Phencyclidine Derivatives –
A new Class of Designer Drugs

Studies on the Metabolism and Toxicological Analysis

Dissertation
zur Erlangung des Grades
des Doktors der Naturwissenschaften
der Naturwissenschaftlich-Technischen Fakultät III -
Chemie, Pharmazie und Werkstoffwissenschaften
der Universität des Saarlandes

von
Christoph Sauer
Saarbrücken
2008

Tag des Kolloquiums:	18. 12. 2008
Dekan:	Univ.-Prof. Dr. U. Müller
Berichterstatter:	Univ.-Prof. Dr. Dr. h.c. H. H. Maurer
	Univ.-Prof. Dr. R. W. Hartmann

Die folgende Arbeit entstand unter der Anleitung von Herrn Professor Dr. Dr. h.c. Hans H. Maurer in der Abteilung Experimentelle und Klinische Toxikologie der Fachrichtung 2.4 Experimentelle und Klinische Pharmakologie und Toxikologie der Universität des Saarlandes in Homburg/Saar von März 2004 bis Dezember 2007.

Mein besonderer Dank gilt:

Herrn Prof. Dr. Dr. h.c. H. H. Maurer für die Aufnahme in seinen Arbeitskreis, die Vergabe eines interessanten und vielseitigen Dissertationsthemas, die Möglichkeit des selbstständigen Arbeitens, zahlreiche Diskussionen zu jeder Tages- und Nachtzeit und die Gelegenheit, im Rahmen weiterer Projekte wertvolle Erfahrungen zu sammeln,

Herrn Prof. Dr. Rolf Hartmann für die Übernahme des Koreferats,

Herrn Dr. Giselher Fritschi vom Hessischen Landeskriminalamt für die Bereitstellung der untersuchten Substanzen und für seine Unterstützung bei speziellen Fragestellungen,

Frau Andrea E. Schwaninger für die biotechnologische Synthese der Hauptmetaboliten im Rahmen ihrer Diplomarbeit,

Herrn Dr. Frank T. Peters für seine moralische Unterstützung und für seine wissenschaftliche Expertise und ständige Diskussionsbereitschaft,

Herrn Dr. Denis S. Theobald und Herrn Markus R. Meyer für die Einführung in die Isoenzym-Bestimmungen und die kritische Diskussion,

meinen Kollegen für die freundschaftliche Atmosphäre, gute Zusammenarbeit und die Unterstützung in schwierigen Situationen in der Dienstbereitschaft,

Herrn Armin Weber für seine ständige Einsatzbereitschaft, Wartung und Reparatur der Messgeräte sowie Rat bei technischen Fragestellungen,

Frau Gabriele Ulrich für gewissenhaft ausgeführte Laborarbeiten,

meinen Eltern, die mich in meinem Tun stets gefördert und unterstützt haben,

meinen Freunden und Bekannten, die in den letzten Jahren des Öfteren ohne mich auskommen mussten.

Meinen Eltern

TABLE OF CONTENTS

1 GENERAL PART 1

1.1 Introduction 1

1.1.1 The Phencyclidine-derived Designer Drugs 1

1.1.2 Drug Metabolizing Enzymes 2

1.1.3 Biotechnological Synthesis of Drug Metabolites 5

1.2 Aims and Scopes 7

2 ORIGINAL PUBLICATIONS 9

2.1 New designer drug *N*-(1-phenylcyclohexyl)-3-ethoxypropanamine (PCEPA): Studies on its metabolism and toxicological detection in rat urine using gas chromatographic/mass spectrometric techniques 9

2.2 Metabolism and toxicological detection of a new designer drug, *N*-(1-phenylcyclohexyl)propanamine, in rat urine using gas chromatography–mass spectrometry 11

2.3 New designer drugs *N*-(1-phenylcyclohexyl)-2-ethoxyethanamine (PCEEA) and *N*-(1-phenylcyclohexyl)-2-methoxyethanamine (PCMEA): Studies on their metabolism and toxicological detection in rat urine using gaschromatographic/mass spectrometric techniques 13

2.4 Metabolism and toxicological detection of the designer drug *N*-(1-phenylcyclohexyl)-3-methoxypropanamine (PCMPA) in rat urine using gas chromatography–mass spectrometry 15

2.5 Identification of Cytochrome P450 Enzymes Involved in the Metabolism of the Designer Drugs *N*-(1-Phenylcyclohexyl)-3-ethoxypropanamine and *N*-(1-Phenylcyclohexyl)-3-methoxypropanamine 17

