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Transplacental Transfer of Monomethyl Phthalate and Mono(2-ethylhexyl) Phthalate in a Human Placenta Perfusion System

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The transplacental passage of monomethylphtalate (mMP) and mono (2-ethylhexyl) phthalate (mEHP) was studied using an ex vivo placental perfusion model with simultaneous perfusion of fetal and maternal circulation in a single cotyledon. Umbilical cord blood and placental tissue collected both before and after perfusion were also analyzed. Placentas were obtained immediately after elective cesarean section and dually perfused in a recirculation system. mMP or mEHP was added to maternal perfusion medium to obtain concentrations at 10 and 25 μg/L, respectively. The placental transfer was followed analyzing samples from fetal and maternal perfusion media by liquid chromatography–mass spectrometry–mass spectrometry (LC-MS-MS). Four perfusions with mMP indicated a slow transplacental transfer, with a feto-maternal ratio (FM ratio) of 0.30 $\pm$ 0.03 after 150 min of perfusion. Four perfusions with mEHP indicated a very slow or nonexisting placental transfer. mEHP was only detected in fetal perfusion media from two perfusions, giving rise to FM ratios of 0.088 and 0.20 after 150 min of perfusion. Detectable levels of mMP, mEHP, monoethylphthalate (mEP), and monobutylphthalate were found in tissue. Higher tissue levels of mMP after perfusions with mMP compared to perfusions with mEHP suggest an accumulation of mMP during perfusion. No tendency for accumulation of mEHP was observed during perfusions with mEHP compared to perfusions with mMP. Detectable levels of mEHP and mEP were found in umbilical cord plasma samples. mMP and possibly other short-chained phthalate monoesters in maternal blood can cross the placenta by slow transfer, whereas the results indicate no placental transfer of mEHP. Further studies are recommended.

Keywords Exposure, Fetal, mEHP, mMP, Perfusion, Placenta

Some phthalates and their metabolites are reproductive and fetal developmental toxicants in animals (Ema and Miyawaki 2001; Gray et al. 2000; Jarfelt et al. 2005; Saillenfait et al. 1998; Tomita et al. 1986). However, the adverse effects of phthalates in humans are still not fully documented (Lottrup et al. 2006). In several studies, phthalates, phthalate monoesters, and oxidized metabolites are used as markers of internal exposure of phthalates (Barr et al. 2003; Kato et al. 2004; Koch et al. 2005; Preuss, Koch, and Angerer 2005). The distribution of phthalates in humans has provided knowledge about the internal exposure and metabolic pathways involved in the excretion of phthalates (Table 1). Phthalates are also found in biological samples from pregnant women, women in the childbearing age, and in the fetus (Adibi et al. 2003; Lashley et al. 2006; Latini et al. 2003a; Mose and Knudsen 2006; Silva et al. 2004a, 2004b), indicating fetal exposure. Recently, many studies in humans have focused on associations between elevated environmental exposures of phthalates and a symptom or a disease, e.g., in utero exposure and duration of pregnancy or prenatal exposure and decreased anogenital distance (Cobellis et al. 2003; Colon et al. 2000; Duty et al. 2004; Latini et al. 2003b; Main et al. 2005; Swan et al. 2005).

Diphthalates are widely used in consumer products, e.g., polyvinylchloride (PVC) products, synthetic leather, and cosmetics from where they eventually leach into the surrounding environment leading to daily exposures. Diphthalates are rapidly

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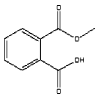
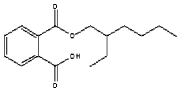
TABLE 1
Chemical properties of monomethyl phthalate and mono-2-ethyl phthalate and their levels in human biological samples

