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# Effects of typical inducers on olfactory xenobiotic metabolizing enzyme, transporter and transcriptional factor expression in rats

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**Running title**: Modulation of olfactory xenobiotic metabolizing enzymes

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**Abbreviations**

AhR, aryl hydrocarbon receptor; AR, Aroclor; ALDH; aldehyde dehydrogenase; AOH3, aldehyde oxidase homolog 3; C, control; CAR, constitutive androstane receptor; CYP, cytochrome P450; CZX, chlorzoxazone hydroxylase; DEX, dexamethasone; EPHX1,

microsomal epoxide hydrolase, EQ, ethoxyquin; EROD, ethoxyresorufin-*O*-deethylase; GR, glucocorticoid receptor; GST, glutathione *S*-transferase; HPLC, high performance liquid chromatography; LB, Luria-Broth; MC, 3-methylcholanthrene; MDR, multidrug resistance protein; MRP, multidrug resistance-associated protein; 4MU, 4-methyl umbelliferone; NO, nifedipine oxidase; NQO1, quinone reductase; Nrf2, nuclear factor E2-related factor 2; O, oil; OAT, organic anion transporter; OCT organic cation transporter; OM, olfactory mucosa; PCR, polymerase chain reaction; PB, phenobarbital; POD, phenacetin-*O*-deethylase; PPAR, peroxisome proliferator-activated receptor; PROD pentoxyresorufin-*O*-dealkylase; PXR, pregnane X receptor; RT-PCR, reverse transcriptase-polymerase chain reaction; SULT, sulfotransferase; UGT, UDP glucuronosyltransferase; XME, xenobiotic metabolizing enzymes.

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## ABSTRACT

Numerous xenobiotic metabolizing enzymes (XMEs) have been identified in the olfactory mucosa (OM) of mammals. However, the molecular mechanisms underlying the regulation of these enzymes have been little explored. In particular, information on the expression of the transcriptional factors in this tissue is quite limited. The aim of the present study was to examine the impact of five typical inducers, Aroclor 1254, 3-methylcholanthrene, dexamethasone, phenobarbital, and ethoxyquin, on the activities and mRNA expression of several XMEs in the OM and the liver in rats. We also evaluated the effects of these treatments on the mRNA expression of transcription factors and transporters. On the whole, the intensities of the effects were lower in the OM than in the liver. Dexamethasone was found to be the most efficient treatment in the OM. Dexamethasone induced the transcription of several olfactory phase I, II and III genes (such as cytochromes P450 (CYP) 2A3 and 3A9, UDP-glucuronosyltransferase (UGT) 2A1 and multidrug resistance-related protein type 1) and increased UGT activities. Surprisingly, we observed that dexamethasone up-regulated sulfotransferase 1C1 expression in the OM but down-regulated it in the liver. Aroclor and ethoxyquin induced the gene expression of CYP1A and quinone reductase in the OM, respectively. The transcription factors AhR, Nrf2, PPARα, PXR and GR were detected in the OM but no CAR expression was observed. Dexamethasone and Aroclor enhanced olfactory Nrf2 expression. These results demonstrate that olfactory XME can be modulated by chemicals and that the mechanisms involved in the regulation of these enzymes are tissue-specific.

**INTRODUCTION**

A large number of xenobiotic-metabolizing enzymes (XMEs) have been characterized in the mammalian olfactory mucosa (OM), including phase I enzymes such as cytochrome P450-dependent monooxygenases (CYPs), aldehyde dehydrogenases (ALDHs) and epoxide hydrolases (EPXHs) as well as phase II enzymes such as UDP-glucuronosyltransferases (UGTs), sulfotransferases (SULTs) and glutathione-S-transferases (GSTs) (Ding and Dahl, 2003; Heydel et al., 2010). Some transporters, also called phase III proteins, such as ATP-binding cassette transporters (MDR1 or MRPs) and organic anion/cation transporters (OATs and OCTs), have also been identified in the OM (Monte et al., 2004; Kudo et al., 2010). The isoforms CYP2A3, CYP2G1, CYP2F4 and UGT2A1 and the transporter OAT6 have been shown to be expressed specifically or preferentially in the olfactory epithelium.

The physiological significance of these proteins is unclear, but the high metabolic capacity of the olfactory mucosa in comparison with other extrahepatic tissues suggests that they could play an important role in maintaining olfactory acuity. These enzymes might provide a first line of defense for the brain. Indeed, during inhalation, the olfactory mucosa can be exposed to potentially toxic compounds. Because the olfactory tract is considered a major entry route of drugs and other xenobiotics within the central nervous system (Minn et al., 2002), XME could constitute a metabolic barrier against these compounds. A role for olfactory XME in the biotransformation and clearance of odorants has also been suggested (Ding and Dahl, 2003). These enzymes could modulate the bioavailability of odorants in the olfactory receptor environment, thereby avoiding over-saturation of the receptors.

Several factors, such as nutritional state, age or gender, can affect XME expression and activity in liver and extra-hepatic tissues. Exogenous compounds such as polycyclic aromatic hydrocarbons, phenobarbital or glucocorticoids can also modulate XME. Little is known about the modulation of XME in nasal tissues. A significant induction of the CYP1A1 protein has been observed in the OM of rats exposed to Aroclor 1254 or cigarette smoke (Voigt et al.,

1993; Wardlaw et al., 1998). Acetone and ethanol were also found to induce CYP2E1 in the rabbit nasal mucosa (Ding and Coon, 1990). Concerning phase II enzymes, olfactory UGT activity has been shown to be up-regulated by phenobarbital and methylcholanthrene in rats (Longo et al., 1988). A minor induction of GST activities by phenobarbital treatment has also been reported (Banger et al., 1996). No data are available on the modulation of transporters in nasal tissues.

The expression of many drug-metabolizing enzymes has been shown to be regulated by the activation of specific transcription factors, including the aryl-hydrocarbon receptor (AhR), the constitutive androstane receptor (CAR), the pregnane X receptor (PXR) and the nuclear factor E2-related factor 2 (Nrf2). These transcription factors act as biosensors for endogenous and xenobiotic chemicals and respond by modulating drug-metabolizing enzyme levels (Xu et al., 2005). The molecular mechanisms underlying the induction of olfactory XME have been poorly explored. Only one study reports the expression of the nuclear receptors PXR and AhR in pig OM (Messina et al., 2009).

The purpose of the present study was to provide original data concerning the modulation of olfactory XME by chemical compounds in rats. We investigated the impact of five typical inducers, Aroclor 1254 (AR), 3-methylcholanthrene (MC), dexamethasone (DEX), Phenobarbital (PB) and ethoxyquin (EQ), on the activities and the mRNA expression of a panel of XMEs in the OM and in the liver. In addition, we evaluated the effects of these treatments on the mRNA expression of several transcription factors and transporters in both tissues. The use of an absolute reverse transcriptase-quantitative polymerase chain reaction (RT-PCR) technique allowed comparison of the olfactory mRNA transcripts steady-state levels between the OM and the liver.

## METHODS

### Chemicals

EQ, MC and DEX were obtained from Sigma Chemical (Saint-Quentin Fallavier, France). AR was purchased from Interchim (Montluçon, France) and sodium PB from the Coopérative Pharmaceutique Française (Melun, France). All other chemicals were of the highest commercial quality available.

### Animals and treatments

Seven-week-old male Wistar rats were purchased from Janvier (Le Genest Saint Isle, France). Animals were housed in conditions of controlled temperature (20–22 °C) and relative humidity (30–70%) and in a 12-h light:dark cycle; animals had free access to water and A04-10 food pellets (Safe, Augy, France). Five groups of 16 animals each were treated with the following chemicals: phenobarbital (group PB, 80 mg/kg/d in 0.9% NaCl solution, i.p., for 3 days), 3-methylcholanthrene (group MC, 20 mg/kg/d in corn oil, i.p., for 3 days), dexamethasone (group DEX, 24 mg/kg/d in corn oil, i.p., for 4 days), ethoxyquin (group EQ, 250 mg/kg/d in corn oil, p.o., for 4 days), Aroclor 1254 (group AR, 500 mg/kg in corn oil, one injection five days before sacrifice). The control rats received either corn oil (group O, i.p., for 3 days) or no treatment (group C). The injection volume for all of the treatments was 4 mL/kg. With the exception of the AR group, the animals were sacrificed 24 h after the last treatment. Their livers and olfactory mucosa were immediately removed, snap-frozen in liquid nitrogen and stored at - 80 °C.

The experiment was carried out in accordance with the French Ministry of Agriculture guidelines for the care and use of laboratory animals.

### Absolute quantitative real-time RT-PCR

*RNA isolation and reverse transcription*

Total RNA was isolated from about 50 mg of tissue after homogenization in a total RNA isolation reagent (Ademtech, Pessac, France) according to the manufacturer's guidelines. The quantity and quality of the RNA samples were assessed using Experion total RNA standard sensitive chips (Bio-Rad). Total RNA (1 µg) from each sample was treated with RNase-Free DNase (Promega) to prevent genomic DNA contamination and reverse-transcribed using the iScript™ cDNA Synthesis Kit (Bio-Rad) according to manufacturer's instructions. The resulting cDNA was then diluted in 80 µL of molecular biology-quality water.

*Primer design*

Primers were designed following the standard real-time PCR parameters using Beacon designer 4 software (Premier Biosoft international, Palo Alto, USA) with reference mRNA sequences obtained from GenBank™. Primer sequences are listed in Supplemental Data Table 1.

*Standard curve constructions*

The PCR products of each target gene were amplified from the cDNA reverse-transcribed from olfactory mucosa and purified using the MinElute PCR Purification Kit (Qiagen). The purified fragments were cloned into a pDrive Cloning Vector using a PCR Cloning Kit (Qiagen). The recombinant plasmids were transformed into competent *Escherichia coli* JM109 cells (Promega) and grown overnight on agar plates containing ampicillin (80 µg/µL) for the selection of recombinant clones. Plasmids were purified from a 50-mL LB culture medium using the QIAfilter Plasmid Midiprep Kit (Qiagen) and stored at -20 °C. All constructs were checked by sequencing (Cogenics, Meylan, France). Quantification of the plasmid DNA was performed using a NanoDrop ND-1000 spectrophotometer (NanoDrop technologies, Wilmington, DE, USA), and the copy number was calculated from the concentration and its molecular weight. A series of 10-fold dilutions in water were made from the purified plasmid DNA to generate external calibration curves.