2.6 Investigations on the cytochrome P450 (CYP) isoenzymes involved in the metabolism of the designer drugs *N*-(1-phenylcyclohexyl)2-ethoxyethan-amine and *N*-(1-phenylcyclohexyl)-2-methoxyethanamine 19

3 CONCLUSIONS 21

4 SUMMARY 23

5 REFERENCES 25

6 ZUSAMMENFASSUNG 29

1 GENERAL PART

1.1 INTRODUCTION

1.1.1 The Phencyclidine-derived Designer Drugs

In the late 1990s, a considerable number of new designer drugs from various (new) drug classes were seized in the German federal state of Hesse and the surrounding federal states. One of these substances was *N*-(1-phenylcyclohexyl)-propanamine (PCPr), a phencyclidine (PCP)-derived compound. Within a short period of time, further members of this new class of PCP-derived designer drugs appeared in the illicit drug market, namely *N*-(1-phenylcyclohexyl)-3-methoxypropanamine (PCMPA), *N*-(1-phenylcyclohexyl)-2-methoxyethanamine (PCMEA), and *N*-(1-phenylcyclohexyl)-2-ethoxyethanamine (PCEEA). The seized preparations contained either one compound alone or in mixtures with other designer drugs.[1] In expectance of its appearance on the illicit drug market, a further homologue, namely, *N*-(1-phenylcyclohexyl)-3-ethoxypropanamine (PCEPA), was synthesized as a reference substance for scientific purposes. Chemical structures of these compounds are shown in Fig. 1.

All compounds have in common the phenylcyclohexylamine structure being substituted with different side chains at the nitrogen. In case of PCPr the side chain consists of a propyl moiety and in the other compounds of combinations of a propyl or ethyl moiety carrying a methoxy or ethoxy moiety. Introducing new substituents allows the drug abusers to create “legal” products which are not scheduled as controlled substances.

Unfortunately, only little information on the pharmacological properties of these compounds is available.[2] Due to structural similarities, they might be assumed to be similar to those of PCP or ketamine, which both act as antagonists at *N*-methyl-D-aspartate (NMDA) receptors and have psychotomimetic as well as anesthetic properties.[3] Furthermore, it has been reported that (1-phenylcyclohexyl)-amine, a known metabolite of PCP and of the above-mentioned PCP-derived compounds,[4-7] produced a long-lasting dose-dependent effect on the efflux of dopamine in the rat.[8] A similar pharmacological profile of the above-mentioned PCP-derived compounds would clearly be in line with their abuse as designer drugs.

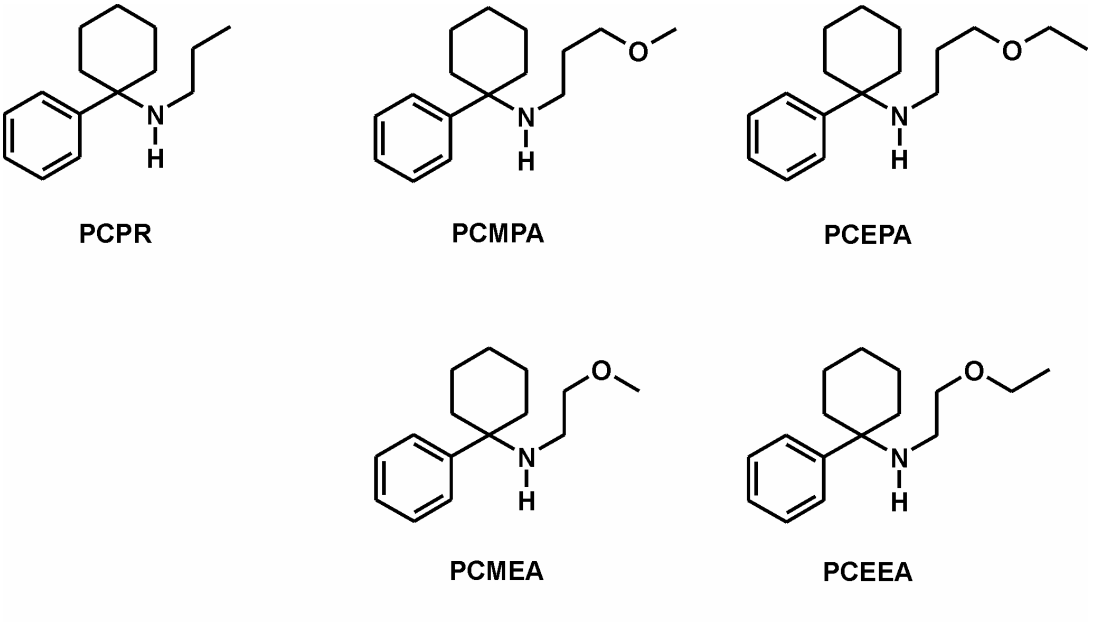

Fig. 1: Chemical structures of PCP-derived designer drugs.

