



## Transplacental transfer of 2-naphthol in human placenta



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### ABSTRACT

**Objective:** To determine the transfer of 2-naphthol (2-NPH) in fullterm human placental tissues.

**Methods:** Six placentas were studied. The ex-vivo dual closed-loop human placental cotyledon perfusion model was used. 2-NPH was added to the perfusate in the maternal compartment. Samples were obtained from the maternal and fetal up to 360 min measuring.

**Results:** The mean fetal weight was  $2880 \pm 304.2$  g. Mean perfused cotyledon weight was  $26.3 (\pm 5.5)$  g. All unperfused placental tissue samples contained NPH with a mean level of  $7.98 (\pm 1.73) \mu\text{g/g}$  compared to a mean of  $15.58 (\pm 4.53) \mu\text{g/g}$  after 360 min perfusion. A rapid drop in maternal 2-NPH concentration was observed; from  $5.54 \mu\text{g/g}$  in the first 15 min and  $13.8 \mu\text{g/g}$  in 360 min. The fetal side increased from  $0.65 \mu\text{g/g}$  in the initial 15 min to  $1.5 \mu\text{g/g}$  in 360 min. The transfer rate of NPH was much lower than that of antipyrine.

**Conclusion:** 2-NPH has the ability to rapidly across the placenta from the maternal to the fetal compartment within 15 min. The placenta seems to play a role in limiting the passage of 2-NPH in the fetal compartment.

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### 1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a large group of chemicals that are widely found in our environment and considered as potent atmospheric pollutants as many of their compounds have been identified as carcinogenic, mutagenic, and teratogenic [14,16,17]. They are released into the environment as a result of incomplete combustion of organic materials and carbon-containing fuels such as wood, coal, and diesel [37].

Naphthalene (NAP), a volatile polycyclic considered as one of the simplest PAH chemicals, detected at large quantities indoors and outdoors due to solvent-related emissions, renovations, household products, and pesticides [6–26]. The initial metabolites are epoxides which are cyclic ether with three ring atoms. They are relatively unstable and undergo spontaneous rearrangement into 1-naphthol or 2-naphthol (1 and 2-NPH), which conjugate to either glucouronides or sulphates and are excreted in the urine [7].

Animal studies have shown that NAP is carcinogenic and embryotoxic [33]. The fetus is especially susceptible to the effects of toxic

agents because of its immature and underdeveloped defense mechanisms. Hence, exposure during pregnancy can have a negative effect on fetal growth and development and even increase the susceptibility to diseases later in life. In-vitro experiments on human cord blood cells suggest that NAP, in particular its metabolites, modifies cord blood gene expression. The result is down-regulation of genes involved in the differentiation of immunocompetent cells, and overexpression of oncogenes in cell proliferation and cell cycle progression, ultimately stimulating cell proliferation [9]. NAP itself does not seem to affect the fetus. Once absorbed, is rapidly distributed throughout the body with little accumulation in any tissue [21]. However, its metabolites (NPH) are toxic. Therefore, NAP is not considered to be cytotoxic or genotoxic without metabolic activation [7,3]. The exact amount of metabolites that can cross the placental barrier has not been determined. The metabolizing enzymes expressed in the placenta and the wide variety of transporters might have significant consequences on the transport of xenobiotics and their effect on the fetus [34]. The placental barrier can limit the delivery of toxic compounds protecting the fetus against their harmful effects [23]. Several mechanisms are suggested, including uptake, storage, and reflux [11].

In this study, we aim to determine the transfer of 2-NPH in full-term human placental tissues. The outcome of this study might

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help in the development of maternal environmental health related guidelines

## 2. Materials & methods

This study was approved by the Al Ain Medical District Human Research Ethics Committee (Al Ain Medical District Human Research Ethics Committee (AAMDHREC) – Protocol No. 11/58 – Naphthalene transfer across the human placenta: A human placenta perfusion study). Written consents were obtained from each pregnant woman.

