kg bw/d	135 (mat) 1.215	405 (mat) 5	(Andrade et al, 2006)
Rat, Sprague Dawley 1, 2, 3, 6, and 12 weeks of age (7-10/group)	5 days, gavage 0, 100, 200, 500, or 1 000 mg/kg bw/day	↓ testis weights at 1000 mg/kg bw per day in 1, 2, 3, & 6-week-old but not in 12-week-old rats; ↓ testis weight in 12 wk rats at 2000 mg/kg; ↓ Sertoli cell nuclei in 1 wk old rats at 500 mg/kg bw & loss of spermatocytes in two- & three-week old rats	200 mg/kg bw	500	(Dostal et al, 1988)
Rat, Sprague Dawley 25, 40, 60 day old (6/group)	14 days, diet 0, 1000, 1700	↓ bwg at 1000 & above all ages; ↓ testicular wt & testicular damage in 25- & 40-day-old rats given 1700 mg/kg bw. No changes in 60-day-old rats	1000 (25, 40 day old)	NA	(Sjoberg et al, 1986a)
marmosets 90–115 days of age	65 weeks, gavage 0, 100, 500, and 2500 mg/kg bw/day	No effect on testicular structure and function.	-	2500 mg/kg bw	(Mitsubishi-Chemical-Safety-Institute*, 2003)

Developmental studies (Dam exposed)					
Species	**Protocol**	**Most sensitive outcome**	**NOAEL (mg/kg bw/day)**	**LOAEL (mg/kg bw/day)**	**Reference**
mouse, 1-CR 30-31 females/group	GD 0-20, diet; 0, 0.025, 0.05, 0.10, or 0.15% 0, 44, 91, 190.6, or 292.5 mg/kg bw/d	↓ maternal bwg from 0.10% (↓ uterine wt, ↓ foetal bw and nb live foetuses/litter); ↑ nb & % of resorptions, late foetal deaths, dead & malformed foetuses, & % malformed foetuses/litter from 0.05% (open eyes, exophthalmia, exencephaly, short, constricted or no tail); visceral malformations & skeletal defects (fused & branched ribs, mis-alignment, and fused thoracic vertebral centra);	91 (maternal) 44 (developmental)	191 (mat) 91 (devp)	(NTIS*, 1984; Tyl* et al, 1988)
mouse, CD-1 15 females/ group	GD 6-15, oral, gavage 0, 40, 200, or 1000 mg/kg bw/day	foetotoxic effects at 200 ↓ nb viable foetuses ↑ nb resorptions & post-implantation losses at 1000 & cardiovascular abnormalities, tri-lobed left lungs, fused ribs, fused thoracic vertebral centres & arches, immature livers, & kidney abnormalities	200 (maternal) 40 (developmental)	100 (maternal) 200 (developmental)	(Huntingdon*, 1997)
Mice, CD-1 28–29 per group	GD 0 – 17, Diet 0, 0.01, 0.025, 0.05% 0, 19, 48, 95 mg/kg bw/day evaluated postnatally	no observed adverse effects on females during pregnancy. ↑ prenatal mortality at ↓ litter size at 95	95□ (maternal) 48 (developmental)	NA (maternal) 95 (developmental)	(Price* et al, 1988)
rat, F344/CrlBr 34-25 females/group	GD 0-20, Diet 0, 0.5, 1.0, 1.5, or 2% 0, 357,666,856,1055	↓ maternal food intake & mean foetal bw from 357; ↓ maternal bwg,	357□ (maternal) 357 (developmental)	666□ (maternal) 666 (developmental)	(NTIS*, 1984; Tyl* et al, 1988)