So far, no information about the metabolism of the PCP-derived designer drugs is available in the literature. However, for developing toxicological screening procedures, especially in urine, it is a prerequisite to know the metabolism of the compounds in question, especially if they are excreted in urine primarily or even exclusively in form of metabolites. Furthermore, data on the metabolism are needed for toxicological risk assessment, because the metabolites may play a major role in the toxicity of a drug.

1.1.2 Drug Metabolizing Enzymes

A wide variety of enzymes catalyze the metabolism of drugs and other xenobiotics. Their metabolites can be more or less toxic than the parent compound. The metabolic profiling of drugs is, therefore, necessary to assess their effects and toxicity.[9] Cytochrome P450 (CYP) enzymes are responsible for oxidative and, to a minor extent, reductive metabolic transformations of drugs, environmental chemicals and natural compounds. CYPs are heme-containing, membrane-bound enzymes (“heme-thiolate proteins”) detected in both prokaryotes and eukaryotes. The enzymes were given their names because their complexes with carbon monoxide under reductive conditions show an absorbance maximum at about 450 nm. In mammals the enzymes can be identified in nearly every tissue, being most abundantly present in the liver. The CYP superfamily

has been classified in different families in accordance to the degree of homology of amino acid sequence in their protein structures. CYP enzymes having ≤ 40% homology in their amino acid sequence are classified in different families which are designated by Arabic numbers, for example, CYP1. Each family is further divided into subfamilies of enzymes. The enzymes within a mammalian subfamily have ≥ 55% sequence homology and are designated by capital letters, for example, CYP1A. An Arabic number is used for designating individual enzymes within a subfamily, for example, CYP1A2.[10] In humans, 18 CYP families with 43 subfamilies and 57 CYP isoenzymes are known so far, of which only 3 families with 7 subfamilies and 12 CYP isoenzymes are relevant for drug metabolism,[11] namely CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C18, CYP2C19, CYP2D6, CYP2E1, CYP3A4, and CYP3A5.[12]

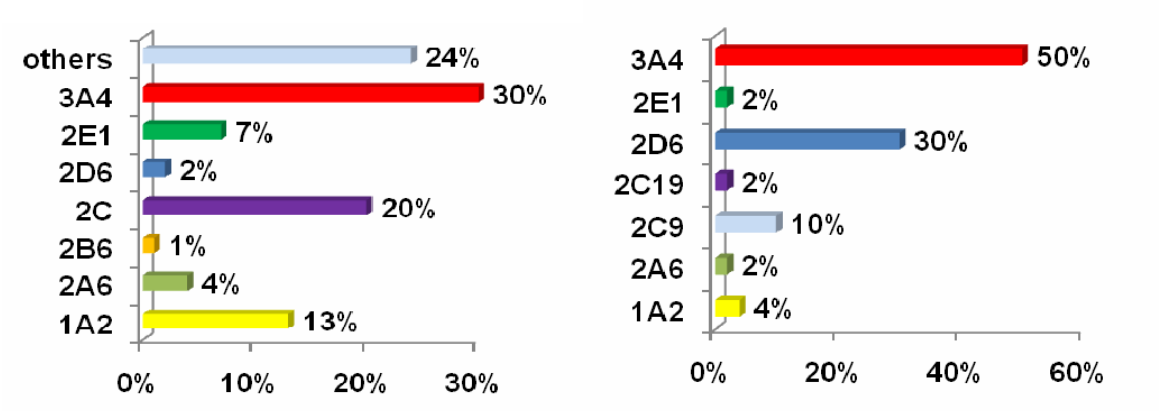

Fig. 2: Relative quantities of CYPs in human liver and their relevance in drug metabolism. Left side: human CYP-expression in the liver. Right side: involvement in xenobiotics metabolism.

The remainder is responsible for the transformation of endogenous biomolecules, for which reason they are called “housekeeping enzymes”. Fig. 2 illustrates the abundances of CYPs in human liver and their importance in xenobiotic metabolism. Some CYP genes are polymorphically expressed, leading to variabilities in patterns of drug metabolism.

