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Challenging the Specificity of Chlorzoxazone and 4-Nitrophenol as Marker Substrates for CYP2E1 Activity in Supersomes TM

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Abstract

Cytochrome P450 2E1 (CYP2E1) plays a pivotal role in the metabolism and detoxification of low molecular-weight xenobiotics, as well as in their bioactivation into toxic and DNA-reactive intermediates. Prominent examples include drugs and chemicals like acetaminophen and N-nitrosamines. Chlorzoxazone and 4-nitrophenol are widely used as probe substrates to assess CYP2E1 activity, however, their specificity remains controversial. This study aimed to reevaluate the selectivity of chlorzoxazone and 4-nitrophenol toward CYP2E1 using recombinant Supersomes™ expressing individual human CYP isoforms. Incubations were performed with chlorzoxazone and 4-nitrophenol at 100 and 500 µM, followed by quantification of 6-hydroxychlorzoxazone and 4-nitrocatechol via UPLC-MS/MS.

Chlorzoxazone turnover was detected across nearly all major liver CYP isoforms, with CYP1A1 showing the highest contribution (39%), followed by CYP2D6 (14%), CYP1A2 (10%), and CYP2E1 (10%). Similarly, 4-nitrophenol hydroxylation was not limited to CYP2E1, with significant contributions from CYP1A2 (20%), CYP2A6 (19%), and CYP2D6 (16%), while CYP2E1 accounted for 26% of total activity.

In conclusion, our results underscore the limited selectivity of chlorzoxazone and 4-nitrophenol and highlight the need for more specific substrates for accurate CYP2E1 enzyme characterization.

Introduction

CYP2E1 is a member of the Cytochrome P450 (CYP) family, a large group of enzymes involved in the metabolism of xenobiotics, mainly through oxidation of low-molecular weight molecules, such as benzene, styrene and chloroform (Yamamura et al., 2015). In addition, CYP2E1 is known to mediate the toxification of various drugs and chemicals, including the conversion of acetaminophen to the reactive metabolite N-acetylbenzo-*p*-quinoneimine, and the activation of N-nitrosamines (Carlsson et al., 2025; Gao et al., 2017a; Guengerich et al., 1991). These metabolic reactions are clinically relevant, as they can lead to drug-induced liver injury following acetaminophen overdose or an increased cancer risk due to the formation of mutagenic metabolites from N-nitrosamines (Fahrer & Christmann, 2023; Gao et al., 2017b; Li et al., 2023; McGill & Hinson, 2020; Ye et al., 2012). As the functional expression of CYP2E1 influences the metabolism and toxicity of drugs primarily oxidized by this enzyme, accurate quantification of CYP2E1 activity is essential for clinical and toxicological evaluations.

The hydroxylation of chlorzoxazone and 4-nitrophenol has traditionally been used as a marker substrate reaction to assess CYP2E1 activity (Hohmann et al., 2019; Koop et al., 1989; Peter et al., 1990). While previous studies have supported the use of chlorzoxazone and 4-nitrophenol as CYP2E1-selective substrates in humans, these investigations often lack isolated enzyme systems (Lucas et al., 1996; Tassaneeyakul, Veronese, Birkett, & Miners, 1993). Subsequent work demonstrated that additional CYP isoforms contribute to their metabolism, with CYP1A1 involved in the formation of 6-hydroxychlorzoxazone (Carriere et al., 1993a), and CYP3A isoforms mediating 4-nitrophenol hydroxylation in rats (Zerilli et al., 1998). Given these uncertainties, this study was designed to systematically evaluate the contribution of major hepatic CYP enzymes using recombinant

Supersomes™ (recombinant CYP enzymes) as a standardized tool. By demonstrating that multiple CYP enzymes are involved in the metabolism of these compounds, this study challenges the long-standing assumption of their selectivity and underscores the need for more reliable and specific markers for CYP2E1 activity.

