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Adverse effects of diisooctyl phthalate on the male rat reproductive development following prenatal exposure

Anne-Marie Saillenfait[a,*], Jean-Philippe Sabaté[a], Alain Robert[a], Benoit Cossec[a], Alain-Claude Roudot[b], Flavien Denis[a], Manuella Burgart[a]

[a] Institut National de Recherche et de Sécurité, Rue du Morvan, CS 60027, 54519 Vandoeuvre Cedex, France
[b] Université de Bretagne Occidentale, UFR des Sciences et Techniques, Brest, France

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ABSTRACT

In a first study, rats were given diisooctyl phthalate (DIOP, CAS 27554-26-3) at 0, 0.1, 0.5, and 1 g/kg/day, by gavage, on gestation days 6–20 (GD). There was a significant increase in resorptions at 1 g/kg/day and a reduction in fetal weights at 0.5 and 1 g/kg/day. Malpositioned testes were observed in fetuses at 1 g/kg/day, and supernumerary lumbar ribs and ossification delay at 0.5 and 1 g/kg/day. In a follow-up study, DIOP administered on GD 12–19 reduced fetal testicular testosterone at 0.1 g/kg/day and above. Finally, postnatal reproductive assessment was conducted in adult male offspring prenatally exposed to DIOP on GD 12–21. Abnormalities of reproductive system (e.g. hypospadias, non scrotal testes, and hypospermatogenesis) were observed in a few adult males at 0.5 g/kg/day, and with a high incidence at 1 g/kg/day. Thus, DIOP displayed an antiandrogenic activity and disrupted the male reproductive development.

1. Introduction

Diisooctyl phthalate (DIOP, CAS RN 27554-26-3) is a phthalic acid diester which is primarily used as a plasticizer for synthetic rubber and vinyl, cellulosic and acrylate resins [1]. There are known uses of DIOP in the manufacture of jackets for building wire, and automotive hoses and parts [2,3]. It was also identified in some children toys in 2000 [4] and in commercial milk products [5]. The production of DIOP was estimated at about 10,000 metric tons in United States and its consumption at 15,000 metric tons in Western Europe in 2008 [3]. DIOP contributed to 1.7% of the total phthalate market. It is listed as a High Production Volume (HPV) chemical by OECD and US EPA. Occupational exposure limits (8 h) to DIOP were set at 5 mg/m^3 in United Kingdom and the Netherlands and at 3 mg/m^3 in Sweden and Denmark. US Food and Drug Administration (FDA) permits the use of DIOP in adhesives for articles in contact with food [6].

DIOP possesses two branched ester side chains with a total length of eight carbons and consists of methylheptyl esters, such as di(6-methylheptyl) phthalate (CAS RN 27554-26-3) [1,3]. Technical DIOP is usually present as mixtures, which may contain isomers with a linear portion (backbone) from four to six carbons in their alkyl chains (e.g. dimethylhexyl phthalate) [7]. The composition of DIOP commercial mixture is commonly 70–75% of isomers with a C4–C6 backbone and less than 25% of isomers with a backbone ≥C7. Di-2-ethylhexyl phthalate (DEHP, CAS RN 117-81-7) which has a backbone of six carbons, and di-*n*-octyl phthalate (DnOP, CAS RN 117-84-0, backbone of 8 carbons) which has straight ester chains, are isomers of DIOP (Fig. 1).

Common reproductive and developmental effects have been associated with several phthalates which predominantly have carbon backbone lengths of C4–C6 (e.g. DEHP). Embryoletality and fetal malformations (e.g. skeletal, cardiovascular, nervous system) have been observed in rats after prenatal exposure to active phthalates, at relatively high doses [8–11]. Moreover, they were shown to impair the male reproductive development in rats when administered during the critical intrauterine period of sexual differentiation. Male offspring displayed reduced anogenital distance

Abbreviations: AGD, anogenital distance; BMD, benchmark dose; DEHP, diethylhexyl phthalate; DIBP, diisobutyl phthalate; DIDP, diisodecyl phthalae; DINP, diisononyl phthalate; DIOP, diisooctyl phthalate; DnOP, di-*n*-octyl phthalate; DBP, di-*n*-butyl phthalate; DnHP, di-*n*-hexyl phthalate; DPP, dipentyl phthalate; GD, gestation day; LOAEL, the lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level; PND, postnatal day; PNW, postnatal week; PVC, polyvinyl chloride; SD, standard deviation.

