4-Fluoroamphetamine (4-FA)

Critical Review Report

Agenda Item 4.3

Expert Committee on Drug Dependence

Thirty-ninth Meeting

Geneva, 6-10 November 2017

World Health Organization

Contents

Acknowledgements

This report has been drafted under the responsibility of the WHO Secretariat, Department of Essential Medicines and Health Products, Teams of Innovation, Access and Use and Policy, Governance and Knowledge. The WHO Secretariat would like to thank the following people for their contribution in producing this review report: Dr. Simon Brandt, United Kingdom (literature review and drafting) Dr. Dilkushi Poovendran, Geneva, Switzerland (questionnaire analysis and report drafting) and Dr. Stephanie Kershaw, Adelaide, Australia (review report editing, questionnaire analysis and report drafting).

WHO would like to thank the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) for providing information on 4-fluoroamphetamine collected from the European Union Early Warning System, which includes data reported by the Reitox National Focal Points in the EU Member States, Turkey, and Norway.

Summary

1-(4-Fluorophenyl)propan-2-amine (4-fluoroamphetamine, 4-FA) underwent a critical review in November 2015 at the 37th meeting of the WHO Expert Committee on Drug Dependence. The Committee recommended that 4-FA not be placed under international control at that time due to insufficiency of data regarding dependence, abuse and risks to public health but be kept under surveillance. This review represents an update.

4-FA is a psychomotor stimulant first synthesized in the early 1940s. The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) received the first formal notification of the detection of 4-FA in Europe in December 2008 although its presence has been noted since at least 2007. In Europe, it has been found in tablets sold as 'ecstasy'/MDMA, paste or powder sold as amphetamine. It has also been detected as an adulterant present in other illicit controlled substances. The EMCDDA has been notified of seizures from various locations in Europe since 2008. In the Netherlands, 4-FA has recently established itself as a drug of choice (powdered form, tablets and capsules) in a subpopulation of recreational drug users associated with clubs and music festivals. 4-FA appears to be most commonly administered orally or by nasal insufflation (snorting).

Data indicate that 4-FA may be able to inhibit monoamine oxidase and that it functions as a substrate-type releasing agent of dopamine, norepinephrine and serotonin. It has been shown to display amphetamine-like features *in vivo* and *in vitro*, which suggests that abuse liability might extend to humans. Further research is warranted to investigate the extent to which 4-FA displays a potential dependence in both animals in humans. Case report data in the scientific literature that unambiguously confirm a causal relationship between adverse effects of 4-FA and its presence in biofluids are limited to only a relatively small number of cases. In cases where this information is available, reported clinical features were associated with those of a sympathomimetic toxidrome. Information about a global perspective is missing. However, reports received from data monitoring systems in the Netherlands indicate increased use and popularity alongside increased numbers of notifications associated with severe adverse drug effects including serious cardiovascular toxicity.

Following a risk assessment carried out by the Dutch Centre for the Assessment and Monitoring of new drugs (CAM), it was concluded that the risk to individual health was small to moderate but with high risk for acute toxicity especially in subpopulations associated with clubbing. The risk to public health was deemed moderate to large based on rising trends in use number of health incidents. The overall risk score of 4-FA was considered high, which led to 4-FA being placed under legislative control.

1. Substance identification

A. International Nonproprietary Name (INN)

Not available.

B. Chemical Abstract Service (CAS) Registry Number

459-02-9 (free base)
64609-06-9 (hydrochloride salt)
72522-20-4 ((α*R*)-free base)
788123-23-9 ((α*S*)-free base)
127515-13-3 ((α*S*)- hydrochloride salt)
72522-24-8 ((α*R*)- hydrochloride salt)
1419922-92-1 (1,2,3,4,5,6-$^{13}C_6$-free base)
59963-24-5 (D-mannopyranose, 1-(hydrogen sulfate))
153506-17-3 (4-(fluoro-^{18}F))
72522-20-4 (*L*-leucine, *N*-acetyl- compd. with (α*R*)-4-fluoro-α-methylbenzeneethanamine, 1:1)
788123-23-9 (*L*-leucine, *N*-acetyl- compd. with (α*S*)-4-fluoro-α-methylbenzeneethanamine, 1:1)
1782279-11-1 (d_5-hydrochloride salt)
1783027-85-9 (d_5-free base)

C. Other Chemical Names

1-(4-Fluorophenyl)-2-propanamine, 1-(*p*-fluorophenyl)-2-aminopropane, 2-amino-1-(4'-fluorophenyl)propane, 2-amino-1-(4'-fluorophenyl)propane, 2-amino-1-(*p*-fluorophenyl)propane, 2-amino-1-(para-fluorophenyl)propane, *p*-fluoro-α-methylphenethylamine, 2-(4-fluorophenyl)-1-methylethylamine, 2-(4-fluoro-phenyl)-1-methyl-ethylamine, α-methyl-β-(4-fluorophenyl)ethylamine, α-methyl-β-(*p*-fluorophenyl)ethylamine, α-methyl-*p*-fluorophenethylamine, 1-(4-fluorophenyl)prop-2-ylamine, 1-(4-fluorophenyl)-2-propylamine, 1-(4-fluorobenzyl)ethylamine, 1-*p*-fluorophenyl-2-propylamine, *p*-fluoro-α-methyl-phenethylamine, *para*-fluoro-α-methyl-phenethylamine, 4-fluoro-α-methyl-phenethylamine, 4-fluoroamphetamine, *p*-fluoroamphetamine, parafluoroamphetamine, PFA, 4-F-A, PAL-303, p-FA, P-FMP, 4-FMP, 4FMP, 4-FA.

D. Trade Names

Not available.

E. Street Names

Flux, Fifa. 4-Fluo, Flo, RDJ, 4-Flava.

F. Physical Appearance

4-Fluoroamphetamine (4-FA) hydrochloride is a white crystalline powder.

G. WHO Review History

4-FA underwent a critical review in November 2015 at the 36th meeting of the WHO Expert Committee on Drug Dependence.[1] The Committee recommended that 4-FA not be placed under international control at that time due to insufficiency of data regarding dependence, abuse and risks to public health but be kept under surveillance.[2]

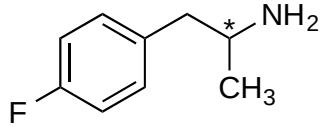
2. Chemistry

A. Chemical Name

IUPAC Name: 1-(4-Fluorophenyl)propan-2-amine
CA Index Name: 4-Fluoro-α-methyl-benzeneethanamine

B. Chemical Structure

Free base:



Note: Asterisk (*) refers to a chiral centre

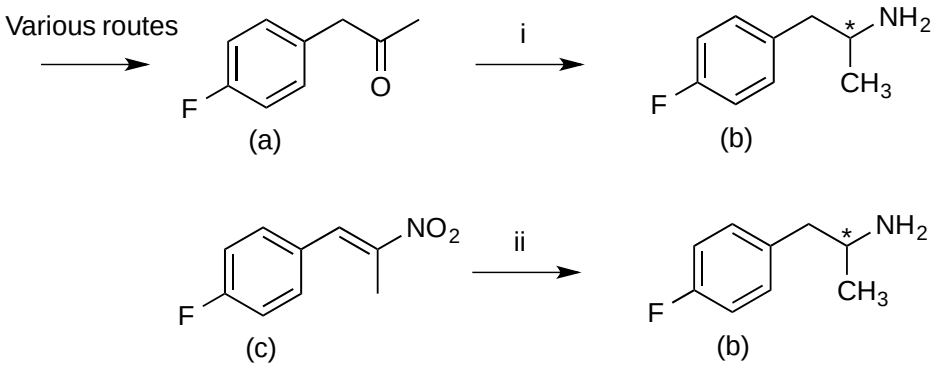
Molecular Formula: $C_9H_{12}FN$ (free base)
Molecular Weight: 153.20 g/mol

C. Stereoisomers

The presence of a chiral center at the α-carbon of the side chain gives rise to the enantiomeric pair of (*S*)-4-FA and (*R*)-4-FA, respectively. However, 4-FA is most likely to be available as the racemic mixture.

D. *Methods and Ease of Illicit Manufacturing*

4-FA may be obtained from a variety of synthetic methods that are commonly employed for the preparation of amphetamine (e.g.[3]). A classic approach might include the use of the 1-(4-fluorophenyl)propan-2-one (4-fluorobenzyl methyl ketone) (a) and exposure to reductive conditions (i) (e.g. using formamide[4]) to give racemic 4-FA (b). Depending on the availability of starting materials, precursor (a) may be obtained from a variety of synthetic procedures. Another common approach includes the reduction of the nitroalkene intermediate 1-fluoro-4-(2-nitroprop-1-en-1-yl)benzene[5-9] (c) where 4-fluorobenzaldehyde may serve as the starting material. Enantiomerically pure forms of 4-FA have been prepared with the help of enzymes[8, 10-18] and a number of chemical methods have also been reported.[6, 9, 19-21] Alternative suggestions for the preparation of 4-FA have been published.[22, 23] The EMCDDA received information in 2009 about some 4-FA samples analyzed by the Forensic Science Service. The presence of specific impurities were associated with the Leuckart synthesis, which pointed to the possible use of 4-fluorobenzyl methyl ketone) (a) as the starting material.[24]



E. Chemical Properties

Melting point

Hydrochloride salt: 156–157 °C (dry acetone)[4]
Hydrochloride salt: 152–154 °C[6]
Hydrochloride salt: 152–154 °C[22]
Hydrochloride salt: 152–154 °C[23]
(α*S*)-Hydrochloride salt: 195–198 °C[6]
(α*R*)-Hydrochloride salt: 195–197 °C[6]
Hydrochloride salt: 156.9 °C[25]

Boiling point

Free base: 95–96 °C (17 mmHg)[4]
Free base: 78 °C (10 mmHg) [6]
Free base: 96 °C (19.5 mmHg)[23]
Free base: 90 °C[22]

Solubility

~Ten mg/ml in phosphate-buffered saline (pH 7.2); ~20 mg/ml in ethanol; ~30 mg/ml in dimethylformamide and dimethyl sulfoxide.[26]

F. Identification and Analysis

Chemical and analytical data are abundantly available. 4-FA has also been employed for analytical purposes and featured in a range of routine methods of analysis associated with forensic and clinical investigations (Annex 2). Analytical challenges, especially when dealing with low concentrations encountered in biological matrices, might arise when attempting to differentiate 4-FA from its other two regioisomers 2-FA and 3-FA. The availability of all three substances used as standard reference material would be recommended.

