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Systemic toxicity of di-2-ethylhexyl terephthalate (DEHT) in rodents following four weeks of intravenous exposure

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ABSTRACT

Background: Di-2-ethylhexyl-terephtalate (DEHT) is a general purpose plasticizer and a structural isomer to di-2-ethylhexyl phthalate (DEHP) being known for its toxicity. Despite the fact that DEHT is used in quite a number of synthetics for medical device production including equipment for intravenous administration, toxicity of DEHT has not been assessed after/during intravenous exposure. Hence we report here the results of a toxicity study in male and female rats with continuous intravenous infusion of DEHT over 4 weeks.
Methods: The study was done according to OECD guidelines under GLP conditions. The dose was infused per day to male and female rats over a period of 4 weeks with saline (control), middle chain triglycerides (vehicle) as well as with 38.2, 114.5 or 381.6 mg DEHT/kg. Each group ($n = 6$) was closely monitored regarding survival, body weight development, food and water consumption. Moreover blood and urine samples were taken and a standardized necropsy as wall as a histological analysis was performed after the investigation period.
Results: DEHT had no effect on survival, body weight development, food and water consumption in the whole dose range investigated. There were no indications as to hematotoxicity or immunotoxicity. Clinical chemistry and histopathology indicated no exposure related effect on hepatic, thyroidal and reproductive functions or organs.
Conclusion: DEHT administered via intravenous infusion was tolerated systemically and locally without adverse effects up to and including 381.6 mg/kg/day (NOAEL = 381.6 mg/kg × day). In particular, there were no effects on reproductive tissues/organs, kidneys, liver hepatocytes and peroxisomes, which are known targets of DEHP-toxicity.

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1. Introduction

Plasticizers are needed to induce and maintain flexibility of certain synthetic materials. Plasticizers are chemically different from the polymer chain, but embedded non-covalently into the polymer matrix and may migrate into materials in contact with the surface. Migration of small plasticizer into foodstuff, consumer products, and medical devices (e.g. infusion line tubing) has led to concern in regard of human exposure.

DEHT and DEHP are structural isomers: DEHT is the benzene-1,4-dicarboxylic acid (para position = terephthalic acid), DEHP the benzene-1,2-dicarboxylic acid (ortho position = phthalic acid) esterified with 2-ethylhexanol. This structural difference is pivotal with regard to metabolism and consequential toxicological effects, which differ markedly for DEHP and DEHT. DEHT is completely hydrolyzed yielding 2 moles of 2-ethylhexanol per mole of terephthalic acid (Barber et al., 1994). Two other terephthalic esters, di-methylterephthalate (DMT) and di-butylterephthalate (DBT) have shown the same complete hydrolysis (Kamendulis et al., 2002). It is thus assumed that the para-position allows for complete metabolism while the ortho-position as in DEHP does not. This difference seems to be pivotal regarding toxicological effects as pointed out and referenced in a preliminary report published by the Scientific Committee on Emerging and Newly-Identified Health Risks (SCENIHR, 2008) on the safety of medical devices containing DEHP-plasticized PVC or other plasticizers on neonates and other groups possibly at risk.

DEHP has been observed to induce teratogenesis in rodents (Faber et al., 2007a; Gray Jr. et al., 2000). Moreover toxic effects on reproductive organs and liver of rats have been described

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after oral administration (Faber et al., 2007b). Conversely, DEHT did not induce lesions in comparable organ/tissue systems after oral administration (SCENIHR, 2008 and references therein). This is probably partially due to fecal elimination of 37% of non-metabolized DEHT after oral administration (Barber et al., 1994) as well as to almost total hydrolysis of DEHT to terephthalic acid and e-ethylhexanol.

Human exposure to plasticizers, however, may occur to a relevant extent also via the intravenous route. Infusion tubing is used to administer various solutions and emulsions for clinical treatment. It may be speculated that this routine clinical procedure – likely to be performed globally millionfold – may release plasticizers and expose patients to these substances. So far, data on potential toxicological effect of DEHT after intravenous administration are scarce.

In the presented rodent study the toxic potential of DEHT for various organ and tissue systems during and after continuous intravenous infusion over 4 weeks was examined in order to define a no-adverse effect level (NOAEL) as based on a theoretical worst case exposure of DEHT in the clinical setting.

2. Theory and calculation

Based on results of a pre-investigational trial worst case estimates of DEHT uptake in the clinical setting were defined. These experiments were performed by BMP laboratory for medical material testing (Aachen, Germany) and Henkel (Dusseldorf, Germany).

In order to mimic the clinical situation sterile infusion tubes (200 cm length, 0.3 cm inner diameter) containing DEHT as plasticizer were perfused with a middle-chain-triglyceride emulsion (Lipofundin MCT 10%. B.Braun), a balanced crystalloid solution (Sterofundin ISO, B.Braun) and the same balanced crystalloid solution also containing 10% ethanol over 0.5 h, 1 h, 2 h and 24 h at room temperature.

Using gas chromatograph mass spectrometry the amount of extracted DEHT was determined. The crystalloid solution did not extract a detectable (threshold < 1 μg) amount of DEHT over any of the investigated time periods and regardless of the addition of ethanol. DEHT was found only in MCT 10% solution. Extraction increased over time yielding a maximum concentration of 13.3 μg/ml Lipofundin over 24 h. Additionally, a threefold standard deviation was applied resulting in a concentration of 17.05 μg/ml.