* Corresponding author. Tel.: +33 3 83 50 20 39; fax: +33 3 83 50 98 46.
E-mail address: anne-marie.saillenfait@inrs.fr (A.-M. Saillenfait).

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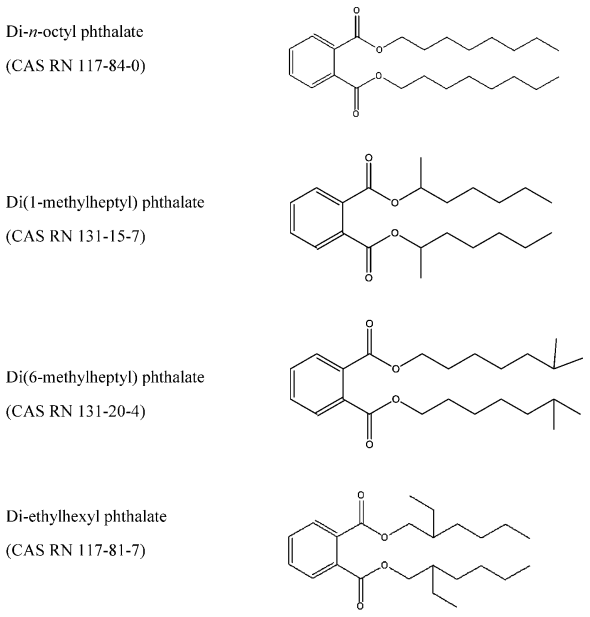

Fig. 1. Chemical structures of selected phthalates.

(AGD), retained nipples, hypospadias, undescended testis, and hypoplasia or absent reproductive organs [12–16]. These effects have been attributed, at least in part, to lowered testosterone production in the rat fetal testis, as a result of decreased expression of genes involved in cholesterol transport and steroidogenesis [17–23]. Comparatively, the linear C8 DnOP had no teratogenic effects after oral administration to Sprague-Dawley rats on GD 6–20 at doses up to 1 g/kg/day [24]. In addition, it did not affect the embryo–fetal survival, intra-uterine growth, AGD of male fetuses, and fetal testicular migration. However, an increased incidence of supernumerary lumbar ribs was observed at 0.25 g/kg/day and higher doses.

No extensive and/or detailed data are available for the reproductive or developmental toxicity of DIOP [2,3]. Thus, our overall objective was to determine if DIOP produces developmental toxic effects in rats after oral administration during pregnancy. Special attention was paid to the development of the male reproductive system. We have therefore conducted three separate studies to (1) evaluate the potential maternal and embryo–fetal toxicity of DIOP; (2) determine its effects on testicular testosterone production by near term fetuses; (3) assess the postnatal consequences of an in utero exposure to DIOP on the male reproductive system.

2. Materials and methods

2.1. Animals

After 1–2 weeks of acclimatization, primiparous female (180–200 g) Sprague-Dawley rats supplied by Charles River Laboratories (Saint-Germain-sur-l'Arbresle, France) were housed overnight with adult males from the same strain and supplier. The day sperm was detected in the vaginal smear was considered to be GD 0. Mated females were individually housed in clear polycarbonate cages with stainless-steel wire lids and virgin loose pulp as bedding (Alpha-Dri®, Dietex, Saint Gratien, France). Food pellets (UAR Alimentation, Villemoisson, France) and filtered tap water were available ad libitum. Animal rooms were maintained at 22 ± 2 °C, a relative humidity of 55 ± 15%, and a 12-h light cycle from 7.00 AM to 7.00 PM. Mated females were randomly assigned to treatment groups by stratified randomization so that the mean body weights on GD 0 did not differ among treatment groups. Time-mated females were administered DIOP, by gavage, once daily, in the morning. The dosing volume was 5 ml/kg. Initial doses were based on maternal weight on the first day of treatment, and adjusted on the most recently recorded body weight of the individual animal (weighed on GD 0, 6, 9, 12, 15, 18, and 21). Three separate studies were conducted. In each, a concurrent control group received the vehicle under the same conditions as the treated groups.