3. Ease of Convertibility Into Controlled Substances

No information available.

4. General Pharmacology

4-FA is a ring-substituted amphetamine with psychostimulant properties. From a contextual perspective, earlier research involving 4-FA is related to work on substances with pressor effects (e.g. ephedrine derivatives), in addition to research on potential appetite suppressants (e.g. fenfluramine or phenmetrazine) and other substances known to interact with the monoaminergic system, such as *L*-deprenyl or 4-chloroamphetamine.

A. Routes of administration and dosage

4-FA appears to be most commonly administered orally or by nasal insufflation (snorting) although the latter has also been associated with intense intranasal burning sensations and pain.[27-29] A case of intravenous injection has also been mentioned in a patient undergoing opioid maintenance treatment.[30] A recent survey of 4-FA users in the Netherlands revealed a range of dosage levels that may be encountered: 50–100 mg (44 participants, 17.6%), 100–150 mg (105 participants, 42.2%), more than 150 mg (48 participants, 19.2%, the remainder did not know).[29] Some anecdotal reports indicate that higher doses might also be used.[27, 31] Somewhat comparable doses have been described for oral administration elsewhere: 'light' 50-70 mg, 'common' 70-110 mg; 'heavy' 115-150.[32] Experience reports, either involving 4-FA alone or in combination with other substances are available, for example, on the Erowid website.[33] Examples can be found in the patent literature where 4-FA was captured as a potential ingredient in pharmaceutical formulations.[34-37] The analysis of three capsules collected in the Netherlands revealed the presence of 112 mg, 156 mg and 179 mg 4-FA.[38]

B. Pharmacokinetics

Information collected from systematic studies in humans is lacking. 4-FA has been identified as one of several metabolites formed in male Sprague-Dawley rat brain in following subcutaneous and intracerebral of *para*-fluoro-deprenyl.[39] A transient, i.e. short-lasting, reduction of serotonin levels was observed in male albino Wistar rat brain homogenates after administration of a 0.1 mmol/kg dose. Based on the analysis of drug concentration levels within the first four hours, a half-life of 3.7 h was calculated for 4-FA which compared to about 1 h in the case of amphetamine in the rat brain.[40] Intravenous injection of 4-[^{18}F]A into female CF-1 mice revealed that uptake into brain tissue was detected after 5 minutes (% does/organ: liver > kidneys > lungs > small intestines > brain > spleen) followed by a rapid decline at the 30 min and 60 min mark.[41] Duration of effects obtained from self-reports of 4-FA users: less than 4 hours (57 participants, 22.9%), 4–6 hours (110 participants, 44.2%); 6–8 hours (54 participants, 21.7%) and more than 8 hours (28 participants, 11.2%).[29] Duration of effects (all routes of administration) have also been estimated as follows: 30-90 min for onset, 4-8 h duration and 1-24 hours after effects.[32]

C. Pharmacodynamics

As summarized in Tables 1 and 2, earlier work pointed toward interactions with the monoaminergic system and data obtained from studies in more recent years supports the suggestion that 4-FA functions as a substrate-type releasing agent of dopamine (DA), norepinephrine (NE) and serotonin (5-HT) and that it displays amphetamine-like features in a number of *in vivo* and *in vitro* assays. 4-FA induces locomotor activity in mice and is associated with DA release in rat striatum and rat nucleus accumbens.[42, 43] Drug discrimination studies performed in rats showed that 4-FA substituted for the 5-HT releaser fenfluramine[44] although this was not confirmed in rats trained to discriminate between the 5-HT releasing agents (+)-MBDB, 5-methoxy-6-methyl-2-aminoindan (MMAI) and saline. In this case, 4-FA fully mimicked (+)-amphetamine instead.[42] It has recently been shown that 4-FA differed from (+)-amphetamine in its ability to result in higher 5-HT dialysate concentrations. These levels were obtained from rat nucleus accumbens and correlated with diminished motor stimulant activity, which led to the hypothesis that 5-HT release might be able to dampen the stimulant effects of amphetamine-type substances that are mediated by DA.[43] Some user reports indicate that 4-FA might show pro-social effects in humans that might share some overlap with MDMA.[29, 45] Recent *in-vitro* investigations involving various monoamine transporter uptake inhibition and release assays suggest that 4-FA displays catecholamine selective properties at NET and DAT with significantly less pronounced interactions with the serotonin transporter SERT (Table 1).

Table 1. 4-FA *in-vitro* uptake and release data

Uptake [a]			Release [b]			Affinity [c]			Ref
DAT IC_{50}/µM	NET IC_{50}/µM	SERT IC_{50}/µM	DAT	NET	SERT	DAT K_i/µM	NET K_i/µM	SERT K_i/µM	
--	--	ID_{50} = 10 [d]	4.10% [d]	1.16% [d]	2.98% [d]	--	--	--	Magyar and Knoll[46]
ID_{50} = 48.7 [d]	--	ID_{50} = 10 [d]	--	--	--	--	--	--	Magyar et al.[47]
0.270	0.356	2.352	--	--	--	--	--	--	Marona-Lewicka et al.[42]
--	--	--	EC_{50}/nM 51.5	EC_{50}/nM 28.0	EC_{50}/nM 939	--	--	--	Wee et al.[7]
0.77	0.42	6.8	EC_{50}/nM 200	EC_{50}/nM 37	EC_{50}/nM 730	--	--	--	Nagai et al.[48]
9.5	10.3	94.83	--	--	--	--	--	--	Rosenauer et al.[49]
3.7	0.2	134	Yes [e]	Yes [e]	Yes [e]	11.0	13.5	32.1	Rickli et al.[50]
0.091	0.0426	3.12	EC_{50}/nM 1400, E_{max} = 96%	EC_{50}/nM 144, E_{max} = 145%	EC_{50}/nM 11,100, E_{max} = 99.5%	33.6	18.5	75.8	Eshleman et al.[51]
21	1.8	205	--	--	--	--	--	--	Zwartsen et al.[52]

Additional *in-vitro* data	
Receptor binding profiles [c]: K_i (µM): 5-HT_{1A} = 4.4; 5-HT_{2A} = 11.3; 5-HT_{2C} = 7.8; α_{1A} > 4.9; α_{2A} = 4.4; D_1 > 12; D_2 > 20; D_3 > 17; H_1 > 13; $TAAR1_{rat}$ = 0.08; $TAAR1_{mouse}$ = 0.32; $TAAR_{human}$ = 2.3; 5-HT_{2B} receptor activation: EC_{50}/µM = 11.4; activation efficacy = 49%.	Rickli et al.[50]

Comparison with MDMA: K_i (μM): 5-HT_{1A} = 12.2; 5-HT_{2A} = 5.9; 5-HT_{2C} > 13; α_{1A} > 6; α_{2A} = 15; D_1 > 12; D_2 = 25; D_3 > 17; H_1 > 13; TA_{1rat} = 0.37; TA_{1mouse} = 2.4; TA_{1human} = 14.6. [50] Cytotoxicity: None detected under conditions used. [f]	
Release studies in mice using radiolabeled cardiac norepinephrine showed that 4-FA cause dose-dependent release. At the 10 mg/kg level, observed release following amphetamine and *d*-methamphetamine administration was comparable. [g]	Benington and Morin [5]
Inhibition of monoamine oxidase (MAO): 40% inhibition at 1 mM. [h]	Beregi et al.[53]
Inhibition of phenethanolamine *N*-methyltransferase studied with pI_{50} = 3.01; comparison with tranylcypromine: pI_{50} = 4.05 [i]	Fuller et al.[54]
Binding to phenylalanyl-tRNA synthetase isolated from E. coli with K_i = 0.48 mM.	Santi et al. [55]
Investigation of MAO inhibition (MAOI) using rat brain mitochondria. I_{50} value for 4-FA = 16 μM compared to 1.9 μM for 4-chloroamphetamine. [j]	Fuller et al.[40]
Study with homologous initiating and non-initiating protein synthesis systems using rabbit reticulocyte lysates. 4-FA and amphetamine were observed to inhibit protein synthesis and aminoacylation under the conditions studied.	Nowak and Munro[56]
Investigation of MAOI using mitochondrial MAO obtained from whole rat brain homogenates. Fixed concentrations of 100 μM ^{14}C-5-HT (MAO-A) and ^{14}C-phenethylamine (MAO-B) were used as substrates. K_i/μM (4-FA) = 28 (MAO-A) and 240 (MAO-B), competitive inhibition. Comparison: K_i/μM (amphetamine) = 8.0 (MAO-A) and 475 (MAO-B).	Fuller et al.[57]
G-protein activation was not observed with 4-FA when using a [^{35}S]GTPγS binding assay.	Nonaka et al.[58]
In contrast to number cathinone derivatives, 4-FA and some other amphetamines, did not react with the tetrazolium-based WST-1 reagent when tested in the absence of SH-SY5Y neuroblastoma cells.	den Hollander et al[59]
4-FA (IC_{50} = 113 μM, reduction to 5% at 300 μM) decreased neuronal activity in multi-well microelectrode array recordings obtained from rat cortical neurons; reduction also observed with other substances, e.g. nicotine, GABA, diazepam, MK-801 and methoxetamine. Cytotoxicity: None detected under conditions used. [l]	Hondebrink et al.[60]
Binding and activity at rat, mouse, and human trace amine-associated receptor 1 (TAAR1).[m] Binding (K_i, μM) / activation (EC_{50}, μM): 0.081 / 0.069 (E_{max} = 78%) (rat); 0.32 / 0.13 (E_{max} = 77%) (mouse); binding not determined / 3.5 (E_{max} = 67%) (human). Comparison with MDMA: 0.37 / 1.0 (E_{max} = 56%) (rat); 2.4 / 4.0 (E_{max} = 71%) (mouse); binding not determined / 35 (E_{max} = 26%) (human).	Simmler et al.[61]