Over its long history of more than 3.5 billion years, the CYP superfamily of enzymes has developed remarkable versatility. The primary catalytic function of CYPs was identified as transfer of one oxygen atom from molecular oxygen into various substrates (Fig. 3). A coenzyme, cytochrome P450 oxidoreductase (OR), is essential for CYP catalytic function, and cytochrome b_5 can stimulate catalytic activities of some enzymes.[13]

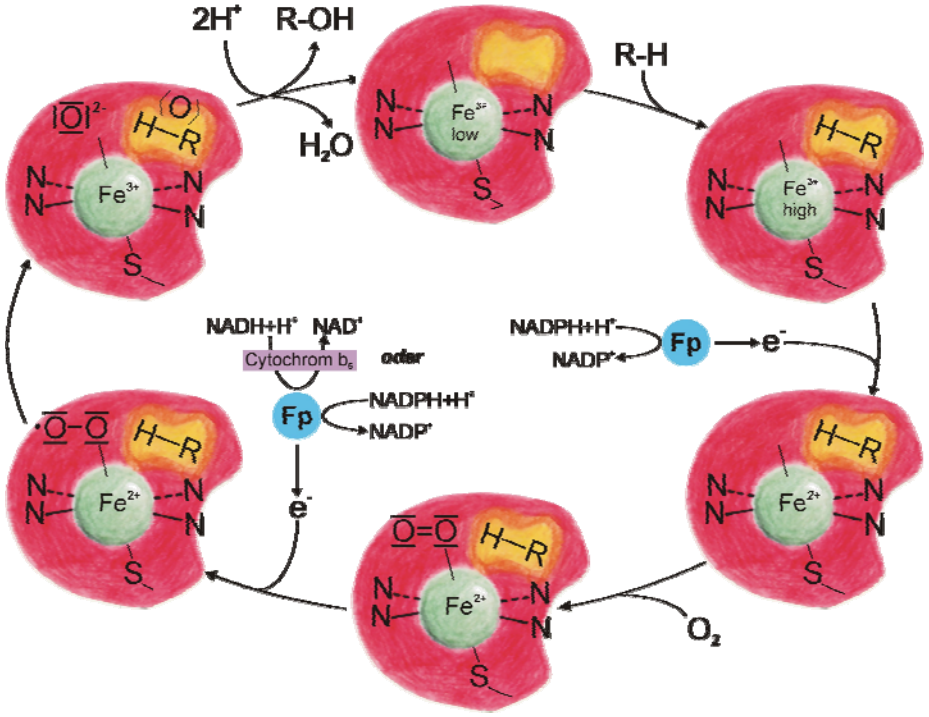

Fig. 3: The cytochrome P450 redox cycle.

Human liver derived enzyme preparations, e.g. human liver microsomes (HLM) contain a natural mixture of CYPs. Chemical inhibitors, immunochemical inhibitors, and/or correlation analyses with marker activities must be used to obtain information on which enzymes are performing specific biotransformations. In contrast, only a single active CYP is present in preparations of cDNA-expressed enzymes. Inhibitors and correlation analyses are not needed, because the mentioned assignments can be performed by direct incubation of the drug with a panel of individual CYPs. However, the balance of enzymes, present in vivo, is lost.[13] Bacteria, yeast, baculovirus and several mammalian cells have been used to produce a wide range of catalytically active CYPs. The baculovirus system offers high-level expression of both the CYP and OR, and are therefore advantageous for metabolism studies of all kinds, especially for low turnover substrates. The development of the cDNA-bearing virus is relatively time-consuming and labor-intensive, but baculovirus infected insect cell microsomes are commercially available. However, because the enzymes are produced transiently in the insect host cells, exact harvest time can have a pronounced effect on the activity of the final preparation.[14]

When a CYP enzyme activity is modified by induction or inhibition, the biological activity of the xenobiotic substrate can be altered considerably. Such effects are called drug-drug, drug-chemical or chemical-chemical-interactions. Such interactions can modify the disposition of xenobiotics.[10,15,16] Hence, identification of the human enzymes involved in the metabolism of specific drugs is becoming increasingly important for drug development. Such identifications should consider two processes involving the new drug: metabolism and inhibition. The identification of enzymes involved in metabolism of the new drug allows prediction, based on knowledge of the ability of co-administered drugs to inhibit the same enzymes, of which co-administered drugs may inhibit the metabolism of the new drug. This information can also be used to predict individual variability based on known metabolic polymorphisms.[13] However, also the new drug can act as an inhibitor what may lead to interactions with co-administered drugs.

