Amphetamine-Derived New Psychoactive Substances:

Metabolic Fate and Toxicological Detectability of Methiopropamine, three Methyl-Amphetamine Isomers, Camfetamine, 5-APB, 6-APB, 5-MAPB, and 6-MAPB in Urine and Human Liver Preparations Using GC-MS, LC-MS^n, and LC-HR-MS^n Techniques

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Dekan:	Univ.-Prof. Dr.-Ing. Dirk Bähre
Berichterstatter:	Univ.-Prof. Dr. rer. nat. Dr. h. c. Hans H. Maurer Univ.-Prof. Dr. rer. nat. Rolf W. Hartmann
Vorsitz:	Univ.-Prof. Dr. rer. nat. Dr. med. habil. Adolfo Cavalié
Akad. Mitarbeiter:	Dr. rer. nat. Matthias Engel

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“The most difficult thing is the decision to act,
the rest is merely tenacity.”

Amelia Earhart (1897 - 1937)

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1 GENERAL PART

1.1 Introduction

1.1.1 New Psychoactive Substances (NPS)

The phenomenon of New Psychoactive Substances (NPS) has increased attention over the last past years. NPS were sold over the internet under terms such as “bath salts”, “research chemicals”, “plant food” or “legal highs” and were primarily synthesized to avoid law enforcements [1, 2]. Most of these substances were not basically new; they were previously described in the (scientific) literature some years ago. For example, Alexander Shulgin synthesized and tested 179 phenethylamines and 55 tryptamines and published the results of his self-testing in his books “Pikhal (Phenethylamines I have known and loved)” in 1991 and “Tikhal (Tryptamines I have known and loved)” in 1995 [3, 4]. However, there are also compounds synthesized by university laboratories or pharmaceutical companies for development of new drugs. One example is 4-methyl-amphetamine (4-MA) that has been designed and synthesized in the 1950s as Aptrol®, an appetite suppressant, but was never released. It was then rediscovered around 2010 when the first seizures were reported. It seems that formerly published data about drugs with the desired effects were consulted before starting to synthesize them in high scale and sell.

Generally, NPS were legally defined by the European Union (EU), as a “new narcotic drug or psychotropic drug in pure form or in a preparation, that is not scheduled under the Single Convention on Narcotic Drugs of 1961 or the Convention on Psychotropic Substances of 1971, but which may pose a public health threat comparable to that posed by substances listed in those conventions” (Council of the European Union decision 2005/387/JHA). Thus, NPS are compounds with similar effects to drugs that are internationally controlled. NPS are not only one class of substances; they can be classified in different categories, depending on the illicit drug they refer to. The group of synthetic cannabinoids (e.g. MAM-2201) mimic the effects of Δ9-tetrahydrocannabinol (THC), the active ingredient in cannabis plants (*cannabis sativa*), and bind to the cannabinoid receptor type 1 (CB1). The highly potent compounds, mainly more potent than THC itself, were often sprayed on various herbal mixtures and sold under brand names like “Spice” or “K2”. The synthetic cathinones (e.g.

mephedrone) should mimic the effects of cathinone, which is present in the leaves of the khat plant (*catha edulis*) as active ingredient, and are (mis-)used as stimulants. The phenethylamines cover a wide range of compounds, which contain the phenethylamine backbone, e.g. amphetamine-type stimulants, which should act like amphetamine, methamphetamine or 3,4-methylenedioxymethamphetamine (MDMA) or compounds of the hallucinogenic 2C-series, which have similar effects to mescaline. Other categories defined by the European Monitoring Centre for Drugs and Drug Addition (EMCDDA) are e.g. piperazines (stimulating drugs) and tryptamines (hallucinogenics). Compounds that did not fit into any of the above mentioned drug families are summarized in the category of “other drugs”. This class can respectively contain plant-based compounds or synthetic cocaine derivatives such as kratom or dimethocaine.