[a] Ref[42]: Uptake inhibition studies carried out using rat whole synaptosomes. Ref [46, 47]: rat brain homogenates obtained from CFY Sprague-Dawley rats following synaptosomal preparation procedure published by Snyder and Coyle for uptake inhibition studies.[62] Concentrations of [^{3}H]5-HT, [^{3}H]DA and [^{3}H]NE were 0.1 nmol/mL. Ref [50]: HEK293-hDAT, HEK293-hNET, HEK293-hSERT; *N*-methyl-[^{3}H]-nisoxetine and indatraline (NET), [^{3}H]citalopram and indatraline (SERT), [^{3}H]WIN35,428 and indatraline (DAT). Ref [42]: whole brain minus cerebellum (male Sprague-Dawley rats) used for synaptosomal preparations; [^{3}H]5-HT, [^{3}H]DA and [^{3}H]NE (10 nM). Ref [49]: HEK293-hSERT, HEK293-hNET and HEK293-hDAT. Cells incubated with test compounds for 5 min before the tritiated substrates were added to incubation buffer: 0.03 μM [^{3}H]5 HT and 0.05 μM [^{3}H]MPP^+. Ref[51]: HEK-hDAT, HEK-hNET and HEK-hSERT); [^{3}H]DA (hDAT), [^{3}H]5-HT (hSERT) or [^{3}H]NE (hNET) (20 nM final concentration). Ref[52]: HEK293-hSERT, HEK293-hNET and HEK293-hDAT; uptake of undisclosed fluorescence substrate, which was not subject to transporter-mediated release when tested using MDMA.

[b] Ref [7]: Synaptosomal preparations: rat caudate (for DA release) or whole brain minus cerebellum and caudate (for NE and 5-HT release); [^{3}H]MPP^+ as radioligand for DA and NE release, [^{3}H]5-HT for 5-HT release measurements. Ref [50]:

100 µM of test compound used. HEK293-hDAT, HEK293-hNET, HEK293-hSERT ([3H]DA, [3H]NE, [3H]5-HT). Ref [48]: Synaptosomal preparations from male Sprague-Dawley rats: striatum for DA and cortex for 5-HT and NE; re-uptake assay initiated by addition of [3H]DA (63 nM), [3H]5-HT (125 nM), and [3H]NE (125 nM); reaction mixture was incubated at 37 °C for 5 min; for release assays: synaptosomes pre-loaded with [3H]DA, [3H]5-HT, and [3H]NE; release terminated after 5 min ([3H]DA) and 30 min ([3H]5-HT, and [3H]NE). Ref[51]: HEK-hDAT, HEK-hNET and HEK-hSERT) using a superfusion approach; cells preloaded with [3H]DA, [3H]5-HT or [3H]NE): normalized to maximal effect of methamphetamine (hDAT, hNET, and hSERT) or *p*-chloroamphetamine (some hSERT).

[c] Ref [50]: [3H]-8-OH-DPAT and indatraline (5-HT_{1A}), [3H]ketanserin and spiperone (5-HT_{2A}), [3H]mesulergine and mianserin (5-HT_{2C}), [3H]prazosin and risperidone (α1 adrenergic receptor), [3H]rauwolscine and phentolamine (α2 adrenergic receptor), [3H]SCH 23390 and butaclamol (DA_{D1}), [3H]spiperone and spiperone (DA_{D2} and DA_{D3}), [3H]pyrilamine and clozapine (H_1) and [3H]-RO5166017 and RO5166017 (TA_1). 5-HT_{2B} activation: HEK293-h5-HT_{2B} and FLIPR assay. Ref[51]: Binding [125I]RTI-55; HEK-hDAT, HEK-hNET and HEK-hSERT).

[d] Ref [46]: release from synaptosomes (rat cerebral cortex, hypothalamus and striatum) determined as a percentage value relative to control following the approach published by Ferris et al.[63] Methamphetamine values: [3H]5-HT (5.23%), [3H]DA (8.85%) and [3H]NE (4.75%).

[e] Ref [50]: Monoamine release expressed as percent reduction of monoamine cell content compared with vehicle (0% = no release; 100% release all monoamines released from the cells). DAT: ~45%, NET: ~40%; SERT: ~50%. Essentially comparable with releasing activity of MDMA.

[f] ToxiLight BioAssay (4 h, 37 °C, incubation with 100 µM test drug).

[g] Ref [5]: Injected DL-norepinephrine-7-3H retained by the heart tissue after drug treatment (tail vein injection of the male Swiss white mice); drugs administered subcutaneously after 1 h and mice were sacrificed after 3 h. At 10 mg/kg levels: 4-FA = 50%, amphetamine = 58% and *d*-methamphetamine = 57%.

[h] Ref [53]: Warburg's technique was employed using tyramine as the substrate. Incubation was for 1 h at pH 7 and 37 °C.

[i] Ref [54]: Enzyme source: homogenate of whole rabbit adrenals; norepinephrine (40 µM) as substrate and measurement of epinephrine formation; enzyme transfer of methyl group using *S*-adenosyl-L-methionine (SAM) as co-factor.

[j] Ref [40]: [14C]Serotonin as substrate (100 µM); incubation with enzyme) for 20 min at 37 °C.

[k] Ref [60]: Cortical neurons from cortex of Wistar rat pups at postnatal day 0-1; cortical cultures consisted mainly of excitatory glutamatergic neurons, inhibitory GABAergic neurons and astrocytes.

[l] Ref [60]: Rat cortical neurons using the Neutral Red assay (30 min, 37 °C, incubation with 300 µM and 1 mM test drug).

[m] Ref [61]: Binding: HEK293 cells; [3H]RO5166017 as TAAR1 radioligand (not determined for human TAAR1); functional activity (cAMP measurements): endogenous TAAR1 ligands β-PEA, *p*-tyramine, and tryptamine (E_{max} = 100%, 94% and 91%).

Table 2. 4-FA *in-vivo* assays

Behaviour	**Neurochemistry / physiological responses / etc.**	**Ref**
--	Toxicity studies in mice; see Section 5. In dogs and guinea pigs pressor activities were noted whereas depressing effects were observed in rabbits.	Suter and Weston[4]
--	Toxicity data from mice; see Section 5; hypertensive effects equivalent to amphetamine; anorectic effects	Beregi et al.[23]

	investigated in the rat at 5 mg/kg; locomotor effects in mice investigated at 10 mg/kg. [a]	
--	Anorectic dose (mg/kg): rat = 3.5; dog = 2.[b] Analgesia (mg/kg): mice = 10. [b] Anticonvulsant action (mg/kg): mice > 20. [b] Vasopressive action. Rat: increase at 0.25 and 0.5 mg/kg. [b]	Beregi et al.[53]
--	No significant impact on whole rat brain serotonin levels compared to saline. [c]	Fuller et al.[64]
Intraperitoneal administration of 5 mg/kg and 10 mg/kg 4-FA in rats trained by the Sidman avoidance conditioning procedure. At the 10 mg/kg dose, bar pressing was disrupted and resulted in death within 6–20 h.	--	Beaton et al.[65]
--	Temporary reduction of whole rat brain serotonin and 5-hydroxyindoleacetic acid levels and tryptophan hydroxylase activity (up to 24 h) whereas 4-chloro and 4-bromoamphetamine caused reductions for up to a week. [d]	Fuller et al.[40] and Gál [66]
--	4-FA administration resulted in reduction of serotonin levels in brainstem and telencephalon when pre-treated with iprindole. No changes observed 24 h without pre-treatment. Two weeks later, reductions were observed in both tissue extracts under both treatment conditions. [e]	Sherman et al.[67]
Unpublished data were mentioned that 4-FA did not show effects on the central nervous system. Details not reported.	Administration of 14 mg/kg (i.p.) resulted in a 11.1% increase of tryptophan levels in rat brain whereas serotonin and 5-hydroxyindoleacetic acid levels did not change significantly. In comparison, a 10 mg/kg dose of amphetamine led to an increase of 96% with respect to tryptophan. Similarly, serotonin and 5-hydroxyindoleacetic acid levels remained unchanged. [f]	Vial et al.[68]
--	Anorectic properties of 4-FA comparable to *d*-amphetamine (ED_{50} = 2.5 vs. 1.8 mg/kg) [g]	Beregi and Duhault[69]
--	4-FA administration (100 µmol/kg) failed to produce serotonin depletion in rat brain sections 3 days after administration. [h]	Harvey et al.[70]
--	Intracerebroventricular injection of 4-FA (200 µg) resulted in increased levels of serum corticosterone, which pointed toward a mechanism without serotonin involvement since serotonergic neurotoxin administration did not impact on elevation. Dexamethasone pre-treatment (4 mg/kg) 4 h before	McElroy et al.[71]

