INDIAN JOURNAL OF SCIENCE AND TECHNOLOGY

RESEARCH ARTICLE

Estimation and Risk Assessment of Di(2-ethylhexyl) Phthalate (DEHP) in Cardiac Catheter Sets

R M Balaje[1], M R Murali[1], T N Sathya[1], Sangeetha V Naveen[1], K R Navaneethakrishnan[1], S S Murugan[1], T S Kumaravel[1]*

1 GLR Laboratories Private Limited, Chennai, India

OPEN ACCESS

Received: 25-11-2022

Accepted: 19-06-2023

Published: 11-07-2023

Citation: Balaje RM, Murali MR, Sathya TN, Naveen SV, Navaneethakrishnan KR, Murugan SS, Kumaravel TS (2023) Estimation and Risk Assessment of Di(2-ethylhexyl) Phthalate (DEHP) in Cardiac Catheter Sets. Indian Journal of Science and Technology 16(26): 1997-2007. https://doi.org/10.17485/IJST/v16i26.Balaje

* **Corresponding author**.

kumaravelts@glrlabs.com

Funding: The research was funded by GLR Laboratories Private Limited, Chennai 600068, INDIA

Competing Interests: None

Published By Indian Society for Education and Environment (iSee)

ISSN
Print: 0974-6846
Electronic: 0974-5645

Abstract

Objectives: To conduct a toxicological risk assessment of di(2-ethylhexyl) phthalate (DEHP) in cardiac catheter (CC) set, following a chemical characterization study and systematic review of toxicity literature. **Methods:** CC set was subjected to chemical analysis study for estimation of DEHP in the PVC containing parts using HPLC-PDA. Toxicity literature on DEHP was collected from online database literature search and reports of international monitoring bodies. From the existing knowledge of DEHP toxicity, tolerable intake for humans was derived from the critical toxicity endpoint. This was compared to the levels of DEHP in CC to make scientific judgement on this risk to patients. **Findings:** During clinical use of medical devices like CC tubing set, intravenous infusion set, storage of blood in blood bags there is a possibility of DEHP leaching in to the patient body via fluids or blood. Based on the chemical analysis of device extract, the content of DEHP in the CC tubing set was estimated to be 38.18 mg per device. The patient exposure of DEHP was calculated assuming clinical use of one device per day. Review of extensive toxicity literature revealed the critical toxicity endpoints of DEHP toxicity. Activation of peroxisome proliferator-activated receptor alpha (PPAR-α) in rodents leading to hepatic tumors and disruption of normal endocrine function of gonads are the primary concerns of toxicity. Human tolerable exposure derived from the NOAEL obtained from a key reproductive toxicity study was used to evaluate the toxicological risk of DEHP. From the assessment, the level of DEHP leaching out from the CC tubing set is concluded to be safe in humans. **Novelty:** This manuscript describes a method for risk assessing DEHP toxicity in medical devices using a case study of CC set. This approach is based on generating patient exposure using chemical analysis and comparing this with toxicology data from literature to make informed decisions on patient safety.

Keywords: DEHP; Risk Assessment; Medical Device; Toxicity

1 Introduction

Phthalate esters are one of the most significant class of plasticizers added in variety of plastic polymers, which are used in a wide range of consumer products and healthcare products. Next to polyethylene and polypropylene, polyvinyl chloride (PVC) is the world's third-most widely produced synthetic plastic polymer [1]. Polyvinyl chloride plastic is used to manufacture a number of medical devices, including IV sets, blood bags and infusion tubing, enteral and parenteral nutrition feeding bags, nasogastric tubes, peritoneal dialysis bags and tubing, and tubing used in devices for cardiopulmonary bypass (CPB), extracorporeal membrane oxygenation (ECMO), and hemodialysis. Unplasticized PVC is hard and brittle at room temperature. As a result, plasticizers are necessary to impart flexibility to the polymer. Various plasticizers (e.g., phthalates, adipates, phosphate esters, etc.) have been used as plasticizers for PVC to modify the properties of final products from soft and, flexible to strong, and tough. Among the phthalate esters the plasticizer of choice for PVC medical devices is di-(2-ethylhexyl) phthalate (DEHP). Di-(2-ethylhexyl) phthalate also has limited use in production of other polymer like nylon, polystyrene, polyurethanes, rubbers, etc. [2,3].

DEHP is considered to exhibit reproductive toxicity, and endocrine disrupting properties and regarded as highly toxic to biological system. European Union has classified DEHP as a substance of very high concern (SVHC) under Article 59(10) of the REACH Regulation [4]. Despite its toxicity, DEHP still plays a significant role in production of PVC based medical plastics. Even though DEHP is used as a plasticizer in PVC, it is not chemically bound to the polymers and thus have the potential to leach out of consumer products and medical devices, when exposed to extreme conditions like high temperatures [5]. Also, plasticizers can migrate within the object and leach out of it over a period of time, subsequently reaching the environment and, frequently, the human body. DEHP has become substance of more concern as its breakdown products are believed to be endocrine disruptors and more toxic. DEHP and its breakdown products have been identified as omnipresent environmental contaminants in soil and water [6].

In spite of the fact, that usage of DEHP containing PVC based medical devices namely, cardiopulmonary bypass set, intravenous infusion sets, etc. can lead to DEHP exposure in patients, DEHP based medical devices are still widely used in healthcare applications and till date there are no published data on risk assessment of DEHP, based on the estimation of actual exposure from the use of a medical device. Also, it is not practically possible to estimate the amount of DEHP leaching from various medical devices. Therefore, in this assessment CC tubing set was selected as a representative worst-case test article, owing to its larger surface area and proportion of PVC/DEHP containing parts. This article emphasizes the review of key toxicity data of DEHP and its use in human risk assessment, following an extractable study by estimation of DEHP in CC tubing set.

Till date no reliable data is available on estimation of DEHP leaching from CC set, following an extractable study according to ISO standard 10093-18 [7]. However, a work on standardized experiment model for measuring the DEHP migration via respiratory medical devices is available [8]. In this investigation we aim at estimating the probable DEHP exposure via parenteral route through the use of blood contact.