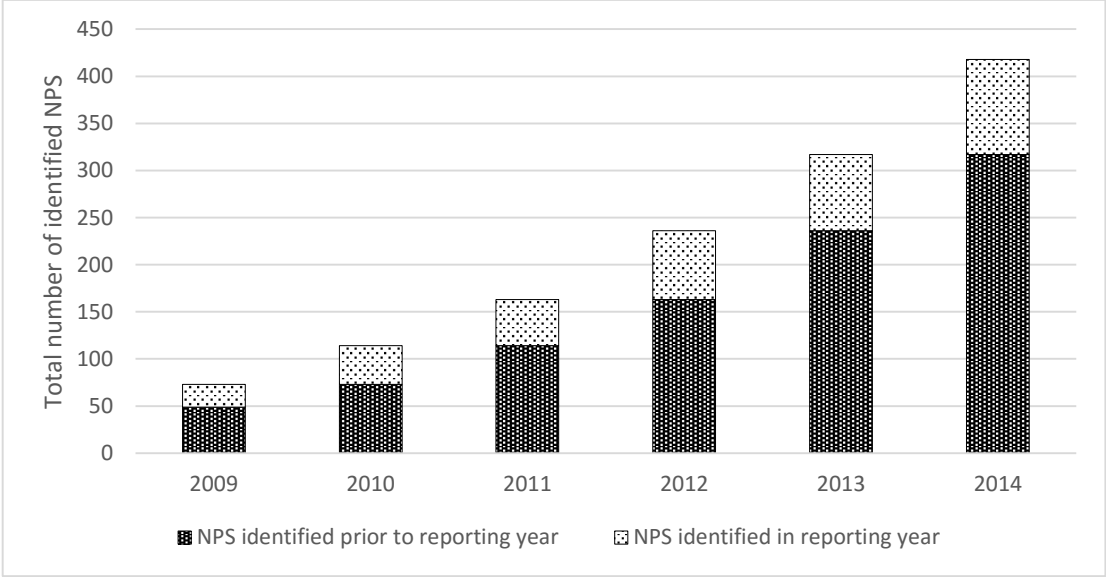

Fig. 1 Number of identified NPS reported to the EU EWS from 2009 to 2014 (data according to refs. [5-12])

To fight this phenomenon or to keep track of the compounds that flood the market, there are several regional, national, and international drug monitoring systems, executed for example by the EMCDDA or the United Nation Organization on Drugs and Crime (UNODC). Every year more and more NPS entered the market and were notified by these Early Warning systems (EWS). The results of these notifications can be found, amongst other information, in the annual reports of the EMCDDA or the UNODC. In 2014 in Europe, 101 new NPS were reported by member states of the EU.

From these, 31 have been synthetic cathinones, 30 synthetic cannabinoids, and 9 phenethylamines. In 2013, 81 new NPS were reported amongst them 29 synthetic cannabinoids, 13 substituted phenethylamines, and 7 synthetic cathinones. In addition, 651 websites offering “legal highs” were identified. All over the world, 97 new NPS have been reported in 2013, whereas 48 have been synthetic cannabinoids, 16 phenethylamines and 8 synthetic cathinones [1, 2, 6-17].

Figure 1 shows the number of identified NPS reported to the EU EWS from 2009 to 2014, it can be seen that the number highly increased in the last six years.

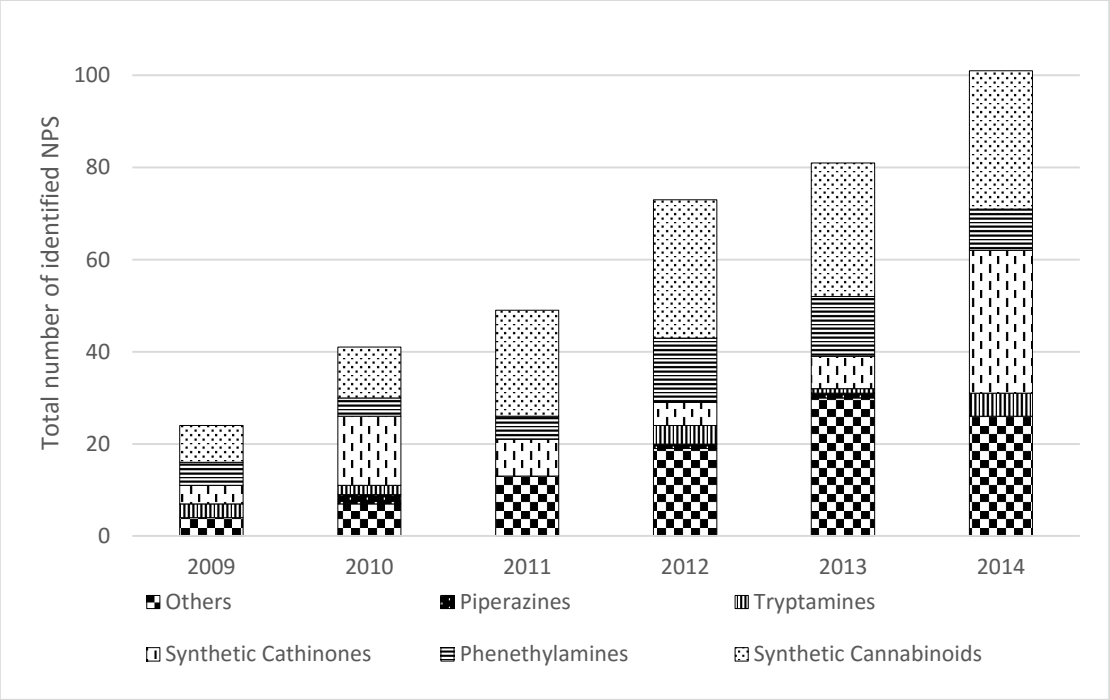

Fig. 2 Number and main groups of newly identified NPS reported to the EU EWS from 2009-2014 (data according to refs. [5-12])

Figure 2 shows the distribution of the newly identified NPS into the different classes. The most predominant groups in the last six years were the synthetic cannabinoids, the phenethylamines, and the synthetic cathinones.

The overall distribution of notified NPS in the last six years is shown in Figure 3. Synthetic cannabinoids represented 35% of the notified NPS, whereas phenethylamines accounted for 14% and synthetic cathinones for 19%.

As the data show, phenethylamines are the third most increasing group in the last six years. Therefore, they should be studied regarding their pharmacological and pharmacokinetic properties, such as receptor affinity, enzyme inhibitory potential, and metabolism including (toxicological) detection in biosamples.