Materials and Methods

Gentest Supersomes™, prepared from transfected insect cells, were purchased from Discovery Life Sciences (Woburn, MA, USA). Each Supersome™ preparation consisted of a single human CYP isoform, co-expressed with human P450 reductase. The following CYP isoforms were used: CYP1A1 (Lot: 7104001, 2408159), CYP1A2 (Lot: 8156001, 2301249), CYP1B1 (Lot: 9155001, 2304109), CYP2A6 + b5 (Lot: 114001, 2311105), CYP2B6 + b5 (Lot: 8120001, 2304112), CYP2C8 + b5 (Lot: 8080001, 2306131), CYP2C9*1 + b5 (Lot: 8114001, 2310201), CYP2C18 (Lot: 1054001, 2401110), CYP2C19 + b5 (Lot: 8121001, 22111294), CYP2D6*1 (Lot: 8045001, 2309291), CYP2E1 + b5 (Lot: 7325004, 2402028), CYP2J2 + b5 (Lot: 1060001, 2211291), CYP3A4 (Lot: 9023001, 2211038), CYP3A4 + b5 (Lot: 1047002, 2304045), CYP3A5 + b5 (Lot: 307004, 2305086) and CYP4A11 (Lot: 9011003, 2409260). An insect cell control Supersome™ (Lot: 90001, 2312016) with no detectable CYP content was included. Chlorzoxazone, 6-hydroxychlorzoxazone, 4-nitrophenol, and NADPH were purchased from Sigma-Aldrich (Steinheim am Albuch, Germany). Salicylamide and 4-nitrocatechol were purchased from TCI (Tokyo, Japan), and telmisartan from Boehringer Ingelheim (Ingelheim am Rhein, Germany).

Incubation mixtures contained 100 mM Tris buffer (pH 7,4), 5 mM $MgCl_2$, 1 mM NADPH and 10 pmol/ml of the respective Supersome™. Chlorzoxazone and 4-nitrophenol were administered at 100 and 500 µM. Solvent content was 0.5%. Reactions were initiated by the addition of NADPH and incubated at 37°C for 20 min. Incubations were stopped by heat treatment at 80°C for 2 min.

Following incubation, probes were added to acidified acetonitrile/water quench solutions. Concentration of the metabolites 6-hydroxychlorzoxazone and 4-nitrocatechol were quantified using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). For each sample 10 µl were injected into an UltiMate 3000 high-performance liquid chromatography (Thermo Fisher Scientific, Dreieich, Germany) and analyzed on a YMC Triart C18 30 x 2 mm, 1.9 µm column (YMC Europe, Dinslaken, Germany). Samples were separated using a gradient of 0.1% formic acid in water and 0.1% formic acid in acetonitrile at a flow rate of 500 µl/min. Analytes were detected using a Triple Quad™ 6500 system (AB Sciex, Marlborough, MA, USA). Both 6-hydroxychlorzoxazone and 4-nitrocatechol were analyzed using a negative ionization mode. Ion transitions [m/z] were as follows: 183.8 to 119.9 for 6-hydroxychlorzoxazone, 513.2 to 287.2 for telmisartan, 154.0 to 123.9 for 4-nitrocatechol and 136.0 to 118.0 for salicylamide. Peak areas of the samples were integrated using the software Analyst 1.7.2 (AB Sciex) with $1/x^2$ weighting. Calibration curves ranged from 0.15–4.50 µM and 0.05–1.0 µM for 6-hydroxychlorzoxazone and 4-nitrocatechol, respectively. Quality control samples (0.23, 0.75 and 3.00 µM for 6-hydrochlozroxazone, 0.175, 0.500 and 0.950 for 4-nitrocatechol) were used to ensure analytical accuracy. To compensate for instrumental variability and matrix effects, telmisartan (2.0 µM) and

salicylamide (0.375 µg/ml) were added as internal standards for 6-hydroxychlorzoxazone and 4-nitrocatechol, respectively.

Experiments were conducted in two independent experiments (N = 2), each in triplicates (n = 3).

Results and Discussion

Substrate specificity for CYP2E1 was assessed by measuring in situ metabolite formation rates of the two probe substrates chlorzoxazone and 4-nitrophenol, following incubation in Supersomes™ at concentrations of 100 and 500 µM.

The metabolite 6-hydroxychlorzoxazone (Fig. 1A) was detected in all Supersome™ preparations, except for CYP4A11, when incubated with 500 µM chlorzoxazone. Reducing the substrate concentration to 100 µM generally led to a decreased metabolite formation rate. Notably, no metabolite was detected in CYP2B6 and CYP3A4 (without b5) Supersomes™ at the lower concentration. The enzyme distribution (Fig. 1B) highlights CYP1A1 (39%) as the major contributor, followed by CYP2D6 (14%), CYP1A2 (10%), and CYP2E1 (10%).