No adequate information about the health impacts of alternative plasticizers or their migration rates is available. Upcoming research shall need to shed some light on the risks and benefits of phthalate replacement with safer alternatives in medical devices, but until that time, phthalates will need to be remain in use [9]. Hence, a more reliable exposure assessment, followed by the use of key toxicity endpoint (safety limit) appropriate for parenteral route of exposure is required for risk assessment of DEHP in CC sets.

Table 1. Chemistry

IUPAC name	**Bis(2-ethylhexyl) phthalate**
Synonyms	Di-(2-ethylhexyl) phthalate; 1,2-Benzenedicarboxylic acid, bis(ethylhexyl) ester
CAS no.	117-81-7
Molecular formula	$C_{24}H_{38}O_4$
Molecular mass	390.6

Di-(2-ethylhexyl) phthalate is a colorless to pale yellow oily liquid, nearly odorless at normal temperature with a boiling point of 231 °C at 5 mm Hg. DEHP is produced by the esterification of phthalic anhydride with 2-ethyl-hexanol [10,11].

1.1 Use & Application of Di-(2-ethylhexyl phthalate)

DEHP based PVC polymer are used in variety of medical devices like medical films, blood bags, colostomy bags, blood tubings, urine collection system, endotracheal tubes, nasogastric tubes, chest tubes, wound drainage system, etc. Children toys and child-care articles like pacifiers, teething rings, squeeze toys, crib bumpers, etc. are also made from PVC [9,12]. A wide variety

of household appliances and objects are made from PVC plastics containing DEHP.

2 Methodology

2.1. Estimation of DEHP in CC sets

Quantity of di(2-ethylhexyl) phthalate leaching out from a CC tubing set was determined in the following case study. CC sets are made from polyvinyl chloride (PVC) plastic. The chosen medical device is a sterile single use product. This is intended for use during cardiopulmonary surgery or any other surgeries involving cardiopulmonary bypass. It is intended for use along with equipment such as blood pumps, oxygenators, reservoirs, filters, heat exchangers and canula. The product is designed for use no more than 6 hours and longer periods should be avoided.

CC tubing set was extracted using exaggerated extraction conditions. A mixture of ethanol/water (4:6) as described in ISO 3826-1:2019[13] (this is an accepted method for determining DEHP from plastic devices) was used as an extraction vehicle to extract DEHP from the components of CC tubing sets. PVC components were cut to be accommodated in the extraction container. The cut pieces were extracted in ethanol/water (4:6) using glass containers at 50 °C for 24 hours with shaking. This exaggerated extraction condition is considered more rigorous (both in terms of extraction time and vehicle used) than the recommended clinical use of around 6 hours. Extraction was carried out on two independent sets of representative samples. For detection and quantification of DEHP, the device extract was analyzed by HPLC-PDA. The blank solutions were prepared and analyzed parallelly. The analytical method was validated using DEHP Certified Reference Material (CRM).

2.2 Review of toxicology data

This toxicological review was conducted by an comprehensive online database literature search from PubMed (pubmed.ncbi.nlm.nih.gov/), ScienceDirect (www.sciencedirect.com/), Google Scholar (scholar.google.com), chemical database like (PubChem (pubchem.ncbi.nlm.nih.gov/), international monitoring bodies' databases such as European Chemicals Agency (ECHA), International Agency for Research on Cancer (IARC), World Health Organization (WHO), European Food Safety Authority (EFSA), European Chemicals Bureau (ECB) and Center for Devices and Radiological Health, U.S. Food and Drug Administration (CDRH - US FDA). Toxicological studies considered were those published in accredited scientific journals and reports from recognized international bodies. The current state of knowledge regarding the mechanism of action and toxicity of di(2-ethylhexyl) phthalate (DEHP) was considered in compiling the toxicological data summarized in results section.

2.3 Toxicological risk assessment of substances of concern

Toxicological risks associated with the exposure to hazardous substances while using a medical device are managed by identifying the potential extractable/leachable substances, quantifying the associated risks and limiting exposure within tolerable levels. In risk assessment for human health, the normal procedure is to compare exposure levels to a population that is exposed or likely to be exposed to those levels at which no toxic effects are expected to occur. This is normally performed by comparing the exposure level, obtained from the exposure assessment with the derived health-based exposure limit, such as tolerable daily intake (TI) or acceptable daily intake (ADI) or reference dose (RfD) or derived no-effect level (DNEL) and converting them to tolerable exposure (TE). The exposure limits can be derived from no observed effect level (NOEL) or no observed adverse effect level (NOAEL) or the lowest observed adverse effect level (LOAEL) values derived from animal studies. Modifying factors and/or uncertainty factors are also utilized to relate the pre-clinical toxicity data to humans for life-time exposure.

ISO guideline-Part 17 "Establishment of allowable limits for leachable substances" provides guidance on establishing tolerable intake (TI) and tolerable exposure (TE), using health-based endpoints such as NOEL/NOAEL. The Threshold of Toxicological Concern (TTC) concept extends the tolerable intake methodology to address substances that have very limited or no toxicity data, but for which reasonable exposure estimates can be made. The TTC is a rational risk assessment tool for assessing substances of unknown toxicity or structural activity relationship will be used wherever necessary. It is based on the principle of establishing a human exposure threshold value for all chemicals, below which there is extremely low probability of an appreciable risk to human health[14–16].

3 Results and Discussion

3.1 DEHP estimation

The amount of DEHP extracted from representative pieces was 3850 μg (Sample 1) and 3150 μg (Sample 2). Average content of DEHP in cut representative sample was calculated as 3500 μg. This amount of DEHP was assumed to leach out from the inner surface, outer surface and cut ends of representative sample. Accordingly, the amount of DEHP leaching out from the inner surface of whole tubing set was estimated to be 38.18 mg per device (i.e., CC set).

3.2 Summary of toxicological data

Bis(2-ethylhexyl) phthalate (DEHP) is readily absorbed and distributed in the body, but there is no evidence of accumulation. Based on the evaluation of pharmacokinetics data from various studies, oral and inhalation absorption rate of DEHP is determined to be around of 75 to 100% in rat and humans [4]. The metabolism of DEHP involves several pathways and yields a variety of metabolites. The major step in the metabolism of DEHP is hydrolysis by lipases to mono(2-ethylhexyl) phthalate (MEHP) and 2-ethylhexanol. The substance is excreted via the urine, mainly as MEHP-metabolites, but some excretion takes place via bile in rodents. Additionally, there are animal and human data showing that DEHP is transferred to mothers' milk. The relative extent to which different metabolites are produced and excreted is very complex and may depend upon the species, the age of the animal, sex, inter-individual differences, nutrition state, prior exposure to DEHP, the amount of DEHP administered, and the route of administration [11].