	Monomethyl phthalate (mMP)	Reference	Mono-2-ethylhexyl phthalate (mEHP)	Reference
Structure		SCIfinder National Center for Environmental Health and Department of Health and Human Services 2005		SCIfinder National Center for Environmental Health and Department of Health and Human Services 2005
Molecular formula	$C_9H_8O_4$		$C_{16}H_{22}O_4$	
Molecular weight (g/mol)	180.16		278.34	
Log *P* (oc/w)	1.130 ± 0.249		4.665 ± 0.253	
pKa	3.323 ± 0.20		3.37 ± 0.20	
Molar solubility (pH 7)	Very soluble		Sligthly soluble	
CAS number	4376-18-5		4376-20-9	
Serum level	Not found		5.4 μg/L	Silva et al. 2003
Umbilical cord blood plasma level	<LOD (0.05 μg/L)	Mose et al. 2006	0.52 mg/L	Latini et al. 2003b
			0.68 mg/L	Latini et al. 2003a
			6.4 μg/L	Mose et al. 2006
Urinary level	4.8 μg/L (gravity adjustment)	Duty et al. 2005	6.3 μg/L (gravity adjustment)	Duty et al. 2005
			5.2 μg/L	Duty et al. 2003
			9.8 μg/L	Preuss et al. 2005
	4.9 μg/L	Duty et al. 2003	4.27 μg/L	National Center for Environmental Health and Department of Health and Human Services 2005
	1.15 μg/L	National Center for Environmental Health and Department of Health and Human Services 2005		
			3.2 μg/L	Silva et al. 2004a
			3.8 μg/L	Silva et al. 2003
			7.18 μg/L in children	Becker et al. 2004
Level in breast milk	0.1 μg/L Denmark 0.09 μg/L Finland	Main et al. 2005	9.5 μg/L Denmark 13 μg/L Finland	Main et al. 2005
Level in amniotic fluid	<LOD (not given)	Silva et al. 2004b	<LOD (0.86 μg/L)	Silva et al. 2004b
Level in salvia	<LOD (0.2 μg/L)	Silva et al. 2005	<LOD (1.0 μg/L)	Silva et al. 2005
Level in placenta	<LOD (0.05 μg/kg)	Mose et al. 2006	13.1 μg/kg	Mose et al. 2006

hydrolyzed to phthalate monoesters, increasing their water solubility and urinary excretion. Hydrophilic phthalate monoesters such as monomethyl phthalate (mMP)[1] and monoethyl phthalate (mEP) are primarily excreted as free monoesters (Silva et al. 2003). The metabolic pathway for mono (2-ethylhexyl) phthalate (mEHP) is much more complex because phase I enzymes are involved in generating many different metabolites. Besides oxidation of the 5th carbon in the monoester chain, hydroxylation can appear in the 6th carbon or in the ethyl side chain, resulting in a variety of hydroxy-phthalates and carboxy-phthalates (Koch et al. 2005; Koch, Preuss, and Angerer 2006).

In this study we only had analytical tools to detect mono(2-ethyl-5-oxohexyl) phthalate (mEOHP), and mono(2-ethyl-5-hydroxy-n-hexyl) phthalate (mEHHP), leaving out the dominant carboxy-metabolites present in serum after di(2-ethylhexyl) phthalate (DEHP) administration (Koch et al. 2005). According to Koch et al. the major metabolite in serum is mEPP (mono[2-ethyl-5-carboxypentyl]phthalate), with a concentration six times

[1]*Abbreviations:* mMP (monomethyl phthalate); mEHP (mono[2-ethylhexyl] phthalate); mEP (monoethyl phthalate); mBP (mono-n-butyl phthalate); mEOHP (mono-[2-ethyl-5-oxohexyl] phthalate); mEHHP mono[2-ethyl-5-hydroxy-n-hexyl] phthalate; DEHP (di [2-ethylhexyl] phthalate); DMP (dimethyl phthalate); DEP (diethyl phthalate); DBP (di-n-butyl phthalate); ACN (acetonitrile).

higher than the level of mEHHP and mMMP (mono[2-carboxymethyl-hexyl] phthalate) 150 min after oral administration of DEHP.

Phase I enzymes are sparingly expressed in human placenta compared to the liver and therefore substrate specificity is very restricted, which limits oxidation and hydroxylation in the perfusion system (Hakkola et al. 1996, 1998; Pasanen 1999; Pasanen and Pelkonen 1994). mEHP or the aforementioned metabolites (mEHHP, mEOHP, and mMMP) undergo phase II metabolism to enhance hydrophilicity and urinary excretion. Silva et al. found that 84% of mEHP in serum was present in the glucuronidated form (Silva et al. 2003). Human placenta contains few forms of phase II enzymes. Uridine 5′-diphosphoglucuronyl transferase (UGT), responsible for glucuronidation is present in the placenta and is shown to be functioning during ex vivo placental perfusion (Schenker et al. 1999; Collier et al. 2004).

As most concerns regard the risks of exposure to the developing fetus, the objective of this study was to use our placental perfusion system to study the placental transfer of selected phthalate monoesters: mMP and mEHP.