2.2. Test chemicals

DIOP (CAS RN 27554-26-3, lot 04817PE, ≥99% pure) was purchased from Aldrich (Regenstauf, Germany). Dosing solutions were formulated in olive oil (Cooper, Melun, France) as the vehicle. Olive oil complied with the quality standards of the European pharmacopoeia. Formulations were prepared weekly and stored in a dark place at room temperature. Stability was established for up to 2 weeks by gas chromatography–mass selective detector analysis.

Analysis by gas chromatography/mass spectroscopy (GC/MS) indicated that linear di-*n*-butyl phthalate, di-*n*-pentyl phthalate, di-*n*-hexyl phthalate (DnHP), di-*n*-heptyl phthalate, di-*n*-octyl phthalate, di-*n*-nonyl phtalate, and di-*n*-decyl phthalate, were absent from the test substance. Additional GC/MS analyses of the alkyl moieties were performed after hydrolysis of the phthalate diester by a simple reaction with a commercially available Grignard reagent [25]. Separation by GC/MS was then carried out on a 450-GC system (Bruker, Wissembourg, France) equipped with a CP-8400 autosampler (Bruker) and a 320 MS triple quadrupole mass spectrometer (Bruker, Wissembourg, France) used in electronic impact (EI) mode (ionization energy at 70 eV). Alcohols were separated on Resteck Rxi 5Sil MS with integra-guard (30 m length × 0.25 mm I.D. × 0.25 μm film thickness) and helium was used as the carrier gas at a constant flow rate of 1.0 ml/min. The sample (1 μL) was injected in splitless mode (injector type: 1177) with an injection temperature set at 250 °C, a split delay and a split ratio respectively set at 0.25 min and 1/50. The oven temperature was held at 60 °C for 1 min, then increased to 240 °C at a rate of 10 °C/min and finally increased to 300 °C at 5 °C/min rate, and finally held at this temperature for 5 min. Ion source temperature and transfer line were set respectively at 200 °C and 250 °C. Acquisitions were performed in Full Scan mode (scan range: 30–400 uma). Linear or branched butanol, pentanol, hexanol, heptanol, nonanol, and decanol were not identified in the crude residue of hydrolysis, nor 2-ethyl-1-hexanol (alkyl moiety of DEHP). Six primary alcohols with a C8 chain were isolated, which did not correspond to 1-octanol, or 2-octanol (1-methyl-1-heptanol). Due to the lack of corresponding analytical standards, their exact structure could not be identified.

2.3. Study 1: prenatal toxicity study

Groups of 10–12 time-mated females (8–12 pregnant) were administered DIOP on GD 6–20, at 0, 0.1, 0.5, or 1 g/kg/day. Cage side observations of females were conducted at least once daily. Food consumption was measured at three-day intervals starting on GD 6. Maternal body weights were recorded on GD 0, 6, 9, 12, 15, 18, and 21. On GD 21, the females were killed by an intrapulmonary injection of T61® (Intervet International, GmbH, Unterschleissheim, Germany) and the uterine horns were removed, trimmed, and weighed. Uterine contents were examined to determine the number of implantation sites, resorptions, and dead and live fetuses. Uteri which had no visible implantation sites were stained with ammonium sulphide (10%) to detect very early resorptions [26]. All live fetuses were individually examined externally, weighed, and euthanized by an injection of sodium phenobarbital. Half of the live fetuses from each litter was preserved in Bouin's solution and examined for internal soft tissue changes [27,28]. The other half was