Mono (2-ethylhexyl) phthalate (MEHP) is the major metabolite when DEHP is degraded in the environment as well as in biological system although the rate of conversion/metabolism differ between different environmental compartments as well as different species. This compound is formed exogenously by lipase enzymes in stored plasma or blood or by hydrolysis in stored and heated IV fluid. As a result, some of the DEHP that is released into stored blood, plasma, or IV fluids will be converted to MEHP before reaching the patient. Exposure to MEHP is important since this compound is thought to be the toxic metabolite of DEHP and because it is more potent than DEHP in producing adverse effects [11,17].

Humans are known to exposed to DEHP via oral, inhalation, dermal, and intravenous routes [18,19]. High exposures to DEHP can occur via the medical devices, during intravenous medical procedures such as hemodialysis, infusion and from storage of blood and its components in PVC based blood bags. Exposures of the general population to DEHP occur mainly through ingestion of residues in food and water. Children may get exposed to higher levels during medical procedures, and drug therapy for certain disease conditions and by use of child-care products and plasticized toys via oral and dermal route [11,17].

3.2.1 Acute toxicity: Oral

The oral LD_{50} of di(2-ethylhexyl) phthalate (DEHP) was found to be > 20,000 mg/kg in F344 rats following an acute oral toxicity study performed equivalent to OECD TG 401 [4].

3.2.2 Acute toxicity: Inhalation

Following an acute inhalation toxicity study in Sprague-Dawley rats, the LC_{50} of DEHP was found to be more than 10,620 mg/m^3 for 4 hours [4].

3.2.3 Acute toxicity: Dermal

In a non-standard study, rabbits were exposed dermally for 24 hours with doses up to 20 mL/kg, which killed 2 of 6 rabbits. The LD_{50} for dermal route was found to be approximately 20 mL/kg i.e., 19800 mg/kg [20].

3.2.4 Irritation: Skin

DEHP was concluded to be slightly irritating to the skin of Little White Russian rabbits, after application of undiluted test substance (0.5 cm^2) in a skin irritation study, performed in accordance with OECD TG 404. Skin lesions were reversible after 8 days [4].

3.2.5 Irritation: Eye

Eye irritation study was conducted as per OECD TG 405, by instillation of 0.1 mL of DEHP (>99% pure) into the eye of Little White Russian rabbits. Conjunctival lesions were observed initially after dosing, but later disappeared. Signs of chemosis, corneal opacity and iris lesions were negative. DEHP was considered as non-irritant for the eye [4].

3.2.6 Sensitization

Di(2-ethylhexyl) phthalate was found to be non-sensitizing to skin of Dunkin-Hartley guinea pig in a guinea pig maximization test performed as per OECD TG 406, in albino Dunkin-Hartley guinea pigs[4]. In another study, DEHP was evaluated for respiratory sensitization in B6C3F1 mice, after topical application and, challenge application at concentrations 0, 25, 50, 100% and followed by estimation of serum IgE, IL-4 or IL-13 levels. Based on the results, DEHP was not considered as a respiratory sensitizer[21].

3.2.7 Repeated dose toxicity: Oral

Di(2-ethylhexyl) phthalate was administered by oral gavage in corn oil to juvenile marmoset at dosage levels of 100, 500, and 2500 mg/kg/day for 65 weeks in a chronic toxicity study, conducted as per OECD TG 452. NOAEL was reported to be 2500 mg/kg/day. The study findings were no treatment-related change was evident in the body weight and general heath signs except for adaptive liver changes. During the treatment period, all males experienced a surge in testosterone, and the testosterone levels in all treated groups were similar to that of the control group. Electron microscopic examination of testis revealed no treatment-related abnormalities. In this study, despite the high dose of 2500 mg/kg/day and adoptive liver change proving absorption, no testicular change was morphologically or functionally noticed[22].

3.2.8 Repeated dose toxicity: Inhalation

In a repeated dose toxicity study conducted according to the OECD TG 412, Wistar rats were exposed in head-nose inhalation systems to DEHP (99.7% pure) aerosols of respirable particles. Exposure duration was 6 hours per day, 5 days per week for 4 weeks at 0, 0.01, 0.05, or 1.0 mg/L (0, 10, 50, or 1,000 mg/m^3). In the highest dose group, a significant increase in relative lung weights was seen in male rats. This was accompanied by foam cell proliferation and thickening of the alveolar septi. All these effects were reversible within the post-exposure observation period. There were no indications of substance-related effects on male reproductive function. Electron microscopical examination of liver samples from all three concentration groups and controls at the end of exposure and after the post-exposure period did not reveal clear ultrastructural changes in hepatocytes that could be attributed to the exposure or to peroxisome proliferation. The NOAEL in this study was 50 mg/m^3 [23].

3.2.9 Repeated dose toxicity: Intravenous

Six intravenous infusions of DEHP at 0, 5, 50 or 500 mg/kg, in emulsion (20% DEHP, fractioned egg yolk phosphatides (1.2%), glycerol (2.2%) and distilled water were administered for 6 days, one infusion daily via the implanted canula were given to 25-day- or 40-day-old rats. In Epon-embedded testicular materials from animals given the highest dose, some altered Sertoli cells (dilated cisternae of endoplasmic reticulum) were observed. No age-related testicular effects were observed. NOAEL was reported to be 50 mg/kg/day[24].

3.2.10 Mutagenicity

With regard to genotoxicity of DEHP, several short-term tests, comparable to guideline studies and performed according to GLP, are available. The results were negative in the majority of the *in vitro* and *in vivo* studies performed with DEHP and its metabolites for detection of gene mutation, DNA damage, and chromosomal effects. The positive results were obtained in the test systems for detection of cell transformation, induction of aneuploidy, and cell proliferation, end-points which were also sensitive to several non-mutagenic substances such as tumour promoters and/or peroxisome proliferators. Based on the above discussion, DEHP and its major metabolites can be considered as non-mutagenic[11].