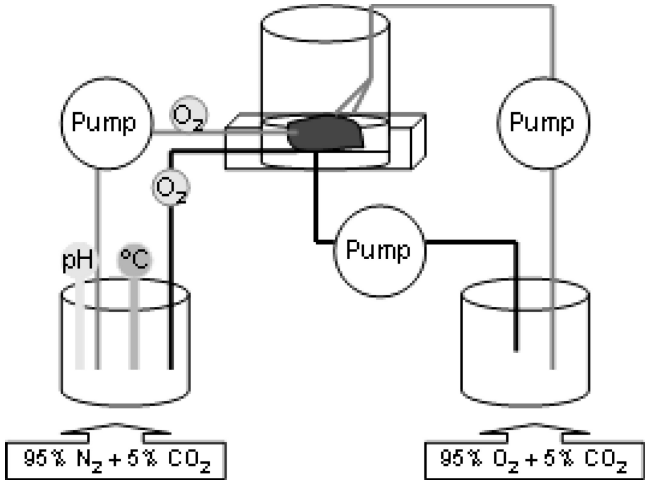


FIGURE 1
A schematic presentation of the double-recirculating placenta perfusion system. Maternal and fetal reservoirs contain 200 ml Krebs–Ringer buffer with added heparin (25,000 IE/ml) and dextran (fetal 30 g/L, maternal 8.5 g/L). Grey lines represent arteries and black lines represent veins.

MATERIALS AND METHODS

The study was approved by the Ethics Committee in Copenhagen and Frederiksberg (j.nr KF 01-145/03 + KF(11) 260063). Fourteen placentas were perfused but only eight perfusions were successful according to our criteria of minimum leak, etc. Placentas were obtained from uncomplicated pregnancies immediately after elective cesarean sections performed at the University Hospital of Copenhagen, Rigshospitalet, Denmark. Of the eight successful perfusions, all mothers were nonsmokers and only one had taken prescribed medicine, lithium.

The perfusion system is described thoroughly in our previous publications (Mose and Knudsen 2006; Mose et al. 2006). Briefly, the placenta was weighed and umbilical cord blood collected (10 ml). Krebs-Ringer buffer with heparin (25,000 IE/L) and glucose (9.0 mM) was injected before transfer to the laboratory. The placenta was inspected for ruptures and the fetal circulation was reestablished by cannulation of an appropriate fetal artery-vein pair, supplying a well-defined and intact cotyledon. The cotyledon was isolated and placed in perfusion chamber and connected to the pump. Two tubes constituting the maternal arteries were gently inserted into the cotyledon (Figure 1). Fetal and maternal flow were 3.5 and 12 ml/min, respectively. The maternal and fetal perfusion media consisted of 200 ml Krebs-Ringer buffer (as above), including 8.5 and 30 g/L dextran, respectively, and were constantly gassed with 95% O_2–5% CO_2 and 95% N_2–5% CO_2. The cotyledon was preperfused approximately 30 min to gain sufficient gas concentration in reservoirs, to restore the tissue with adequate oxygen, and to ensure a stable venous outflow from the fetal circulation. Antipyrine (1000 μl of 20,000 μg/ml, 98%; Aldrich-Chemcie, Germany) and mMP (500 μl of 4000 μg/l in acetonitrile [ACN]; Cambridge Isotope Laboratories, USA) or mEHP (500 μl of 10,000 μg/l in can; Cambridge Isotope Laboratories) were added to the maternal side to obtain initial concentrations of 100 μg/mL, 10 μg/L, and 25 μg/L of antipyrine, mMP, and mEHP, respectively. Antipyrine was used as a positive-reference compound for the connection between maternal and fetal circulation.

Samples (1.5 ml) were collected from the maternal and fetal perfusion medium at 0, 15, 30, 60, 90, 120, and 150 min after addition of test compound and antipyrine. In addition, a sample was collected just before connection to the tissue and just before addition of test compound and antipyrine. The volume collected was replaced with perfusion buffer. The proteins were precipitated by centrifugation at 4000 $\times$ g in 5 min. Supernatant (1.0 ml) was added 1.2 M H_3PO_4 (100 μl) before storage (-20°C). Excess supernatant was stored at -20°C until analysis of antipyrine. The umbilical cord blood was processed like the samples. Tissue samples before and after perfusion were cut into small pieces, homogenized, and stored at -20°C.

To limit contamination of phthalates liberated from laboratory equipment, all utensils were made of glass and were flushed with methanol (MeOH) before use. Tubes and dispenser tips were made of polypropylene, all tubing was PVC-free, and either latex or nitril gloves were used.

Eight perfusions succeeded according to our criteria: constant venous outflow (3.5 ml/min), leakage from fetal circulation $<$20 ml/180 min (preperfusion + perfusion), FM ratio of antipyrine diffusion $>$0.75, pH 7.2 to 7.5 in fetal reservoir, time from birth to laboratory $<$30 min, uptake of oxygen in the placenta, and temperature 35°C to 39°C. The eight reported perfusions consisted of four with mMP and four with mEHP, respectively.