The incubations with two concentrations of 4-nitrophenol showed significant activity across multiple hepatic CYP isoforms (Fig. 2A). The highest activity was observed in CYP2E1 Supersomes™ independent of the substrate concentration. Using 100 µM led to a decreased 4-nitrocatechol formation. Compared to the incubation with 500 µM, no metabolite was detected in CYP1B1 and CYP2C8. For the 500 µM incubation (Fig. 2B), the primary formation of the metabolite was observed in Supersomes™ expressing CYP2E1 (26%), CYP1A2 (21%), CYP2A6 (21%) and CYP2D6 (15%).

The 6-hydroxylation of chlorzoxazone is widely considered a selective marker for CYP2E1, but this is not supported by the results in this study. Shortly after chlorzoxazone was first proposed as a specific CYP2E1 substrate (Peter et al., 1990), additional studies further evaluated its selectivity. Carriere et al. reported that chlorzoxazone was hydroxylated not only by CYP2E1, but also by CYP1A1 and CYP3A4 in yeast expression systems (Carriere et al., 1993a). Ono et al. further demonstrated CYP1A2's contribution in HepG2 cells expressing individual CYP enzymes (Ono et al., 1995a). In a later study Carriere et al. showed that CYP1A1 had the highest 6-hydroxylation activity towards chlorzoxazone in human CYP enzymes expressed in yeast, yet the in vivo relevance of this finding was considered minimal by the authors, as CYP1A1 was regarded as being expressed only at very low levels in the liver (< 1 pmol/mg) (Carrière et al., 1996). However, more recent findings indicate that CYP1A1 exerts a greater influence in human liver than previously assumed. Induced CYP1A1 reaches similar activity levels as uninduced CYP1A2 in HepaRG cells and primary human hepatocytes (Hammer et al., 2021; Lenich et al., 2025). Given that CYP1A1 is readily induced by cigarette consumption via the aryl hydrocarbon receptor (Kroon, 2007; Lenich & Ruez, 2025a, 2025b), and considering that approximately 20 % of the global population smokes, this should be taken into consideration (WHO, 2025). In contrast to these studies Lucas et al.

demonstrated high selectivity of chlorzoxazone forCYP2E1 using purified CYP enzymes expressed in human B-lymphoblastoid cell lines (Lucas et al., 1999a). We could not confirm these results in our study, which might be due to differences in the expressing system. Furthermore, chlorzoxazone has been used as a selective probe to measure the activity of CYP2E1 in vivo (Ernstgård et al., 2004; Küttner et al., 2023).

Another proposed substrate for assessing CYP2E1 activity is 4-nitrophenol, as initially suggested by (Tassaneeyakul, Veronese, Birkett, Gonzalez, et al., 1993). However, subsequent findings challenge its specificity. Zerilli et al. (1997a) demonstrated that 4-nitrophenol hydroxylation is not restricted to CYP2E1 but also catalyzed by CYP3A4 in both rat and human microsomes, as well as in recombinant enzyme systems. Their work, which included a smaller panel of enzymes (CYP2A6, CYP2E1, CYP3A4 and CYP3A5), indicates that 4-nitrophenol might not be a selective substrate for CYP2E1. These results are consistent with the study's findings. Although CYP2E1 showed the highest activity in the present study, CYP2D6 contributed more strongly than CYP3A4, further supporting that multiple enzymes participate in the hydroxylation of 4-nitrophenol.

Most previous studies on the selectivity of substrates for CYP2E1 (Carriere et al., 1993b; Lucas et al., 1999b; Ono et al., 1995b) used purified CYP enzymes expressed in yeast, HepG2 or B-lymphoblastoid cell lines (except for CYP3A4 and CYP3A5 in the study of Zerilli et al. (1997b) where these enzymes were expressed in baculovirus insect cells). In contrast, our study applied a commercially available Supersome™ system, which offers highly standardized enzyme production, minimal batch to batch variation, and high-catalytic activity (Gentest® Supersomes®, 2025). These properties align with FDA requirements for reaction phenotyping (FDA, 2024) and support the suitability of this system for robust and reproducible assessment of CYP-specific metabolism. In addition, metabolite formation was quantified using UPLC-MS/MS, which compared to HPLC alone provides greater sensitivity and selectivity for analyte identification and quantification (Churchwell et al., 2005) .