### 2.1. Placental perfusion

Six placentas were obtained from women undergoing elective Caesarean section or vaginal delivery at term.

The placentas were transported to the laboratory within 30 min. We adopt methods described by Schneider et al. [15,25]. In summary, a suitable cotyledon was selected and the fetal vessels were catheterized with a French catheter. The perfusate which has been used composed of a synthetic tissue culture medium M199 Medium Sigma, circulate through the fetal side at a flow rate of 4 ml/min delivered by a digitally controlled pump at a pressure not higher than 60 mmHg. For the delivery of maternal perfusate, three catheters were directly introduced into the intervillous space at a depth of 0.5–1.0 cm. Perfusion of the intervillous space was started at a flow rate of 12 ml/min. Maternal perfusate was equilibrated with 95% O<sub>2</sub> and 5% CO<sub>2</sub> and fetal perfusate was equilibrated with 95% N and 5% CO<sub>2</sub> after 20 min both circuits were closed. The perfusate in the maternal side was replaced by fresh perfusate contains 2-NPH Sigma–Aldrich and antipyrine at 0.05 g/l. The concentration of 2-NPH used for perfusion is based on the recommendation of the Human Biomonitoring Commission of the German Federal Environment Agency, where an upper margin of reference value of exposure for adult non- smokers to 2-NPH in urine should be less than 20 µg/l [35]. The perfusion was then continued for six hours. Samples were obtained from the maternal and fetal circulations at 15, 30, 60, 120, 240, and 360 min and stored at –80 °C. The glucose and lactate levels were measured using Rx Monza clinical analyzer, Randox, laboratories; UK and β-human chorionic gonadotropin (β-hCG) secretion was measured using DRG diagnostics kits, DRG Instrument GMBH, Germany 2-NPH transfer and antipyrine transfer were measured by HPLC.

### 2.2. Placental tissue extraction

Three samples from the placenta, weighing 1 g each, were taken from both unperfused and perfused cotyledon and stored at –20 °C until analysis. Tissues samples were homogenized (Ultra Turrax® IKA, Werke, Germany) for 1 min at 15,000 rpm on ice. With addition of 2 mL of ACN –20% acetonitrile: water. The homogenate were then centrifuged at 13,000 rpm and the supernatant were collected and stored in –20 °C for further analysis using HPLC [19].

### 2.3. HPLC analysis

All reagents used were HPLC grade Merck, Germany HPLC method as described [18]. was used for antipyrine The dried extract was dissolved in 200 µl of the mobile phase. Chromatographic separation were performed on HPLC system Waters Alliance, USA. For 2NH detection, a stock solution of concentration of 1 µg/ml, 100 µg/ml, and 1 mg/ml of 2NPH in acetonitrile HPLC grade were prepared and stored at –20 °C until the assay. For each run 20 µl was injected.

**Table 1**

shows glucose consumption, lactate production, HCG secretion as evidence of placenta viability and metabolic activity.

	Maternal	Fetal
Glucose consumption(µmol/g/min)	0.42 ± 0.04	0.35 ± 0.04
Lactate production (µmol/g/min)	0.49 ± 0.14	0.31 ± 0.06
HCG (mU/g/min)	40.8 ± 7.3	

### 2.4. Calculations

Statistical analysis was performed using the one-way or two-way ANOVA. Values of *p* less than 0.05 were taken as statistically significant. The transfer percentage from maternal to fetal circulation was calculated by the following formula [2]:

$100 \times Fc \times Fv / [(Fc \times Fv) + (Mc \times Mv)]$ , where Fc = fetal concentration, Mc = maternal concentration, Fv is fetal perfusate volume, and Mv = maternal perfusate volume.

FM ratio, calculated for the figures, is the ratio of fetal concentration to maternal concentration.

Transfer index was calculated by dividing the transfer percentage of 2-NPH with that of antipyrine. All values are expressed as a mean ± sd.