	measurement prevented corticosterone elevation. [i]	
Drug discrimination: [j] Rats trained to discriminate fenfluramine (3 mg/kg) and saline. 4-FA (1.0–4.0 mg/kg) was found to substitute; 100% lever selection at 4 mg/kg.	--	McElroy and Feldman[44]
--	Tissue distribution following 4-[^{18}F]A administration (i.v.) in mice was detected after 5 min (% does/organ: liver > kidneys > lungs > small intestines > brain > spleen) followed by a rapid decline at the 30 min and 60 min mark. [k]	Shiue et al.[41]
Drug discrimination: [l] 4-FA displayed (+)-amphetamine-like discriminative stimulus effects; ED50: 0.23 mg/kg, 1.25 µmol/kg for training drug; 0.43 mg/kg, 2.11 µmol/kg for 4-FA; no substitution observed for (+)-MBDB and MMAI.	Microdialysis (rat striatum): [l] At 7 mg/kg (i.p.) of 4-FA, increase of extracellular DA levels (849%) 1 h after administration and non-significant decrease in DOPAC; when DA returned to baseline 3 h later, DOPAC and HVA concentrations still decreased. No effects of the 1.75 mg/kg dose. DA levels increases ~250% at 3.5 mg/kg (at 90 and 120 min post-injection). (+)-Amphetamine (2.0 mg/kg, i.p.) significantly increased dialysate DA levels from 30 to 120 min. DOPAC concentration not altered but HVA significantly decreased in DA dialysates at 1.5 h and 2 h following amphetamine injection.	Marona-Lewicka et al.[42]
Self-administration: [m] In rhesus monkeys, 4-FA functioned as a positive reinforcer under fixed-ratio (FR) 25 schedule (biphasic dose-response) and progressive-ratio (PR) conditions; reinforcing efficacy (PR schedule) lower than that of *d*-amphetamine. Potency (FR): 0.3 mg/kg; potency (PR): ED_{50} = 0.26 µmol/kg/injection; *d*-amphetamine: ED_{50} = 0.04 µmol/kg/injection.	--	Wee et al.[7]
Evaluation of anti-Parkinson effects: [n] 4-FA reported to significantly reduce haloperidol-induced catalepsy in rats. Conditioned place preference: [n] 4-FA did not in behaviour associated with drug dependence.	--	Nagel and Schmidt[34, 35]
--	Cortical EEG and EMG: [o] 5 mg/kg (i.p.); wakefulness period: power spectral patterns revealed an increase in frequency range of 7.0–8.5 Hz and a decrease in that of 11.5–19.0 Hz for the first 7 h and decreased power spectra in the range 6.5–30.5 Hz for 8 h during non-REM sleep. 4-FA led to increased wakefulness for 7 h after administration; REM and non-REM sleep not detected in 2 h to 6 h following administration.	Uchiyama et al.[72]

Locomotor activity: [p] Dialysate DA correlated positively with ambulation and stereotypy whereas dialysate 5-HT correlated positively with stereotypy but not ambulation.	Microdialysis (rat nucleus accumbens): [p] Intravenous administration of 4-FA led to dose-related increases in dialysate: DA ~ 12-fold and 5-HT ~15-fold.	Baumann et al.[43]

[a] Ref [23]: male Sprague-Dawley rats; female NMRI mice.

[b] Ref [53]: Anorexia test. Rats: dose at which food intake was reduced by 50% for 2 h when drug administered orally one hour previously; dogs: oral minimum dose delaying food ingestion for 2 h. Analgesia (Haffner's method in mice): intraperitoneal dose which inhibits reflex of biting the artery clip placed on tail by 50%. Anticonvulsant action: oral dose, which protects 50% of mice from tonic extension; bucco-occipital electroshocks were given (7.5 to 30 V lasting 1 s). Vasopressive action: blood pressure variation in pithed rat in mm Hg following intravenous administration.

[c] Ref [64]: Total radioactivity in the brain of male white Harlan rats was measured 20 min after i.p. injection of 5 µmol DL-5-hydroxytryptophan-3-^{14}C/kg.

[d] Ref [40]: male albino Wistar-derived rats; drugs administered intraperitoneally; drug levels in whole brain determined by methyl orange assay; tryptophan hydroxylase activity in whole brain homogenates assayed spectrofluorometrically; monoamine oxidase inhibition also determined, see Table 2. Serotonin and 5-hydroxyindoleacetic acid levels in whole brain assayed spectrofluorometrically following derivatization.

[e] Ref [67]: Sprague-Dawley rats; intraventricular injection (600 µg) with and without iprindole (10 mg/kg, i.p. one hour before drug administration) treatment; cerebellum was removed to dissect into brainstem and telencephalon-cortical sections.

[f] Ref [68]: female Wistar rats; brain levels of tryptophan, serotonin and 5-hydroxyindoleacetic acid were determined following sacrifice after 60 min (amphetamine) and 90 min (4-FA), respectively.

[g] Ref [69]: male Long-Evans rats; inhibition of food consumption determined 2 h after oral administration of test drug. Values expressed as percentage of food consumed the preceding day.

[h] Ref [70]: Male albino rats (i.p. drug administration); brain sections B-7, B- and B-9 were isolated three days following administration and stained to determine abnormal staining and extent of neurotoxicity.

[i] Ref [71]: Male albino rats; trunk blood collected one hour after injection (i.c.v.); dexamethasone treatment (inhibition of pituitary ACTH secretion): 4-FA given 1 h before rats were sacrificed (i.p.) and either after dexamethasone (1 mg/kg i.p.) or 4 h after dexamethasone (4 mg/kg i.p.); chronic depletion of brain 5-HT induced by pre-treatment with *p*-chlorophenylalanine or 5,7-dihydroxytryptamine.

[j] Ref [44]: male albino rats.

[k] Ref [41]: Female CF-1 mice; lateral tail vein injection (0.3-1.5 mCi); tissues removed after 5, 30 and 60 min.

[l] Ref [42]: Male Sprague-Dawley rats; trained with (+)-amphetamine (1 mg/kg, 5.4 µmol/kg (i.p.), (+)-MBDB (1.75 mg/kg, 7.18 µmol/kg (i.p.), MMAI (1.71 mg/kg, µmol/kg (i.p.). Microdialysis: dialysate collected every 30 min (striatum); i.p. injections of 4-FA HCI at 1.75, 3.5, and 7.0 mg/kg followed by determination of dopamine, DOPAC, and HVA; dialysates collected 1.5 h before and extended 3 h after injection of 4-FA or (+)-amphetamine.

[m] Ref [7]: Seven male rhesus monkeys; FR schedule: baseline dose of cocaine = 0.03 mg/kg/injection; test drugs 0.003–1.0 mg/kg; PR schedule: baseline dose of cocaine maintaining maximum injections = 0.1 or 0.3 mg/kg/injection; test drug available at doses of 0.003–1.0 mg/kg. For FR schedule, mean dose that maintained responding at the peak of the biphasic curve was calculated for potency. For PR schedule, ED_{50} dose of dose-response function obtained in individual monkey and averaged across monkeys for mean.

[n] Refs [34, 35]: Sprague-Dawley rats; several fluorinated amphetamines and MDMA were included in these investigations. 1-(3,4-Difluorophenyl)-*N*-ethylpropan-2-amine was chosen as a representative example and it was claimed that 4-FA, amongst others, gave similar results. Haloperidol (i.p.) was administered at 0.5 mg/kg; quantitative assessment of descent latencies (in seconds) included comparisons between haloperidol and test drug. Conditioned place preference: daily dose of test drug was 5 mg/kg following FDA protocols; further details were not provided.

[o] Ref [72]: Male Sprague-Dawley rats (10 weeks old); electromyogram (EMG) and electroencephalogram (EEG) recorded for 48 h (24 h after saline treatment and 24 h following i.p. drug administration of 5 mg/kg); Cortical EEG and EMG signals were amplified, filtered (EEG, 0.5–35 Hz; EMG, 16–128 Hz) and recorded using 'SleepSign' software.

[p] Ref [43]: Male Sprague-Dawley rats; microdialysis probe tips implanted in nucleus accumbens; locomotor activity: sensor ring lined with photobeams spaced 2.54 cm apart positioned in horizontal plane; activity monitored in 20 min bins, starting 60 min before intravenous drug injections and continuing for 120 min; ambulation and stereotypy quantified separately; ambulation is defined as the total distance travelled in the horizontal plane (measured in cm); rats received intravenous injection of 1 mg/kg of drug at time 0, followed by 3 mg/kg at 60 min and dialysate samples were analysed for 5-HT and DA.

5. Toxicology

Oral administration (white female Cartworth mice): LD_0 = 15 mg/kg, LD_{50} = 25 mg/kg, LD_{100} = 50 mg/kg.[4] Intraperitoneal administration in mice: LD_{50} = 46.4 mg/kg[23, 73] and 46 mg/kg, respectively.[53] Oral administration (male CD Servier mice): LD_{50} = 150 mg/kg (comparison to *d*-amphetamine under identical conditions: 120 mg/kg).[69] A cytolysis test with 4-FA did not lead to observations of cytotoxic effects under the conditions studied (4 h incubation at 37°C, drug concentration 100 μM).[50] Similarly, another cell viability test studying the exposure of 4-FA to cortical neurons did not affect cell integrity under the investigated conditions (30 min incubation at 37°C, drug concentration 300 μM and 1 mM).[60] Data on the effects of 4-FA metabolites are not available.

6. Adverse Reactions in Humans

Tables 3 and 4 provide an overview of fatal and non-fatal intoxications obtained from the scientific literature and from reports received by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). Case report data reported in the scientific literature that unambiguously confirm a causal relationship between adverse 4-FA and presence in biofluids are limited to a relatively low number of cases. In cases where this information is available (e.g.[74]), sympathomimetic features, typically encountered with some amphetamine-type stimulants, have been observed. A recent study carried out in the Netherlands,[29] which included a systematic survey of 4-FA users, who identified the main positive effect and the three most frequent adverse effects, confirmed that adverse effects of 4-FA were consistent psychomotor/entactogen stimulant profile (Table 5).