3.2.11 Genetic toxicity: In vitro

Diethylhexylphthalate was tested in an *in vitro* gene mutation assay using mammalian cells cultures both in the absence and presence of metabolic activation (S9 mix), according to the protocol similar to the OECD TG 476. In this mammalian cell gene mutation assay (TK+/-), mouse lymphoma L5178Y cells cultured *in vitro* were exposed to DEHP in ethanol for 4 hours at concentrations namely 125, 250, 500, 750, 1000, 2000, 3000, 5000 nL/mL) in the presence and absence of metabolic activation. Under the test conditions, DEHP did not induce any increase in mutant colonies and was not considered as mutagenic[25].

3.2.12 Genetic toxicity: In vivo

The ability of DEHP to induce DNA damage or repair was examined in rat hepatocytes *in vivo*. Unscheduled DNA synthesis was measured by incorporation of 3H-thymidine into primary hepatocyte cultures immediately isolated from treated animals. DNA damage was measured by alkaline elution of cellular DNA from the same cultures. No chemically induced DNA damage or repair was observed *in vivo* in rat hepatocytes under any of the conditions employed. However, an increase in the percentage

of cells in S-phase in the animals given DEHP was observed. These data indicate that DEHP does not exhibit, direct genotoxic activity in the animals even with a treatment regimen which eventually produced tumors in a long-term bioassay, and that both rat and human hepatocytes were similar in their lack of a genotoxic response to DEHP exposure in culture[26].

In a study performed according to GLP principles, DEHP, mono-(2-ethylhexy1) phthalate (MEHP) and 2-ethylhexanol (2-EH) were tested for their ability to induce chromosomal damage in male Fischer rats after oral administration. Five rats per group were given by gavage in corn oil 0.5, 1.7, 5.0 ml/kg/day of DEHP (purity: 99.9%), 0.01, 0.05, 0.14 ml/kg/day of MEHP (purity: 94.7%), or 0.02, 0.07, 0.21 ml/kg/day of 2-EH (purity: 99.7%) for 5 consecutive days. No significant increase in chromatid and chromosome breaks or structural rearrangements were noted and the mitotic index was also unaffected[27].

3.2.13 Carcinogenicity

DEHP was found to be carcinogenic in rats and mice. A statistically significant increase in the incidence of hepatocellular tumours with a dose-response relationship was observed in rats and mice of both sexes and a significant dose-related increase in the incidence of Leydig cell tumours was observed in male rats. It was also noted that low doses did not cause hepatocellular tumours, which suggests a threshold for this effect[4].

In a combined chronic/carcinogenic study realized according the OECD TG 453, groups of 70 male and female B6C3F1 mice received in their diets DEHP concentrations of 0, 100, 500, 1500 or 6000 ppm (0, 19.2, 98.5, 292.2, or 1,266.1 mg/kg/day, respectively, for males, and 0, 23.8, 116.8, 354.2, or 1,458.2 mg/kg/day, respectively, for females) for 104 weeks. In an additional recovery group, the mice (55 males and 55 females) were administered 6,000 ppm DEHP for 78 weeks, followed by a 26-week recovery period in which they were fed basal diet alone. In addition to the study of all relevant parameters for a mouse carcinogenicity study, DEHP-induced cell proliferation and peroxisome proliferation in the livers of dosed mice was evaluated. The LOAEL and the NOAEL for tumour induction (total male mice with hepatocellular neoplasms) in this study were 1500 and 500 ppm DEHP in the diet, respectively (corresponding to 292 and 98 mg/kg/day for males of the two dose groups respectively). The LOAEL and the NOAEL for non-neoplastic effects on the liver in this study was 98 mg/kg/day and 19 mg/kg/day of DEHP in the diet, respectively for males of the two dose groups[28,29].

In a combined chronic/carcinogenic study realized according to the OECD guideline 453, groups of F-344 rats (70 - 85 males and females per group) received in their diets DEHP concentrations of 0, 100, 500, 2500 or 12500 ppm (0, 5.8, 28.9, 146.6 or 789.0 mg/kg/day, respectively, for males, and 0, 7.3, 36.1, 181.7, or 938.5 mg/kg/day, respectively, for females) for 104 weeks. In an additional recovery group, rats (55 males and females/group) were administered 12,500 ppm DEHP for 78 weeks, followed by a 26-week recovery period in which they were fed basal diet alone. In addition to the study of all relevant parameters for a carcinogenicity study, DEHP-induced cell proliferation and peroxisome proliferation in the livers of dosed rats was evaluated. The LOAEL for tumour induction (total male rats with hepatocellular neoplasms and MCL) and for the effects on the liver, kidney and testis in this rat study was 2500 ppm DEHP in the diet (147 mg/kg/day for males). An overall NOAEL for the tumour induction and for the effects on the liver, kidney and testis was established as 28.9 mg/kg/day for male rats[28,29].

In the above rodent studies, liver tumours, Leydig cell tumours, and leukaemia had been observed. The liver tumours are most likely caused by peroxisome proliferation and are therefore not considered relevant for humans. As to the other two tumour types, a relevance to humans cannot be ruled out, although the evidence is inconclusive for this endpoint[11].

3.2.14 Reproductive toxicity

To assess the potential reproductive effects over multiple generations, diethylhexylphthalate (DEHP) was administered in the diet at concentrations of 1.5 (control), 10, 30, 100, 300, 1,000, 7500, and 10,000 ppm to groups of 17 male and 17 female Sprague-Dawley rats. The control dose level was set at 1.5 ppm as this was the amount of DEHP found in the control feed. Animals in the F0 generation began exposure as adults and were bred to produce the F1 generation (F1a, 1b, 1c), the F1 adults were bred to produce the F2 generation (F2a, 2b, 2c), and the F2 adults were bred to produce the F3 generation (F3a, 3b, 3c). Additional non-mating males (up to three per litter) were selected from the F1c, F2c, and F3c litters, and were maintained following similar procedures as those for mating males, except they were not cohabited with females. The 10,000 ppm animals only completed the F1 generation and were terminated due to the inability to produce any F2 generation animals. Parameters evaluated over the course of the study included body weights, feed consumption, clinical observations, reproductive performance, anogenital distance, pup survival, sexual development, estrous cyclicity, sperm endpoints, gross pathology, organ weights, and limited/selected histopathology. Based on measured feed consumption, mg/kg daily doses were calculated to be 0.12, 0.78, 2.4, 7.9, 23, 77, 592, and 775 mg/kg/day in the F0 animals; 0.09, 0.48, 1.4, 4.9, 14, 48, 391, and 543 mg/kg/day in the F1 animals; and 0.1, 0.47, 1.4, 4.8, 14, 46, 359 mg/kg/day in the F2 animals. The no-observed adverse effect level (NOAEL) for testicular toxicity in this study was 100-ppm (equivalent to approximately 8 mg DEHP/kg/day in the F0 animals and 4.8 mg DEHP/kg/day in the F1 and F2 animals)[30].