In conclusion, the present study demonstrates that chlorzoxazone and 4-nitrophenol are not selectively metabolized by CYP2E1 but rather by multiple CYP isoforms. Although these compounds have been widely used as probe substrates for assessing CYP2E1 activity, their limited specificity significantly restricts their utility in accurately quantifying functional enzyme expression. While chlorzoxazone and 4-nitrophenol may still be applicable in certain experimental contexts, such as in systems with dominant CYP2E1 expression or in combination with selective inhibitors, the identification and validation of more selective probe substrates is suggested for precise characterization of CYP2E1 activity.

Abbreviations

CYP = Cytochrome P450

UPLC-MS/MS =Ultra-performance liquid chromatography- tandem mass spectrometry

Declarations

Declaration of interests

The authors declare no conflict of interest.

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Author Contribution

Anna Hellmann was responsible for conducting the experiments and drafting the manuscript. The study was designed by Anna Hellman, Ann-Kathrin Lenich, Stephanie Ruez, and Michelle Schaefer. Ann-Kathrin Lenich, Stephanie Ruez, Jasmin Lott and Jörg Fahrer critically reviewed the manuscript and provided valuable input. Michelle Schaefer is designated as senior author due to her overall supervision, conception of the study, substantial contribution to data interpretation, and final manuscript oversight and approval.

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Data Availability

The authors declare that all the data supporting the findings of this study are available within the paper.

References

1. Carlsson MJ, Herzog N, Felske C, Ackermann G, Regier A, Wittmann S, Cereijo RF, Sturla SJ, Küpper J-H, Fahrer J (2025) The DNA Repair Protein MGMT Protects against the Genotoxicity of N-Nitrosodimethylamine, but Not N-Nitrosodiethanolamine and N-Nitrosomethylaniline, in Human HepG2 Liver Cells with CYP2E1 Expression. Chem Res Toxicol 38(6):1134–1146. https://doi.org/10.1021/acs.chemrestox.5c00133

2. Carrière V, Berthou F, Baird S, Belloc C, Beaune P, de Waziers I (1996) Human cytochrome from genotype to phenotype. Pharmacogenetics 6(3):203–211. https://doi.org/10.1097/00008571-199606000-00002
3. Carriere V, Goasduff T, Ratanasavanh D, Morel F, Gautier JC, Guillouzo A, Beaune P, Berthou F (1993a) Both cytochromes P450 2E1 and 1A1 are involved in the metabolism of chlorzoxazone. Chem Res Toxicol 6(6):852–857. https://doi.org/10.1021/tx00036a015
4. Carriere V, Goasduff T, Ratanasavanh D, Morel F, Gautier JC, Guillouzo A, Beaune P, Berthou F (1993b) Both cytochromes P450 2E1 and 1A1 are involved in the metabolism of chlorzoxazone. Chem Res Toxicol 6(6):852–857. https://doi.org/10.1021/tx00036a015
5. Churchwell MI, Twaddle NC, Meeker LR, Doerge DR (2005) Improving LC–MS sensitivity through increases in chromatographic performance: Comparisons of UPLC–ES/MS/MS to HPLC–ES/MS/MS. J Chromatogr B 825(2):134–143. https://doi.org/10.1016/j.jchromb.2005.05.037
6. Ernstgård L, Warholm M, Johanson G (2004) Robustness of chlorzoxazone as an in vivo measure of cytochrome P450 2E1 activity. Br J Clin Pharmacol 58(2):190–200. https://doi.org/10.1111/j.1365-2125.2004.02132.x
7. Fahrer J, Christmann M (2023) DNA Alkylation Damage by Nitrosamines and Relevant DNA Repair Pathways. Int J Mol Sci 24(5):4684. https://doi.org/10.3390/ijms24054684
8. FDA (2024) *M12 Drug Interaction Studies*
9. Gao J, Wang G-J, Wang Z, Gao N, Li J, Zhang Y-F, Zhou J, Zhang H-X, Wen Q, Jin H, Qiao H-L (2017a) High CYP2E1 activity correlates with hepatofibrogenesis induced by nitrosamines. Oncotarget 8(68):112199–112210. https://doi.org/10.18632/oncotarget.22937
10. Gao J, Wang G-J, Wang Z, Gao N, Li J, Zhang Y-F, Zhou J, Zhang H-X, Wen Q, Jin H, Qiao H-L (2017b) High CYP2E1 activity correlates with hepatofibrogenesis induced by nitrosamines. Oncotarget 8(68):112199–112210. https://doi.org/10.18632/oncotarget.22937
11. Gentest® Supersomes® (2025) https://go.dls.com/l/997171/2024-05-24/2dntk/997171/1716556294ZkWo1CMv/DLS_Supersomes_Brochure_52424.pdf
12. Guengerich FP, Kim DH, Iwasaki M (1991) Role of human cytochrome P-450 IIE1 in the oxidation of many low molecular weight cancer suspects. Chem Res Toxicol 4(2):168–179. https://doi.org/10.1021/tx00020a008
13. Hammer H, Schmidt F, Marx-Stoelting P, Pötz O, Braeuning A (2021) Cross-species analysis of hepatic cytochrome P450 and transport protein expression. Arch Toxicol 95(1):117–133. https://doi.org/10.1007/s00204-020-02939-4
14. Hohmann N, Blank A, Burhenne J, Suzuki Y, Mikus G, Haefeli WE (2019) Simultaneous phenotyping of CYP2E1 and CYP3A using oral chlorzoxazone and midazolam microdoses. Br J Clin Pharmacol 85(10):2310–2320. https://doi.org/10.1111/bcp.14040
15. Koop DR, Carmen, TIERNEY DJ (1989) THE UTILITY OF pNITROPHENOL HYDROXYLATION IN PWIIE1. Drug Metab Rev 20(2–4):541–551. https://doi.org/10.3109/03602538909103560