	mg/kg bw/d	↓ foetal wt from 666 ↑ foetal wt at 357 nb resorptions, nonlive dose-related & stat sign at 1055;			
Rats, Fischer 344	GD 0 - 20 diet 0, 0.25, 0.5, or 1.0% 0, 164, 313, 573 mg/kg bw/d evaluated: postnatal	↓ food consumption during the treatment period in 313, 573 ↓ maternal bwg was reduced at 573. ↓ litter size in a dose-related manner (stat sign at 573) ↓ pup bw in a dose-related manner (stat sign at 573) Pup wts in the high-dose group recovered by PND4.	164 (developmental)	313 (developmental)	(Price* et al, 1988)
Rat, Long Evans 12 rats/group	GD 1-21 Oral, drinking water 0, 3.0-3.5, 30-35 mg/kg bw/d	↓ pup kidney wts were observed at both doses, ↓ testicular wts with only a few elongated spermatids in tubules at low dose level and a generalized disorganization of tubular epithelium at high dose level.		3.5	(Arcadi* et al, 1998)
rat, Wistar 9-10 females/group	GD 6-15 gavage, oil 0, 40, 200, 1000	↓ maternal bw at 1000 ↑ maternal relative kidney and liver wts at 1000 ↓ nb live foetuses/dam at 1000 ↓ foetal body weights at 1000 ↑ nb malformed foetuses/dam (tail, brain, urinary tract, gonads, vertebral column, and sternum) at 1000	200 (maternal) 200 (developmental)	1000 (maternal) 1000 (developmental)	(BASF AG*, 1995; Hellwig* et al, 1997)
Rat, Sprague-Dawley 3-9/group	GD 7–18 Gavage, oil 0, 125, 250, 500 Examined: GD 20, 5 or 10 weeks (Exp 2)	No effect on mat bw ↑ pup bw at 250, 500 at PND4 no malformations at any dose Interstitial hyperplasia in 250, 500; Leydig cell hyperplasia in the 500. 5 and 10 weeks of age, no testicular	125	250	(Shirota* et al, 2005)

		abnormalities			
Rat, Wistar 8/gp	Gavage 0, 10, 30, 100, 300 GD7-21	↑ test path at 100 ↓ test prodn at 300	30	100	(Borch et al, 2006)
Rat, Sprague-Dawley 6 dams/group	Gavage, oil 0, 500, 1000 GD 7–18 Evaluated: GD 12, 14, 16, 18, 20, 7 weeks	↓ Dam bw at 1000 ↓ Foetal wt at 1000 ↑ mortality at 1000. Sporadic malformations at both doses On GD 18 and 20, small foetal testes at 500, 1000 (no wts given) and showed hyperplasia of interstitial cells At 7 weeks of age, testicular histology rel normal at 500; epididymal atrophy, atrophic tubules at 1000	250 (developmental)	1000 (maternal) 500 (developmental)	(Shirota* et al, 2005)
Rat, Long-Evans 7/group	GD 12–21 Gavage, oil 0, 100 mg/kg bw Evaluated: PND 21, 35, or 90	no effects on dam bw or bwg no effect on offspring bw. no effect on testis and seminal vesicle wts ↓ Serum testosterone and LH at PND 21- and 35 but not at 90 days Testicular histology was normal	NA	100 (developmental)	(Akingbemi et al, 2001)
Rat, Wistar 18/dose group	GD 7-21, gavage 0, 750 mg/kg bw/day	Sertoli vacuolisation of cells enlarged tubules Leydig cell hyperplasia DNA laddering	750	NA	(Borch et al, 2005)

Pre- and postnatal exposure (Dam exposed)					
Species	**Protocol**	**Most sensitive outcome**	**NOAEL (mg/kg bw/day)**	**LOAEL (mg/kg bw/day)**	**Reference**
Rat, Wistar 10/gp	Gavage 0, 0.015, 0.045, 0.135, 0.405, 1.215, 5, 15, 45, 135, 405 mg/kg bw/day GD6-PND21	↑ dam liver wt ↑ nipple retention at 405 mg/kg bw/d ↓ AGD at 405 mg/kg bw/d delayed preputial separation in males above 1.215; ↑ testes weight 5-135 mg/kg bw/d but ↓ at 405 ↑ test pathology above 135 mg/kg bw/d	1.215	5	(Andrade et al, 2006)
Rat, Sprague-Dawley 11 rats/dose	Gavage 0, 750 mg/kg bw/d GD 14-PND2	↓ mat wt gain no effect on litter size ↓ testes wt at GD 20, PND2 ↓ body weight at PND2 not GD20 ↓ testosterone and anogenital distance from GD17 ↑ Leydig cell hyperplasia and multinucleated gonocytes were observed in testes.	NA	750	(Parks* et al, 2000)
Rat, Sprague-Dawley 5-19/dose	GD 14-PND 3 Gavage 0, 750	No overt maternal toxicity or reduced litter sizes. ↓ maternal weight gain ↓ pup weights in male pups; ↓ anogenital distance and reduced testis weights. Retention of female-like areolas/nipples; ↑ incidence of reproductive malformations such as complete agenesis of the ventral prostate,	750	NA	(Gray* et al, 2000)