3.2.15 Developmental toxicity / teratogenicity

The potential of DEHP to induce developmental toxicity after maternal exposure during the critical period of organogenesis was evaluated in rat according to OECD TG 414 and in compliance with Good Laboratory Practices. DEHP was administered by aerosol inhalation (nose only) to four groups of 25 bred female Wistar rats, 6 hours daily from gestation days 6 through 15. Dosage levels were 0, 0.01, 0.05 and 0.3 mg/L. Animals were observed daily for mortality and morbidity. On gestation day 20, a laparohysterectomy was performed on 20/25 female. The uteri, placenta and ovaries were examined, and the number of foetuses, early and late resorptions, total implantations, and corpora lutea were recorded. Gravid uterine weights were recorded, and net body weights and net body weight changes were calculated. The foetuses were weighted, sexed and examined for external, visceral and skeletal malformations and developmental variations. All animals survived to the scheduled necropsy; No maternal toxicity had been observed. No clinical signs that could be attributed to DEHP were observed in the treated groups. Intrauterine growth and survival were unaffected by DEHP administration at all dose levels. The developmental variations expressed in the treated groups were generally similar to those present in the control group or occurred in a manner that was not dose related. Under these experimental conditions, the no observed adverse effect level (NOAEL) was 0.3 mg/L for maternal and foetal toxicity[31].

Results from a two-generation study in Wistar rats indicate effects on fertility and developmental toxicity Wistar rats (F0 generation = 10 rats/sex) were exposed to dietary levels of 0, 1,000, 3,000 and 9,000-ppm DEHP (equivalent to 0, 110, 339 and 1,060 mg/kg/day). F1 pups were raised and mated to produce a F2 generation, which was sacrificed two days after birth. The mean relative liver weight was significantly increased in F0 parental males at 3,000 and 9,000-ppm (at the higher dose level also the absolute liver weight). No treatment related histopathological changes were, however, noted. There was a reduced total number of delivered F1 pups and the viability index was reduced on post-partum day 0 and 4 at 9,000-ppm. In F1 male pups a treatment related loss of spermatocytes was found at 3,000 and 9,000-ppm (2/10 and 7/9, respectively). At the highest dose level, the presence of areolas/nipple anlagen was significantly increased and the male sexual maturation was significantly retarded. A reduced anogenital distance was observed in F2 male pups at 9,000-ppm (not investigated in F1 pups). Mortality occurred in F1 parental males (3/9) at 9,000-ppm in the premating phase, initially also reduced food consumption and reduced mean body weights were noted. At this dose level, the fertility was also reduced in the males (fertility/mating index 83%). The effects found in F1 parental males indicate that DEHP exerts a specific action on male genital organs such as the testicle and the epididymis, when males were exposed during early development. This was strengthened by the fact that female gonads were unaffected. No effects were observed on Sertoli cells in F1 parental male rats[32].

The Baxter Healthcare Corporation made public the results of an unpublished study (2000) in which neonatal male rats or rabbits were injected either with DEHP or 4% bovine serum albumin during postnatal days 3 to 21 in rats and days 14 to 42 in rabbits. Histopathological examination of the testes and other organs of DEHP-exposed animals revealed no histologic alterations that could be attributed to the test material administered at a dose of 62 mg/kg/day[17].

AdvaMed, or the Advanced Medical Technology Association made available the results of a 21-day repeat dose study (2001) of DEHP in neonatal (3 to 5 days old) rats to the FDA. 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 and hepatomegaly were observed in neonatal rats following daily intravenous exposure to DEHP at a dose of 300 mg/kg/day. Histopathological examination of the testes of animals in the 300 mg/kg/day dosing group revealed a decrease in the diameter of the seminiferous tubules and a mild depletion of germinal epithelial cells. Although testicular atrophy persisted at the end of the recovery period, histopathological changes were not seen in the recovery group previously exposed to a DEHP dose of 300 mg/kg/day for 21 days. The NOAEL in the study was 60 mg/kg/day; consistent with the results reported previously by Baxter study[17].

An investigation was conducted for determining the potential effects of Di (2-ethylhexyl) phthalate (DEHP) on the changes of ovarian miRNA expression profile, during mouse primordial follicle assembly using miRNAs-seq analysis. The ovaries of newborn CD1 mice were collected and in vitro cultured with different concentration of DEHP for 72 h and were prepared for miRNAs-seq analysis. The results indicated that DEHP exposure altered ovarian miRNA expression profile of newborn mice and it was concluded that DEHP exposure increased ROS and oxidative stress responsive miRNAs, then influenced the key genes in the PI3K/AKT1/mTOR signaling pathway and induced cell apoptosis in the newborn mouse ovaries, thereby influencing cyst breakdown and primordial follicle assembly[33].

3.2.16 Neurotoxicity

The neurobehavioural effects were tested in rats by a functional observational battery (FOB) and motor activity measurements before exposure, at specified times after a single dose exposure, and during and after a 14-day repeated dose exposure. Female Fischer 344 rats (number not given) were administered 150, 500, 1,500 or 5,000 mg/kg/day of DEHP (> 99% pure) (single dose study), or 50, 150, 500 or 1,500 mg/kg/day of DEHP (repeated exposure, 14 days) in corn oil by gavage. The FOB included

following measures: autonomic, activity, excitability, neuromuscular, sensorimotor, and physiological measures. Motor activity was measured in a maze. The FOB was performed on each rat just prior to the first dose. Thereafter, the FOB followed by motor activity assessments was conducted at 4 and 24 hours after exposure (single dose study), and on day 4 and 9 (before the daily dose) and 24 hours after the last dose. No lethality occurred. A single administration of the highest dose produced pronounced signs of general debilitation in two rats 24 hours after dosing. No changes in body weight were observed in either study. No functional domain was overall affected in either study. The NOAEL of 1,500 mg/kg/day was derived for neurotoxicity[34].