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*Absolute real-time quantification*

The real-time PCR runs were performed in duplicate using a MyiQ5™ instrument (Bio-Rad). Each reaction mixture was prepared using the iQ™ SYBR Green Supermix in a total volume of 25 µL: 5.5 µL water, 1 µL of each primer (final concentration 0.3 µM), 12.5 µL Supermix and 5 µL of template cDNA or diluted plasmid. The thermal cycling was as follows: initial denaturation for 3 min at 95 °C followed by 40 cycles of 10 s at 95 °C and 30 s at 60 °C. After amplification, a melt peak analysis was performed to confirm that only the specific products were amplified and to check the absence of primer dimers. Amplification data were analyzed using iQ5™ software.

**Preparation of microsomal and cytosolic fractions**

Microsomes and cytosols were prepared by differential centrifugation as described previously (Haber et al., 1994; Minn et al., 2005) and stored in small aliquots at - 80°C. In each group, four pools of four OM were set up in order to have enough material to perform all of the enzyme assays on the same samples. The protein levels of the microsomal and cytosolic fractions were measured according to the method used by Bradford (Bradford, 1976) adapted for the use of a Cobas Fara II centrifugal analyzer (Roche Instruments) using bovine serum albumin as a standard.

**Enzyme assays**

Phenacetin O-deethylase (POD), nifedipine oxidase (NO) and chlorzoxazone hydroxylase (CZX) activities were determined by HPLC. The measurements of ethoxyresorufin O-deethylation (EROD), pentoxyresorufin O-deethylation (PROD), quinone reductase (NQO1), GST and ALDH activities were adapted for the use of a Cobas Fara II centrifugal analyzer (Roche Instruments). UGT activities using *p*-nitrophenol, 1-naphthol and eugenol as substrates were determined by spectrophotometry. UGT activity using 4-methylumbelliferone as a substrate was determined by fluorimetry. Detailed information and references for these assays are given in Supplemental Data Table 2.

**Statistical analyses**

The non-parametric Mann-Whitney test was used to assess the difference between the different groups. The PB group data were compared with the C group data while the data of the other groups (AR, MC, DEX and EQ) were compared with the O group data. The level of significance was $P \leq 0.05$. The statistical analyses were carried out using Statistica software, version 8 (StatSoft, Maisons-Alfort, France).

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## RESULTS

### Absolute quantification of XME mRNA

#### *Basal expression of transcripts in the liver and the OM*

In order to compare the steady-state levels of mRNA transcripts in the liver and in the olfactory mucosa of untreated rats, the expression patterns of XME genes in these tissues were quantified using an absolute real-time RT-PCR procedure. Transcripts of seven phase I enzymes (CYP1A1, 1A2, 2A3, 2E1, 2G1, 2F4 and 3A9); a dozen transcripts of phase II enzymes, including glutathione-S-transferases (mGST, GSTA3, M1 and M2), UDP-glucuronosyltransferases (UGT1A6, 2A1 and 2B1), a sulfotransferase (SULT1C1), an epoxide hydrolase (EPHX1), a quinone reductase (NQO1), two aldehyde-dehydrogenases (ALDH1 and 3) and five phase III proteins (OAT6, MRP1, MRP3, MRP5 and MDR1a) were analyzed. We also measured the transcript levels of the transcription factors AhR, CAR, Nrf2, PXR, PPARα and GR in both tissues.

Figure 1 shows that the basal expression profiles of the liver and the OM differ substantially. CYP2A3, CYP2F4 and CYP2G1 mRNA transcripts were expressed in higher amounts in the OM, whereas CYP1A2, CYP2E1 and CYP3A9 mRNA were as abundant in the OM as in the liver. CYP1A1 was moderately expressed in both tissues, but its expression level was greater in the OM.

Analysis of the expression of the phase II enzyme genes also exhibited notable differences between tissues. Aldehyde oxidase homolog 3 (AOH3) and UGT2A1 transcripts were highly expressed in the OM, but there was little or no expression in the liver. Conversely, the UGT2B1 gene was only expressed in the liver. The expression level of ALDH3 was a thousand-fold higher in the OM than in the liver. EPHX1, NQO1, ALDH1, GST and SULT1C1 mRNA levels were equivalent in the OM and in the liver.

With respect to the transcripts coding for transporters, OAT6 mRNA was detected for the first time in rat OM. This transporter was not expressed in the liver. The basal expression of MRP transcripts in the OM was superior to that observed in the liver. MDR1a was detected in both

tissues, whereas MDR1b was expressed in the liver and barely detectable in the OM (data not shown).

The quantification of transcription factor mRNA in the liver and the OM also revealed an organ-specific expression profile. We observed no expression of CAR in the OM, while the other studied transcription factors were detected in this tissue. The expression level of Nrf2 was slightly higher in the OM compared to the liver. Conversely, PXR and PPARα mRNA expression was higher in the liver. AhR mRNA levels were similar in both tissues.

***Modulation of XME expression in the liver by enzyme inducers***

As expected, CYP1A1 and CYP1A2 were strongly induced by both AR (3000- and 20-fold, respectively) and by MC (7000- and 25-fold, respectively) (Figure 2). CYP3A9 mRNA expression was strongly induced by DEX treatment (60-fold) and, to a lesser extent, by PB (17-fold) and AR (6-fold). Interestingly, CYP2F4 and CYP2A3 mRNA, which were expressed weakly at the basal level in the liver, were also significantly induced by DEX.

Hepatic expression of SULT1C1, ALDH1 and UGT1A6 was significantly decreased by DEX treatment (Figure 3). GSTM1 and GSTM2 mRNA transcripts were up-regulated by EQ, AR, PB and MC. mGST expression was not affected by any treatment. NQO1 mRNA expression was significantly enhanced by AR and PB treatments. Expression of UGT1A6 mRNA was induced by MC, AR and EQ treatments (10-, 6- and 5-fold, respectively). UGT2A1 and ALDH3, which were barely or not expressed at the basal level in the liver, were strongly induced by DEX and by the AR and MC treatments, respectively. Hepatic induction of EPHX1 and UGT2B1 mRNA was observed in response to PB, AR and EQ.

The level of MRP3 transcripts was strongly up-regulated by EQ treatment (30-fold) and by AR and PB treatments (10-fold) (Figure 4). EQ and PB treatments also tended to induce MDR1a hepatic expression, but these effects were not significant. MRP1 and MRP5 mRNA expression was not affected by any treatment (MRP5 data not shown).

AR treatment significantly induced Nrf2 and AhR hepatic expression (Figure 4). A slight decrease in PPARα transcripts was also provoked by MC treatment in the liver. DEX

administration led to a decrease in GR expression and to a non-significant increase in the expression of the nuclear receptor PXR. CAR expression was not significantly affected by any treatment.

***Modulation of XME expression in the OM by enzyme inducers***

DEX treatment significantly induced the expression of CYP1A2, CYP2A3, CYP2G1, CYP2F4 and CYP3A9 mRNA (2- to 4-fold) in the OM (Figure 5). AR treatment led to an increase in CYP1A1 mRNA (6-fold) and, to a lesser extent, to an increase in CYP1A2 mRNA (2-fold). CYP1A1 and CYP1A2 transcript levels were doubled by MC and EQ treatments, respectively. Concerning the modulation of olfactory phase II enzymes, SULT1C1 expression was found to be enhanced by the DEX, EQ and AR treatments (1.8- to 3-fold) (Figure 6). DEX also significantly induced UGT2A1 and GSTM2 mRNA levels. NQO1 mRNA was significantly up-regulated by EQ (3-fold). Conversely, EQ provoked a decrease in ALDH3 transcripts, but this effect was not significant.

Compared to their basal expression, olfactory mRNA levels of the transporters OAT6, MDR1a and MPR1 were doubled by DEX treatment (Figure 7). EQ induced expression of MRP3 mRNA by 2.5-fold. The other treatments (PB, MC and AR) had no effect on transporter expression.

Concerning transcription factors, only Nrf2 expression was modulated by chemical treatments in the OM (Figure 7); this factor was significantly induced by DEX and AR (about 2-fold). The other transcription factors detected in the OM (PXR, GR, PPARα and AhR) were not affected by any treatment.

**Phase I and phase II enzyme activities**

***Basal enzyme activities in the liver and the OM***

The basal activities of ALDH3, POD and CZX were considerably higher in the OM than in the liver of untreated rats (32-, 5.3- and 4.5- fold higher, respectively) (Figure 8). ALDH1 activity and UGT activity measured using 4-MU as substrate were also higher in the OM, but to a

lesser extent (1.6-fold higher). Conversely, GST, NQO1 and eugenol-UGT activities were significantly greater in the liver than in the OM (1-3 to 1.6-fold). UGT activity measured using 1-naphthol as a substrate did not exhibit tissue differences.

***Modulation of XME activities in the liver by enzyme inducers***

In the liver, as expected, all of the chemical treatments were able to modulate one or several XME activities (Figure 9). AR treatment significantly induced all of the hepatic enzyme activities measured. High inductions of EROD, PROD and ALDH3 activities were provoked by this treatment (53-, 50- and 105-fold, respectively). MC elicited a strong induction of hepatic EROD (39-fold) and ALDH3 (43-fold) activities. POD, CZX, UGT, GST, NQO1 and ALDH1 activities were also significantly increased by MC, but to a lesser extent. Conversely, hepatic NO activity was decreased by 38% in the MC group. PB greatly increased hepatic PROD activity (38-fold) and, more modestly, EROD, POD, UGT, GST, NQO1 and ALDH1 activities. A 33% decrease in hepatic NO activity was observed in the PB group. Hepatic CYP-dependent activities were significantly stimulated by DEX (1.4 to 2.7-fold). Conversely, DEX treatment reduced UGT, GSTM and NQO1 activities in the liver. EQ strongly induced PROD and NQO1 activities (8- and 4.7-fold, respectively), and to a lesser extent, POD, UGT, GST and ALDH activities (2- to 3-fold). On the other hand, NO and CZX activities were reduced by 25-35% in the liver of EQ-treated rats.

***Modulation of XME activities in the OM by enzyme inducers***

With respect to the control oil group, DEX significantly increased ALDH1, ALDH3, 1-naphthol- and eugenol-UGT activities by 1.4- to 1.7-fold (Figure 10). EQ strongly reduced CZX and POD activities (by about 80%), ALDH3 activity (25%) as well as 1-naphthol- and 4-MU-UGT activities (25-40%). Conversely, olfactory NQO1 activity was significantly enhanced by EQ treatment (1.4-fold). AR increased the activities of 1-naphthol- and eugenol-UGT (1.4-fold) and ALDH activities (about 1.5-fold), whereas a 50% reduction of CZX activity was observed in the OM from AR-treated rats. MC significantly enhanced olfactory NQO1 and

ALDH activities (1.2 to 1.7-fold). PB treatment resulted in a significant increase in eugenol-UGT activity (1.7-fold) and in a decrease in ALDH activities (by about 20-25%).

