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Pregnancy-associated changes in plasma concentration of the endocrine disruptor di(2-ethylhexyl) phthalate in a sheep model

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Abstract

The plasticizer di(2-ethylhexyl)phthalate (DEHP), used for producing polyvinyl chloride (PVC), acts as an endocrine disruptor with toxic effects on reproductive and developmental processes. Exposure to DEHP in humans is mainly by environment and food. Thus, our aim was to determine plasma levels in livestock animals using the ewe (*Ovis aries*) as a model. In a first trial, 150 samples from ewes of different ages (2 to 7 yr) and reproductive status (pregnant and nonpregnant) were analyzed by high-performance liquid chromatography (HPLC). DEHP was detected in 34.7% of the samples, with a mean level of 0.45 ± 0.01 μg/mL (range, 0.05 to 2.81 μg/mL). The percentage of nonpregnant animals with DEHP traces was higher in animals older than 4 yr (n = 66, 37.9%) than in younger animals (n = 69, 17.4%; $P < 0.05$), although the mean levels in ewes with residues were similar (0.16 ± 0.01 vs. 0.16 ± 0.02 μg/mL). All the pregnant ewes (n = 15) showed presence of DEHP, with higher plasma levels than that in nonpregnant females (1.42 ± 0.18 vs. 0.16 ± 0.01 μg/mL; $P < 0.0001$). For confirming the effect of pregnancy on mobilization of DEHP from body fat, 101 ewes of the same age were sampled in a second trial at a different farm. The percentage of animals with DEHP traces was higher in pregnant ewes (n = 32, 71.9%; $P < 0.005$) than in nonpregnant ewes (n = 37, 35.1%) or in ewes that recently gave birth (n = 32, 21.9%), although mean levels were similar (0.42 ± 0.02, 0.33 ± 0.02, and 0.34 ± 0.05 μg/mL, respectively). In conclusion, current results indicate a high incidence of ewes reared in the field showing accumulation of phthalates; percentage of animals with presence of DEHP increases with age, due to an extended period of exposure, but mainly during pregnancy, due to the mobilization of body reserves.

Keywords: DEHP; Endocrine disruptors; Ewe; Exposure; Phthalate; Pregnancy

1. Introduction

Currently, the deleterious influence of some environmental chemical contaminants, termed endocrine disrupting chemicals or contaminants (EDCs), on reproduction in mammals and other living organisms is well known. The list of EDCs is a heterogeneous group of xenobiotics including pesticides (e.g., organochlorines, organophosphates, and carbamates), industrial substances (e.g., alkyl phenols, polychlorinated biphenyl ethers, phthalates, parabens, and bisphenol A), derivatives from industrial processes (e.g., dioxins and

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furans), some metals (e.g., arsenic and cadmium), and natural compounds (e.g., phytoestrogens and mycotoxins). However, incessantly, new compounds are being added to the list.

The plasticizer di(2-ethylhexyl)phthalate (DEHP), used for producing polyvinyl chloride (PVC), acts as an EDC with toxic effects on reproductive and developmental processes [1–3]. DEHP can be found in different PVC toys, devices, and items [4,5] and even in the dust surrounding the objects [6]. DEHP is a lipophilic compound and, therefore, it is not chemically combined to PVC; thus, DEHP can be released from plastic [7,8]. Experimental exposure to DEHP has mainly been analyzed in rodent models [9], and non-experimental exposure has mainly been studied in humans [4,10,11]. Knowledge of animal exposure to DEHP is important for understanding the potential risk to animal health [12] as well as the risk to human health, as the animal-derived products represent one of the most important sources of human exposure to many organic pollutants. Nevertheless, until very recently, studies of plasma levels in domestic animals have been limited [13]; thus, our first aim was to determine non-experimentally induced plasma levels in ruminants reared in the field using the sheep as a model.

The second objective was to evaluate possible influences of variables like age and reproductive status that can be extrapolated to other species including humans. It has been described for other EDCs (pesticides) that circulating levels in peripheral blood are increased during periods of metabolic demand, like pregnancy, due to release from fat depots [14]. In the case of DEHP, increases in the exposure to the compound in pregnant females may affect the concept [5,15]. Currently, the sheep is widely considered to be a good model for evaluation of human reproductive endocrinology [16–19]. The ewe is indeed a suitable model for purposes of studies on pregnancy [20], carrying one or two fetuses that are born mature after a long gestation. Conversely, there are differences in placentation (hemochorial in humans and epitheliochorial in ruminants), but this seems not to be a critical point [21], certainly not in studies involving long-term changes in exposure and metabolism [22].

2. Materials and methods

2.1. Animals and experimental procedure

This study involved a total of 251 ewes. In a first trial, 150 samples from ewes (*Ovis aries*) of three different breeds (84 Manchega, 35 Rubia del Molar, and 31 Negra Colmenareña), diverse age (2 to 7 yr), and different reproductive status (15 pregnant and 135 nonpregnant ewes) were analyzed. The second experiment arose from the results of the first trial for confirming the effect of pregnancy on DEHP levels and included 101 ewes, born and reared at a different farm, of the same breed (Manchega × Assaf) and age (4 to 5 yr) but different reproductive status; 32 were pregnant, 37 were nonpregnant, and 32 were ewes that had recently given birth less than a month before sampling.

2.2. Sampling

In all the animals, jugular blood samples (5 mL) were obtained with heparinized vacuum blood evacuation tubes (Vacutainer Systems Europe, Becton Dickinson, Meylan Cedex, France). Blood samples were immediately centrifuged at 2000 × *g* for 15 min, and the plasma was stored at –20 °C until assayed for DEHP.

2.3. DEHP analysis

2.3.1. Chemicals

DEHP, acetonitrile, and tetrahydrofuran were of HPLC grade and obtained from Sigma-Aldrich (Madrid, Spain). Other reagents were obtained from commercial sources and were of analytical grade. DEHP standards were prepared by dissolving the materials in acetonitrile and storage at 4 °C.

2.3.2. Sample Preparation

Plasma sample (1 mL) was placed in a glass test tube and mixed with 1 mL NaOH (1 M) solution, 500 μL acetonitrile, and 2 mL *n*-hexane. The mixture was vigorously vortex-mixed for 2 min and centrifuged at 2500 × *g* for 5 min. The upper phase was transferred to another glass test tube, and the lower phase was extracted again with 2 mL *n*-hexane. The upper organic phase was added to the first one and dried under nitrogen atmosphere at 40 °C. The dried extracts were reconstituted in 250 μL acetonitrile, vortexed, mixed for 20 sec, and transferred to autosampler vials for HPLC analysis.

2.3.3. Chromatographic conditions

Measurement of the DEHP concentrations in plasma samples was performed by a reversed-phase HPLC-UV method modified from the method of Kambia et al. [23]. The HPLC system consisted of Spectra-physic Series (Thermo Scientific, Essex, UK) components including a pump (P100), an autosampler (AS1000), and a variable UV wavelength detector (UV100) set at 210 nm. The detector signals were recorded with a Spectra-physic