3.3 Key toxicity endpoints

Diethylhexylphthalate was known to cause tumors in hepatic tissue by activation of peroxisome proliferator-activated receptor alpha (PPAR-α). The critical role of PPAR-α in peroxisomal proliferation and carcinogenicity in mice was clearly established by the lack of either response in mice genetically modified to remove the PPAR-α[19]. Rats and mice are responsive to carcinogenic effects of peroxisome proliferators, while guinea pigs, dogs, non-human primates, and humans are non-responsive. This difference is due to marked interspecies variations in the expression of PPAR-α[18,28,29]. Therefore, evidence for occurrence of carcinogenicity in humans is very low.

Review of reproductive and developmental toxicity studies suggested, that DEHP is a potent reproductive toxin which affects the reproductive performance of F0 individuals, in-utero fetal growth and, development of F1 off springs and their reproductive performance when matured. Also, DEHP was more potent in affecting the male reproductive organs (testis, epididymides, seminal vesicle, and prostate) in rodents[17,30,32]. The primary endocrine disrupting function is that, in-utero DEHP exposure diminishes mineralocorticoid receptor (MR) expression in adult rat Leydig cells, which affects aldosterone-induced androgen formation, which in turn probably decreases production of testosterone and thus results in malformation of male sex organs[35]. Another possible mechanism may be due to its potential to disturb synthesis, regulation, and action potential of thyroid hormones, which plays key role in growth, differentiation, and metabolism in early embryonic stages of zebrafish[36,37].

Based on the above discussion of toxicity end points, reproductive/developmental toxicity was considered as a critical endpoint and therefore, parenteral NAOEL value[17] was recommended for derivation of human safety limit, whereas the earlier published risk assessments had utilized oral acceptable intake values for deriving the safety margin[38,39]

Table 2. Summary of toxicity endpoints

Safety limit descriptor	Study type	Critical end point	Value
NOAEL (Rat) - Intravenous route	21-day repeat dose study	Testicular atrophy, decrease in diameter of seminiferous tubules and depletion of germinal cells in testes, hepatomegaly	60 mg/kg/day[17]
NOAEL (Rat) - Oral route	Two-generation reproductive toxicity study (OECD TG 416)	Reproductive tract malformations in F1 and F2 generations	4.8 mg/kg/day[30]
NOAEL (Rat) - Intravenous route	Repeated dose toxicity: Six IV infusions	Alteration in Sertoli cells	60 mg/kg/day[24]

Due to the fact that DEHP is metabolized in gastro intestinal tract to MEHP, a more potent toxic substance[11,17], the oral NOAEL was found to be 10-fold lesser than parenteral NOAEL. For the risk assessment of DEHP in CC set, the relevant route of exposure is parenteral, hence the NOAEL value of 60 mg/kg/day was used for deriving the tolerable intake in this study.

3.4 Risk assessment of DEHP

3.4.1 Derivation of patient exposure (PE)

During clinical use, generally one CC set will be used per person in single day. Thus, amount of DEHP exposed to patients will be 42.18 mg/person/day.

3.4.2 Calculation of tolerable exposure (TE)

The NOAEL from the selected reproductive toxicity studies were used for the determination of tolerable intake as per ISO guideline part 17 "Establishment of allowable limits for leachable substances"[15]. Based on the study design, data quality and relevance the NOAEL was converted to tolerable intake (TI) value, which will be applicable to humans.

UF1: Uncertainty factor for interindividual variability in the human population; UF2: Uncertainty factor for interspecies extrapolation; UF3: Uncertainty factor for data quality and relevance

Table 3. Derivation of tolerable intake

Route	Endpoint	UF1	UF2	UF3	Modifying Factor	Tolerable Intake
Parenteral	NOAEL = 60 mg/kg/day	10	3	3	100	0.6 mg/kg/day
Oral	NOAEL = 4.8 mg/kg/day	10	5	1	50	0.1 mg/kg/day

Parenteral tolerable intake of 0.6 mg/kg/day derived, based on the key toxicity endpoint (reproductive toxicity) was used for toxicological risk assessment of DEHP exposure via blood contact medical devices. The tolerable intake (0.1 mg/kg/day) derived for oral route can be used for the toxicological risk assessment of DEHP via oral route of exposure. Since the patient exposure to DEHP via the CC tubing set is through parenteral route, tolerable intake of 0.6 mg/kg/day was utilized for this risk assessment.

Tolerable exposure in males was calculated as follows: (TE) = TI x bodyweight x utilization factor (UTF) = 0.6 x 70 x 1 = 42 mg/person/day. (Adult body weight = 70 kg; UTF = 1, since less than 5 devices are used during single procedure). Tolerable exposure in females (58 kg) was calculated as 34.8 mg/person/day.

3.4.3 Safety evaluation

To evaluate the biological risk, (PE) patient clinical exposure level of these extractable substance is compared to the tolerable daily exposure; TE and the margin of safety (MOS) is estimated. Bigger the MOS value, better the safety. When the MOS is less than 1.0, that would raise concerns about the adequacy of patient safety. Hence, minimum requirement is > 1. Margin of safety (MOS) is the ratio of tolerable exposure obtained from animal toxicology studies to the predicted or estimated patient exposure level or dose of extractable compound. Margin of safety (MOS) = TE/PE. Margin of safety in male and female patients were estimated to be 1.0 (42/38.18) and 0.8 (34.8/38.18), respectively.

It should be noted that the tolerable intake and therefore, tolerable exposure for DEHP was calculated based on the repeated exposures (i.e., NOAEL). From the above evaluation, the amount of DEHP extracted from the CC tubing set is approximately equal to the tolerable exposure. However, it should be noted that DEHP leaching during clinical use of CC tubing will be much lower than the extracted DEHP, due to lower clinical exposure duration (6 hours in clinical v/s 24 hours in exaggerated extraction) and usage of less rigorous extraction medium (i.e., 4:6 ethanol/water in DEHP estimation study v/s blood in clinical use). Moreover, the CC tubing set will typically be used once during the life time of patient and repeated exposure is rarely expected. Under these circumstances, it can be concluded that the amount of DEHP leaching from CC tubing set will pose low or negligible toxicological risk to the patients during the intended clinical use.