Analysis of Antipyrine

Supernatant (200 μL) was mixed with 0.5 M H_3PO_4 (20 μl) containing 10 ng/ml phenacetin (97%; Acros Organics, Belgium) as internal standard. Antipyrine and phenacetin were analyzed using a reverse phase LaChrom HPLC system (Merck, Hitachi). The stationary phase was a C18 column (Nucleosil, ODS; 20 × 4.6 mm, 5-μm particles) with a Security Guard precolumn (Phenomexes, ODS; 4 × 3 mm ID). Mobil phase was a degassed methanol and water (45/55 v/v) solution adjusted to a flow rate of 1 ml/min. Injections volume was 25 μl, oven temperature 30°C to 32°C and detection absorbance 254 nm.

Analysis of Phthalate Monoesters

The phthalate monoesters were analyzed by a liquid chromatography–mass spectrometry–mass spectrometry (LC-MS-MS) method developed to measure phthalate monoesters in breast milk (Mortensen et al. 2005). This method consisted of a liquid extraction and two solid-phase extractions. The same method was used to measure phthalate monoesters in placenta samples as described previously (Mose and Knudsen 2006). Perfusion samples and plasma samples (1.1 ml) were analyzed without the liquid extraction but with the same two solid-phase extractions.

Internal standards (75 μl of 0.1 to 1.0 μg/ml C^{13}-labeled monoesters) were added to all samples. For recovery experiments, native standards (60 or 120 μl, 0.2 to 0.6 μg/ml) were added to the sample. The linearity of the compounds was tested in the range from 0.5 to 1000 μg/L. For calibration curves the concentrations were selected in order to include the concentration level of the actual compound in the final extract. In general, the stability and response were stable and the calibration curves were linear with a correlation coefficient (r^2) >.995. Calibration standards were measured before and after sample were taken, and all measurements were used for the quantitation. Internal standards were used for the actual compounds and the relative ratio determined and used in the calculation. Recoveries in duplicate ranging from 6 to 18 μg/L were included using both maternal and fetal buffers for each experiment together with with blank samples. For placenta samples, duplicate recoveries ranging from 4 to 12 μg/kg were included. Validation parameters for selected phthalate monoesters measured in the perfusate, plasma from umbilical cord blood, and placenta tissue are shown in Table 2.

TABLE 2
Analytical detection limits and recoveries for phthalate monoester detection

	Detection limits		Recoveries	
Monoesters	Perfusate (μg/L)	Placenta (μg/kg)	Perfusate (%)	Placenta (%)
mMP	0.05	0.05	109 ± 7.9	106 ± 7.1
mEHHP	0.5	0.05	94 ± 1.5	92 ± 7.1
mEHP	0.5	0.5	104 ± 10.5	98.8 ± 10.4
mEOHP	0.5	0.5	93 ± 3.3	98 ± 5.4

RESULTS

Placental Transfer of Antipyrine

Antipyrine (100 μg/ml) was added to the maternal perfusion medium together with mMP (10 μg/L) or mEHP (25 μg/L) as a positive control for proper connection between the established maternal and fetal circulation. After 150 min of perfusion, the mean fetal/maternal concentration ratio (FM ratio) was 0.85 ± 0.07 (Figure 2). The mean antipyrine transfers were comparable between perfusions with mMP and mEHP. The antipyrine kinetics was similar in all perfusions but the FM ratio was lower in two perfusions with mEHP (data not shown).

Placental Transfer of mMP

Similarly to antipyrine, mMP was detectable in fetal perfusion medium from all four perfusions, although a slower transfer rate was observed. The mean FM ratio after 150 min of perfusion was 0.30 ± 0.03 (Figure 2), with mean maternal and fetal values of 6.4 ± 0.41 and 1.7 ± 0.26 μg/L, respectively. Except for one perfusion, mMP was detected in fetal perfusion medium within 5 min. This perfusion had undetectable levels (limit of detection [LOD] = 0.05 μg/L) of mMP in fetal perfusion medium during the first 15 min and had a slightly lower FM ratio of mMP transfer, but an unaffected FM ratio of antipyrine diffusion. The mean recovery of mMP in maternal and fetal perfusion media together after completion of the perfusion was 8.1 ± 0.37 μg/L: 80.9% ± 3.7% of the 10 μg/L initially added to the maternal perfusion medium (n = 4).

Placental Transfer of mEHP

Contrary to mMP, mEHP was not detectable (LOD = 0.5 μg/L) in two of four fetal perfusion media. Therefore, the mean maternal and fetal concentrations instead of the FM ratio are illustrated in Figure 3. In two perfusions, 1.8 and 3.0 μg/L mEHP were found in fetal perfusion medium after completion of the perfusion, giving rise to FM ratios of 0.088 and 0.20, respectively. In the two perfusions where mEHP was detectable, antipyrine had a faster transfer rate and the leakage from fetal perfusion medium was additionally higher, indicating a better connection between maternal and fetal circulation, together with a more permeable or ruptured membrane. The mean recovery of mEHP in maternal and fetal perfusion media together after completion of the perfusion was 18.7 ± 3.4 μg/L: 74.7% ± 13.7 % of the 25 μg/L initially added to the maternal perfusion medium (n = 4).