**DISCUSSION**

Because little information is available on the regulation of XME and xenobiotic transporter expression in mammal olfactory tissues, we investigated the modulation of a panel of XMEs and transporters by using prototypical inducers in both the liver and the OM in rats. For this purpose, five chemicals known to induce different regulation pathways (PB, AR, MC, DEX and EQ) were administered to animals.

First, this study allowed us to compare the basal expression of numerous XME, transcription factors and transporters in the OM and in the liver. Our results showed that the basal mRNA expression and the activity of several phase I and phase II enzymes were of the same order of magnitude or higher in the OM than in the liver. This result is in good agreement with previous observations (Longo et al., 1988; Ben-Arie et al., 1993; Minn et al., 2005). In the present study, we show, for the first time, a significant expression of SULT1C1 mRNA in rat OM. SULT1C1 has been shown to be weakly or not expressed in rat non-hepatic tissues, but olfactory tissues were not examined until the present study (Dunn and Klaassen, 1998). This finding is consistent with the recently reported detection of the SULT1C1 protein in rat olfactory sensory cilia (Mayer et al., 2008). In addition, SULT1C1 mRNA was detected in human and mouse nasal epithelia (Beckmann et al., 1995; Tamura et al., 1998). A number of transcription factors and transport proteins were also found to be expressed in the olfactory mucosa. We detected transcripts of the receptors AhR, PPAR$\alpha$, PXR and GR. However, in agreement with a recent report (Messina et al., 2009), no CAR expression was detected in this tissue. The transcription factor Nrf2 was found to be expressed in high amounts in the OM. This high expression of Nrf2 could play a key role in the defense mechanisms against oxidative stress, electrophiles or environmental toxicants that might alter the olfactory neuroepithelium (Osburn and Kensler, 2008). In this study, we also detected the presence of several transporters, OAT6, MDR1a, MRP1, MRP3 and MRP5, in the OM. This finding confirms and supplements recent observations made by Kudo and collaborators (Kudo et al., 2010). The role of these proteins in the OM needs to be further explored. It has been shown

that the mouse OAT6 transporter may interact with odorant organic anions (Kaler et al., 2006), which suggests that transporters play a role in the availability of odorants in the receptor environment.

Considering the effects of chemicals in the liver, as expected, we observed that administration of these chemicals resulted in a subsequent increase in mRNA expression and the activity of numerous hepatic XMEs. For example, AR and MC treatments were found to strongly induce CYP1A1/2, Aldh3 and UGT1A6 mRNA expression, whereas DEX and EQ increased NO and NQO1 activities. It is interesting to note that UGT2A1, CYP2A3 and CYP2F4, which have been characterized as specific olfactory or respiratory forms (Su et al., 1996; Shelby et al., 2003; Baldwin et al., 2004), were significantly expressed in the liver after DEX treatment. These isoforms might be involved in the metabolism of DEX. Indeed, UGT2A1 has been shown to contribute especially to the glucuronidation of steroids and phenolic compounds (Jedlitschky et al., 1999). CYP2A3 has also been reported to catalyze the hydroxylation of testosterone and a number of xenobiotics (Liu et al., 1996).

In the olfactory mucosa, all treatments elicited significant effects on the expression and activity of XMEs. However, the intensity of the observed effects was lower than those observed in the liver. In our study, DEX was found to exert pleiotropic effects and to be the most efficient treatment in the OM. This treatment induced the transcription of a number of phase I, II and III genes (in particular CYP3A9, CYP2A3, CYP2F4, UGT2A1, SULT1C1 and MDR1a) and provoked the augmentation of UGT and ALDH activities. The nuclear receptors PXR and GR, which have been shown to be implicated in the DEX-mediated induction of hepatic XMEs (Quattrochi and Guzelian, 2001), were not affected by DEX treatment in the OM. Interestingly, unlike in the liver, DEX elicited a significant induction of the Nrf2 transcription factor in the OM, suggesting that DEX effects could occur through mechanisms involving this transcription factor. Induction of CYP is in agreement with a previous study that reported that DEX treatment potentiates the olfactory toxicity of 3-methylindole, a chemical

known to be activated by CYP (Kratskin et al., 1999). This potentiation could be the result of the induction of CYP2A3 and CYP2F4 because human orthologs of these CYPs have been known to metabolize 3-methylindole (Thornton-Manning et al., 1996).

Previous studies reported that 2,3,7,8-tetrachlorodibenzo-p-dioxin, an AhR agonist, induces CYP1A1 and CYP1A2 proteins and activities in the OM (Voigt et al., 1993; Genter et al., 2002). Similarly, in the present study, the hepatic CYP1A inducers AR and MC were found to significantly enhance the expression of CYP1A1 in the OM. The expression of CYP1A2 in the OM was also induced by AR. We observed that Nrf2 expression was induced by AR in the OM, whereas AhR expression was not affected. Functional interactions between Nrf2 and AhR have been reported recently (Miao et al., 2005). It has been shown that expression of Nrf2 can be directly modulated by AhR or by electrophilic metabolites generated by CYP1A1.

In contrast to its activity in the liver, PB had little effect in the OM. Only a slight enhancement of eugenol-UGT activity was noted, although UGT2A1 and UGT1A6 expression were not affected by this treatment. None of the enzymes induced in the liver (CYP3A9, EPHX1, UGT2B1 or MRP3) were modulated by PB in the OM. This finding could be due to the absence of CAR expression in this tissue, as it is well-known that CAR regulates the gene expression of the enzymes induced by PB (Honkakoski and Negishi, 1998). These observations are in keeping with previous reports that showed low effects of PB in the OM (Longo et al., 1988).

EQ significantly increased NQO1 expression and activity in both the OM and in the liver. The induction of olfactory NQO1 is all the more noteworthy because basal expression and activity of this enzyme is four-fold higher in the OM than in the liver. The transcriptional factors Nrf2 and AhR have been shown to be implicated in NQO1 induction in the liver (Ma et al., 2004). Whether these factors are also involved in NQO1 induction in the OM remains to be determined. Elsewhere, it was found that EQ treatment resulted in a decrease in CZX, POD and Aldh3 activities in the OM. While the Aldh3 activity decrease can be explained by the reduction of Aldh3 transcripts, the mechanisms resulting in the decrease of CYP-dependent

activities remain unclear. One possible explanation might be the inhibition of CYP-activities by either EQ or its metabolites. Indeed, although the CYP induction capacities of EQ are well known, inhibitory effects of EQ on CYP activities have also been reported (Stohs and Wu, 1982). Because CZX and POD activities are sustained by several CYP, such as CYP2E1, CYP2G1, 2A3, 1A1 and 1A2 (Minn et al., 2005), it is difficult to determine which enzymes are affected by EQ.

This study clearly showed that there are quantitative and qualitative differences in the ability of chemicals to modulate enzyme expression and activity in the OM and in the liver. Taken as a whole, the treatments provoked less-important effects in the OM compared to the liver. Because the chemicals were administered by oral or intraperitoneal routes, the hepatic first-pass metabolism is likely responsible for a reduction in the bioavailability of the chemicals in the extra-hepatic tissues such as the OM. Another explanation for the moderate induction capacity of the OM is that a full activation of XMEs would occur rapidly following birth, resulting in an enzyme basal expression level higher in the OM than in the liver (Ding et al., 1992). Moreover, in several instances, the chemical treatments caused opposite effects in the liver and OM. In the liver, SULT1C1 was significantly down-regulated by DEX treatment, whereas it was up-regulated in the OM. The hepatic DEX-induced lowering of SULT1C1 transcripts observed in our study is in agreement with published findings (Liu and Klaassen, 1996). Little information is available on the mechanisms of XME gene regulation in olfactory tissues. Previous studies demonstrated that enzymes such as CYP1A2 and CYP2A3 are regulated by complex mechanisms in the OM. A splicing variant of the nuclear factor I-A2 (NFI-A2) was shown to control the constitutive expression of these CYPs (Zhang et al., 2000; Ling et al., 2004).

In conclusion, the current study increases our understanding of the mechanisms that regulate XME expression in the olfactory mucosa. Our results demonstrate that typical hepatic inducers can modulate the activities and the expression of olfactory XMEs and

suggest that transcriptional factors such as Nrf2 are likely involved in the modulation of olfactory XME genes. Nevertheless, because noticeable differences have been observed between the OM and the liver (for example, concerning the expression and modulation of SULT1C1), it can be assumed that specific regulation mechanisms occur in the OM. These mechanisms would merit further consideration. Among the different chemicals studied, dexamethasone was found to be the most efficient treatment. It would be interesting to examine the impact of other glucocorticoids on XME expression because these drugs are usually administered to treat certain olfactory dysfunctions such as postviral olfactory loss (Stenner et al., 2008).

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**FOOTNOTES**

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**FIGURE LEGENDS**

**Figure 1**: Steady-state levels of transcripts coding for phase I and phase II enzymes, transporters and transcription factors in the liver and the olfactory mucosa.

Total mRNA was quantified by an absolute quantitative real-time RT-PCR assay.

Data are presented as means ± s.e.m. (n = 5 rats).

**Figure 2**: Effects of typical inducers on phase I enzyme mRNA expression in the liver.

Male Wistar rats were treated with the following chemicals: phenobarbital (PB), 3-methylcholanthrene (MC), dexamethasone (DEX), ethoxyquin (EQ) and Aroclor 1254 (AR). Control rats received either corn oil (group O) or nothing (group C).

Data are presented as means ± s.e.m. (n = 5 rats). Asterisks (*) indicate significant differences between the treatment groups and their respective control ($p < 0.05$, Mann-Whitney test).

**Figure 3**: Effects of typical inducers on phase II enzyme mRNA expression in the liver.

Male Wistar rats were treated with the following chemicals: phenobarbital (PB), 3-methylcholanthrene (MC), dexamethasone (DEX), ethoxyquin (EQ) and Aroclor 1254 (AR). Control rats received either corn oil (group O) or nothing (group C).

Data are presented as means ± s.e.m. (n = 5 rats). Asterisks (*) indicate significant differences between the treatment groups and their respective control ($p < 0.05$, Mann-Whitney test).