4 Conclusion

During the clinical use of medical devices like CC tubing set, intravenous infusion set, storage of blood in blood bags, there is a possibility of DEHP leaching in to the patient body via fluids or blood. To understand the patient clinical exposure scenario of DEHP, this case study using CC tubing set was designed to estimate the maximum probable amount of DEHP getting released into the patients. Owing to its large surface area and number of DEHP containing parts, CC tubing set represents a worst-case scenario of clinical exposure. Following the toxicological risk assessment of DEHP considering the health-based exposure limit, it was established that exposure to DEHP at the current estimated level will not cause any toxicological risk to patients. Until comprehensive safety information is gathered on alternative (non-DEHP) plasticizers, this safety assessment study can be considered for deciding on the use of di(2-ethylhexyl) phthalate (DEHP) based medical devices.

Acknowledgement

The research was funded by GLR Laboratories Private Limited, Chennai 600068, INDIA.

References

1) Allsopp MW, Vianello G. Poly(Vinyl Chloride). 2000. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/14356007.a21_717.
2) Cadogan DF, Howick CJ. Plasticizers. *Ullmann's Encyclopedia of Industrial Chemistry*. 2000. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/14356007.a20_439.
3) Sastri VR. Plastics in Medical Devices. Oxford. William Andrew Publishing. 2014. Available from: https://doi.org/10.1016/B978-1-4557-3201-2.00001-X.

4) Bis(2-ethylhexyl) phthalate, EC number. *European Chemicals Agency*;p. 204–211. Available from: https://echa.europa.eu/registration-dossier/-/registered-dossier/15358/7/1.
5) National Research Council (US) Committee on the Health Risks of Phthalates. 2008. Available from: https://pubmed.ncbi.nlm.nih.gov/25009926/.
6) Erythropel HC, Maric M, Nicell JA, Leask RL, Yargeau V. Leaching of the plasticizer di(2-ethylhexyl)phthalate (DEHP) from plastic containers and the question of human exposure. *Applied Microbiology and Biotechnology*. 2014;98(24):9967–9981. Available from: https://pubmed.ncbi.nlm.nih.gov/25376446/.
7) International Organization for Standardization. *Chemical Characterization of Medical Device Materials Within a Risk Management Process (ISO Standard No ISO 10993-18:2020)*. 2020;18. Available from: https://www.iso.org/standard/64750.html.
8) Bouattour Y, Wasiak M, Bernard L, Pinguet J, Richard D, Rouzo-Grèves ML, et al. Quantification of bis(2-ethylhexyl) phthalate released by medical devices during respiratory assistance and estimation of patient exposure. *Chemosphere*. 2020;255:126978. Available from: https://doi.org/10.1016/j.chemosphere.2020.126978.
9) Šimunović A, Tomić S, Kranjčec K. Medical devices as a source of phthalate exposure: a review of current knowledge and alternative solutions. *Archives of Industrial Hygiene and Toxicology*. 2022;73(3):179–190. Available from: https://pubmed.ncbi.nlm.nih.gov/36226817/.
10) IARC. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Lyon: IARC Press. 1982. Available from: https://publications.iarc.fr/Book-And-Report-Series/Iarc-Monographs-On-The-Identification-Of-Carcinogenic-Hazards-To-Humans/Some-Industrial-Chemicals-And-Dyestuffs-1982.
11) Pakalin S, Aschberger K, Cosgrove O, Lund B, Perez P, Vegro A, et al. European Union Risk Assessment Report - bis (2-ethylhexyl) phthalate (DEHP). . Available from: https://publications.jrc.ec.europa.eu/repository/handle/JRC45705.
12) Sastri VR. Plastics in Medical Devices . Elsevier. 2014;p. 1–8. Available from: https://www.sciencedirect.com/science/article/pii/B978145573201200001X.
13) International Organization for Standardization, Plastics collapsible containers for human blood and blood components - Part 1: Conventional containers (ISO Standard No. ISO 3826-1:2019). 2019. Available from: https://www.iso.org/standard/70726.html.
14) International Organization for Standardization, Biological Evaluation of Medical Devices - Application of the Threshold of Toxicological Concern (TTC) for Assessing Biocompatibility of Medical Device Constituents (ISO Standard No. ISO/ TS 21726:2019). 2019. Available from: https://www.iso.org/standard/71514.html.
15) International Organization for Standardization. Biological Evaluation of Medical Devices - Part 17: Establishment of Allowable Limits for Leachable Substances (ISO Standard No. ISO 10993-17:2002), 2002. 2002. Available from: https://www.iso.org/standard/23955.html.
16) Kroes R, Kleiner J, Renwick A. The threshold of toxicological concern concept in risk assessment. *Toxicology Letters*. 2006;164(2):S48–S48. Available from: https://pubmed.ncbi.nlm.nih.gov/15829616/.
17) FDA (U.S. Food and Drug Administration). Safety assessment of di(2- ethylhexyl)phthalate (DEHP) released from PVC medical devices. 2002. Available from: http://icpe.in/pdf/Safety_assessment_Diphthalate.pdf.
18) Huber WW, Grasl-Kraupp B, Schulte-Hermann R. Hepatocarcinogenic Potential of Di(2-Ethylhexyl)phthalate in Rodents and its Implications on Human Risk. *Critical Reviews in Toxicology*. 1996;26(4):365–481. Available from: https://pubmed.ncbi.nlm.nih.gov/8817083/.
19) Doull J, Cattley R, Elcombe C, Lake BG, Swenberg J, Wilkinson C, et al. A Cancer Risk Assessment of Di(2-ethylhexyl)phthalate: Application of the New U.S. EPA Risk Assessment Guidelines. *Regulatory Toxicology and Pharmacology*. 1999;29(3):327–357. Available from: https://pubmed.ncbi.nlm.nih.gov/10388618/.
20) Shaffer CB, Carpenter CP, Smyth HF. Acute and Subacute Toxicity of Di (2-Ethylhexyl) Phthalate with Note upon its Metabolism. *The Journal of industrial hygiene and toxicology*. 1945;27:130–135. Available from: https://www.cabdirect.org/cabdirect/abstract/19462700110.
21) Butala JH, David RM, Gans G, Mckee RH, Guo TL, Peachee VL. Phthalate treatment does not influence levels of IgE or Th2 cytokines in B6C3F1 mice. *Toxicology*. 2004;201(1-3):77–85. Available from: https://pubmed.ncbi.nlm.nih.gov/15297022/.
22) Tomonari Y, Kurata Y, David RM, Gans G, Kawasuso T, Katoh M. Effect of Di(2-Ethylhexyl) Phthalate (DEHP) on Genital Organs from Juvenile Common Marmosets: I. Morphological and Biochemical Investigation in 65-Week Toxicity Study. *Journal of Toxicology and Environmental Health, Part A*. 2006;69(17):1651–1672. Available from: https://pubmed.ncbi.nlm.nih.gov/16854791/.
23) Klimisch HJJ, Gamer AO, Hellwig J, Kaufmann W, Jäckh R. Di-(2-ethylhexyl) phthalate: A short-term repeated inhalation toxicity study including fertility assessment. *Food and Chemical Toxicology*. 1992;30(11):915–919. Available from: https://pubmed.ncbi.nlm.nih.gov/1473784/.
24) Sjöberg P, Lindquist NG, Montin G, Plöen L. Effects of repeated intravenous infusions of the plasticizer di-(2-ethylhexyl) phthalate in young male rats. *Archives of Toxicology* . 1985;58(2):78–83. Available from: https://pubmed.ncbi.nlm.nih.gov/4091660/.
25) Myhr B, Bowers L, Casparay W. Assays for the Induction of Gene Mutations at the Thymidine Kinase Locus in L5178Y Mouse Lymphoma Cells in a Culture. 1985. Available from: https://hero.epa.gov/hero/index.cfm/reference/details/reference_id/783191.
26) Butterworth BE, Bermudez E, Smith-Oliver T, Earle L, Cattley R, Martin J, et al. Lack of genotoxic activity of di(2-ethylhexyl)phthalate (DEHP) in rat and human hepatocytes. *Carcinogenesis*. 1984;5(10):1329–1335. Available from: https://pubmed.ncbi.nlm.nih.gov/6488454/.
27) Putman DL, Moore WA, Schechtman LM, Hodgson JR. Cytogenetic evaluation of di-(2-ethylhexyl)phthalate and its major metabolites in fischer 344 rats. *Environmental Mutagenesis*. 1983;5(2):227–231. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/em.2860050211.
28) David RM, Moore MR, Finney DC, Guest D. Chronic Toxicity of Di(2-ethylhexyl)phthalate in Mice. *Toxicological Sciences*. 2000;58(2):377–385. Available from: https://pubmed.ncbi.nlm.nih.gov/11099649/.
29) David RM, Moore MR, Cifone MA, Finney DC, Guest D. Chronic peroxisome proliferation and hepatomegaly associated with the hepatocellular tumorigenesis of di(2-ethylhexyl)phthalate and the effects of recovery. *Toxicological Sciences*. 1999;50(2):195–205. Available from: https://pubmed.ncbi.nlm.nih.gov/10478855/.
30) Blystone CR, Kissling GE, Bishop JB, Chapin RE, Wolfe GW, Foster PMD. Determination of the Di-(2-Ethylhexyl) Phthalate NOAEL for Reproductive Development in the Rat: Importance of the Retention of Extra Animals to Adulthood. *Toxicological Sciences*. 2010;116(2):640–646. Available from: https://pubmed.ncbi.nlm.nih.gov/20484383/.
31) Merkle J, Klimisch HJ, Jackh R. Developmental toxicity in rats after inhalation exposure of di-2-ethylhexylphthalate (DEHP). *Toxicology Letters*. 1988;42(2):215–223. Available from: https://pubmed.ncbi.nlm.nih.gov/3406961/.
32) Schilling K, Gembardt C, Hellwig J. Reproduction toxicity of di-2-ethylhexyl phthalate (DEHP). 1999. Available from: https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/1334141.
33) Zhang JNN, Zhang RQN, Liu JCC, Li L, Shen W, Sun XFF. Di (2-ethylhexyl) Phthalate Exposure Impairs the microRNAs Expression Profile During Primordial Follicle Assembly. *Frontiers in Endocrinology*. 2019;10(2). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6923199/.