Dutch drug monitoring systems have received reports on increasing numbers of acute toxicity cases among young people attending large-scale events (median age 23 years). Between January and September 2016, 16% of reported acute toxic effects were associated with 4-FA, which compared to no reports between 2009 and 2011, less than 1% in 2012 and 2013, 2% in 2014, and 11% in 2015. The Dutch Poisons Information Center was also

reported to receive increasing numbers of reports of 4-FA intoxications that increased from two in 2011 to 44 in 2015. Between January and September 2016, 33 reports were received, which included increasing number of reports of adverse drug reactions beyond those commonly observed with amphetamine-like substances (e.g. agitation, tachycardia, hypertension, and hyperthermia), such as pronounced cardiovascular toxicity, severe headache and cerebral hemorrhage but also deaths (analytical only available in some cases).[75, 76] Following a risk assessment carried out by the Dutch Centre for the Assessment and Monitoring of new drugs (CAM), it was concluded that the risk to individual health was small to moderate but with high risk for acute toxicity especially in subpopulations associated with clubbing. The risk to public health was deemed moderate to large based on rising trends in use number of health incidents. The overall risk score of 4-FA was considered high.[38]

Table 3. Fatal case reports associated with 4-FA published in scientific literature.

Year	Cases	Patient, age	Context/clinically related comments	Notes	Reference
2012	1	F, 44	HIV-positive, 18 years history of drug dependence undergoing methadone treatment. Death considered due to high level of methadone in combination with 4-FA and amphetamine.	Whole blood analysis: 4-FA 0.58 mg/kg; amphetamine 0.30 mg/kg; diazepam 0.029 mg/kg; oxazepam 0.043 mg/kg, methadone 0.65 mg/kg.	Johansen and Hansen[77]
2013	1	Not reported	Not reported.	Detection of 4-FA in one post-mortem blood sample.	Rosano et al.[78]
2017	2	Not reported	See also Table 4 (Nugteren-Van Lonkhuyzen et al.[75]) Analytical confirmation (in blood/urine) in 4 cases. Three of these patients ingested one 4-FA capsule (plus cannabis in 1 patient) and developed severe headache and cerebral hemorrhage. One of these patients died. The fourth patient died due to extensive bowel ischemia following chronic 4-FA use. In this patient and in one of the patients with cerebral hemorrhage, pronounced cardiovascular toxicity was also observed. See also Wijers et al.[76]	Analytical confirmation (blood/urine; details not reported).	Nugteren-Van Lonkhuyzen et al.[75]

Table 4. Non-fatal case reports associated with detection of 4-FA reported in the scientific literature.[a]

Year[b]	Cases	Patient, age	Context/clinically related comments (examples)	Notes	Reference
2012	14	12 x M, 19-38; 2 x F, 17 and 21	Case history details not reported. Amphetamine also detected in most cases; whole blood 0.049 mg/kg – 0.70 mg/kg; in nine cases, additional drugs were detected, such as several benzodiazepines, tetrahydrocannabinol (THC), ketamine, 4-methylamphetamine and lidocaine.	Cases observed between 2009 – 2011. One rape case and 13 DUID cases. Whole 4-FA blood concentrations in DUID cases between	Johansen and Hansen[77]

				0.006 – 0.43 mg/kg (mean 0.087 mg/kg, median 0.021 mg/kg).	
2012	2	Not reported	Case 1: police traffic check and medical examination indicative of sympathomimetic drug use: pupils dilated and delayed contraction in response to light; fingertips trembling; Romberg test showed tremor and swaying; restless behavior. Case 2: symptoms associated with psychostimulants, such as slow pupil light reflex, tremor and restlessness.	DUID cases. 4-FA serum concentrations 350 ng/mL and 475 ng/mL.	Röhrich et al.[74]
2012	14	Not reported	Not reported.	4-FA was detected in 12 cases subjected to hair analysis.	Rust et al.[79]
2013	14	Not reported	Not reported. Urine drug screening of authentic samples obtained from 'addiction treatment clinics' during one year in Sweden.	In combination with 4-FMC (3), 3-FMC (1) and MDPV (1). Total number of urine samples: 1335.	Al-Saffar et al.[80]
2013	2	Not reported	Not reported. In 2010, 103 cases received from patients presenting at emergency departments across Sweden.	4-FA detection reported in 2 cases.	Helander et al.[81]
2013	5	Not reported	Not reported. Analysis of 1335 DUID cases.	4-FA detected in whole blood and considered below the legal limit.	Pedersen et al.[82]
2014	1	M, 18	Abrupt onset of nausea, vomiting, shortness of breath and chest tightness 5 h after drug consumption; received intramuscular naltrexone two days prior to admission as part of opioid addiction treatment program; fluoxetine and trazodone were also taken. 'Reverse takotsubo cardiomyopathy': cardiogenic shock developed requiring invasive management and life support. Acute cardiomyopathy caused by 4-FA catecholamine- induced myocarditis and/or small vessel myocardial ischemia was suggested. It has been suggested that the medication used during treatment might have contributed to triggering the Takotsubo syndrome.[83]	4-FA urine and serum levels 64,000 ng/mL and 118 ng/mL. Also detected in urine: naproxen, fluoxetine, trazodone, naltrexone, nicotine, and cotinine in urine; in serum: 4-FA, naproxen, trazodone, and cotinine.	Al-Abri et al.[84]
2015	1	M, 27	Patient with history of polysubstance dependence; agitated with non-sensible speech, diaphoresis, dilated pupils, and hyperreflexia without clonus. Vital signs included: heart rate 156 beats/min and rectal temperature 41.4 °C; treatment: dextrose (50 g, i.v.), midazolam (multiple boluses, i.v., 28 mg in total), and submerged in ice water.	Urine tests with EMIT positive for amphetamines and PCP; qualitative serum and urine analysis confirmed presence of 4-FA. Poklis et al.[85]: Serum 23 h post-admission (ng/mL): 4-FA 1,400; PCP 4.7; diazepam 170;	Laskowski et al.[86] Quantiative data reported by Poklis et al.[85]

				nordiazepam 83 (treatment-related). Urine 4 h post-admission (ng/mL): 4-FA 285,000; PCP 107 (diazepam and metabolite not detected). Urine 23 h post-admission (ng/mL): 4-FA 124,000; PCP 12; diazepam 420; nordiazepam 2,000.	
2015	1	M, 35	Routine traffic control; slow coordination, deficiency in concentration, washed-out pronunciation, agitation, restlessness, dry mouth, eyes reddened, and glassy, and pupil abnormalities (slow reaction to light, enlarged pupils). Orders had to be repeated multiple times and the man could not follow long sentences.	Blood sample taken 1 h and 55 min afterwards: THC 0.9 ng/mL; 11-OH-THC < 0.8 ng/mL; THC-COOH 6.8 ng/mL; 4-FA 90.0 ng mL.	Maas et al.[87]
2015	4	Not reported	Tachycardia, headache, dizziness, restlessness, visual disturbances, tremors, agitation, tachypnea, confusion, nausea, vomiting, abdominal pain, dysphagia, neck pain, feeling of fainting.	Reported to the Dutch Poisons Information Centre in 2013.	Hondebrink et al.[88]
2016	1	Not reported	Retrospective analysis of 200 urine samples collected consecutively between October 2013 and April 2014 from 82 different opioid-dependent patients.	4-FA, amphetamine, alprazolam, THC-COOH, and zolpidem detected.	Heikaman et al.[30] Unclear if same case mentioned in Sundström et al.[89] (below).
2016	1	Not reported	Details not reported. 4-FA detected in one out of 34 irregular attendees of drug treatment centre.	4-FA detected, no further details.	Sundström et al.[89]
2017	3	M, 21 F, 19 M, 22	Self-reported use of 4-FA. One patient exhibited rhabdomyolysis; hyperthermia, hypertension and tachycardia were commonly observed; one patient consumed 14 tablets as a suicide attempt.	Analytical confirmation not available.	Knippels et al.[90]
2017	20	Not reported	See also Table 3 (Lonkhuyzen et al.[75]). Between January-September 2016, the Dutch Poisons Information Center was consulted about thirty-six 4-FA exposures; follow-up performed in 22 cases with either physician and/or patient. observed symptoms included restlessness (77%), headache (68%), anxiety (59%), tachycardia (59%), hypertension (50%), confusion, tachypnea, chest pain (all 41%), seizures and coma (both 14%). More pronounced cardiovascular toxicity observed in 11 cases (2 analytically confirmed). These included conduction abnormalities (prolonged QTc or QRS interval, right bundle branch block), acute heart failure and arrhythmias (including bigeminy). One of these patients developed inverted	Analytical confirmation in 6 cases (blood/urine; details not reported)	Nugteren-Van Lonkhuyzen et al.[75]

			Takotsubo cardiomyopathy following intake of two 4-FA capsules with a 30-minute interval (plus 5 units of alcohol, exposures not analytically confirmed). Analytical confirmation (in blood/urine) in 4 cases. Three of these patients ingested one 4-FA capsule (plus cannabis in 1 patient) and developed severe headache and cerebral hemorrhage. One of these patients died. The fourth patient died due to extensive bowel ischemia following chronic 4-FA use. In this patient and in one of the patients with cerebral hemorrhage, pronounced cardiovascular toxicity was also observed. See also Wijers et al.[76]		
2017	4	Not reported	Analysis of 558 blood samples and 199 oral fluid samples obtained during traffic controls in Belgium between January and August 2015. In one case, blood-red eyes, dry mouth, trembling arms and legs but also equanimity was observed (amphetamine and THC-COOH also detected).	4-FA detected in 3 blood samples and 1 oral fluid sample. All samples also contained other drugs.	Wille et al.[91]
2017	1	F, 18	Presented to emergency department (ED) after drinking two capfuls of "Molly's Mosquito cap"; also reportedly insufflated 110 mg methylphenidate and ingested 800 mg modafinil. Experienced headache, nausea, vomiting, lightheadedness, and diaphoresis. On arrival to emergency department, only complaint was anxiety; clinical feature features were associated with acute dilated cardiomyopathy and myocardial injury.	4-FA detected in urine at 37,000 ng/mL. Concentrations of methylphenidate and modafinil not reported.	Wolf et al.[92]

[a] The EMCDDA also received reports about positive identifications of 4-FA in biofluids although case level data are not reported, see Table 7. [b] Year of publication with allocation of volume and page number; some cases have been encountered before that.