16. Kroon LA (2007) Drug interactions with smoking. Am J Health-System Pharm 64(18):1917–1921. https://doi.org/10.2146/ajhp060414
17. Küttner J, Grzegorzewski J, Tautenhahn H-M, König M (2023) A physiologically based pharmacokinetic model for CYP2E1 phenotyping via chlorzoxazone. bioRxiv. 2023.04.12.536571 https://doi.org/10.1101/2023.04.12.536571
18. Lenich A-K, Nilles J, Scherer V, Li F, Ruez S (2025) Assessing granisetron as a specific CYP1A1 substrate in primary human hepatocytes: A comprehensive evaluation for drug development studies. Drug Metab Dispos 53(4):100064. https://doi.org/10.1016/j.dmd.2025.100064
19. Lenich A-K, Ruez S (2025a) Evaluating CYP enzyme induction by cigarette smoking: a new in vitro approach using primary human hepatocytes. Arch Toxicol 1–18. https://doi.org/10.1007/s00204-025-04135-8
20. Lenich A-K, Ruez S (2025b) Insights from a novel in vitro cigarette smoke induction model: comparison of 24 primary human hepatocyte donors of diverse demographics and multiple sources. Arch Toxicol 1–7. https://doi.org/10.1007/s00204-025-04238-2
21. Li X, Ni J, Chen L (2023) Advances in the study of acetaminophen-induced liver injury. Front Pharmacol 14:1239395. https://doi.org/10.3389/fphar.2023.1239395
22. Lucas D, Ferrara R, Gonzalez E, Bodenez P, Albores A, Manno M, Berthou F (1999a) Chlorzoxazone, a selective probe for phenotyping CYP2E1 in humans. Pharmacogenetics 9(3):377–388. https://doi.org/10.1097/00008571-199906000-00013
23. Lucas D, Ferrara R, Gonzalez E, Bodenez P, Albores A, Manno M, Berthou F (1999b) Chlorzoxazone, a selective probe for phenotyping CYP2E1 in humans. Pharmacogenetics 9(3):377–388. https://doi.org/10.1097/00008571-199906000-00013
24. Lucas D, Menez J-F, Berthou F (1996) [12] Chlorzoxazone: An in vitro and in vivo substrate probe for liver CYP2E1. Methods Enzymol 272:115–123. https://doi.org/10.1016/s0076-6879(96)72014-1
25. McGill MR, Hinson JA (2020) The development and hepatotoxicity of acetaminophen: reviewing over a century of progress. Drug Metab Rev 52(4):472–500. https://doi.org/10.1080/03602532.2020.1832112
26. Ono S, Hatanaka T, Hotta H, Tsutsui M, Satoh T, Gonzalez FJ (1995a) Chlorzoxazone is metabolized by human CYP1A2 as well as by human CYP2E1. Pharmacogenetics 5(3):143–150. https://doi.org/10.1097/00008571-199506000-00002
27. Ono S, Hatanaka T, Hotta H, Tsutsui M, Satoh T, Gonzalez FJ (1995b) Chlorzoxazone is metabolized by human CYP1A2 as well as by human CYP2E1. Pharmacogenetics 5(3):143–150. https://doi.org/10.1097/00008571-199506000-00002
28. Peter R, Boecker R, Beaune PH, Iwasaki M, Guengerich FP, Yang CS (1990) Hydroxylation of chlorzoxazone as a specific probe for human liver cytochrome P-450IIE1. Chem Res Toxicol 3(6):566–573. https://doi.org/10.1021/tx00018a012
29. Tassaneeyakul W, Veronese ME, Birkett DJ, Gonzalez FJ, Miners JO (1993) Validation of 4-nitrophenol as an in vitro substrate probe for human liver CYP2E1 using cDNA expression and