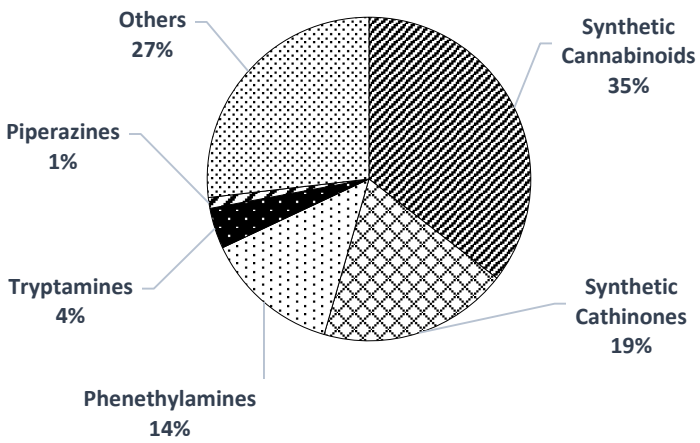

Fig. 3 Distribution of identified NPS reported to the EU EWS from 2009-2014 (data according to refs. [5-12])

Well-known illicit phenethylamine representatives are, as already mentioned, amphetamine, methamphetamine, and MDMA. NPS derived from these compounds contain the phenylisopropylamine backbone (e.g. 4-MA). Also bioisosteric substitution of the benzene ring was observed, e.g. by a thiophene ring (methiopropamine, 2-MPA). Amphetamine-derived NPS mimic the dopaminergic, noradrenergic, and serotonergic effects of their illicit companions and thus have psychoactive, stimulating effects.

1.1.2 Pharmacology and Toxicology of Amphetamine-derived NPS

As already described above, amphetamine-derived NPS are intended to mimic the effects of amphetamines and methamphetamines. Both are indirect sympathomimetics and the mechanism of action is based on a reuptake transporter inhibition rather than to a direct release of these monoamines [18]. Amphetamine derivatives act as

competitive substrates of dopamine (DAT), noradrenaline (NAT), and serotonin (SERT) reuptake transporters and thus inhibit the reuptake of dopamine (DA), noradrenalin (NA), and serotonin (5-HT) in different potencies [19]. In addition, this leads to the reverse transport of monoamines in the synaptic cleft, resulting in higher concentrations of the respective monoamines. Thereby, DA produces the desired euphoric effects, whereas NA is responsible for the central stimulation. MDMA affects more the 5-HT release in contrast to amphetamine and methamphetamine, which have higher effects on DA and NA release. Therefore, the described effects when (mis-) using MDMA include, beside euphoria, stimulation, and increased wakefulness, also an increased sociability, extraversion, sharpened sensory perception, and an increased sense of closeness to other people. These MDMA effects were also summarized under the term of “empathogen" or “entactogen” [20].

Unfortunately, the pharmacological effects of most new designer drugs were not studied, as they were not used as medication. The only – non-scientific – source of information on the respective effects of new drugs of abuse (DoA) are trip reports of drug users, published e.g. on www.bluelight.org or www.land-der-traeume.de. These trip reports are very popular among drug users and serve as information source and platform for the exchange of information. For example, information on dosing or routes of administration can be found as well as discussions about the general potential of a particular NPS. However, these reports have to be interpreted with caution since the users cannot be sure about what they consumed due to the variable content of internet purchased products [21, 22]. Unfortunately, only few scientific information is available on the pharmacology of amphetamine-derived NPS. For more details, see Chapters 2.1 – 2.5.

There are also no data available on the toxicity of these compounds. Nevertheless, several case reports were published after ingestion of NPS [23-29]. In general, side effects described in these case reports were nausea, insomnia, hyperthermia, and cardiovascular symptoms, all typical for amphetamine-type stimulants. After intake of 5-APB and 6-APB, respectively, hallucinations or acute psychosis have been described indicating more serotonergic effects. Unfortunately, due to poly-drug use in most of the described cases, it is not sure, which compound is responsible for the observed effects. Poly-drug use is always a health threat as no one could estimate the additive effects of DoA mixtures.

As already mentioned, there is only little information about the pharmacology and toxicology of new psychoactive substances. Indeed, the effects of NPS could be estimated through known compounds with similar structures. The studies of Iversen et al. [30], who tested several NPS for their *in vitro* ability to inhibit NAT, DAT, and SERT, are of great importance when trying to estimate the effects and health threat of NPS. Green and Nutt also require that pharmacology studies should be in the center of clinical studies even for NPS [31]. Nevertheless, there is need of more studies, such as long-term toxicity and the risk of combining several NPS because of enzyme inhibition potentials.

1.1.3 General Information and Structures of the Tested Stimulants

1.1.3.1 Methiopropamine

2-Methiopropamine, 1-(thiophen-2-yl)-2-methylaminopropane (2-MPA), is a thiophene analogue of methamphetamine, where the benzene ring was bioisosterically substituted. Therefore, it is not a classic phenethylamine. Figure 4 shows the chemical structures of 2-MPA compared to amphetamine and methamphetamine. Blicke and Burkhalter first synthesized 2-MPA in 1942 and described its pharmacological properties (e.g. pressor activity) being similar to the corresponding benzene analogues [32].