34) Moser VC, Cheek BM, Macphail RC. A multidisciplinary approach to toxicological screening: III. Neurobehavioral toxicity. *Journal of Toxicology and Environmental Health*. 1995;45(2):173–210. Available from: https://pubmed.ncbi.nlm.nih.gov/7783252/.
35) Martinez-Arguelles DB, Culty M, Zirkin BR, Papadopoulos V. In Utero Exposure to Di-(2-Ethylhexyl) Phthalate Decreases Mineralocorticoid Receptor Expression in the Adult Testis. *Endocrinology*. 2009;150(12):5575–5585. Available from: https://pubmed.ncbi.nlm.nih.gov/19819939/.
36) Bereketoglu C, Pradhan A. Plasticizers: negative impacts on the thyroid hormone system. *Environmental Science and Pollution Research*. 2022;29(26):38912–38927. Available from: https://link.springer.com/article/10.1007/s11356-022-19594-0.
37) Jia PPP, Ma YBB, Lu CJJ, Mirza Z, Zhang W, Jia YFP, et al. The Effects of Disturbance on Hypothalamus-Pituitary-Thyroid (HPT) Axis in Zebrafish Larvae after Exposure to DEHP. *PLOS ONE*. 2016;11(5):e0155762. Available from: https://pubmed.ncbi.nlm.nih.gov/27223697/.
38) Eckert E, Müller J, Höllerer C, Purbojo A, Cesnjevar R, Göen T, et al. Plasticizer exposure of infants during cardiac surgery. *Toxicology Letters*. 2020;330:7–13. Available from: https://pubmed.ncbi.nlm.nih.gov/32387387/.
39) Saab Y, Oueis E, Mehanna S, Nakad Z, Stephan R, Khnayzer RS. Risk Assessment of Phthalates and Their Metabolites in Hospitalized Patients: A Focus on Di- and Mono-(2-ethylhexyl) Phthalates Exposure from Intravenous Plastic Bags. *Toxics*. 2022;10(7):357. Available from: https://pubmed.ncbi.nlm.nih.gov/35878262/.