1.1.3 Biotechnological Synthesis of Drug Metabolites

Reference standards of drug metabolites are needed for studies on their pharmacokinetic characterization of their formation. However, such metabolite standards are often not commercially available, particularly in the case of new therapeutic drugs or drugs of abuse. The classical chemical synthesis of drug metabolites can be cumbersome and stereochemically demanding and hence go beyond the possibilities of most biochemistry or pharmacology/toxicology oriented laboratories. Custom made metabolite standards are a possible but usually time-consuming and very expensive solution.

Recently Peters et al.[17] demonstrated that biotechnological synthesis of drug metabolites using CYP enzymes can be a versatile alternative to classical chemical synthesis. They used the pyrrolidinophenone-type designer drug 4'-methyl-α-pyrrolidinobutyrophenone (MPBP) as model substrate and human CYP2D6 heterologously expressed in strain CAD58 of the fission yeast *S. pombe* as model enzyme. Furthermore Peters et al.[18-20] demonstrated biotechnological synthesis of drug metabolites of phencyclidine-derived designer drugs. These drugs metabolites were used as reference standard for the characterization of the kinetic profile of *O*-dealkylation of PCEPA, PCMPA, PCEEA, and PCMEA.

1.2 AIMS AND SCOPES

The knowledge of the metabolic steps of a drug (of abuse) is a prerequisite for developing toxicological screening procedures, especially if the compounds are excreted in urine only in form of their metabolites.

The knowledge of the involvement of particular CYP isoenzymes in the biotransformation of a new drug is a prerequisite to predict possible drug-drug-interactions, inter-individual variations in pharmacokinetic profiles and increased appearance of side effects and serious poisonings.[21,22] However, such risk assessment is typically performed for substances intended for therapeutic use, but not for drugs of the illicit market. In addition, there is good evidence that genetic variations in drug metabolism have important behavioral consequences that can alter the risk of drug abuse and dependence.[23] The phencyclidine derived compounds were not yet investigated in any of these respects, so that the aims of the presented studies were:

- Identification of the metabolites
- Development of a urine screening procedure
- Identification of the cytochrome P450 isoforms involved in the main metabolic steps
- Characterization of the kinetic profile of the involved CYP isoenzymes in the main metabolic steps

2 ORIGINAL PUBLICATIONS

2.1 NEW DESIGNER DRUG *N*-(1-PHENYLCYCLOHEXYL)-3-ETHOXYPROPANAMINE (PCEPA): STUDIES ON ITS METABOLISM AND TOXICOLOGICAL DETECTION IN RAT URINE USING GAS CHROMATOGRAPHIC/MASS SPECTROMETRIC TECHNIQUES[6]

(DOI:10.1002/JMS.1058)

2.2 METABOLISM AND TOXICOLOGICAL DETECTION OF A NEW DESIGNER DRUG, *N*-(1-PHENYLCYCLOHEXYL)PROPANAMINE, IN RAT URINE USING GAS CHROMATOGRAPHY–MASS SPECTROMETRY[4]

(DOI:10.1016/J.CHROMA.2007.11.002)

2.3 NEW DESIGNER DRUGS *N*-(1-PHENYLCYCLOHEXYL)-2-ETHOXYETHANAMINE (PCEEA) AND *N*-(1-PHENYLCYCLOHEXYL)-2-METHOXYETHANAMINE (PCMEA): STUDIES ON THEIR METABOLISM AND TOXICOLOGICAL DETECTION IN RAT URINE USING GASCHROMATOGRAPHIC/MASS SPECTROMETRIC TECHNIQUES[7]

(DOI:10.1002/JMS.1312)

2.4 METABOLISM AND TOXICOLOGICAL DETECTION OF THE DESIGNER DRUG *N*-(1-PHENYLCYCLOHEXYL)-3-METHOXYPROPANAMINE (PCMPA) IN RAT URINE USING GAS CHROMATOGRAPHY–MASS SPECTROMETRY[5]

(DOI:10.1016/J.FORSCIINT.2008.09.001)

2.5 Identification of Cytochrome P450 Enzymes Involved in the Metabolism of the Designer Drugs *N*-(1-Phenylcyclohexyl)-3-ethoxypropanamine and *N*-(1-Phenylcyclohexyl)-3-methoxypropanamine[24]

(DIO: 10.1021/tx8001302)

2.6 INVESTIGATIONS ON THE CYTOCHROME P450 (CYP) ISOENZYMES INVOLVED IN THE METABOLISM OF THE DESIGNER DRUGS *N*-(1-PHENYLCYCLOHEXYL)2-ETHOXYETHANAMINE AND *N*-(1-PHENYLCYCLOHEXYL)-2-METHOXYETHANAMINE[25]