microsomal kinetic techniques. Biochem Pharmacol 46(11):1975–1981. https://doi.org/10.1016/0006-2952(93)90639-e

30. Tassaneeyakul W, Veronese ME, Birkett DJ, Miners JO (1993) High-performance liquid chromatographic assay for 4-nitrophenol hydroxylation, a putative cytochrome P-4502E1 activity, in human liver microsomes. J Chromatogr B Biomed Sci Appl 616(1):73–78. https://doi.org/10.1016/0378-4347(93)80473-h
31. WHO (2025) *WHO report on the global tobacco epidemic, 2025*
32. Yamamura Y, Koyama N, Umehara K (2015) Comprehensive kinetic analysis and influence of reaction components for chlorzoxazone 6-hydroxylation in human liver microsomes with CYP antibodies. Xenobiotica 45(4):353–360. https://doi.org/10.3109/00498254.2014.985760
33. Ye Q, Lian F, Chavez PRG, Chung J, Ling W, Qin H, Seitz HK, Wang X-D (2012) Cytochrome P450 2E1 inhibition prevents hepatic carcinogenesis induced by diethylnitrosamine in alcohol-fed rats. Hepatobiliary Surg Nutr 1(1):58–18. https://doi.org/10.3978/j.issn.2304-3881.2012.11.05
34. Zerilli A, Lucas D, Dreano Y, Picart D, Berthou F (1998) Effect of Pyrazole and Dexamethasone Administration on Cytochrome P450 2E1 and 3A Isoforms in Rat Liver and Kidney: Lack of Specificity of p-Nitrophenol as a Substrate of P450 2E1. Alcoholism: Clin Experimental Res 22(3):652–657. https://doi.org/10.1111/j.1530-0277.1998.tb04307.x
35. Zerilli A, Ratanasavanh D, Lucas D, Goasduff T, Dréano Y, Menard C, Picart D, Berthou F (1997a) Both Cytochromes P450 2E1 and 3A Are Involved in the O-Hydroxylation of p-Nitrophenol, a Catalytic Activity Known To Be Specific for P450 2E1. Chem Res Toxicol 10(10):1205–1212. https://doi.org/10.1021/tx970048z
36. Zerilli A, Ratanasavanh D, Lucas D, Goasduff T, Dréano Y, Menard C, Picart D, Berthou F (1997b) Both Cytochromes P450 2E1 and 3A Are Involved in the O-Hydroxylation of p-Nitrophenol, a Catalytic Activity Known To Be Specific for P450 2E1. Chem Res Toxicol 10(10):1205–1212. https://doi.org/10.1021/tx970048z