**Figure 4**: Effects of typical inducers on transporter and transcription factor mRNA expression in the liver.

Male Wistar rats were treated with the following chemicals: phenobarbital (PB), 3-methylcholanthrene (MC), dexamethasone (DEX), ethoxyquin (EQ) and Aroclor 1254 (AR). Control rats received either corn oil (group O) or nothing (group C).

Data are presented as means ± s.e.m. (n = 5 rats). Asterisks (*) indicate significant differences between the treatment groups and their respective control ($p < 0.05$, Mann-Whitney test).

**Figure 5**: Effects of typical inducers on phase I enzyme mRNA expression in the olfactory mucosa.

Male Wistar rats were treated with the following chemicals: phenobarbital (PB), 3-methylcholanthrene (MC), dexamethasone (DEX), ethoxyquin (EQ) and Aroclor 1254 (AR). Control rats received either corn oil (group O) or nothing (group C).

Data are presented as means ± s.e.m. (n = 5 rats). Asterisks (*) indicate significant differences between the treatment groups and their respective control ($p < 0.05$, Mann-Whitney test).

**Figure 6**: Effects of typical inducers on phase II enzyme mRNA expression in the olfactory mucosa.

Male Wistar rats were treated with the following chemicals: phenobarbital (PB), 3-methylcholanthrene (MC), dexamethasone (DEX), ethoxyquin (EQ) and Aroclor 1254 (AR). Control rats received either corn oil (group O) or nothing (group C).

Data are presented as means ± s.e.m. (n = 5 rats). Asterisks (*) indicate significant differences between the treatment groups and their respective control ($p < 0.05$, Mann-Whitney test).

**Figure 7**: Effects of typical inducers on transporter and transcription factor/nuclear receptor mRNA expression in the olfactory mucosa.

Male Wistar rats were treated with the following chemicals: phenobarbital (PB), 3-methylcholanthrene (MC), dexamethasone (DEX), ethoxyquin (EQ) and Aroclor 1254 (AR). Control rats received either corn oil (group O) or nothing (group C).

Data are presented as means ± s.e.m. (n = 5 rats). Asterisks (*) indicate significant differences between the treatment groups and their respective control ($p < 0.05$, Mann-Whitney test).

**Figure 8**: Basic activities of phase I and phase II enzymes in rat liver and olfactory mucosa.

Data are means ± s.e.m (n = 4 pools of 4 rats).

**Figure 9**: Effects of typical inducers on phase I and phase II enzyme activities in the liver.

Male Wistar rats were treated with the following chemicals: phenobarbital (PB), 3-methylcholanthrene (MC), dexamethasone (DEX), ethoxyquin (EQ) and Aroclor 1254 (AR). Control rats received either corn oil (group O) or nothing (group C).

Data are presented as means ± s.e.m (n = 4 pools of 4 rats)

Asterisks (*) indicate significant differences between the treatment groups and their respective control ($p < 0.05$, Mann-Whitney test).

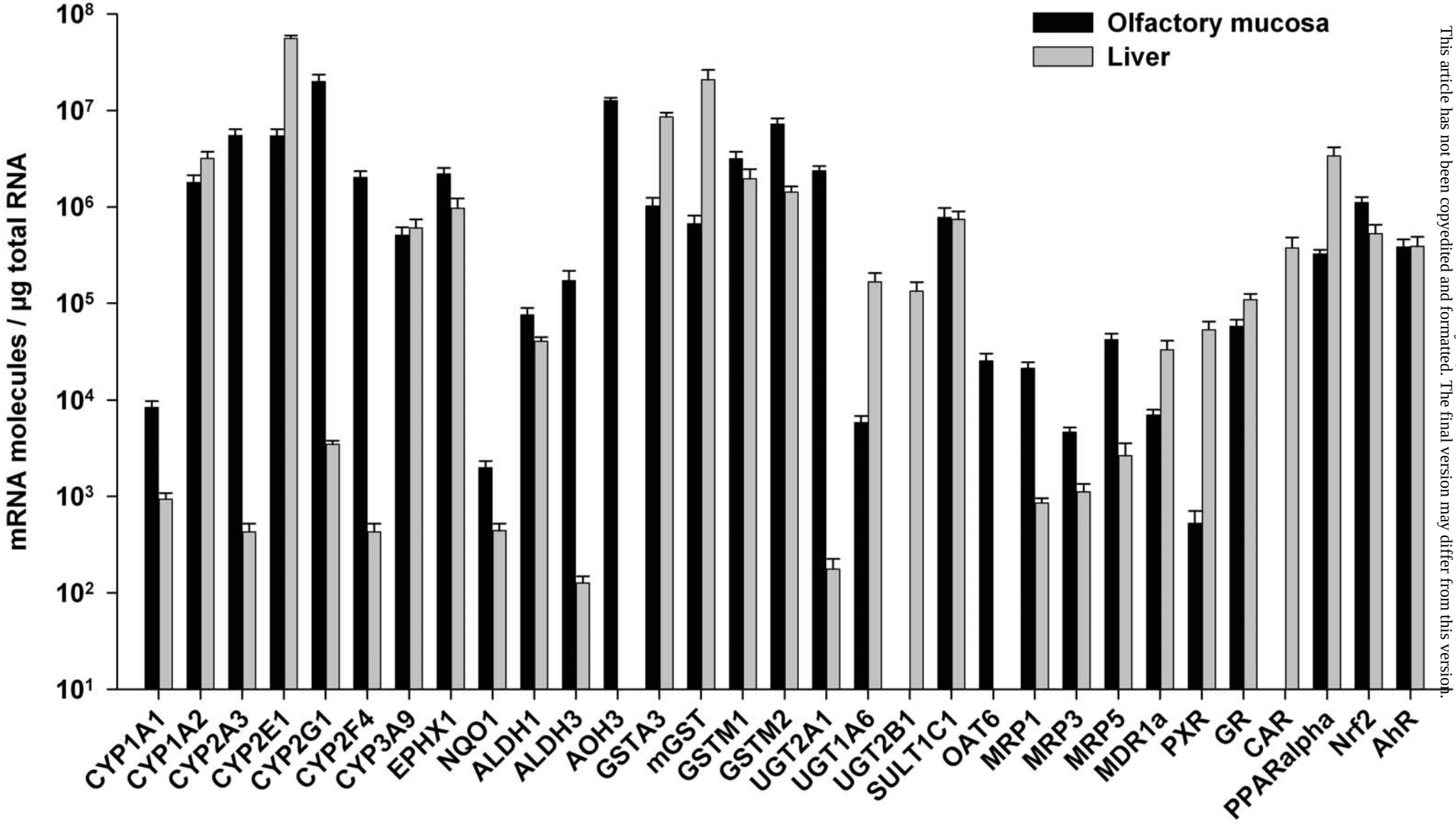
**Figure 10**: Effects of typical inducers on phase I and phase II enzyme activities in the olfactory mucosa.

Male Wistar rats were treated with the following chemicals: phenobarbital (PB), methylcholanthrene (MC), dexamethasone (DEX), ethoxyquin (EQ) and Aroclor 1254 (AR). Control rats received either corn oil (group O) or nothing (group C).

Data are presented as means ± s.e.m (n = 4 pools of 4 rats)

Asterisks (*) indicate significant differences between the treatment groups and their respective control ($p < 0.05$, Mann-Whitney test).