(DOI: 10.1016/J.BCP.2008.10.024)

3 CONCLUSIONS

The metabolism studies presented here showed that the designer drug PCEPA, PCMPA, PCEEA, PCMEA, and PCPr were mainly metabolized by *O*-dealkylation, hydroxylation of the cyclohexyl ring in different positions, hydroxylation of the phenyl ring, oxidation to the carboxylic acid, *N*-dealkylation, and combinations of these steps. As metabolic phase II reactions partial glucuronidation and sulfation were observed. The developed screening procedures allowed the detection of the studied compounds in rat urine after administration of common drug abusers' doses mainly via their metabolites. Assuming the similar metabolism in rats as in human, it can be concluded that the procedure should also be applicable for human urine screening in clinical or forensic cases.[4-7]

In vitro studies showed that in the case of PCEPA, PCMPA, PCEEA, and PCMEA the *O*-dealkylation was the main metabolic step. This metabolic reaction was catalyzed by different CYPs. It could be concluded that the decreasing side-chain length of the compounds was associated with a higher contribution of CYP2B6. In accordance with the decreasing involvement of CYP2B6, the number of CYPs involved and their contributions increased with increasing side-chain length.[25] The chemical inhibitor 4-(4-chlorobenzyl)pyridine (CBP) reduced metabolite formation in pooled HLMs in the case of PCEPA, PCMPA, PCEEA, and PCMEA. The chemical inhibitor quinidine reduced metabolite formation in pooled HLMs in case of PCEPA and PCMPA. Examinations with HLMs from CYP2D6 poor metabolizers showed a reduction of metabolite formation for PCEPA and PCMPA.

In the case of the *O*-dealkylation of PCMEA and PCPr in humans, CYP2B6 showed a major or even exclusive involvement. Considering this major involvement of CYP2B6, simultaneous intake of potent CYP2B6 inhibitory drugs, e.g. triethylene-thiophosphoramide (thiotepa), ticlopedine, clopidogrel,[26,27] or several antidepressants[28] might lead to a decreased clearance of PCMEA or PCPr and, consequently, to elevated plasma concentrations. However, the clinical relevance of such interactions remains to be established.[24,25]

4 SUMMARY

In the presented studies, the phencyclidine-derived designer drugs PCEPA, PCMPA, PCEEA, PCMEA, and PCPr were investigated regarding their metabolism and their toxicological analysis in urine. Furthermore, CYP isoform dependence of the main metabolic step and characterization of their kinetic profile were elucidated. The phencyclidine derived compounds were mainly metabolized by *O*-dealkylation, followed by oxidation to the corresponding acid, hydroxylation of the cyclohexyl ring in different positions, hydroxylation of the phenyl ring, *N*-dealkylation, and combinations of these steps. Phase II reactions consisted of partial glucuronidation and/or sulfation of some phase I metabolites. In the case of PCEPA, PCMPA, PCEEA, and PCMEA, the target analytes for the toxicological analysis were the derivatized *O*-dealkyl metabolites and their hydroxy isomers. In case of PCPr they were the mono-hydroxy metabolites, dihydroxy isomers and *N*-dealkylated mono-hydroxylated isomers. The main metabolic step of PCEPA, PCMPA, PCEEA, and PCMEA was catalyzed by different CYP isoforms. In case of the *O*-dealkylation of PCMEA in humans simultaneous intake of potent CYP2B6 inhibitory drugs, might lead to a decreased clearance of PCMEA.