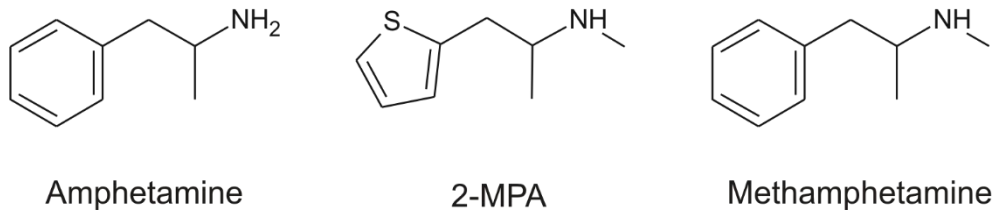

Fig. 4 Structure of amphetamine, 2-MPA, and methamphetamine

In 2010, it achieved attention as “legal high” when it appeared on several websites offering NPS. Iversen et al. [30] showed that 2-MPA acts as noradrenaline (NA) and dopamine (DA) reuptake inhibitor, less potent than amphetamine but with similar potencies. This is in accordance to users’ reports on internet forums (e.g. www.bluelight.org and www.land-der-traeume.de) describing as desired effects stimulation and mild euphoria and as adverse effects hypertension, tachycardia, and

increased sweating. Several seizures and the occurrence in forensic case work lead to controlling of the compound in some European countries, e.g. Switzerland (2012) and Germany (2013). Nevertheless, it is still available on the internet and continuing discussions on drug users' forums showing its ongoing relevance. For more details about 2-MPA, see Chapter 2.1. So far, no data were available on its metabolism or detectability in biosamples. Therefore, studies on its metabolism and the toxicological detectability in biosamples are of great interest.

1.1.3.2 2-, 3-, and 4-Methyl-Amphetamine

4-Methyl-amphetamine (1-(4-methylphenyl)propane-2-amine, 4-MA), the para-methyl analogue of amphetamine, was developed in the 1950s when it was studied as appetite suppressant under the trade name Aptrol®, but was never sold as such. It reachieved importance in 2010 when it was found in a seized powder sample in Germany labelled as amphetamine [33]. The EMCDDA joint report on 4-MA also showed that 4-MA was sold on the illicit market as amphetamine in several other countries [26]. In addition, several severe poisonings and fatalities most probably due to overdosing have been reported after use of contaminated "speed" [26, 29].

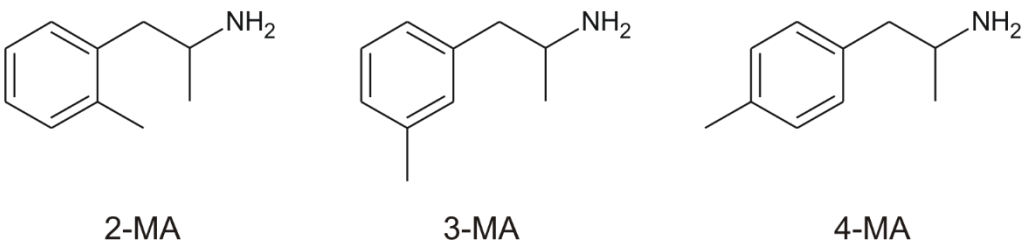

Fig. 5 Structures of 2-MA, 3-MA, and 4-MA

The chemical structures of 4-MA and its 2-methyl (2-MA) and 3-methyl isomers (3-MA) are depicted in Figure 5. For more details, see Chapter 2.2. In March 2013, the EU Council decided an EU-wide control of 4-MA based on the EMCDDA joint report. However, the other isomers, 2-MA and 3-MA, are uncontrolled, therefore differentiation of the three isomers after ingestion is an important analytical task in forensic toxicology. Until now, no data were available on the metabolism or the selective detectability in biological samples and only 4-MA was integrated either in a LC-TOF screening in whole blood or in an LC-MS/MS screening in oral fluid [34, 35]. Therefore, studies should be performed on the metabolism and detectability of all of these compounds.

1.1.3.3 Camfetamine

Camfetamine (*N*-methyl-3-phenyl-norbornan-2-amine, CFA) is not substituted at the ring system like the other tested compounds but at the alkyl chain. CFA has a phenethylamine backbone and belongs to the group of amphetamine-derived NPS. CFA is structurally related to fencamfamine (*N*-ethyl-3-phenyl-norbornan-2-amine; FCF), which is the corresponding *N*-ethyl analogue and both compounds can be seen as camphor derivatives with a norbornane ring system (Figure 6).

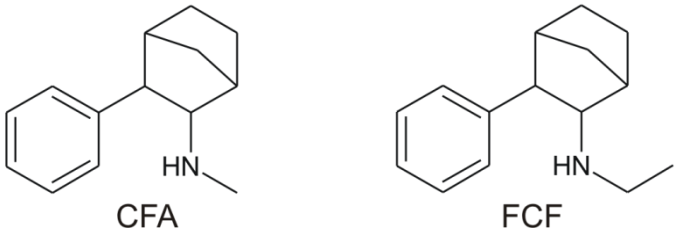

Fig. 6 Structures of camfetamine (CFA) and fencamfamine (FCF)

No data are available on CFA, but FCF was developed in the 1960s as appetite suppressant and was tested for its pharmacological effects [36-43]. For more details about CFA, see Chapter 2.3. CFA is, in contrast to FCF, not scheduled in Germany, therefore it is quite probable that it is still used as “legal high”. Cinosi et al. also supposed ongoing use of CFA [44]. For toxicological detection of CFA metabolism studies should be performed.