**Figure 1**



## Figure 2

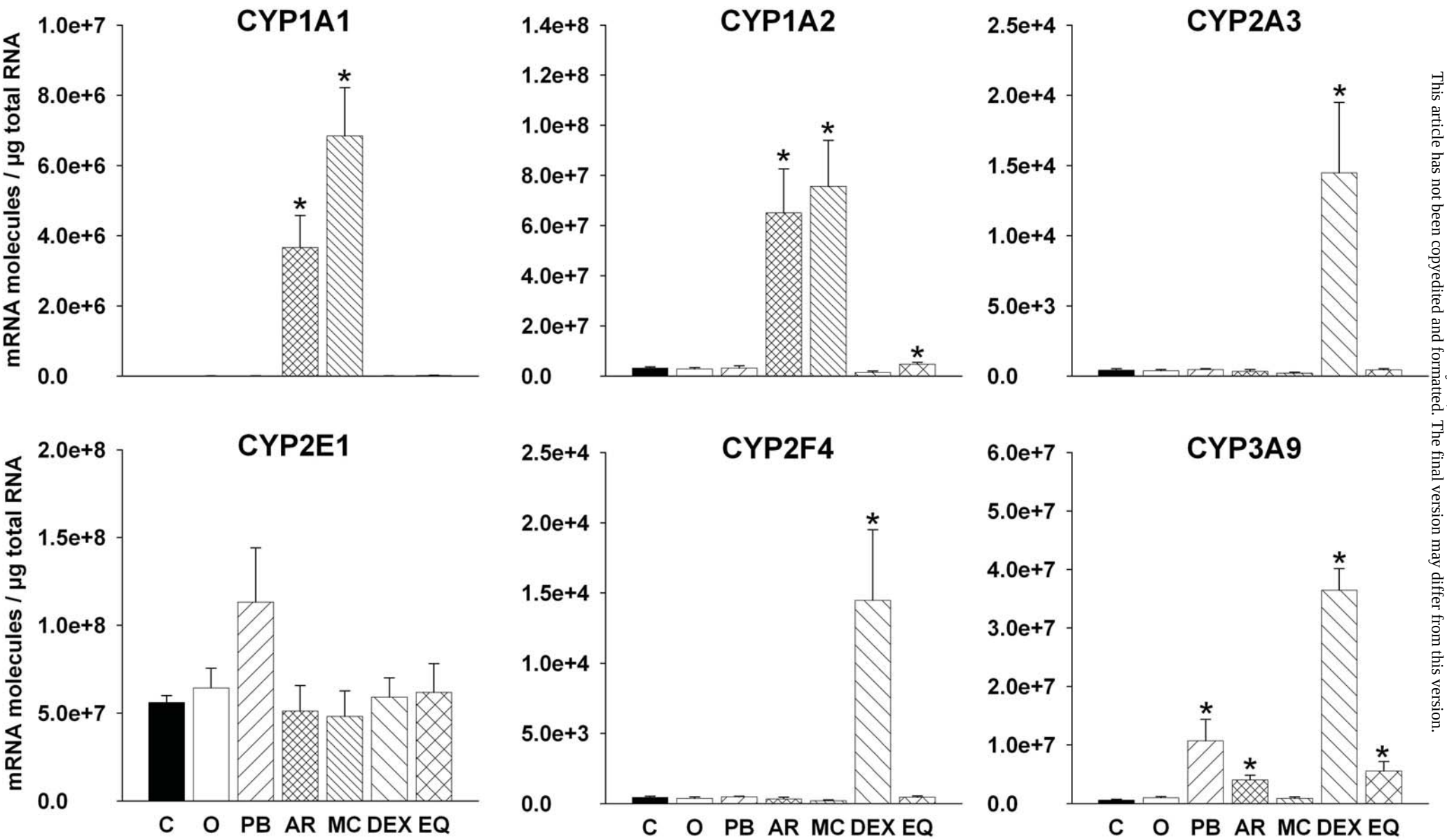


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**Figure 3**

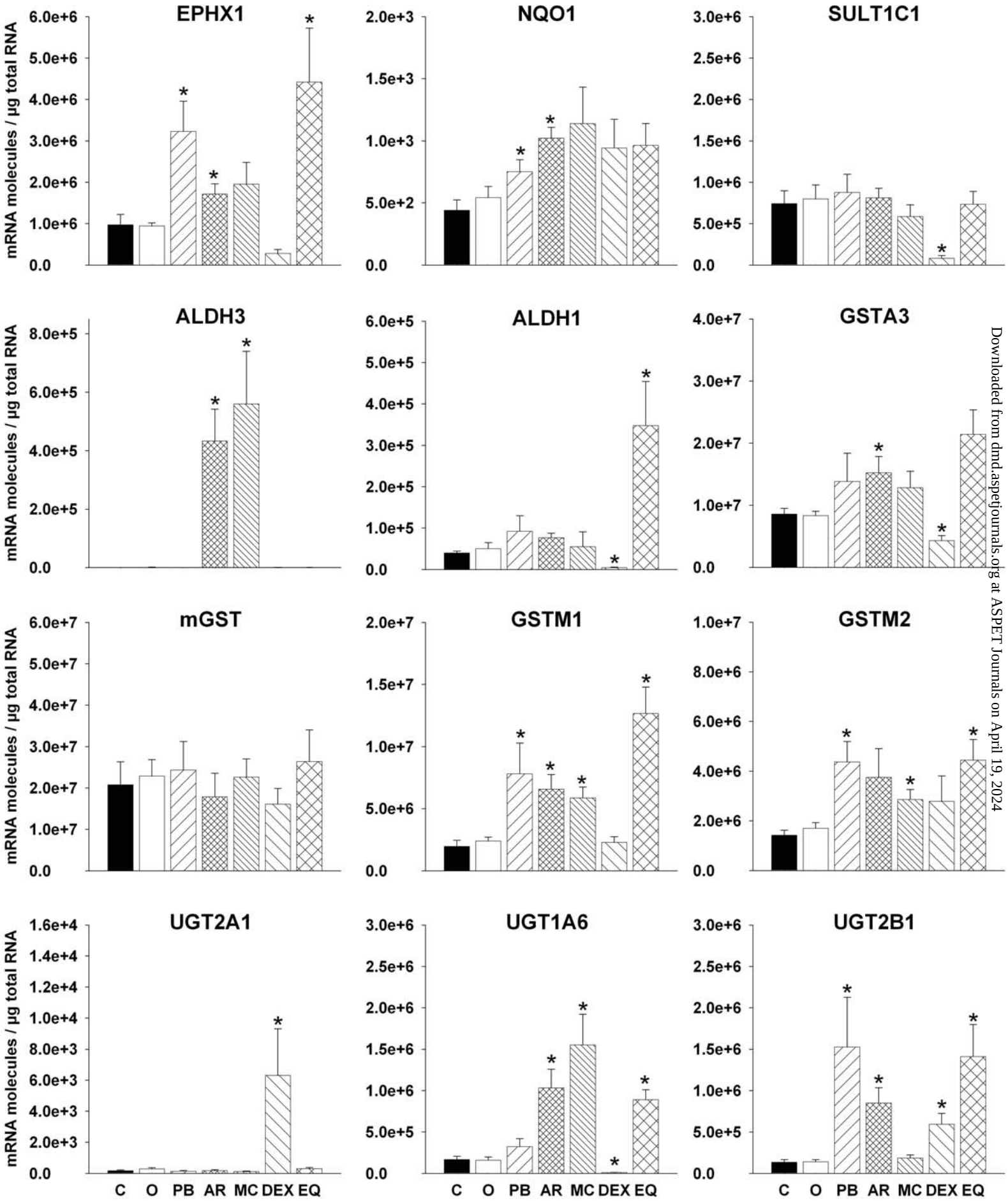

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Figure 4

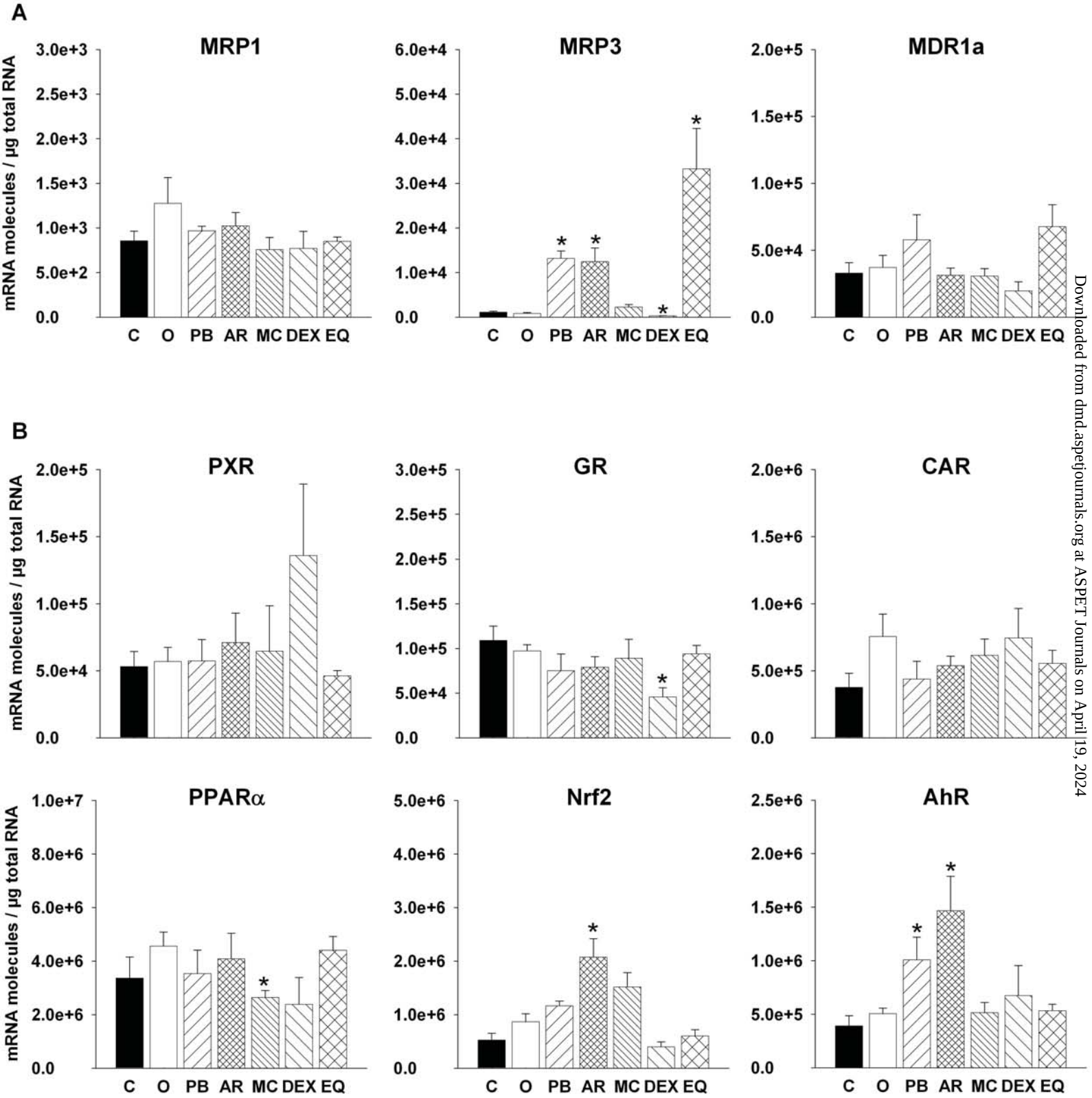