5 REFERENCES

1. Roesner P, Junge T, Fritschi G, Klein B, Thielert K, Kozlowski M. Neue synthetische Drogen: Piperazin-, Procyclidin- und alpha-Aminopropiophenon-derivate. *Toxichem. Krimtech.* 1999; **66**: 81.
2. Maddox VH, Godefroi EF, Parcell RF. Synthesis of Phencyclidine and Other 1-Arylcyclohexylamines. *J. Med. Chem.* 1965; **8**: 230.
3. Rang HP, Dale MM, Ritter JM, *Pharmacology,* 4 Churchill Livingston: London 1999;
4. Sauer C, Peters FT, Staack RF, Fritschi G, Maurer HH. Metabolism and toxicological detection of a new designer drug, *N*-(1-phenylcyclohexyl)propan-amine, in rat urine using gas chromatography-mass spectrometry. *J. Chromatogr. A* 2008; **1186**: 380.
5. Sauer C, Peters FT, Staack RF, Fritschi G, Maurer HH. Metabolism and toxicological detection of the designer drug *N*-(1-phenylcyclohexyl)-3-methoxypropanamine (PCMPA) in rat urine using gas chromatography-mass spectrometry. *Forensic Sci. Int.* 2008; **181**: 47.
6. Sauer C, Peters FT, Staack RF, Fritschi G, Maurer HH. New designer drug (1-(1-phenylcyclohexyl)-3-ethoxypropylamine (PCEPA): Studies on its metabolism and toxicological detection in rat urine using gas chromatography/mass spectrometry. *J. Mass Spectrom.* 2006; **41**: 1014.
7. Sauer C, Peters FT, Staack RF, Fritschi G, Maurer HH. New designer drugs N-(1-phenylcyclohexyl)-2-ethoxyethanamine (PCEEA) and N-(1-phenylcyclohexyl)-2-methoxyethanamine (PCMEA): Studies on their metabolism and toxicological detection in rat urine using gas chromatographic/mass spectrometric techniques. *J. Mass Spectrom.* 2008; **43**: 305.
8. Takeda H, Gazzara RA, Howard SG. Phenylcyclohexylamine: effect of a metabolite of phencyclidine on the efflux of dopamine in the rat. *Neuropharmacology* 1986; **25**: 1341.
9. Ono S, Hatanaka T, Hotta H, Satoh T, Gonzalez FJ, Tsutsui M. Specificity of substrate and inhibitor probes for cytochrome P450s: evaluation of in vitro metabolism using cDNA-expressed human P450s and human liver microsomes. *Xenobiotica* 1996; **26**: 681.

10. Rendic S, Di Carlo FJ. Human cytochrome P450 enzymes: a status report summarizing their reactions, substrates, inducers, and inhibitors. *Drug Metab. Rev.* 1997; **29**: 413.
11. Ortiz-de-Montellano PR, *Cytochrome P450 - Structure, Mechanism, and Biochemistry,* 3rd Kluwer Academic/Plenum Publishers: New York 2005;
12. Aktories K, Förstermann U, Hofmann F, Starke K, *Allgemeine und spezielle Pharmakologie und Toxikologie,* 9 Urban & Fischer: München 2004.
13. Crespi CL, Penman BW. Use of cDNA-expressed human cytochrome P450 enzymes to study potential drug-drug interactions. *Adv. Pharmacol.* 1997; **43**: 171.
14. Crespi CL, Miller VP. The use of heterologously expressed drug metabolizing enzymes-state of the art and prospects for the future. *Pharmacol. Ther.* 1999; **84**: 121.
15. Sesardic D, Boobis AR, Edwards RJ, Davies DS. A form of cytochrome P450 in man, orthologous to form d in the rat, catalyses the O-deethylation of phenacetin and is inducible by cigarette smoking. *Br. J. Clin. Pharmacol.* 1988; **26**: 363.
16. Distlerath LM, Reilly PE, Martin MV, Davis GG, Wilkinson GR, Guengerich FP. Purification and characterization of the human liver cytochromes P-450 involved in debrisoquine 4-hydroxylation and phenacetin O-deethylation, two prototypes for genetic polymorphism in oxidative drug metabolism. *J. Biol. Chem.* 1985; **260**: 9057.
17. Peters FT, Dragan CA, Wilde DR, Meyer MR, Bureik M, Maurer HH. Biotechnological synthesis of drug metabolites using human cytochrome P450 2D6 heterologously expressed in fission yeast exemplified for the designer drug metabolite 4'-hydroxymethyl-alpha-pyrrolidinobutyrophenone. *Biochem. Pharmacol.* 2007; **74**: 511.
18. Peters FT, Schwaninger AE, Dragan CA, Sauer C, Fritschi G, Bureik M, Maurer HH. Isolation and Purification of the Designer Drug Metabolite O-Deethyl-(1-phenylcyclohexyl)-3-ethoxypropanamine (O-Deethyl-PCEPA) - Biotechnologically Synthesized using Fission Yeast Expressing CYP2D6. In: *Current Contributions to Forensic and Clinical Toxicology*, Pragst F, Aderjan R (eds). GTFCH: Bad Vilbel (Germany) 2008; 204.
19. Peters FT, Schwaninger AE, Dragan CA, Sauer C, Bureik M, Maurer HH. Isolation and purification of the designer drug metabolite *O*-demethyl-*N*-(1-phenylcyclo-hexyl)-3-ethoxyethanamine (*O*-demethyl-PCMEA) biotechnologically synthesized

using a new fission yeast strain expressing human CYP2B6. In: *Proceedings of the 45th International TIAFT/ICADTS Meeting in Seattle*, TIAFT2007: 2008; in press.