1.1.3.4 5-APB, 6-APB, 5-MAPB and 6-MAPB (“Benzofuries”)

“Benzofury” is a trade name related to 6-(2-aminopropyl)benzofuran (6-APB), but is also used for several benzofuran-containing compounds. 6-APB, its 5-isomer 5-APB (5-(2-aminopropyl)benzofuran) and their *N*-methyl derivatives 5-MAPB (*N*-methyl-5-(2-aminopropyl)benzofuran) and 6-MAPB (*N*-methyl-6-(2-aminopropyl)benzofuran) are benzofuran analogues of amphetamine and methamphetamine by “adding” a furan ring to the benzene ring. Considering the benzofuran ring, they can also be seen as analogues of 3,4-methylenedioxy-amphetamine (MDA) and 3,4-methylenedioxy-methamphetamine (MDMA), where one oxygen (-O-) in the methylenedioxy ring was replaced by methine (-CH=). Due to the structural similarities, it is not a surprise that the reported effects on drug users’ forums are also more MDMA- and MDA-like. MDA

is the *N*-demethyl metabolite of MDMA, but is also misused as stimulant and thus controlled in Germany. Figure 7 shows the chemical structures of the tested benzofuran-containing compounds, MDA and MDMA.

Since 2010, these compounds appear consecutively on the internet and users started to discuss their effects. More details can be found in Chapter 2.4 and 2.5.

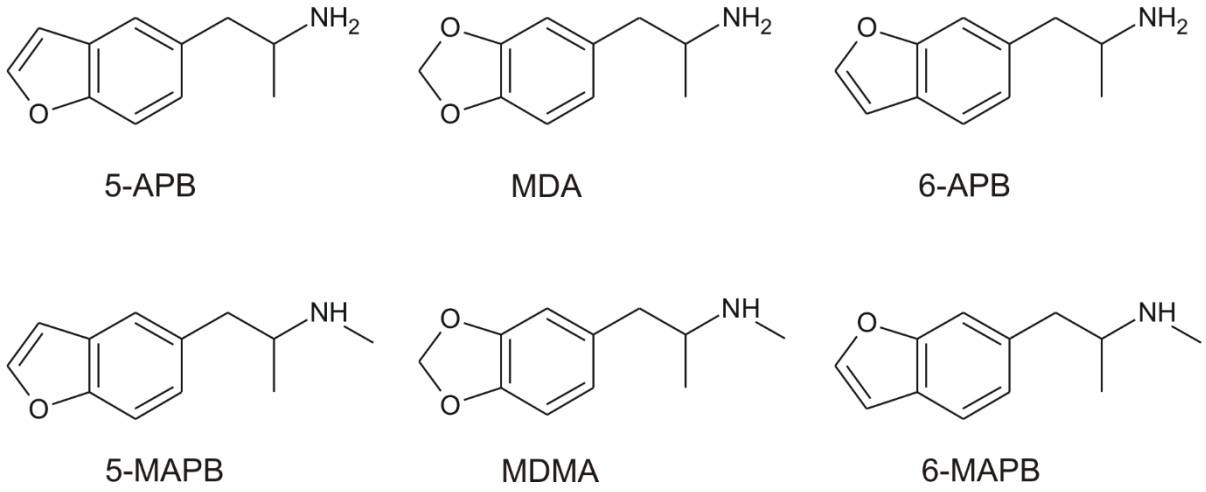

Fig. 7 Structures of 5-APB, 6-APB, MDA, MDMA, 5-MAPB and 6-MAPB

In Germany, 5-APB and 6-APB were controlled since 2013, but the corresponding *N*-methyl derivatives are not. A few data on analytical characterization have been published by Casale et al. and Stanczuk et al. but only applied on internet purchased products [45, 46]. They separated the positional isomers of APB with GC-MS with or without derivatization. Data on analytical separation in biosamples are not available so far, and the metabolism of these compounds has not been studied yet. However, this is of great interest as these compounds are still discussed and sold on the internet and several case reports have been published showing that these compounds are also a possible public health threat [23, 25, 28, 29]. Another interesting question could be if the position of the oxygen in the furan ring makes any difference in metabolism caused by different fitting in the active center of involved enzymes.

1.1.4 Metabolism of Amphetamine-derived NPS

In general, metabolic reactions should facilitate the (renal) excretion of lipophilic endogenous or exogenous compounds. Therefore, several metabolizing enzyme systems catalyze different reactions resulting in metabolites that are either pharmacologically and toxicologically active or inactive. The metabolic reactions can be divided into different phases. Phase I metabolizing enzymes create nucleophilic or electrophilic compounds, e.g. by oxidative or reductive reactions. This step is also called functionalization. An important phase I enzyme system are the cytochrome P450 monooxigenases (CYP), which are not selective and transform several different compound classes [47]. These activated compounds were then conjugated e.g. with glucuronic acid, sulfuric acid (nucleophilic compounds) or with glutathione (electrophilic compounds) by the corresponding phase II metabolizing enzymes. The main metabolic steps observed for amphetamine- or methamphetamine-derived compounds are aromatic hydroxylation, aliphatic hydroxylation, *N*-demethylation, and oxidative deamination [48, 49]. MDMA-derived compounds show *O*-demethylenation to dihydroxy derivatives followed by methylation of one hydroxy group, *N*-demethylation, and oxidative deamination [50]. The knowledge of the metabolic fate of compounds is important for assessing the risk of pharmacokinetic interactions and pharmacogenomic variations.