Figure 5

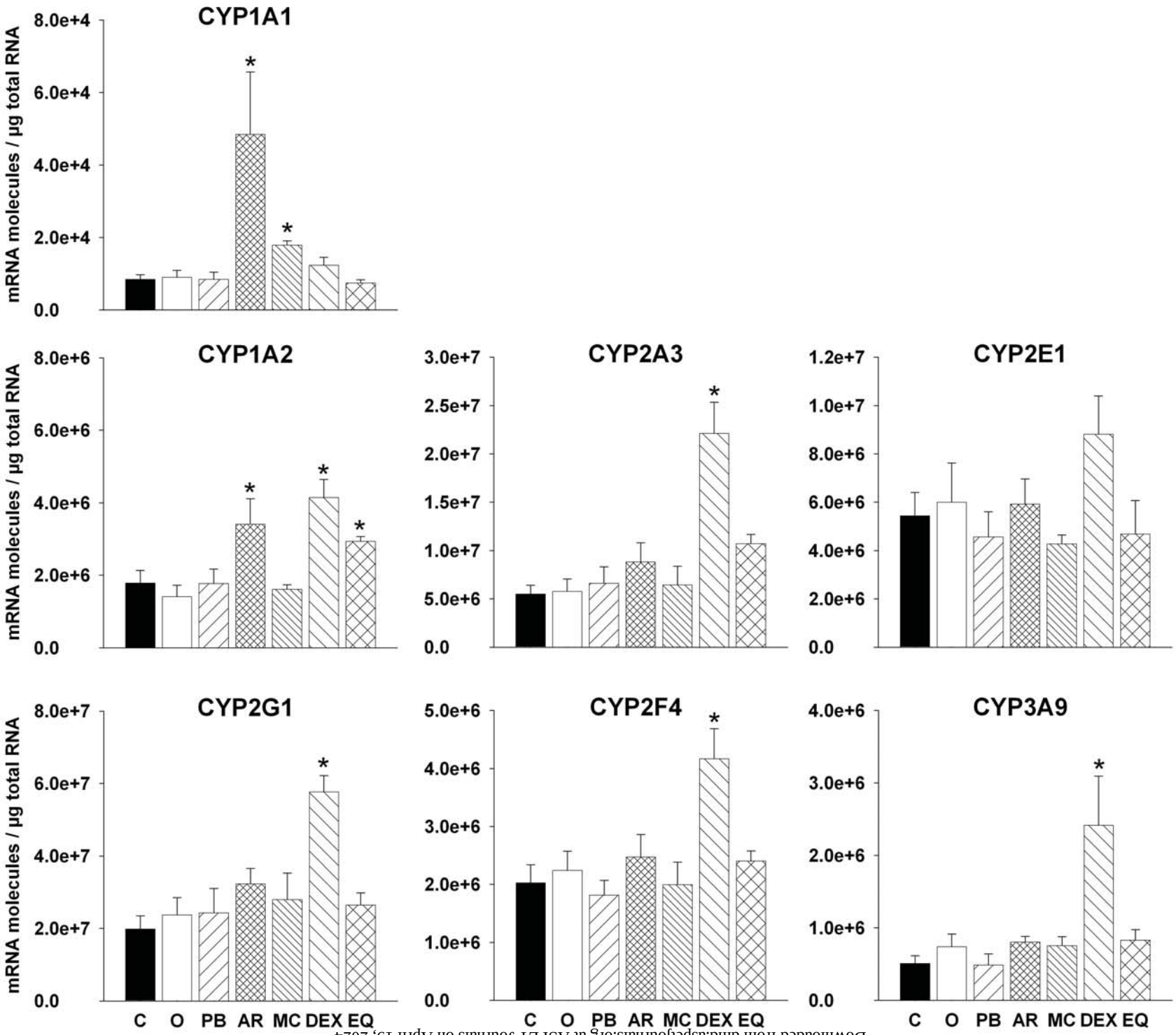

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**Figure 6**

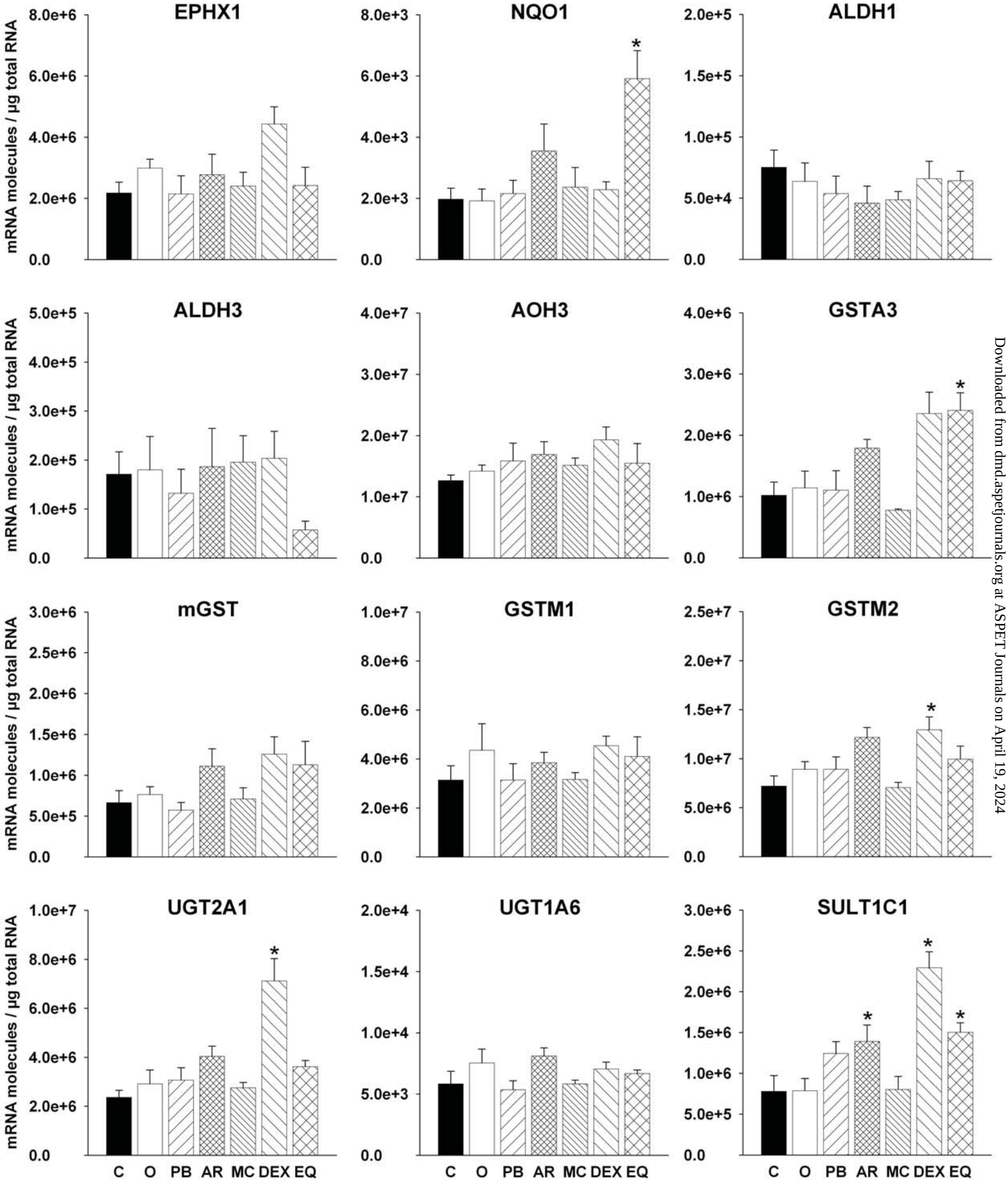

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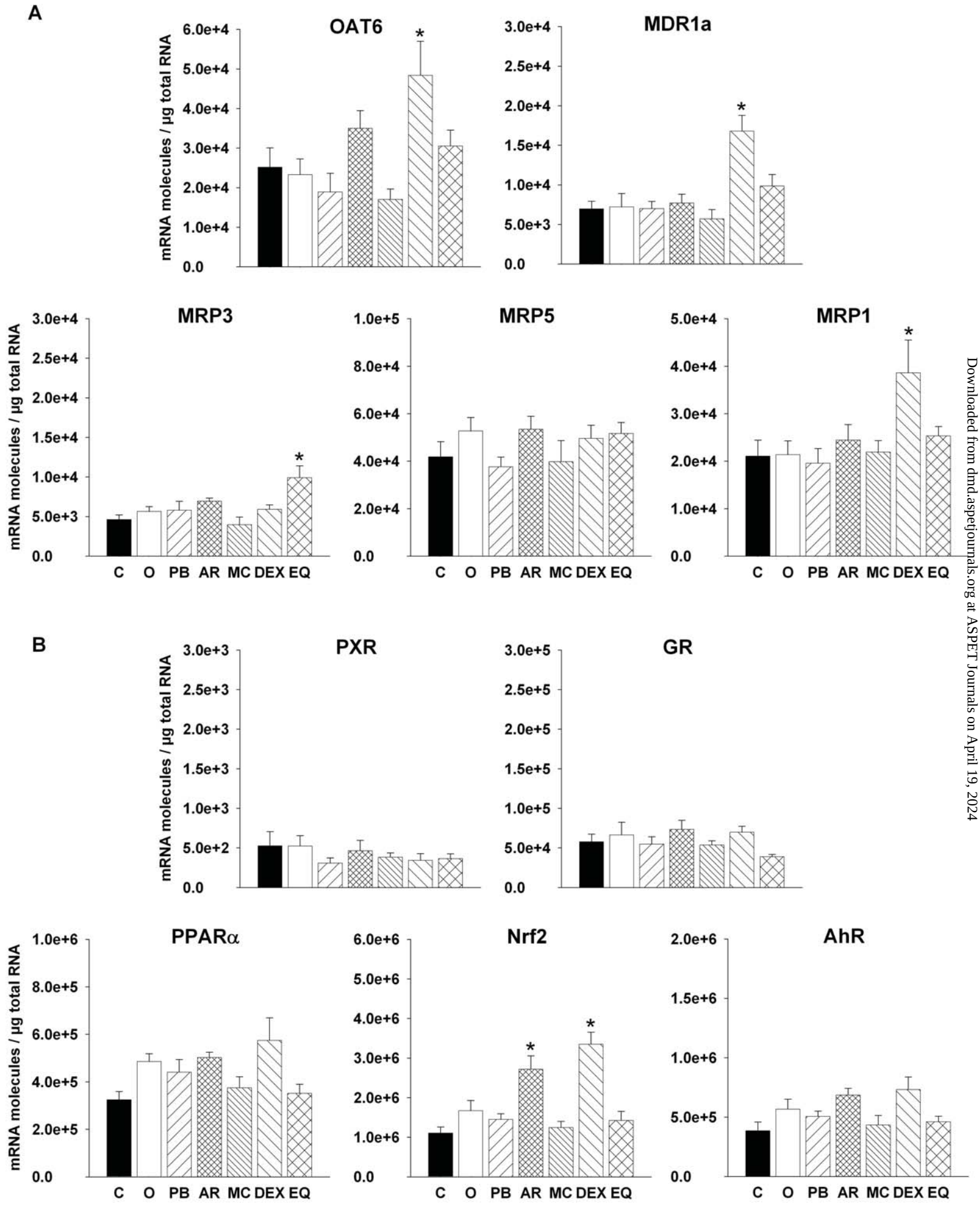
Figure 7