Table 5. Reported positive and adverse effects of 4-FA, adapted from Linsen et al.[a,29]

Effects	***n***	**%**	***95% CI***
Positive			
Stimulatory	145	58.2	51.4–64.3
Euphoria	69	27.9	22.1–33.3
Empathic	24	9.6	6.0–13.3
Sedative	11	4.3	1.1–5.6
Adverse			
Difficulty falling asleep	133	53.4	47.4–59.0
Dry mouth	109	43.8	37.4–50.2
Jaw tension/cramp	106	42.6	36.2–49.0
Elevated heartbeat	92	36.9	30.9–43.4
Sweating/high body temperature	83	33.3	27.7–39.4
Lowered mood in the days after use	49	19.7	14.9–24.5
Muscle weakness in days after use	46	18.5	13.7–23.3
Nausea	17	4.8	3.6–10.0
The drug had no effect	16	6.4	3.6–9.6
Tachycardia	29	11.6	8.0–15.7

Headache	21	8.4	5.2–12.4
Loss of memory (while intoxicated)	17	6.8	4.0–10.4
Unpleasant hallucinations	3	1.2	0.0–2.8
Difficulty breathing	2	0.8	0.0–2.0
Tolerance (higher dose needed after first use)	16	6.4	3.6–9.6
Other	6	2.4	1.0–4.6
[a] Multiple answers were possible for adverse effects; CI = confidence interval. Users of 4-FA have identified pro-social effects similar to MDMA, which was not associated with effects induced by amphetamine.			

7. Dependence Potential

A. Animal Studies

As shown in Table 2, self-administration studies in rhesus monkeys indicated that 4-FA functioned as a positive reinforcer under fixed-ratio (FR) 25 schedule (biphasic dose-response) and progressive-ratio (PR) conditions. The reinforcing efficacy (PR schedule) was lower than that of *d*-amphetamine and it was hypothesized that the 5-HT releasing effects of 4-FA might have negatively impacted on the potency as a reinforcer compared to *d*-amphetamine. Correspondingly, it was suggested that a DA/5-HT ratio might serve as a potential predictor. Taking into account the EC_{50} values obtained from rat brain synaptosome release assays, the DA/5-HT ratio obtained for 4-FA was 0.05 compared to 0.004 for *d*-amphetamine, which reflected the higher 5-HT releasing potency of 4-FA.[7] An example was found in the patent literature where it was claimed that 4-FA did not display signs of dependence potential based on experiments that assessed conditioned place preference (Sprague-Dawley rats).[34, 35] Further studies seem warranted to determine the extent of dependence potential in animals.

B. Human Studies

Data from clinical studies in humans are not available.

8. Abuse Potential

A. Animal Studies

As shown in Tables 2 and 3 and Section 4, limited information is available. From the available data it appears that 4-FA shows classic features associated with other psychomotor stimulants. The available information related to amphetamine-like properties suggests that abuse liability might be extendable to humans. Further studies seem warranted to clarify the similarities and differences that might exist between 4-FA and amphetamine and other amphetamine-type substances.

B. Human Studies

Data from clinical studies in humans are not available.

9. Therapeutic Applications and Extent of Therapeutic Use and Epidemiology of Medical Use

Not applicable.

10. Listing on the WHO Model List of Essential Medicines

4-FA is not listed on the WHO Model List of Essential Medicines (20th List) or the WHO Model List of Essential Medicines for Children (6th List).

11. Marketing Authorizations (as a Medicinal Product)

4-FA was never marketed as a medicinal product.

12. Industrial Use

4-FA has no recorded industrial use.

13. Non-Medical Use, Abuse and Dependence

Use of 4-FA appears to be limited to recreational substance users rather than the general population. The mode of use may involve the combinational use (intentionally or unintentionally) of other drugs and users may be unaware of the exact dose or compound being ingested (by whatever route). Dependence-producing properties in humans have not been studied. The appearance of 4-FA in Europe has been observed since at least 2007. Over the years, it has been found in products sold as 'ecstasy'/MDMA tablets, amphetamine powder but also as adulterants present in other illicit controlled substances.[28, 29, 45, 88, 93-95] However, 4-FA is also available in its own right, either from on-line or off-line retailers and specifically sought after.[38, 96]

Also see Annex 1: Report on WHO questionnaire for review of psychoactive substances.

14. Nature and Magnitude of Public Health Problems Related to Misuse, Abuse and Dependence

Surveys that systematically assess the prevalence of 4-FA use within the general population are not available. Research carried out in the Netherlands suggest that 4-FA, which originally appeared as one of many new psychoactive substances (NPS) (e.g. as an adulterant or as an alternative to reduced availability of substances such as MDMA) has established itself as a substance of choice in a subpopulation of recreational substance users, especially those who might prefer using this substance in a social context.[29, 38, 88] In The Netherlands, 4-FA has become increasingly popular amongst users of new psychoactive substances who are associated with participation at music festivals and clubs, which led to increased numbers of incidents with adverse effects and high toxicity, thus, potentially leading to an in increased risk to public health.[38] Further studies are indicated to investigate the prevalence of use in other countries. Some detections of 4-FA in biofluids related to roadside testing have been described (Table 3). Dependence-producing properties in humans have not been studied.

Also see Annex 1: Report on WHO questionnaire for review of psychoactive substances.

15. Licit Production, Consumption and International Trade

No data available.

16. Illicit Manufacture and Traffic and Related Information

Information provided to Europol and EMCDDA by European Member States, Turkey and Norway is summarized in Table 6.

Table 6. Information available on the EMCDDA's European database on new drugs (EDND). The European Union Early Warning System, the Reitox National Focal Points in the EU Member States, Turkey and Norway, as well as the Europol National Units and their networks, provided data. [a]

Information provided to EMCDDA

Seizures 2008:

- Belgium – 2 seizures of powder.
- Denmark – 2 seizures; 1 sample seized (4 g) containing 1% amphetamine.
- Netherlands – 4 collected samples of powder; 1 collected sample of capsule; 1 collected sample of liquid; 3 seizures totalizing 212 g of powder and 1 seizure of liquid containing also 2C-B and BZP.

Seizures 2009 (January – June) plus update from 9th Annual EWS meeting, 4-5 June 2009, Lisbon (A. Gallegos and R. Sedefov):

- Austria – 2 seizures of powder totaling 707 g and 4 collected samples of powder.
- Belgium – 6 seizures of 33.9 g powder; 1 seizure of 49.79 g of paste/sticky powder and 1 seizure of tablets.
- Croatia: 4 seizure of white powder also containing amphetamine traces, caffeine, mannitol and creatine.
- Denmark – 5 seizures; one seizure of 4 g white powder that also contained 1% amphetamine.
- Estonia – 1 seizure of 0.32 g powder.
- Finland – 1 seizure of 12 tablets (Forensic Laboratory) and 2 seizures of powder/capsule, 5g/3units (Customs)
- France – 8 seizures of powder, 3115 g
- Germany – seizure between November 2008 and June 2009: in one case, 60 kg of amphetamine were seized, 14 of which were a mixture of amphetamine and 4-FA; two seizures of a 'few grams' of powder and one seizure of 1 kg.
- Hungary – 2 seizures of powder, 2.5 g (powder in 36 capsules); 1 collected sample of 638.2 g powder; 4 seizures of 211 tablets (from 2008), one seizure of 700 g homogenous powder.
- Netherlands – From DIMS: 60 collected samples of powder, 6 collected samples of tablets and 1 collected sample of liquid from NFI: 1 seizure of powder > 340 kg; 98 seizures of powder, > 100 kg, 98 seizures of powder > 100 kg in combination with amphetamine.
- Slovakia – 1 seizure of 16 mg powder.
- Sweden – 105 seizures of 6174 g powder; 7 seizures of 21 mL liquid; 24 biological samples of urine; 71 biological samples of blood and 57 seizures of 1460.4 g powder.
- United Kingdom – 2 seizures of 0.9 mL liquid; 102 seizures (93 kg powder); 4 seizures (6.67 kg powder); 6 seizures of paste (6.355 kg); 1 seizure of 300 g powder; 1 seizure of 7.627 kg paste containing also amphetamine. UK National Focal Point also reported a seizure of 371 mg of a pale pink powder in Guernsey.

Seizures 2010:

- Belgium – 1 seizure of 112.5 tablets containing also caffeine and piperonal; 1 seizure of 3.18 g powder containing also caffeine and amphetamine; 3 biological samples of urine.
- Denmark – 13 seizures.