20. Peters FT, Dragan CA, Schwaninger AE, Sauer C, Zapp, J., Bureik M, Maurer HH. Use of Fission Yeast Heterologously Expressing Human Cytochrome P450 2B6 in Biotechnological Synthesis of the Designer Drug Metabolite 2-[(1-phenylcyclohexyl)amino]ethanol. *Forensic Sci. Int.* 2008; submitted.
21. Krishnan S, Moncrief S. An evaluation of the cytochrome p450 inhibition potential of lisdexamfetamine in human liver microsomes. *Drug Metab. Dispos.* 2007; **35**: 180.
22. Evans WE, McLeod HL. Pharmacogenomics-drug disposition, drug targets, and side effects. *N. Engl. J. Med.* 2003; **348**: 538.
23. Howard LA, Sellers EM, Tyndale RF. The role of pharmacogenetically-variable cytochrome P450 enzymes in drug abuse and dependence. *Pharmacogenomics* 2002; **3**: 185.
24. Sauer C, Peters FT, Schwaninger AE, Meyer MR, Maurer HH. Identification of Cytochrome P450 Enzymes Involved in the Metabolism of the Designer Drugs *N*-(1-Phenylcyclohexyl)-3-ethoxypropanamine (PCEPA) and *N*-(1-Phenylcyclohexyl)-3-methoxypropanamine (PCMPA). *Chem. Res. Toxicol.* 2008; **21**: 1949.
25. Sauer C, Peters FT, Schwaninger AE, Meyer MR, Maurer HH. Investigations on the cytochrome P450 (CYP) isoenzymes involved in the metabolism of the designer drugs *N*-(1-phenylcyclohexyl)-2-ethoxyethanamine and *N*-(1-phenylcyclohexyl)-2-methoxyethanamine. *Biochem. Pharmacol.* 2008; in press (DOI:10.1016/j.bcp.2008.10.024).
26. Turpeinen M, Raunio H, Pelkonen O. The functional role of CYP2B6 in human drug metabolism: substrates and inhibitors in vitro, in vivo and in silico. *Curr. Drug Metab* 2006; **7**: 705.
27. Rae JM, Soukhova NV, Flockhart DA, Desta Z. Triethylenethiophosphoramide is a specific inhibitor of cytochrome P450 2B6: implications for cyclophosphamide metabolism. *Drug Metab Dispos.* 2002; **30**: 525.
28. Hesse LM, Venkatakrishnan K, Court MH, von Moltke LL, Duan SX, Shader RI, Greenblatt DJ. CYP2B6 mediates the in vitro hydroxylation of bupropion: potential drug interactions with other antidepressants. *Drug Metab. Dispos.* 2000; **28**: 1176.

6 ZUSAMMENFASSUNG

In dieser Dissertation wurden der Metabolismus und die Nachweisbarkeit der neuen Designerdrogen des Phencyclidin-Typs PCEPA, PCMPA, PCEEA, PCMEA und PCPr im Urin untersucht. Ferner wurden die die Hauptmetabolismusschritte katalysierenden Cytochrom P450 (CYP) Isoenzyme und ihr kinetisches Profil aufgeklärt. Die PCPs wurden hauptsächlich durch *O*-Dealkylierung gefolgt von Oxidation zur Carbonsäure, Hydroxylierung des Cyclohexylringes in unterschiedlichen Positionen, Hydroxylierung des Phenylringes, *N*-Dealkylierung und Kombinationen aus diesen Schritten metabolisiert. Als Phase-II-Reaktionen wurden partielle Glucuronidierung oder Sulfatierung einiger Phase-I-Metaboliten gefunden. Die derivatisierten Metaboliten der Drogen aus den Hauptstoffwechselwegen (*O*-Dealkylierung, *O*-Dealkylierung und Hydroxylierung bei; PCEPA, PCMPA, PCEEA, PCMEA; Monohydroxylierung, Dihydroxylierung, *N*-Dealkylierung und Monohydroxylierung bei PCPr) waren die Zielsubstanzen im toxikologischen Nachweisverfahren. Die metabolischen Hauptschritte von PCEPA, PCMPA, PCEEA, und PCMEA wurde von unterschiedlichen CYPs katalysiert. Im Falle der *O*-Dealkylierung des PCMEA im Menschen könnte eine gleichzeitige Einnahme von Substanzen, die CYP2B6 hemmen, zu einer verminderten Clearance von PCMEA führen.