## Figure 8

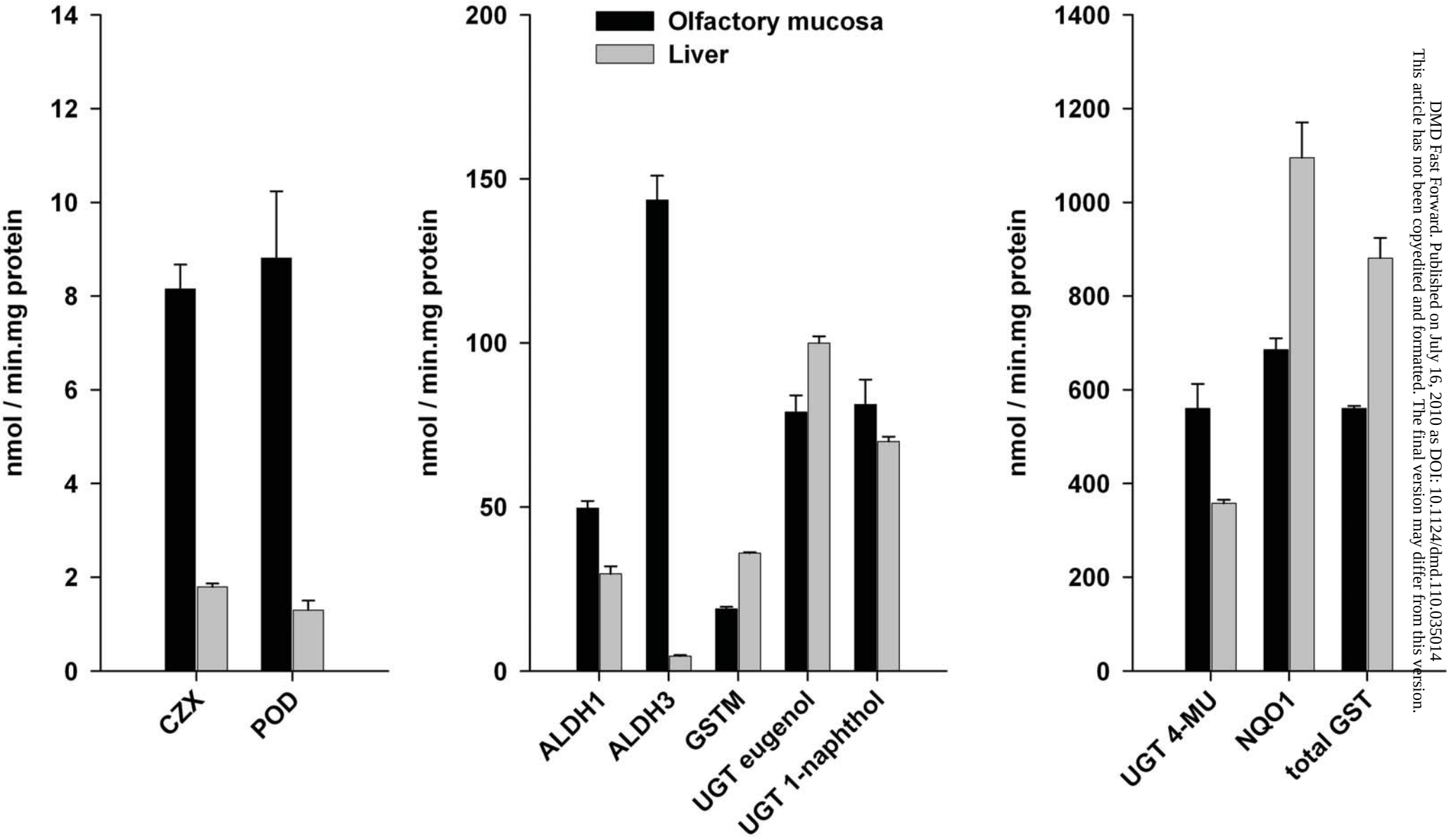


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Figure 9

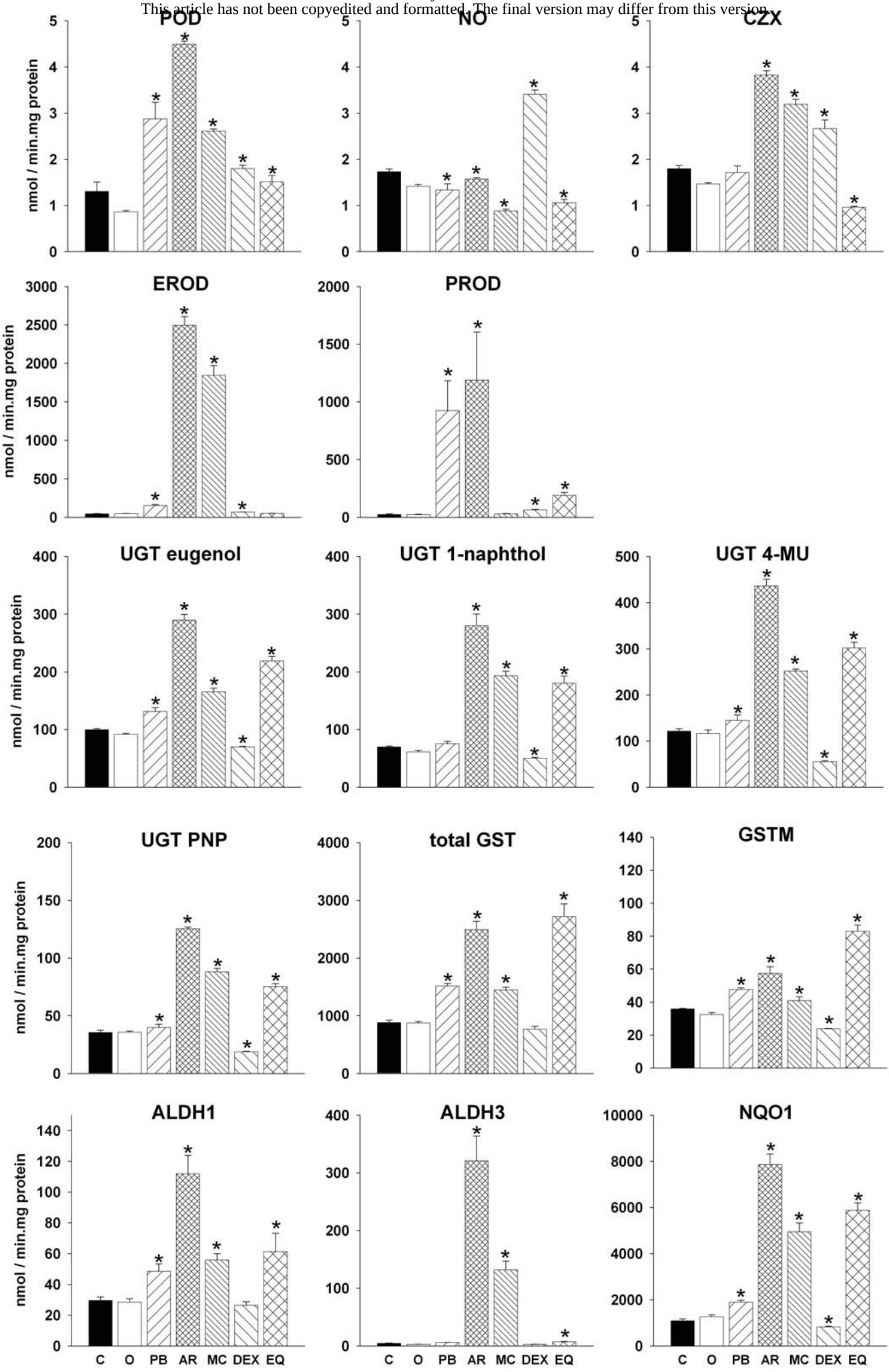
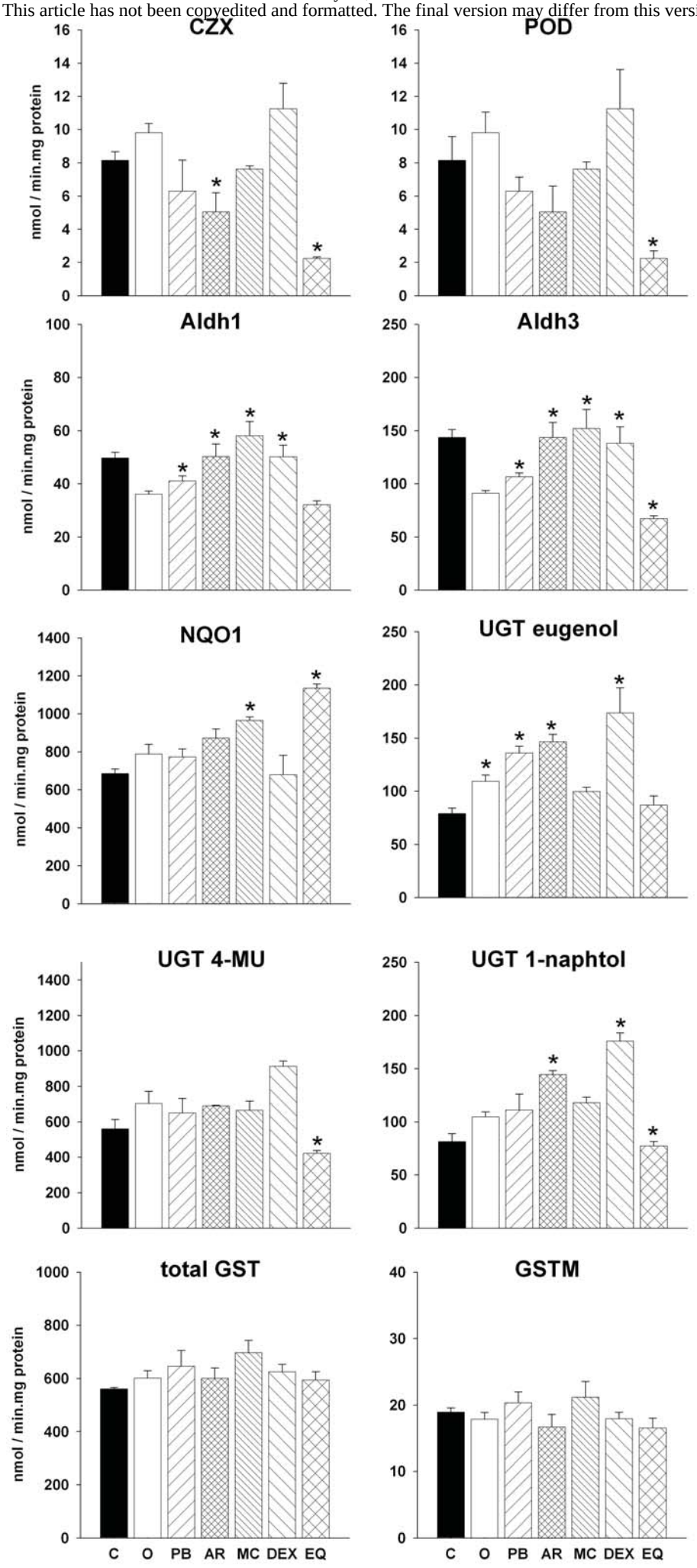


**Figure 10**



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Drug Metabolism and Disposition

**Supplemental Data Table 1:** Primer sequences used for the absolute quantification of XME, transporter and transcription factor mRNA.