Umbilical Cord Plasma

Umbilical cord blood was collected before perfusion from seven of the eight placentae. A mean level of 9.4 ± 4.8 μg/L

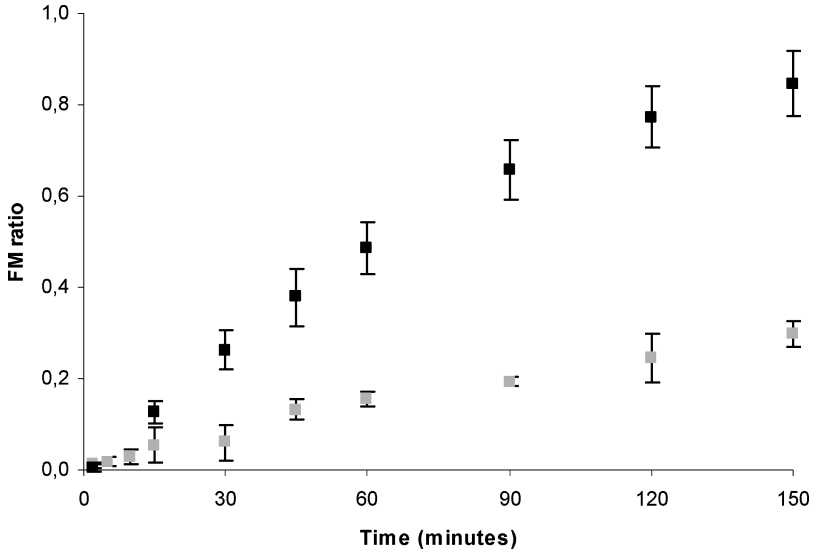


FIGURE 2
The mean fetomaternal concentration ratios (FM ratios) of antipyrine (*black*, $n = 8$) and mMP (*grey*, $n = 4$) during perfusions lasting 150 min. When the ratio is 1, equal amounts of compound are in fetal and maternal circulation.

mEHP ($n = 7$) and 4.6 $\pm$ 5.3 μg/L mEP ($n = 6$) was found, whereas mEHHP and mEOHP were detectable in the plasma sample with the highest level of mEHP (18.4 μg/L). mMP, mBP, mBzP, and mNP were undetectable in all plasma samples.

Placental Tissue

mEP, mBP, and mEHP were found in all placenta samples before perfusions. The mean levels were 4.02 ± 3.09, 12.89 ± 4.17, and 8.48 ± 4.33 μg/kg, respectively. mBzP was detected in five placentas, mean level 0.943 ± 0.629 μg/kg. The levels of mMP and mEHP before perfusion and after perfusion are shown in Figure 4. In addition, accumulated levels of mMP and mEHP after perfusions with either compound are illustrated. mMP was only detected in one placenta sample before perfusion and in one sample after perfusion with mEHP. The level of mMP increased by nearly 10-fold in the perfusions where mMP was added, showing an accumulation of mMP in placental tissue. The total accumulated level in tissue represents 42% of the added mMP (10 μg/L). No accumulation of mMP was observed in the experiments with mEHP. After completion of the perfusion, the levels of mBP and mEHP were increased from 12.9 to 44.3 μg/kg and