Toxicological detection in forensic and clinical cases is often performed with metabolite-based screening-approaches and for the development of such screening approaches metabolism studies should be executed [51-53]. As already mentioned above, for NPS or other DoA, such studies are not performed before marketing in contrast to drugs used in medicine. To test for the metabolic fate of drugs, different approaches are possible. *In vitro* assays using human recombinant enzymes, liver preparations or hepatocytes can be used as well as animal models, e.g. with rats whose qualitative metabolic profiles turned out to be similar to that of humans [54-56]. Combining rat animal studies with human liver microsomes incubations allowed a good, at least qualitative prediction of human metabolism [57-69].

1.1.5 Involvement of CYP Isoenzymes in the Metabolism

As already mentioned above, one of the main metabolizing enzyme systems are the CYP isoenzymes. These isoenzymes were not only involved in the metabolism of NPS or DoA, but in the metabolism of several endogenous and exogenous compounds [47]. To estimate or predict the risk of drug-drug interactions and pharmacogenomics variations, an initial CYP activity screening might help. The ten most abundant CYP isoenzymes are tested for their involvement in the main metabolic steps. The results can help to give a preliminary hint of potential drug-drug interactions with medicaments or other DoA. However, the real distribution of the respective isoenzymes in the liver and affinities of a substrate to different isoenzymes is not considered. Therefore, kinetic studies should be performed if reference standards of the metabolites, which are needed for the quantification, are available [70-74]. Unfortunately, especially for new DoA, such reference standards are rarely commercially available. One possibility to perform kinetic studies without a reference standard is the substrate depletion approach [75, 76]. However, this approach only allows the determination of K_m (Michaelis-Menten constant) and not V_{max} values [74-76]. Once, V_{max} and K_m values are determined, calculation of the real contribution of the particular enzyme to hepatic excretion is possible and thus estimation of the risk of drug-drug interactions.

1.1.6 Analytical Challenge and Toxicological Detectability

As one could imagine, the rapid increase of NPS on the market also challenges the respective forensic or clinical laboratories. It is like the race between the hare and the hedgehog, once a new reference standard was synthesized or a new method was validated, several other compounds appear on the market and the competition restarts [77]. For identification of pure compounds in seized material, some approaches have been published [21, 45, 77-81].

Nevertheless, toxicological detection in biosamples, such as blood or urine, is even more challenging because of the small detection window in blood and the possible absence of the parent compounds in urine samples due to extensive metabolism. Two reviews have been published recently, focusing on the analytical challenge covering NPS [77, 82]. Peters summarized the method developments in urinalysis that were published since 2008 and summarizes the different techniques regarding formation of the metabolites, sample preparation, or analytical detection, including various

instrumentations (e.g. GC-MS, LC-MS) and detection modes (e.g. targeted or general unknown). Especially, when using the targeted or the general unknown mode in urinalysis, knowledge of the excretion products or the metabolites of a respective compound is of great importance. Therefore, metabolism studies should be performed and a metabolite-based library should be created and used for identification as described previously [52, 53, 83, 84]. In this work, the detectability studies were performed in rat urine after application of a low dose, which was calculated according to the reported single doses on drug forums. If human urine samples were available, those have been additionally investigated even if the ingested doses were not known, for comparison of the metabolic patterns between humans and rat.

1.1.7 Isomer Differentiation

Some of the compounds were available as different positional isomers, e.g. the three methyl-amphetamines and the benzofurans. As some of these compounds were controlled substances in Germany (4-MA, 5-APB and 6-APB), isomer differentiation could be necessary.

For isomer differentiation, solid phase extraction was used followed by derivatization via heptafluorobutyrylation according to a published procedure for plasma by Peters et al. [85]. For the results of the separation studies on the three methyl-amphetamine isomers or the benzofuries, see chapter 2.2 or 2.5, respectively.

1.2 Aims of the dissertation and resulting publications

So far, no studies were available on the metabolism and toxicological detectability of the above-mentioned compounds. Therefore, the aims of this dissertation and the resulting publications are given below:

- Identification of the phase I and II metabolites in rat (and human) using GC-MS and LC techniques coupled to either low or high resolution mass spectrometry (LC-MS^n or LC-HR-MS^n)
  (Chapters 2.1 – 2.5)
- Confirmation of the phase I metabolites in human liver microsomes preparations and identification of the involved cytochrome P450 (CYP) isoenzymes
  (Chapters 2.1 – 2.5)
- Investigation of the detectability in urine within standard urine screening approaches (SUSA) using GC-MS and LC-MS^n
  (Chapters 2.1 – 2.5)
- Determination of human plasma concentrations after ingestion of 5-MAPB
  (Chapter 2.4)
- Quantitative *in vitro* enzyme kinetics of 5-MAPB *N*-demethylation and determination of the *in vivo* contribution
  (Chapter 2.4)
- Separation of the methyl-amphetamine isomers or the benzofuran-containing isomers in urine using GC-MS
  (Chapters 2.2 and 2.5)