Based on an inner volume of 14.14 ml (length 200 cm, inner diameter 0.3 cm) the maximum amount extracted and possibly infused to a patient from the tube over a 24 h period were 241 μg of DEHT by the application of Lipofundin MCT 10%.

All dose levels chosen for the following safety study in rats were high multiples of this potential worst case daily exposure of patients: 158times, 475times and 1580 times and per kg bodyweight in ascending dosage.

3. Materials and methods

3.1. Sponsoring, conducting and location of the study

The study was sponsored by B.Braun (Melsungen, Germany). Experiments were conducted by and at Bayer Schering Pharma Toxicology, Germany according to guideline B7 Directive 67/548/EEC, OECD 407 and Note for Guidance on Repeated Dose Toxicity in full compliance with GLP. The study was approved and complied with governmental regulations including animal welfare legislation.

3.2. Chemicals and formulations

BIS(2-ethylhexyl) terephthalate (DEHT, Eastman 168 CAS 6422-86-2) was obtained from Eastman and used for manufacturing of sterile infusion formulation with concentrations of 1590, 4770 and 15900 μg DEHT/ml Lipofundin MCT10 (the vehicle) at B.Braun Melsungen.

3.3. Experimental animals and housing conditions

Male and female Wistar rats (SPF bred, strain (WI) BR from Charles River, Sulzfeld, Germany) with a mean bodyweight of 199 g (females) and 260 g (males) used in this study were housed under standardized, conventional conditions.

Polyurethane catheters were implanted under general anesthesia in the femoral vein, the catheter tip positioned in the vena cava at the level of kidneys.

3.4. Experimental design

Three experimental groups (six male and 6 female rats each per group) were infused with 38.2, 114.5 or 381.6 mg DEHT/kg daily within 12 h at a flow rate of 2 ml/kg/h (defined as exposure). As controls the same number of male and female rats each were infused with Lipofundin MCT 10% (vehicle control group) or with saline (saline control group) at a flow rate of 2 ml/kg/h for 12 h daily. In between exposures saline was administered at a flow rate of 1 ml/h in order to maintain catheter patency. The total exposure period was 28 days.

Animals were identified by cage labels stating the test item, animal no., group no., dose, sex, and study number as well as by transponders stating animal and study number. Body weights and a detailed clinical assessment including behavioral observation in a standard open field (OFO) were recorded before exposure start. During the exposure period body weight, food and water, vehicle and saline or DEHT infusion formulation intake were determined weekly from the weight difference of containers supplied and not used. From these data group means, weekly intake, intake per kg body weight, per rat and cumulative consumptions were calculated.

Rats were inspected visually for morbidity and mortality (twice daily,) and generally examined for clinical symptoms daily (home cage). In addition, a detailed physical examination including palpation and behavioral observations in a standard open field was done once weekly in order to monitor general health.

On study day 28 after an overnight fasting with free access to water urine and blood (retro-bulbar vein plexus of anesthetized rats) samples were collected and analyzed at room temperature. Blood samples were investigated for differential blood count, erythrocyte morphology and indices, erythrocyte, reticulocyte, leukocyte and platelet count, hemoglobin concentration, hematocrit, thromboplastin time (Hepato-Quick) as well as for plasma activity of alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, glutamate dehydrogenase, lactate dehydrogenase, creatine kinase, gamma glutamyl transferase, the concentration of albumin, bilirubin, cholesterol, creatinine, total protein, triglycerides, urea, glucose, chloride, calcium, inorganic phosphate, potassium, sodium, thyroid hormones (T3, T4, TSH). In urine samples density, volume and protein (quantitatively), blood, bilirubin, glucose, ketone bodies, pH, protein, urobilinogen and microscopy of sediment (semiquantitatively) were assessed.

On day 29 animals were sacrificed by exsanguination under deep general anesthesia. Post mortem the following analyses were performed:

3.5. Metabolic activity of liver tissue

Liver tissue liver specimen were collected during necropsy (day 29) and stored frozen until determination of: cytochrome P-450, triglyceride content, activities of aminopyrine-N-demethylase (N-DEM), p-nitroanisole-O-demethlyase (O-DEM), 7-ethoxycoumarin deethylase (ECOD), 7-ethoxy-resorufin deethylase (EROD), aldrin epoxidase (ALD) epoxide hydrolase (EH), UDP-glucuronyltransferase (GLU-T), Glutathione-S-transferase (GST), testosterone hydroxylation.

3.6. Immunotoxicological assessment

In blood and spleen samples collected at necropsy (day 29) antibody titers, splenic cell count, subpopulation composition and size distribution were determined according to validated methods.

3.7. Pathology

All organs and tissues were examined by a veterinary pathologist with extensive experience in evaluating laboratory animal tissues, especially of rats infused continuously via implanted catheter. At necropsy a systematic gross examination of each animal's general physical condition, body orifices as well as external and internal organs and tissues was performed.

The following organs and tissues were weighed: liver, spleen, kidneys (both), thymus, adrenal glands (both), heart, popliteal lymph node, prostate, ovaries, uterus, testes (both), and ductus epididymides (both). In addition organ weights were related to terminal body weight (relative organ weights). An extensive range of tissue and organ samples as required by OECD guideline 407 were fixed and evaluated.

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