Glucose consumption was measured by the following formula:

Glucose consumption = glucose level of perfusate at 360 min – glucose level at initial time and normalized with time and tissue weight. Lactate production was measured using the same formula [8].

## 3. Results

A total of 6 term placentas were perfused. The mean pregnant women age was 27.6 ± 6.0 years, with a mean maternal weight at delivery of 83.1 ± 18.3 and mean fetal weight was 2880 ± 304.2 g. The mean weight of the perfused cotyledon was 26.3 ± 5.5 g. The perfusate pH ranged from 7.2 to 7.4, and the fluid shift between the fetal and maternal compartments was less than 3 ml/h. Evidence of placenta viability and metabolic activity throughout the perfusion was assured by the constant glucose and lactate production (Table 1). The glucose consumption is calculated according to the method described by Di Santo et al. [8]:

Glucose consumption = glucose level of perfusate at 360 mins – glucose level at initial time and normalized with time and tissue weight

All unperfused placental tissue samples contained NPH with a mean level of 7.98 ± 1.73 µg/g compared to a mean of 15.58 ± 4.53 µg/g after 360 min of perfusion.

The initial drop in the concentration of NPH in the maternal compartment was rapid. It dropped 5.54 µg/g in the first 15 min and 13.8 µg/g in 360 min. However, the increase of NPH concentration on the fetal side was much slower. The increase in the initial 15 min was as low as 0.65 µg/g and increased only to 1.5 µg/g in 360 min (Fig. 1).

The transfer rate of NPH was much lower than that of antipyrine. The highest transfer index was at 120 min (Table 2).

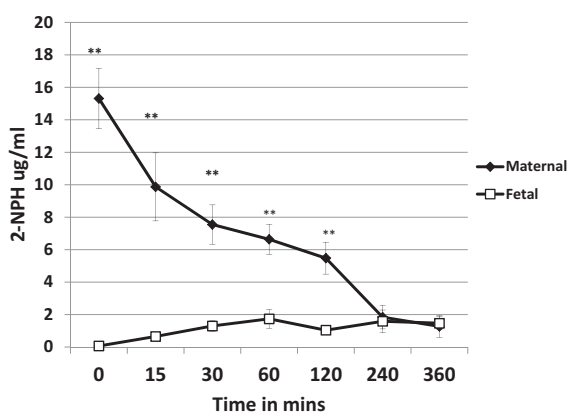
## 4. Discussion

Pregnant women exposed to PAH during pregnancy show a significant increase in the levels of chromosomal aberrations in neonatal cord blood samples [20]. NPHs (both 1 and 2 isomers) are used as biomarkers for polycyclic aromatic hydrocarbons exposure [29]. In our study we used 2-naphthol as it seems to be related to atmospheric pollutants. In particular, that related to Cigarette consumption [27]. Animal studies have shown that NAP and its metabolites, are embryotoxic and abortifacient [13].

**Table 2**

Shows the transfer percentage and transfer index of 2-naphthol. Transfer percentage and transfer index were calculated up to 3 h of perfusion, data are presented as mean  $\pm$  SEM ( $n = 6$ ).

Perfusion time (min)	Antipyrine Transfer percentage	2-Naphthol Transfer percentage	Transfer index
15	12.05 $\pm$ 2.79	4.46 $\pm$ 1.33	0.38 $\pm$ 0.12
30	25.55 $\pm$ 7.41	11.87 $\pm$ 2.36	0.46 $\pm$ 0.26
60	40.35 $\pm$ 6.32	11.82 $\pm$ 1.60	0.30 $\pm$ 0.09
120	30 $\pm$ 3.89	16.68 $\pm$ 2.67	0.55 $\pm$ 0.05
240	52.10 $\pm$ 6.18	15.89 $\pm$ 3.26	0.29 $\pm$ 0.06
360	48.15 $\pm$ 3.10	17.01 $\pm$ 3.26	0.35 $\pm$ 0.05



**Fig. 1.** Shows the mean ( $\pm$ sem) transfer of 2-naphthol ( $\mu\text{g/ml}$ ) across the placenta showing the rapid drop from the maternal compartment and slow increase in the fetal compartment ( $n = 6$ ).