- Finland – 3 seizures of 5g powder; 1 biological sample of blood; 13 seizures of 46 g powder and 1 seizure of 2 tablets (last two, both reported as 3-FA or 4-FA).
- France – 4 seizures of 340 g liquid; 7 seizures of tablets.
- Hungary –10 seizures of 35 g powder and 7 seizures of 14 tablets (reported as fluoramphetamine).
- Netherlands – 13 seizures: 2775 tablets and 65 g powder (from NFI).
- Norway – 8 seizures of 106 g powder.
- Poland – 1 seizure of 10 capsules; 1 seizure of 550 tablets containing piperonal and caffeine.
- Slovenia – 5 seizures of 9.21 g white powder, most of the samples containing caffeine.
- United Kingdom – From a variety of forensic providers. Key Forensics: 3 seizures of powder, 157.7 g; 1 seizure of powder, 23.5 g in an amphetamine sample. From LGC Forensics: 6 seizures of powder, 136.3 g; 13 seizures of powder, 520 g. From FSNI: 1 seizure of 16.76 g powder containing traces of mephedrone; 1 seizure of 102 g white powder. From FSS: 3 seizures of liquid, 1.6 mL; 152 seizures of powder, 46.4 kg; 1 seizure of 5 tablets. From Scotland: 9 seizures of powder, 529.5 g. From Key Scientifics: 3 seizures of powder, 14.46 g; 1 seizure of 2 tablets, 0.46 g; 3 seizures of powder, 5.73 g.

Seizures 2011:

- Austria –1 seizure of 9.4 g of powder.
- Bulgaria – 3 seizures of 5.60 g of powder
- Denmark – 5 (19)* seizures of 442.2 g of powder (*The number in brackets is the total number of individual exhibits in all seizures).
- Finland – 38 seizures of 198 g of powder; 4 seizures of 20 tablets/blotters; 7 seizures of 82 g of powder; 2 biological samples of blood (all reported as 2-, 3- or 4-FA).
- France – 17 seizures of tablets.
- Hungary – 18 seizures of 15 g of powder, 60 seizures of 3235 tablets; 27 seizures of 26 g powder also containing amphetamine; 1 seizure of 3 tablets also containing 2C-D; 462 biological samples.
- Italy – 1 seizure of 1.18 g of tablets; 2 seizures of powder (one seizure also contained MDPV, 4-MEC, lidocaine or procaine and the other also contained mephedrone and MDPV, lidocaine and propanamide were also identified).
- Netherlands – 7 seizures of 279 tablets and 16 g powder (from NFI); 61 collected samples of powder (from DIMS); 9 collected samples of tablets (from DIMS); 3 collected samples of liquid (from DIMS); 1 collected sample of capsules (from DIMS).
- Norway – 3 seizures of 517 g of powder.
- Spain – 1 seizure of powder.
- United Kingdom – From FSNI: 1 seizure of 2.05g of powder; from FSS: 13 seizures of 400.1 g powder.

Seizures 2012:

- Austria – 1 seized powder sample.
- Belgium – 1 kg white powder seized by customs at airport, package sent from China.
- Denmark – 3 cases of seized powder, 9.5 g.
- Spain – Seizure of 7760 tablets and 15 tablets where 4-FA was present in combination with other substances; seizure of 210 g powder.
- Finland – Customs seizures of 243.8 g powder (53 cases), 18 tablets/blotter and 28.8 g 'other'. Police seizure of 28 g powder and 10 cases of biological analyses with positive identification. Note: not clear what positional isomer might have been identified.
- France – seizures of tablets (5 cases) and two liquids (customs); police seizure of 100 tablets.
- Hungary – seizures of 421 tablets (13 cases), 2 g powder (2 cases); presence of additional substances: 7 g of powder (4-FA + amphetamine, 4 cases), 38 g of powder (4-FA + MDPV, 1 case), 33 tablets (4-FA + methoxetamine, 1 case), 30 tablets (4-FA + 4-MEC + methylone, 1 case); 43 samples of biological origin tested positive for 4-FA but details not reported.
- Netherlands – seizure of 1060.2 g (27 cases), 7 tablets and 11 tablets (4-FA + PMMA + 3,4-dimethoxymethamphetamine).
- Norway – seizure of materials in three cases and positive identification in one biofluids sample.
- Poland: 1 g powder and 2.99 g powder also containing amphetamine and caffeine.

- Slovakia – seizure of 367 g powder (see also Reitox National Focal Point Slovakia, below).
- Slovenia – 1 powdered sample (1.3 g).
- United Kingdom – Seizures of 1010 g powder (18 cases), 1488.57 mL liquid (3 cases), 1 tablet and 1 'other' (6.34 g).

Seizures 2013:

- Denmark – 2 cases of collected powder (1.9 g)
- Finland – Powder (141.6 g, 34 cases); positional isomer not specified.
- France – seizure of tablets (6378 g, 37 cases), liquid (9312 g, 40 cases), and 97 g powder (8 cases).
- Greece – seizure of 2.4 g powder
- Italy – seizure of 1.69 g powder containing 4-FA + 4-MEC + methedrone (bk-PMMA) + methylone + 5-MeO-MIPT + 5-MeO-DALT.
- Latvia – seizure of 49.42 g powder
- Poland – seizure of 796.66 g powder (64 cases)
- Spain – 50 g powder (12 cases) and 11 g powder (18 cases) containing additional substances.

Information provided to Europol

Seizures 2009 (January – June) plus update from 9th Annual EWS meeting, 4-5 June 2009, Lisbon (A. Gallegos and R. Sedefov) and newsletter related to Europol's SYNERGY project[97]

- Sweden: Two seizures of 700 g and 340 g.
- Netherlands: found at two illegal amphetamine laboratories.
- From SYNERGY newsletter: 'A total of 19 member states had replied to Europol's request for information, with four member states (Finland, Germany, the Netherlands and the United Kingdom) reporting seizures of the substance, both in powder and tablet form. Additionally, insofar not yet officially provided, the Slovak Republic has also forensically identified this substance amongst submitted seizures. Equally, reports submitted to the EMCDDA from the NFP's reveal that seizures of this substance have taken place in Belgium, Croatia, Denmark, Estonia, France and Hungary.'

  'In fact, the Dutch National Crime Squad reported that this substance was increasingly emerging on the national users market. Also, two production sites had been dismantled, with one in January 2009 where both this substance and its precursor chemical were seized; the other took place in February 2009, where traces of both this substance and amphetamine were found. Besides, one additional seizure totalling 169 kg of the substance was made in January 2009.'

Reitox National Focal Point Slovakia

- Rented premises were used for 4-FA manufacturing, packing and distribution. Mixing equipment and facilities for pills processing were encountered, including 367 g powdered 4-FA.

[a] Some of the data reported to Europol and EMCDDA may overlap. Data were drawn from bi-annual data gathering (EU EWS progress and final reports) and from individual Reporting forms submitted on an *ad hoc* basis to EMCCDA. Positive identifications of 4-FA in various biofluids have also been reported but further details are not available.

17. Current International Controls and Their Impact

4-FA is not controlled under the 1961, 1971 or 1988 United Nations Conventions.

18. Current and Past National Controls

The EMCDDA's European database on new drugs (EDND) lists that the following countries have taken legislative measures to control 4-FA: Belgium, Czech Republic, Denmark,

Finland, France, Germany, Hungary, Italy, Latvia, Lithuania, Norway, Poland, Portugal, Slovakia, Slovenia, Sweden, Turkey, United Kingdom. 4-FA is not a controlled Substance in Greece. Confirmation from all Reitox European National Focal Points, however, might be needed to obtain an update. Also see Annex 1: Report on WHO questionnaire for review of psychoactive substances.

19. Other Medical and Scientific Matters Relevant for a Recommendation on the Scheduling of the Substance

Not applicable.

References

1. World Health Organization.4-Fluoroamphetamine (4-FA). Critical review report. Agenda item 5.4. Expert Committee on Drug Dependence. Thirty-seventh Meeting. Geneva, Switzerland, 16-20 November 2015. Available at: http://www.who.int/medicines/access/controlled-substances/5.4_4-FA_CRev.pdf?ua=1

2. World Health Organization. WHO Expert Committee on Drug Dependence: thirty-seventh report. WHO Press, Geneva, Switzerland. Available at: http://apps.who.int/iris/bitstream/10665/206452/1/WHO_TRS_998_eng.pdf?ua=1.

3. Allen A, Cantrell TS. Synthetic reductions in clandestine amphetamine and methamphetamine laboratories - a review. *Forensic Sci Int* 1989;42:183-99. doi:10.1016/0379-0738(89)90086-8.

4. Suter CM, Weston AW. Some fluorinated amines of the pressor type. *J Am Chem Soc* 1941;63:602-5. doi:10.1021/ja01847a069.

5. Benington F, Morin RD. The chemorelease of norepinephrine from mouse hearts by substituted amphetamines. *J Med Chem* 1968;11:896-7. doi:10.1021/jm00310a048.

6. Plenevaux A, Dewey SL, Fowler JS, Guillaume M, Wolf AP. Synthesis of (*R*)-(-)- and (*S*)-(+)-4-fluorodeprenyl, (*R*)-(-)- and (*S*)-(+)-[N-^{11}C-methyl]-4-fluorodeprenyl and PET studies in baboon brain. *J Med Chem* 1990;33:2015-19. doi:10.1021/jm00169a034.

7. Wee S, Anderson KG, Baumann MH, Rothman RB, Blough BE, Woolverton WL. Relationship between the serotonergic activity and reinforcing effects of a series of amphetamine analogs. *J Pharmacol Exp Ther* 2005;313:848-54. doi:10.1124/jpet.104.080101.

8. Muñoz L, Rodriguez AM, Rosell G, Bosch MP, Guerrero A. Enzymatic enantiomeric resolution of phenylethylamines structurally related to amphetamine. *Org Biomol Chem* 2011;9:8171-7. doi:10.1039/c1ob06251d.

9. Lichtenberger O. Fluorphenyl- und Fluormethoxyphenylalkylamine und deren Anwendung. Patent No. DE102011015842A1, 2012.

10. Svedendahl M, Branneby C, Lindberg L, Berglund P. Reversed enantiopreference of an ω-transaminase by a single-point mutation. *ChemCatChem* 2010;2:976-80. doi:10.1002/cctc.201000107.