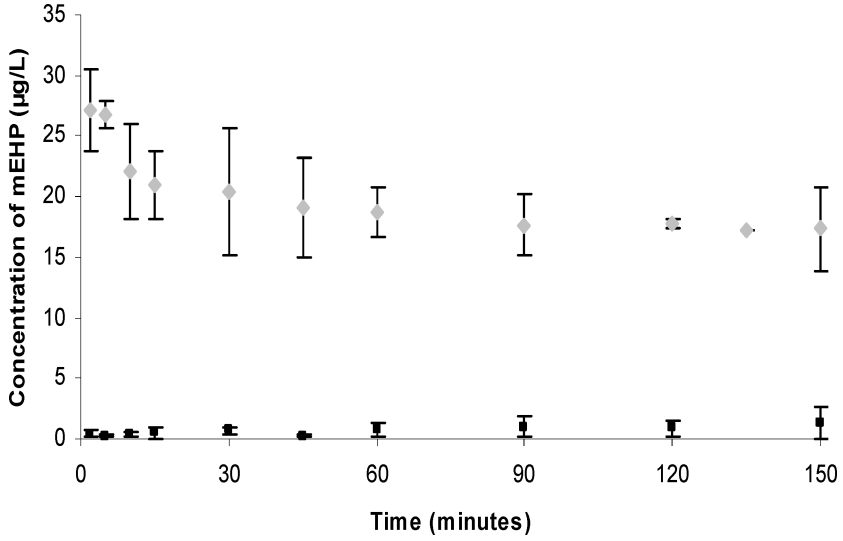


FIGURE 3
The mean concentrations of mEHP from maternal circulation *(grey)* and fetal circulation *(black)* during 150 min of human placental perfusion. The initial concentration in the maternal perfusion medium is 25 μg/L.

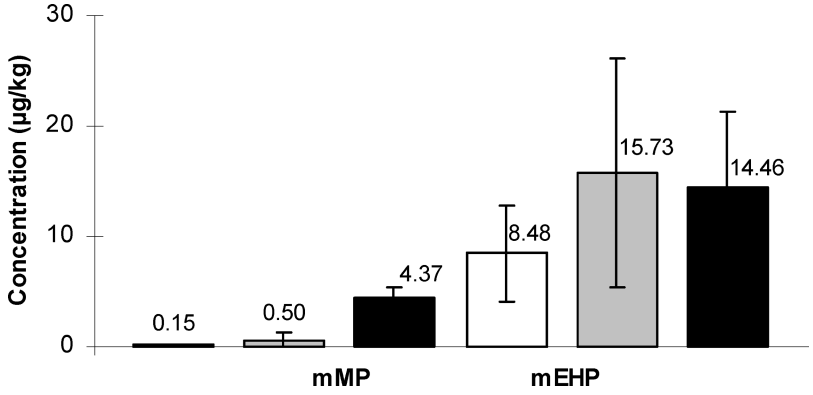


FIGURE 4

The levels (μg/kg) of mMP and mEHP in placental tissue. Tissue samples from before perfusion (*semiwhite*, $n = 8$) and perfused cotyledon (*grey* and *black*) are illustrated. The grey columns are with perfusion but without actual phthalate monoester ($n = 4$). The black columns represent the level in perfused cotyledon after perfusion with phthalate monoester ($n = 4$). mMP is only detectable (LOD = 0.05 μg/kg) in one sample before and one sample after perfusion without mMP. The mean is calculated from LOD/2.

8.5 to 15.3 μg/kg, respectively, even though these compounds were not added to the system. The accumulated level of mEHP was approximately 15 μg/kg. There were no differences between mEHP accumulation in the mEHP and mMP perfusion studies, suggesting that the tissue was saturated with mEHP. mEHHP, mNP, and mEOHP were not detected in any of the placenta tissue samples. No association was found between levels of mEP, mBP, mEHP, and mBzP in placenta tissue and umbilical cord plasma.

Affinity and Exchange of mMP and mEHP to the Perfusion System

The affinity of mMP (10 μg/L) and mEHP (25 μg/L) to the perfusion system was tested using the maternal circulation through an empty perfusion chamber. The affinity was insignificant because the concentration was only slightly increasing for mEHP ($y = 0.0048x + 22.1$) and slightly decreasing for mMP ($y = -0.0063x + 8.2$) during the 150-min perfusion test (Figure 5).

Background Levels of Nonspiked Phthalate Monoesters in Perfusion Media

mEP and mBP were detectable in maternal and fetal perfusion media after perfusion without these phthalate monoesters added to the system. The highest concentrations were found in maternal perfusion media. mEHP and mBzP were only detectable in two of the four mMP-spiked studies, whereas mMP was undetectable in the mEHP-spiked studies. mNP, mEHHP, and mEOHP were undetectable in all perfusion samples. No association was found between the levels of mEP, mBP, mEHP, and mBzP in perfusion media and umbilical cord plasma, or perfusion media and placental tissue, which supports earlier findings (Mose et al. 2006).

DISCUSSION

Human placental transfer depends on the functionality of the placenta, uterine and umbilical blood flow, transporters located in the membrane, chemical gradients across the membrane, and the physiochemical properties of the compound (Audus 1999; Pacifici and Nottoli 1995). Physiochemical properties such as molecular weight, lipophilicity [log P(oc/w)], degree of ionization (pKa), and protein binding can affect the rate of placental transfer (Audus 1999). In general, neutral compounds with a molecular mass below 1000 g/mol can freely diffuse across placenta. Both mMP and mEHP have molecular masses below 300 g/mol and are nearly 100% ionized at physiological pH (Table 1). mEHP is more lipophilic than mMP, which should have facilitated the placental transfer rate and/or the accumulation in the tissue. Differences in molecular weight, lipophilicity, and metabolic pathways were determining factors in selection