Figures

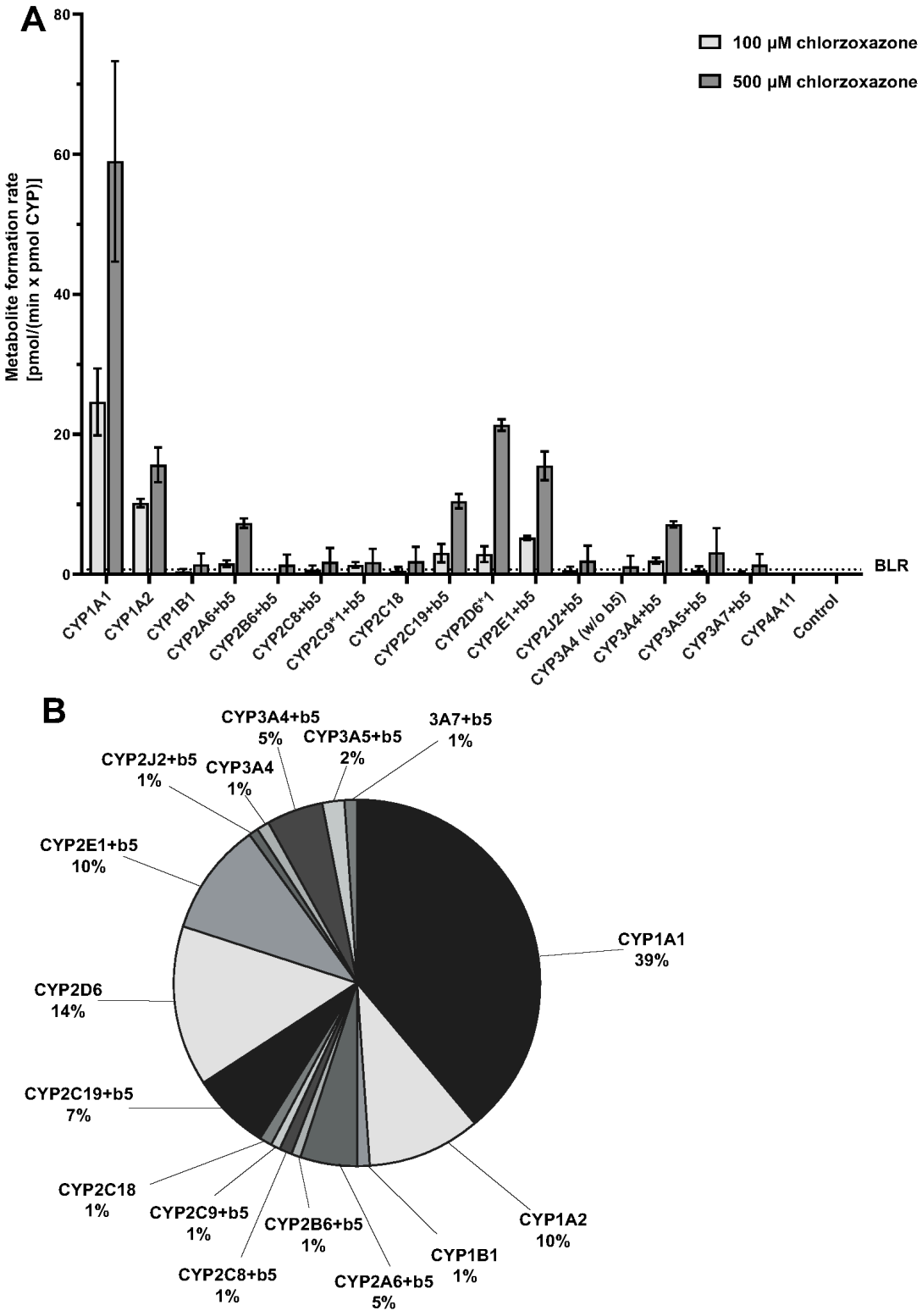

Figure 1

Incubation of chlorzoxazone in CYP Supersomes™

A: Metabolite formation rate in recombinant CYP Supersomes™ incubated with 100 µM and 500 µM chlorzoxazone. Shown is the mean ± SD of 2 independent experiments, each with 3 biological replicates. **B:** Relative contribution of CYP isoforms to 6-hydroxychlorzoxazone formation incubated with 500 µM chlorzoxazone. CYP1A1, CYP2D6, CYP1A2 and CYP2E1 showed the highest activity. Data represents the

mean of 2 independent experiments, each with 3 technical replicates. Metabolite formation rate was normalized to pmol/(min x pmol CYP). BLR (dotted line): Below linear range of the UPLC-MS/MS assay