2 PUBLICATION OF THE RESULTS

The results of the studies were published in the following papers:

2.1 2-Methiopropamine, a thiophene analogue of methamphetamine: studies on its metabolism and detectability in the rat and human using GC-MS and LC-(HR)-MS techniques [57]

(DOI: 10.1007/s00216-013-6741-4)

2.2 Studies on the metabolism and the detectability of 4-methyl-amphetamine and its isomers 2-methyl-amphetamine and 3-methyl-amphetamine in rat urine using GC-MS and LC-(high-resolution)-MS^n [86]

(DOI: 10.1007/s00216-013-7595-5)

2.3 GC-MS and LC-(high-resolution)-MS^n studies on the metabolic fate and detectability of camfetamine in rat urine [87]

(DOI: 10.1007/s00216-014-7796-6)

2.4 Benzofuran analogues of amphetamine and methamphetamine: studies on the metabolism and toxicological analysis of 5-APB and 5-MAPB in urine and plasma using GC-MS and LC-(HR)-MS^n techniques [58] (DOI: 10.1007/s00216-014-8360-0)

2.5 Metabolic fate, mass spectral fragmentation, detectability, and differentiation in urine of the benzofuran designer drugs 6-APB and 6-MAPB in comparison to their 5-isomers using GC-MS and LC-(HR)-MS^n techniques [88]

(DOI: 10.1007/s00216-015-8552-2)

3 CONCLUSIONS

The presented studies provide systematic data on the metabolism and toxicological detection of several amphetamine-derived compounds belonging to the phenethylamine group of new psychoactive substances. They were performed using rat urine, human liver preparations and, if available, human urine. These data show that the methamphetamine-derived compounds, 2-MPA, 5-MAPB, and 6-MAPB, were only metabolized to a minor extent. *N*-Demethylation was the predominant metabolic step, resulting in the corresponding nor metabolites. These nor metabolites were beside the parent compounds the second most important target for urinalysis. For 2-MPA and 5-MAPB, these results could also be confirmed in human urine. The CYP isoenzymes involved in the *N*-demethylation were more or less the same for each compound, namely CYP2B6, CYP2C19, CYP2D6, and CYP3A4. The other two benzofuran-containing compounds, 5-APB and 6-APB, were metabolized even less than the methamphetamine-derived compounds. The main excretion products and thus the targets for urinalysis were 5-APB and 6-APB themselves. In the metabolism studies, at least some metabolic steps could be detected, such as ring cleavage, but only to a minor extent and only in the high dose rat urine. Evaluation of the initial CYP activity screening was also not possible due to small formation rates or multiple step reactions (e.g. ring cleavage).

The methyl-amphetamine isomers 2-MA, 3-MA, and 4-MA showed different degradation. Although they are amphetamine derivatives, the main excretion products in urine were the hydroxy-aryl metabolites beside the parent compounds. These isomers also show that the position of the methyl group in the aromatic ring affects the metabolism, as 2-MA was metabolized more extensive than the other isomers. As already mentioned, aromatic hydroxylation was the main metabolic step observed for the methyl-amphetamine isomers and this step was catalyzed only by CYP2D6, which is accordance to published results about amphetamine [89]. For CFA, which was the only investigated compound with the variation in the side chain, the observed metabolism was very extensive. This resulted in the excretion of several different metabolites even in the low dose rat urine, namely, hydroxy-aryl, nor-hydroxy-aryl, and nor CFA. CFA itself was excreted only in a minor concentration. Therefore, the main targets for urinalysis were the hydroxy-metabolites. The CYP isoenzymes involved in

the aromatic hydroxylation were CYP2C19 and CYP2D6 and those involved in the *N*-demethylation were CYP2B6, CYP2C19, CYP2D6, and CYP3A4.

In summary, it can be seen, that the *N*-demethylations for all tested compounds were more or less catalyzed by the same CYP isoenzymes, namely CYP2B6, CYP2C19, CYP2D6, and CYP3A4. And also the aromatic hydroxylation was mainly catalyzed by CYP2D6, even if for CFA also CYP2C19 was additionally observed as catalyzing enzyme.

Concerning phase II metabolism, for all tested compounds, glucuronidation and sulfation of the corresponding functionalized metabolites could be observed, even if these steps play only a minor role, with the exception of CFA, 2-MA, and 3-MA, where the hydroxy-aryl glucuronides could also be detected in the LC-MS^n standard urine screening approaches (SUSA) after the low dose. Hydroxy-aryl CFA glucuronide was even a main target in the low dose rat urine.

Separation of the methyl-amphetamine isomers or the benzofuran isomers has been performed with an additional work-up because they were not distinguishable within the used GC-MS or LC-MS^n SUSAs. The sample preparation consisted of a solid-phase extraction (SPE) and a following heptafluorobutyrylation according to published procedures for plasma [85]. Separation was then performed using GC-MS with longer HP5 columns compared to routine conditions and special temperature gradients.

In the case of 5-MAPB, six human samples have been send to the laboratory for toxicological diagnostic reasons after ingestion of 5-MAPB. Therefore, quantification of the plasma concentrations of 5-MAPB and its main metabolite 5-APB was possible. The determined concentrations ranged between 5 and 124 µg/L for 5-MAPB and from 1 to 38 µg/L for 5-APB. Considering similar doses for MDMA and 5-MAPB, as described on drug users' internet forums, these concentrations are comparable to published data for MDMA (1–514 µg/L) and MDA (1–67 µg/L) [50, 90].