Interestingly, NPH was detected in placental tissue samples obtained prior to perfusion, indicating a significant NAP environmental exposure during pregnancy. The accumulation of 2-NPH seems related to the gestational age. Singh et al. [28] observed higher levels of PAH, including NPD, in pre-term placenta compared to term placenta. This might be due to structural changes occurring during the early second half of pregnancy mainly the partial disappearance of the cytotrophoblast layer which reduce the placenta barrier thickness [10]. Stabenau et al. [30], suggested that the accumulation of NAP has greater effect on the functions of the liver that have higher metabolic activities, and those that have gaseous exchange function. The placenta is known to have a high metabolic activity [31]. Hence, the presence of NPH in unperfused placental tissue and its rapid accumulation observed in our study might affect placental function. However, all neonates of pregnant women involved in this study had a normal birth weight and no structural anomalies. Nevertheless, there is increasing evidence that prenatal exposure to PAH might have long term effects on neonates and infants. Rosa et al. [22] suggested that prenatal exposure to PAH is associated with development of asthma later in life. More worrying are the reports suggesting associated chromosomal aberrations in the newborn and potential risk for cancer development later in life [20].

Our results illustrate NPH appearance in the fetal compartment within 15 min. This could be due to lipophilic nature and low molecular weight of NPH [24]. PAH components with structures similar to NPH rapidly pass from food to milk through the mammary epithelium [5]. Several factors might influence the rate of transfer of any xenobiotic compound, including placental membrane permeability, placental blood flow, pH differences between the maternal and fetal circulations, and protein binding properties of the compound itself. In addition, the placenta possesses xenobiotic-metabolizing capacity to metabolize a number of foreign compounds [12] which may have an impact on the exposure

of the fetus to foreign compounds. Coordinated induction of estrogen hydroxylase (EH) and catechol-O-methyl transferase (COMT) by xenobiotics in first trimester human placental explants, and COMT enzyme activities appear to undergo a coordinated induction in cultured placental explants in the first trimester [1]. The limiting factor in sulphation reaction seems to be the activity of sulphotransferase (ST) towards 2-naphthol.

In our study we observed that NPH transfer across the placenta was at a rate significantly lower than that of antipyrine throughout the period of perfusion. From 60 min the transfer reached a steady state. This suggests that the method of transfer of NPH may differ from that of antipyrine, which is transferred across the placenta by simple diffusion. This might suggest NPH is transferred by active transport via the host of transporters expressed in the placenta.

Furthermore, the rate by which NPH increased in the fetal compartment was at a much lower rate than its drop from the maternal compartment. This might be due to the accumulation of NPH in placental tissue evident by the significant higher NPH levels observed in the perfused placenta cotyledon compared to levels in unperfused placental tissues. This finding supports the protective role of the placenta against fetotoxic materials [32]. Animal studies have shown that NPH accumulates in different body tissues such as the liver, lung, and muscle at different rates [4,30]. However, its elimination from the body seems to be more rapid from organs that have high metabolic activity such as the liver. This is believed to be related to the activity of cytochrome P450 enzymes [30], which seem to play the main role in metabolizing foreign toxins and chemicals from the placenta. They are found throughout pregnancy [10]. Similarly, other PAHs are metabolized by a group of Cytochrome P450 oxidoreductase enzymes including CYP 1A2, 3A4 [1,36].

## 5. Conclusion

Our study suggests that pregnant women are exposed to high levels of environmental NAP, and its metabolites accumulate in term placentas. NPH has the ability to rapidly cross the placenta from maternal to fetal compartment within 15 min. The placenta seems to play a role in limiting the passage of NPH in the fetal compartment by acting as a chemical barrier. Our findings recommend further studies to better understand how the placenta metabolizes the 2-naphthol.

## Conflict of interest

No conflict of interest to report.

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