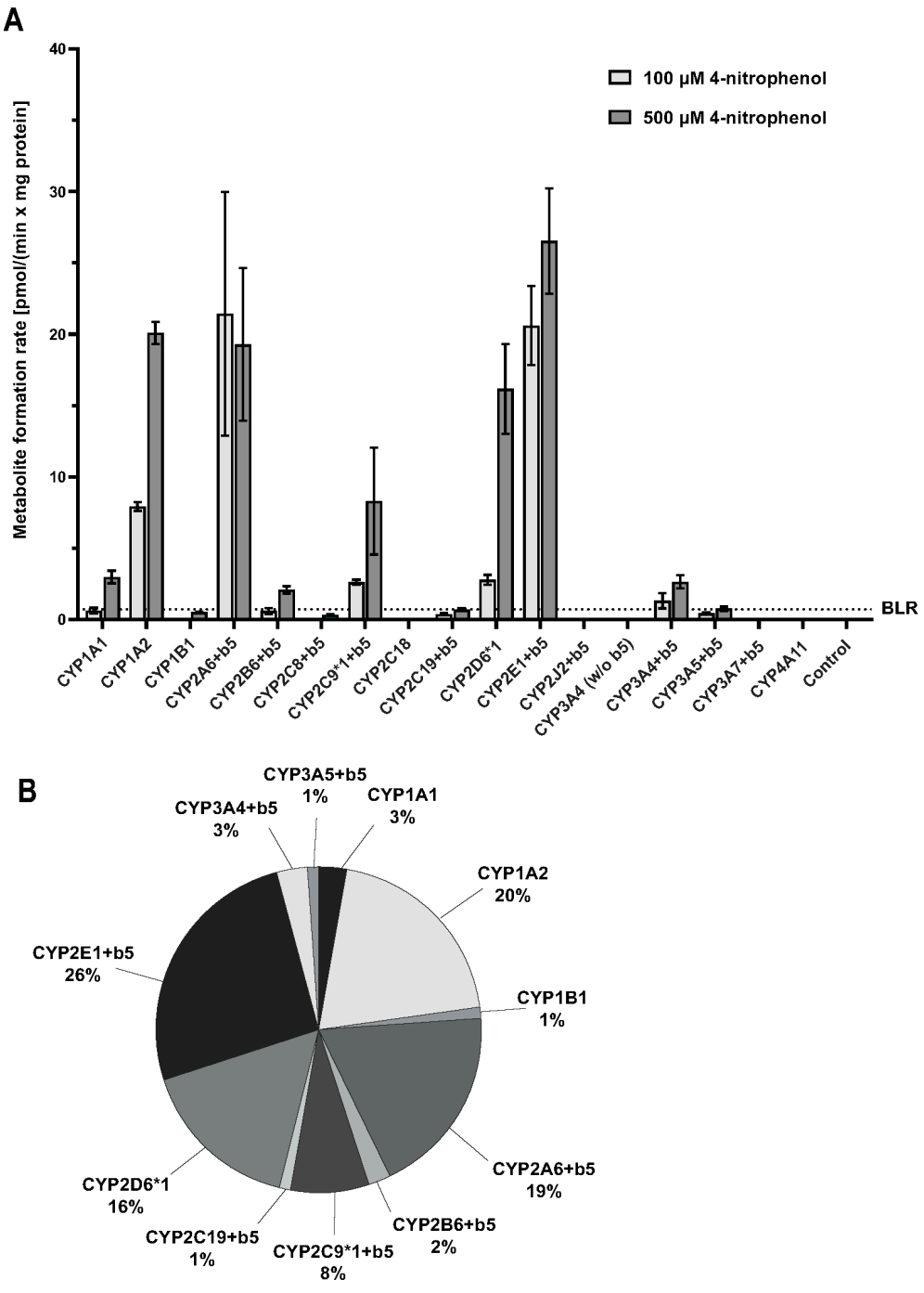

Figure 2

Incubation of 4-nitrophenol in CYP Supersomes™

A: Metabolite formation rate in recombinant CYP Supersomes™ incubated with 100 µM and 500 µM 4-

nitrophenol. Shown is the mean ± SD of 2 independent experiments, each with 3 technical replicates. **B:** Proportional contribution of CYP enzymes to the formation of 4-nitrocatechol incubated with 500 µM 4-nitrophenol. Data represents the mean 2 independent experiments, each with 3 biological replicates. Metabolite formation rate was normalized to pmol/(min x pmol CYP). BLR (dotted line): Below linear range of the UPLC-MS/MS assay