| Gene | NCBI Accession number | | Primer sequences 5'→3' | Tm (°C) | Amplified fragment length (bp) |
|---|---|---|---|---|---|
| **AhR** | NM_013149 | Sense | CCACAGCCAGCGGTCTACTAC | 59,7 | 182 |
| | | Anti-sense | GCTCCGTCTTTCCCTTTCTTGTTC | 60,0 | |
| **ALDH1a1** | NM_022407 | Sense | TTTCCTCCTGGCGTGGTGAAC | 60,1 | 283 |
| | | Anti-sense | GCAATGTCCAAGTCGGCATCTG | 59,9 | |
| **ALDH3a1** | NM_031972 | Sense | CTGCTGGAGAAGGCTGTGTAGG | 60,1 | 119 |
| | | Anti-sense | CAACTGCTGGATTCGGAACTGC | 59,8 | |
| **AOH3 (AOX2)** | AY665588 | Sense | AGTTACTGGAGGAAGAGAGGCATTG | 60,1 | 112 |
| | | Anti-sense | AGCCGTCGGTGTAGATGTGAAC | 60,1 | |
| **CAR (NR1i3)** | NM_022941 | Sense | CATTTCCATGCCCTGACTTGTG | 57,6 | 133 |
| | | Anti-sense | AGGCTGGACAATGGCGTCTC | 59,5 | |
| **CYP1A1** | NM_012540 | Sense | TAATGTGTCACCAGGCGAGAAGG | 60,1 | 115 |
| | | Anti-sense | GAGATGCTGAGGACCAGAAGACC | 60,0 | |
| **CYP1A2** | NM_012541 | Sense | GTGGAATCGGTGGCTAATGTCATC | 59,7 | 103 |
| | | Anti-sense | CAAAGTCCTTGCTGCTCTTCACG | 60,1 | |
| **CYP2A3** | NM_012542 | Sense | AAGGACTGGCAAGGATGGAACTC | 60,1 | 168 |
| | | Anti-sense | TGCTGGCTCAACGGGACAAG | 60 | |
| **CYP2E1** | NM_031543 | Sense | TCTGCTCCTGTCTGCTATTCTGC | 60,0 | 101 |
| | | Anti-sense | GGATACTGCCAAAGCCAACTGTG | 59,9 | |
| **CYP2F4** | NM_019303 | Sense | GACCCAAGCCTCTCCCAATCC | 59,6 | 109 |
| | | Anti-sense | TACACCGTGAACACTGACCCATAG | 60 | |
| **CYP2G1** | M33296 | Sense | GAAGTCGCTCTCATTGCTGTGC | 59,9 | 94 |
| | | Anti-sense | ACTGTGGCTAGAACCCTGCTTG | 59,9 | |
| **CYP3A9** | NM_147206 | Sense | TGTACCTGCCCTTTGGGAATGG | 59,9 | 166 |
| | | Anti-sense | TCTGGTTGAAGAAGTCCTTGTTTGC | 59,9 | |
| **EPHX1** | NM_001034090 | Sense | CTTACACATCCAAGCCACCAAGC | 59,9 | 136 |
| | | Anti-sense | GCCTCCATCCTCCAGTTCACG | 60 | |
| **GR** | M14053 | Sense | TCCGTGTGTGGCTTCATACCG | 59,9 | 79 |
| | | Anti-sense | TCATACAGGCATGTCACCGAGTC | 59,9 | |
| **GSTA3** | NM_031509 | Sense | TACCCACAAAGCCAGCCTTCG | 60,1 | 145 |
| | | Anti-sense | GCATCCCAGCAATTCCTCATCAG | 59,5 | |
| **GSTM1 (GST mu1)** | NM_017014 | Sense | GTTACAACCCCGACTTTGAGAAGC | 60,0 | 89 |
| | | Anti-sense | CTTGCCCAGGAACTCAGAGTAGAG | 60,0 | |
| **MDR1a (Abcb1)** | NM_133401 | Sense | TCAGTGTTGGGCAGGCATCTC | 59,9 | 144 |
| | | Anti-sense | TTCCTTGTATGTTGTCGGGTTTGTG | 60,0 | |
| **mGST** | NM_134349 | Sense | ATCGTTCCCTTTCTCGGTATCGG | 59,8 | 206 |
| | | Anti-sense | CTCCTGAGCAGCCTGTAAGCC | 60,0 | |
| **MRP1 (Abcc1)** | NM_022281 | Sense | CACACTGAATGGCATCACCTTCG | 60,0 | 83 |
| | | Anti-sense | GAGATGACTTCCCACAGCCTACC | 60,0 | |
| **MRP3 (Abcc3)** | NM_080581 | Sense | GCGTTCACAACTCACCATCATCC | 59,9 | 150 |
| | | Anti-sense | GGCTGGCTGCTCACAAATGC | 59,8 | |
| **MRP5 (Abcc5)** | NM_053924 | Sense | GGCAGTGAAGTCGGAGGAAGG | 59,9 | 132 |
| | | Anti-sense | AGGACCATGATGACCAGGAAAGC | 60,1 | |
| **Nrf2** | NM_031789.1 | Sense | TGCCTTCCTCTGCTGCCATTAG | 60,1 | 173 |
| | | Anti-sense | GGATGCTCGGCTGGGACTTG | 60,2 | |
| **OAT6 (Slc22a20)** | NM_001106327 | Sense | GTTTGCTGTGTCTGCTCCTTTCC | 60,0 | 147 |
| | | Anti-sense | TCCGCCTTCCTGCCATTCATC | 60,0 | |
| **PPARa** | NM_013196 | Sense | TCTTCACGATGCTGTCCTCCTTG | 60,2 | 116 |
| | | Anti-sense | CATGATGTCGCAGAATGGCTTCC | 60,1 | |
| **PXR** | NM_052980 | Sense | ATCCCCACCTCAGAAGACAAAGC | 60,1 | 141 |
| | | Anti-sense | TTCCAGTGACGCACAGAACCC | 60,0 | |
| **NQO1** | M58495 | Sense | AAAGGCGATTTCCCACATCTGAC | 59,1 | 125 |
| | | Anti-sense | AATTCAACTGCTCCCTGTCCAAG | 58,7 | |
| **SULT1C1** | NM_031732 | Sense | GAAAGGGATGCCTGGAGACTGG | 60,1 | 142 |
| | | Anti-sense | GGGCTTCTCTCTTCCTCACTGC | 60,1 | |
| **UGT1A6** | AF461737 | Sense | CTGGGTGTGTGAGAGGGAGTAG | 58,9 | 92 |
| | | Anti-sense | GAAGAAGGCAAGCCATCCTAGTG | 58,9 | |
| **UGT2A1** | NM_022228 | Sense | TCCTGCAATCTTGTCAGAACTC | 55,8 | 147 |
| | | Anti-sense | GTGGGTCTTCCTAAGGCTTTAC | 55,8 | |
| **UGT2B1** | M13506 | Sense | TGTTGGTATTCCCTTGTTTGC | 54,2 | 256 |
| | | Anti-sense | GTGCTTGGCTCCTTTGTGACG | 59,6 | |
| **T7 (pDrive Cloning Vector)** | | Sense | GTAATACGACTCACTATAG | - | - |

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**Supplemental Data Table 2**: Substrate probes and incubation conditions used to assess phase I and phase II enzyme activities in rat liver and olfactory mucosa (OM).

| Activity* | Organ | Substrate | Substrate concentration | Protein concentration (mg/ml) | Reference |
|---|---|---|---|---|---|
| **POD (CYP1A)** | Liver<br>OM | Phenacetin | 4 mM<br>500 µM | 0.5<br>0.05 | Kobayashi et al, 1999. Drug Metab. Disp., 27, 860-865. |
| **CZX (CYP2E1)** | Liver<br>OM | Chlorzoxazone | 1 mM<br>1 mM | 0.5<br>0.25 | Chittur and Tracy, 1997. J. Chromatogr B Biomed Sci Appl.,693, 479-483. |
| **EROD (CYP1A)** | Liver | Ethoxyresorufin | 5 µM | 0.25 | Burke et al., 1985. Biochem. Pharmacol., 34, 3337-3345. |
| **PROD (CYP2B)** | Liver | Pentoxyresorufin | 10 µM | 0.25 | Burke et al., 1985. Biochem. Pharmacol., 34, 3337-3345. |
| **NO (CYP3A)** | Liver | Nifedipine | 200 µM | 1 | Guengerich et al, 1986. J. Biol. Chem., 261, 5051-5060. |
| **UGT** | Liver<br>OM<br>Liver<br>OM<br>Liver<br>OM<br>Liver<br>OM | Paranitrophenol<br><br>1-Naphthol<br><br>Eugenol<br><br>4-methylumbelliferone | 200 µM<br>200 µm<br>1 mM<br>1 mM<br>1 mM<br>400 µM<br>1 mM<br>1 mM | 0.1<br>1<br>0.04<br>0.04<br>0.02<br>0.02<br>0.1<br>0.1 | Mulder and Vandoom, 1975. Biochem J 151: 131-140.<br><br><br><br><br><br>Lilienblum et al., 1982. Biochem Pharmacol 31:907-913. |
| **total GST** | Liver<br>OM | 1-chloro-2,4-dinitrobenzene | 2 mM<br>2 mM | 0.2<br>0.6 | Habig et al., 1974. J. Biol. Chem., 249, 7130-7139. |
| **GSTM** | Liver | 1,2-dichloro-4-nitrobenzene | 2 mM | 0.2 | Habig et al., 1974. J. Biol. Chem., 249, 7130-7139. |
| **NQO1** | Liver<br>OM | Menadione | 10 µM<br>20 µM | 0.2<br>0.6 | Lind et al, 1990. Methods Enzymol., 186, 287-301. |
| **ALDH1** | Liver<br>OM | Propionaldehyde | 5 mM<br>40 mM | 0.15<br>0.15 | Vasiliou and Marselos, 1989. Pharmacol. Toxicol., 64, 39-42. |
| **ALDH3** | Liver<br>OM | Benzaldehyde | 5 mM<br>2.5 mM | 0.7<br>0.15 | Vasiliou and Marselos, 1989. Pharmacol. Toxicol., 64, 39-42. |

Abbreviations : ALDH; aldehyde dehydrogenase; CZX, chlorzoxazone hydroxylase; EROD, ethoxyresorufin-*O*-deethylase; GST, glutathione *S*-transferase; NO, nifedipine oxidase; NQO1, quinone reductase; POD, phenacetin-O-dethylase; PROD pentoxyresorufin-*O*-dealkylase; UGT, UDP glucuronosyltransferase.
*The main CYP isoforms associated with the activities are mentioned parenthetically. Other CYP isoforms can be involved, namely in OM (see Minn et al., 2005).