The results presented here show that metabolism studies on new psychoactive substances are of great necessity. The targets for urinalysis are not always the parent compounds; depending on the extent of metabolism the unchanged drug is only excreted in a minor concentration. Therefore, the metabolic fate of such compounds should be tested systematically. The information obtained from these studies can help to create a library-based approach for the identification of NPS in urine. With the results from the studies presented here, the intake of each tested compound could be proved using the established SUSAs.

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5 ABBREVIATIONS

2-MA	2-methyl-amphetamine
2-MPA	2-methiopropamine
3-MA	3-methyl-amphetamine
4-MA	4-methyl-amphetamine
5-APB	5-aminopropylbenzofuran
5-HT	serotonin
5-MAPB	*N*-methyl-5-aminopropylbenzofuran
6-APB	6-aminopropylbenzofuran
6-MAPB	*N*-methyl-6-aminopropylbenzofuran
CB1	cannabinoid receptor type 1
CFA	camfetamine
CYP	cytochrome P450
DA	dopamine
DAT	dopamine transporter
DoA	drugs of abuse
EMCDDA	European Monitoring Centre for Drugs and Drug Addiction
EU	European Union
EWS	Early Warning System
FCF	fencamfamine
GC	gas chromatography
HLM	human liver microsomes
HR	high resolution
LC	liquid chromatography
MDA	3,4-methylenedioxyamphetamine
MDMA	3,4-methylenedioxymethamphetamine
MS	mass spectrometry
NA	noradrenalin
NAT	noradrenalin transporter
NPS	new psychoactive substances
SERT	serotonin transporter
SPE	solid-phase extraction
SUSA	standard urine screening approach

THC	Δ9-tetrahydrocannabinol
TOF	time-of-flight
UN	United Nations
UNODC	United Nations Office on Drugs and Crime

6 SUMMARY

The number of new psychoactive substances identified each year by the EMCDDA is increasing rapidly, as in 2014, 101 new compounds have been identified. One important structural group of NPS are the phenethylamines. In the presented studies, the metabolic fate and the toxicological detection of different amphetamine-derived compounds belonging to the phenethylamine group of NPS have been investigated. Using GC-MS and/or LC-High-Resolution-MS^n, the main phase I metabolic step observed for the compounds containing the methamphetamine backbone, such as 2-MPA, 5-MAPB, and 6-MAPB, was *N*-demethylation, whereas for the three methyl-amphetamine isomers and CFA aromatic hydroxylation was the predominant step. 5-APB and 6-APB were metabolized only to a minor extent, resulting in the unchanged drug being the main target for urinalysis. The isoenzymes mainly involved in the *N*-demethylation steps were CYP2B6, CYP2C19, CYP2D6, and CYP3A4, whereas the aromatic hydroxylations were catalyzed by CYP2D6 and CYP2C19. The intake of each tested compound could be proved within the established SUSAs, either with the unchanged drugs or the nor metabolites being the main targets in urine, or in the case of CFA and the three MAs, the hydroxy-aryl metabolites. Separation of isomers was successfully accomplished with an additional work-up, including heptafluorobutyrylation. For 5-MAPB, plasma concentrations determined in authentic cases were in the same range as published for MDMA.

7 ZUSAMMENFASSUNG

Die Anzahl neuer psychoaktiver Substanzen, die jedes Jahr von der EMCDDA identifiziert wird, steigt stetig an. Alleine 2014, wurden 101 neue Substanzen identifiziert. Die Phenethylamine stellen dabei eine wichtige Gruppe dar, deshalb wurde im Rahmen dieser Dissertation der Metabolismus und die Nachweisbarkeit von verschiedenen Phenethylamin-Abkömmlingen mittels GC-MS- und LC-(HR)-MS^n-Verfahren untersucht. Für Substanzen, mit einer Methamphetamin-verwandten Struktur, wie 2-MPA, 5-MAPB und 6-MAPB, war der nachgewiesene Hauptstoffwechselschritt die *N*-Demethylierung, während es für die drei Isomere des Methyl-Amphetamins und CFA, die aromatische Hydroxylierung war. 5-APB und 6-APB wurden insgesamt nur wenig verstoffwechselt, was dazu führt, dass hauptsächlich der unveränderte Stoff im Urin nachgewiesen wurde. CYP2B6, CYP2C19, CYP2D6 und CYP3A4 waren an der *N*-Demethylierung beziehungsweise CYP2D6 und CYP2C19 an der aromatischen Hydroxylierung beteiligt. Eine Einnahme der untersuchten Substanzen konnte im Urin nachgewiesen werden mithilfe der im Labor durchgeführten Standard Urinscreening-Verfahren. Der Fokus für die Nachweisbarkeit lag dabei entweder auf den unveränderten Substanzen, und den Nor-Metaboliten, oder im Fall von CFA und den Methyl-Amphetaminen auf den Hydroxy-Aryl-Metaboliten. Eine Isomeren-Trennung wurde erfolgreich durchgeführt. 5-MAPB-Plasmakonzentrationen, die in authentischen Fällen bestimmt wurden, waren vergleichbar mit bekannten MDMA-Konzentrationen.