		the seminal vesicles and coagulating glands, hypospadias, complete unilateral testicular agenesis/atrophy or absence of both testes, fluid-filled or undescended testes.			
Rat, Wistar 20/group	GD 7-PND17 Gavage 0, 300, 750	↓ birth wt (males) at 750 mg/kg bw/d no change maternal wt ↓ anogenital distance at PND3 (not dose-dependent)	300	750	(Jarfelt* et al, 2005)
Rat, Sprague-Dawley 5-8 /group	GD3-PND21 gavage 0, 375, 750, 1500	↓ litter size at 1500 ↓ anogenital distance at PND1 at: 750	375	750	(Moore* et al, 2001)
Rat, Wistar 10/group	GD 1 – PND21 Gavage 0, 20, 100, 500	↓ litter size, ↓ prostate wt at, ↓ sperm no & production at 500;	100	500	(Dalsenter et al, 2006)

Inhalation					
Species	**Protocol**	**Most sensitive outcome**	**NOAEL (mg/kg bw/day)**	**LOAEL (mg/kg bw/day)**	**Reference**
rat, Wistar 25 females/ group	head-nose, 0, 0.01, 0.05, or 0.3 mg/litre (0, 10, 50, or 300 mg/m^3) 6h/d GD 6-15	↓ nb live foetuses at 50 mg/m^3 but not 300 mg/m^3 ↑ percentage of resorptions at 50 mg/m^3 but not 300 mg/m^3			(Merkle* et al, 1988)
Parenteral					
rats	IV DEHP plasma extracts of PL-146 and PL-130 0, 1.3-1.4, 4.7-5.3 mg/kg/d GD 6–15.	No effect on maternal body weight, No effect on embryo/ foetal viability No effect on foetal weights	4.7-5.3	NA	(Lewandowski* et al, 1980)
Rat, Sprague-Dawley 5/group	IP injections 0, 4930 and 9860 mg/kg bw GD 5, 10, and 15.	↓ implantations at 4930, and 9860 ↓ in foetal weights at . 4930 and 9860 Gross anomalies at 9860 only	NA	4930	(Singh* et al, 1972)
Rat, Sprague-Dawley 5/group.	IP injection 0, 1972, 3944 mg/kg/day GD3, 6, and 9 Examined at weaning	↓ implantation at 1972 and 3944 ↓ number of weaned pups most dams died during delivery	NA	1972	(Peters* & Cook, 1973)
Rat, Sprague-Dawley 5/group.	IP injection 0 or 1972 mg/kg bw/day GD 1, 3, 6, 9 or GD 3 and 6, or GD 6 and 9, or GD 3, 6, and 9.	One or more dams died in days 6; 9; 6 and 9; and 3, 6 and 9, groups. ↓ implantations in groups injected prior to day 6 not day 1 group. ↓ number of live pups in all treated groups not day 6 and 9 group.	NA	1972	(Peters* & Cook, 1973)

Table adapted from (National Chemical Inspectorate, 2005)