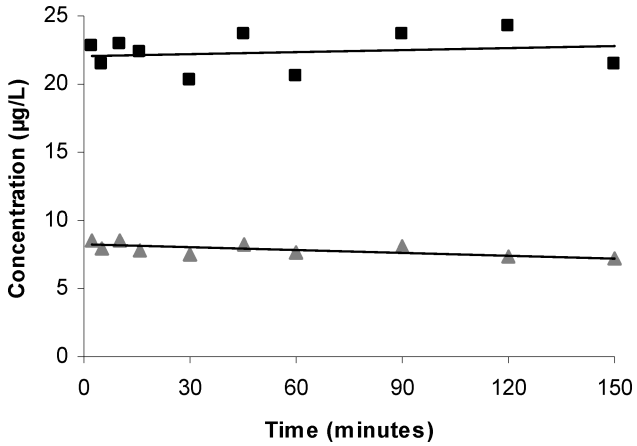


FIGURE 5

Time-dependent exchange of mMP *(grey triangles)* and mEHP *(black squares)* with an empty perfusion system during 150 min of circulation in a maternal system connected to a perfusion chamber. The initial concentration in a maternal perfusion medium is 25 μg/L mEHP and 10 μg/L mMP.

of mMP and mEHP instead of other phthalate monoesters. Another important aspect in the decision was the low or steady background level of mMP and mEHP present after 150 min of perfusion with antipyrine (Mose et al. 2006). In this study, however, mMP was intended to represent the short-chained phthalate monoesters whereas mEHP would represent the more lipophilic long-chained monoesters.

Fetal exposure to mMP was minimal because either undetectable or very low levels were found in plasma isolated from umbilical cord blood. Our results may be utilized to predict placental transfer of mEP because mMP and mEP are chemically very similar. mEP is additionally found in human serum (Silva et al. 2003) and umbilical cord plasma, probably because diethyl phthalate (DEP) is more extensively used compared to dimethyl phthalate (DMP).

mEHP disappears from maternal circulation but is not transferred to fetal circulation or accumulated in tissue. Metabolism and protein binding or attachment to the perfusion system can reduce the concentration of mEHP during the perfusion. Glucuronidation in the placenta during perfusion can reduce the amount of free mEHP and the degree of placental transfer because the molecule enlarges. However, placental transfer can proceed even when a molecule is glucuronidated (Schenker et al. 1999). Besides metabolism, protein binding may reduce the percentage of available free compound in a physiological solution. Plasma protein carriers such as albumin are sometimes necessary to facilitate placental transfer (Kihlstrom 1983). Protein binding probably was insignificant in our perfusion medium due to lack of protein carriers. mMP and mEHP did not attach to the perfusion equipment (Figure 5), eliminating this as a possible route of removal or contamination of compounds.

Our hypothesis was that mMP and mEHP would cross the placenta (1) because mEHP was found in umbilical cord blood and (2) because of the low molecular mass and relatively high lipophilicity of mMP and mEHP. We assumed mMP was undetectable in umbilical cord blood because of low exposures and rapid metabolism in vivo. Acetylsalicylic acid is chemically similar to mMP, with the same molecular formula ($C_9H_8O_4$), log P (oc/w) 1.19, pKa 3.49, and a transfer at steady state of 15.4% in fetal circulation (Jacobson et al. 1991). The loss of acetylsalicylic acid from maternal circulation exceeded the accumulation in fetal circulation by 75% and the authors presumed an accumulation in tissue and/or metabolism. According to Pacific and Nottoli, strongly dissociated acid molecules will have an incomplete transfer (Pacifici and Nottoli 1995). At physiological pH, the mono phthalates are fully dissociated, which most likely is associated with the slow or no placental transfer observed in our study. However, the transfer of anions in vivo is enhanced by the pH gradient between fetal and maternal circulation. Fetal blood is more acidic, making trapping of anions possible. We only monitored pH in the fetal compartment, but a gradient is most likely because pH usually is lower in fetal compartments due to saturation with N_2.

The incomplete transfer of anions may involve anion transporters located at the maternal-fetal interface (Ganapathy and Prasad 2005; St Pierre et al. 2002; Young, Allen, and Audus 2003). Several anion transporters are expressed in the syncytiotrophoblast at the brush border membrane closest to the maternal circulation and at the basal membrane closest to the fetal circulation. Organic anion transporters like OAT-4 and OATP-B are located in the basal syncytiotrophoblast and cytotrophoblast, and facilitate transfer of molecules such as steroid metabolites. In contrast, the multidrug resistance-associated proteins MRP1, MRP2, and MRP3 are located in the brush border membrane (St Pierre et al. 2000). These transporters are ATP-dependent efflux pumps responsible for the removal of molecules such as bilirubin from the fetal circulation (Ganapathy and Prasad 2005). Most of these transporters can function both as efflux or influx transporters into the syncytiotrophoblast depending on the ionic gradient driving forces and concentration gradient of the anion

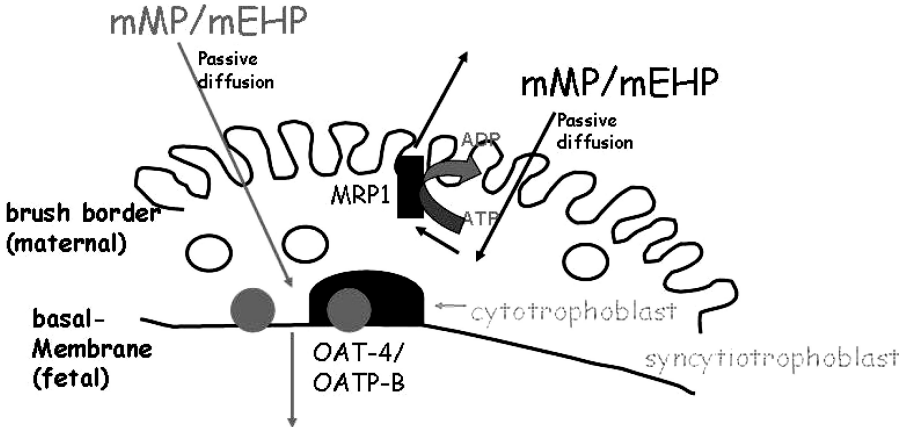


FIGURE 6

Schematic presentation of selected transporter systems located in the human placenta that may be involved in the transfer of anions. Päivi Myllynen and Kirsi Vähäkangas originally made the drawing. Multidrug resistance–associated proteins (MRP1–3) are located in the brush border membrane closest to maternal circulation and organic anion transporters (OAT-4 and OATP-B) located in cytotrophoblast and syncytiotrophoblast in the basal membrane closest to fetal circulation. MRP transfer is active and requires adenosine triphosphate (ATP).

(Ganapathy et al. 2000). If ionized mMP and/or mEHP are substrates for these transporters, they can enter the placenta and fetal circulation by diffusion or via influx transporters (OATs) as shown in Figure 6. However, activated efflux transporters (MRP1) can transfer ionized mMP and/or mEHP from the syncytiotrophoblast at the brush border membrane back to the maternal circulation. Perfusion studies with depletion of ATP or inhibition of MRP1 can clarify whether mMP and/or mEHP are substrates, possibly in the presence and absence of OATs enhancers. Depletion of ATP or inhibition of MRP1 would possibly inhibit the removal of anions from the placenta back to maternal circulation thereby increasing the amount in fetal circulation.

After perfusion, the perfused area contained considerably higher amounts of mBP and mEHP than the tissue sample collected before perfusion. This increase in compound during perfusion without added compound was also observed for mBP in our previous study (Mose et al. 2006). Samples collected from the isolated tissue surrounding the perfused cotyledon showed the same elevated levels (data not shown), ruling out diffusion of mEHP from an unperfused to a perfused area during perfusion as a possible reason. Hydrolysis of residing DEHP and DBP may explain the higher levels after end perfusion. DEHP is found in high amounts in the human placenta (Poole and Wibberley 1977), supporting this explanation.

Lashley et al. found mEHP in higher levels in umbilical cord serum compared to maternal serum levels but concentration levels are not provided (Lashley et al. 2006). mEHP is in the fetal compartment, but how does it get there?

CONCLUSION

Our data show a slow transfer of mMP across the placenta, no detectable levels in umbilical cord blood, and very low levels in placental tissue. However, mMP is accumulated in placental tissue during perfusions with mMP. Recovery of mMP in perfusion media alone was 81%.

For mEHP, our data suggest no placental transfer because the levels found in fetal perfusion medium was close to LOD. Detectable levels were found in umbilical cord blood, and in placental tissue. Recovery of mEHP in perfusion media alone was around 75%. The low recovery was not due to accumulation in placental tissue, metabolism to mEHHP and mEOHP, or adherence to the perfusion system.

Further investigations on transplacental transfer of phthalates are needed. Some phthalates and/or markers of internal phthalate exposure are present in maternal and fetal blood. It is essential to study the transplacental kinetics of these compounds because information on the distribution among the fetal, the maternal, and the placental compartments is necessary to assess fetal risks associated with a maternal exposure to phthalates.

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