Table 17. Key reproductive studies with MEHP in laboratory animals

Species and dosing	Protocol	Most sensitive outcome	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Reference
ORAL					
Guinea pigs Five weeks old male	gavage single dose; 2000 mg/ml	Testicular pathology	NA	2000	(Awal et al, 2005)
Rats 28-day old male	single dose of 400	Testicular pathology	NA	400	Dalgaard et al, (2001)
Rats, Wistar male 26 days old, 6 /group	single dose 2800 DEHP	Reduced testicular wt; testicular pathology	NA	2800	(Teirlynck et al, 1988).
Rats, Wistar male 26 days old, 6 /group	single dose 400 or MEHP	Reduced testicular wt; testicular pathology	NA	400	(Teirlynck et al, 1988).
Rats, Fischer male 28-day-old	Gavage, single dose 2000	testicular pathology	NA	2000	(Richburg* & Boekelheide, 1996).
Rats, Sprague-Dawley males, 4-6 weeks old 6/group	intubation 5 days 1000	Reduced testicular wt testicular pathology	NA	1000	(Gray et al, 1982)
Rats, Sprague-Dawley males, 4-6 weeks old 6/group	intubation 5 days 2800 DEHP	Reduced testicular wt testicular pathology	NA	2800	(Gray et al, 1982)
Syrian Hamsters, DSN 8/group	Intubation 9 days 1000	Reduced testicular wt testicular pathology	NA	1000	(Gray et al, 1982)
Syrian Hamsters, DSN 8/group	9 days 4200		NA	> 4200	(Gray et al, 1982)
PARENTERAL					
Rats, Sprague-Dawley	IP every other day for 20 days 0, 50, 100	reduced prostate & gonadal zinc levels	NA	50	(Curto* & Thomas, 1982)

Rats, Sprague-Dawley	IP DEHP every other day for 20 days 0, 50, 100	reduced prostate & gonadal zinc levels	50	100	(Curto* & Thomas, 1982)
Swiss-Webster mice	SC 5 days 0, 1, 5, 10 or SC 10 days 0, 5, 10, 20	No adverse effects	10	NA	(Curto* & Thomas, 1982)
Swiss-Webster mice	IP 5 days MEHP or DEHP 0, 50, 100 mg/kg bw for; IP every other day for 20 days MEHP or DEHP 0, 50, 100	no effects on reproductive organ weights or zinc levels, but death occurred in 3/6 mice exposed IP to 100 mg/kg bw/day for 5 days	50	100	(Curto* & Thomas, 1982)

APPENDIX C

Collection of information on DEHP in PVC containing medical devices

The major route of DEHP from medical procedures is intravenous, through infusion of saline, drugs, blood, blood products or lipid solutions. DEHP migrates from the PVC bag during storage and from the tubing used during infusion. Exposure to DEHP may also occur by inhalation (e.g., ventilators) and by ingestion (e.g., nasogastric tubes).

Factors that influence the concentration of DEHP delivered to the patient include:
Device:

- DEHP content of the medical device eg. storage bags, transfusion lines and tubes for intravenous, intraperitoneal, inhalation and oral delivery of saline, drugs, blood products (packed red blood cells, platelets, cryoprecipitate, plasma) and other solutions.

Procedure:

- Type of solution delivered: Composition of the solution is critical. The more hydrophobic the solution, the more DEHP is leached.
- Storage time and conditions
- Volume delivered:
- Duration/frequency:

The following questions pertain to the **device**.

If more than one please copy this page and complete this form for each product type.

Company Name:

Device General Classification (eg. Infusion set, urethral catheter)	
Volume of infusate (bags only)	
Length and internal diameter (tubing only)	
% DEHP (list separately for bag and tubing if part of set)	
DEHP Leaching rate (if known) (list rates into each known phase: gas or liquid, and specific liquid type eg. 10% interlipid, packed RBC):	
Storage (recommended): (infusate containing bags only)	

The following questions pertain to the intended **procedure** for this device.

Note: If more than one. Please list separately.

Procedure 1

Type of procedure:	
Route of exposure (Please circle)	Intravenous/ intraperitoneal / inhalation / oral
Solution delivered If a mixture, name each component and state proportions. For TPN state % lipid content.	
Volume delivered (Total volume at end of procedure)	
Duration/frequency (duration of each procedure, number of procedures/treatment)	

Procedure 2

Type of procedure:	
Route of exposure (Please circle)	Intravenous/ intraperitoneal / inhalation / oral
Solution delivered If a mixture, name each component and state proportions. For TPN state % lipid content.	
Volume delivered (Total volume at end of procedure)	
Duration/frequency (duration of each procedure, number of procedures/treatment)	

The following question pertain to the intended **patient.**

Please circle intended patient group.

Age group: adult/ pregnant females/ peripubertal child/neonates.

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