11. Bommarius AS, Abrahamson MJ, Bommarius B. Engineered amine dehydrogenases and methods of use thereof. US 20130309734A1. Georgia Tech Research Corporation, USA., 2013.

12. Abrahamson MJ, Wong JW, Bommarius AS. The evolution of an amine dehydrogenase biocatalyst for the asymmetric production of chiral amines. *Adv Synth Catal* 2013;355:1780-6. doi:10.1002/adsc.201201030.

13. Au SK, Bommarius BR, Bommarius AS. Biphasic reaction system allows for conversion of hydrophobic substrates by amine dehydrogenases. *ACS Catal* 2014;4:4021-6. doi:10.1021/cs4012167.

14. Green AP, Turner NJ, O'Reilly E. Chiral amine synthesis using ω-transaminases: an amine donor that displaces equilibria and enables high-throughput screening. *Angew Chem, Int Ed* 2014;53:10714-7. doi:10.1002/anie.201406571.

15. Mutti FG, Knaus T, Scrutton NS, Breuer M, Turner NJ. Conversion of alcohols to enantiopure amines through dual-enzyme hydrogen-borrowing cascades. *Science* 2015;349:1525-9. doi:10.1126/science.aac9283.

16. Gomm A, Lewis W, Green AP, O'Reilly E. A new generation of smart amine donors for transaminase-mediated biotransformations. *Chem - Eur J* 2016;22:12692-5. doi:10.1002/chem.201603188.

17. Martínez-Montero L, Gotor V, Gotor-Fernández V, Lavandera I. But-2-ene-1,4-diamine and but-2-ene-1,4-diol as donors for thermodynamically favored transaminase- and alcohol dehydrogenase-catalyzed processes. *Adv Synth Catal* 2016;358:1618-24. doi:10.1002/adsc.201501066.

18. Pushpanath A, Siirola E, Bornadel A, Woodlock D, Schell U. Understanding and overcoming the limitations of *Bacillus badius* and *Caldalkalibacillus thermarum* amine dehydrogenases for biocatalytic reductive amination. *ACS Catal* 2017;7:3204-9. doi:10.1021/acscatal.7b00516.

19. Gschwend HW, Huebner CF. 2-Aminoethyl-1,4-benzodioxans. Patent No. US4187313A. Ciba-Geigy Corp., USA, 1980.

20. Miriyala B, Bhattacharyya S, Williamson JS. Chemoselective reductive alkylation of ammonia with carbonyl compounds: synthesis of primary and symmetrical secondary amines. *Tetrahedron* 2004;60:1463-71. doi:10.1016/j.tet.2003.12.024.

21. Oslob J, Anderson R, Aubele D, Evanchik M, Fox JC, Kane B et al. Pyrimidinedione compounds against cardiac conditions. Patent No. WO 2014205223A1. MyoKardia, Inc., USA, 2014.

22. Patrick TM, McBee ET, Hass HB. Synthesis of arylpropylamines. I. From allyl chloride. *J Am Chem Soc* 1946;68:1009-11. doi:10.1021/ja01210a032.

23. Beregi L, Hugon P, Douarec JCL, Schmitt H. Nouveux produits anorexigènes et euphoriques. Patent No. FRM1658. Science Union et C[ie], Société Francaise de Recherche Médicale, 1963.

24. European database on new drugs (EDND). 4-Fluoroamphetamine (4-FA). EMCDDA, Lisbon (accessed 13 September 2015).

25. SWGDRUG. 4-Fluoroamphetamine Monograph. Latest revision 14 June 2013. Available at: http://www.swgdrug.org/Monographs/4-FA.pdf.

26. Safet Data Sheet. para-Fluoroamphetamine (hydrochloride). Revision: 21 August 2014. Cayman Chemical Company, Ann Arbor, MI. Available at: https://www.caymanchem.com/msdss/11156m.pdf.

27. Drugs-Forum. 4-fluoroamphetamine (4-FA / PFA) Experiences. Accessed https://drugs-forum.com/forum/showthread.php?t=90535&page=4 (15 September 2015).

28. Brunt TM, Niesink RJ, Poortman A, van den Brink W. Instability of the illicit psychostimulant market leads to the rise of "Legal Highs" in the Netherlands. *J Psychopharmacol* 2010;24:A60.

29. Linsen F, Koning RPJ, van Laar M, Niesink RJM, Koeter MW, Brunt TM. 4-Fluoroamphetamine in the Netherlands: more than a one-night stand. *Addiction* 2015;110:1138-43. doi:10.1111/add.12932.

30. Heikman P, Sundström M, Pelander A, Ojanperä I. New psychoactive substances as part of polydrug abuse within opioid maintenance treatment revealed by comprehensive high-resolution mass spectrometric urine drug screening. *Human Psychopharmacology Clinical and Experimental* 2016;31:44-52. doi:10.1002/hup.2512.

31. Erowid Experience Vaults. 4-Fluoroamphetamine Reports. Accessed https://www.erowid.org/experiences/subs/exp_4Fluoroamphetamine.shtml (15 September 2015).

32. Tripsit Factsheet. 4-FA. Available at: http://drugs.tripsit.me/4-FA.

33. Erowid Experience Reports. 4-Fluoroamphetamine Reports. Available at: https://erowid.org/experiences/subs/exp_4Fluoroamphetamine.shtml.

34. Nagel U, Schmidt WJ. Fluorsubstituierte Amphetamine und Amphetaminderivate und deren Verwendung. Patent No. DE 102007014286 A1. Universität Tübingen, Germany, 2008.

35. Nagel U, Schmidt WJ. Fluorsubstituierte Amphetamine und Amphetaminderivate und deren Verwendung. Patent No. WO 2008113565A1. Universität Tübingen, Germany, 2008.

36. Bird P. Compositions and methods for treating psychiatric disorders. Patent no. WO2010015029A1. Gosforth Centre Pty Ltd, Australia, 2010.

37. Bird P. Combination of pharmaceutical compositions for treatment of neurological disorders. Patent No. WO 2013007698A1. Gosforth Centre Holdings Pty Ltd, Australia, 2013.

38. Rapport Risicobeoordeling 4-fluoramfetamine. Rijksinstituut voor Volksgezondheid en Milieu. Ministerie van Volksgezondheid, Welzijn en Sport. Coördinatiepunt Assessment en Monitoring nieuwe drugs. Bilthoven, The Netherlands, 01 December 2016.Available at: https://www.rijksoverheid.nl/documenten/rapporten/2016/12/07/rapport-risicobeoordeling-4-fluoramfetamine-4-fa.

39. Lajtha A, Sershen H, Cooper T, Hashim A, Gaal J. Metabolism of (-)-deprenyl and PF-(-)-deprenyl in brain after central and peripheral administration. *Neurochem Res* 1996;21:1155-60. doi:10.1007/BF02532389.

40. Fuller RW, Baker JC, Perry KW, Molloy BB. Comparison of 4-chloro-, 4-bromo-, and 4-fluoroamphetamine in rats. Drug levels in brain and effects on brain serotonin metabolism. *Neuropharmacology* 1975;14:739-46. doi:10.1016/0028-3908(75)90099-4.

41. Shiue CY, Shiue GG, Rysavy JA, Pleus RC, Huang H, Bai LQ et al. Fluorine-18 and carbon-11 labeled amphetamine analogs-synthesis, distribution, binding characteristics in mice and rats and a PET study in monkey. *Nucl Med Biol* 1993;20:973-81. doi:10.1016/0969-8051(93)90098-F.

42. Marona-Lewicka D, Rhee GS, Sprague JE, Nichols DE. Psychostimulant-like effects of *p*-fluoroamphetamine in the rat. *Eur J Pharmacol* 1995;287:105-13. doi:10.1016/0014-2999(95)00478-5.

43. Baumann MH, Clark RD, Woolverton WL, Wee S, Blough BE, Rothman RB. In vivo effects of amphetamine analogs reveal evidence for serotonergic inhibition of mesolimbic dopamine transmission in the rat. *J Pharmacol Exp Ther* 2011;337:218-25. doi:10.1124/jpet.110.176271.

44. McElroy JF, Feldman RS. Discriminative stimulus properties of fenfluramine: evidence for serotonergic involvement. *Psychopharmacology* 1984;83:172-8. doi:10.1007/Bf00429730.

45. Brunt TM, Koeter MW, Niesink RJM, van den Brink W. Linking the pharmacological content of ecstasy tablets to the subjective experiences of drug users. *Psychopharmacology* 2012;220:751-62. doi:10.1007/s00213-011-2529-4.

46. Magyar K, Knoll J. *Para*-substituted amphetamines and brain serotonin. *Pol J Pharmacol Pharm* 1975;27, Suppl.:139-43.

47. Magyar K, Tekes K, Knoll J. The effect of para-halogenated amphetamines on brain monoamines. *Pol J Pharmacol Pharm* 1978;30:245-53.

48. Nagai F, Nonaka R, Satoh Hisashi Kamimura K. The effects of non-medically used psychoactive drugs on monoamine neurotransmission in rat brain. *Eur J Pharmacol* 2007;559:132-7. doi:10.1016/j.ejphar.2006.11.075.

49. Rosenauer R, Luf A, Holy M, Freissmuth M, Schmid R, Sitte HH. A combined approach using transporter-flux assays and mass spectrometry to examine psychostimulant street drugs of unknown content. *ACS Chem Neurosci* 2013;4:182-90. doi:10.1021/cn3001763.

50. Rickli A, Hoener MC, Liechti ME. Monoamine transporter and receptor interaction profiles of novel psychoactive substances: *para*-halogenated amphetamines and pyrovalerone cathinones. *Eur Neuropsychopharmacol* 2015;25:365-76. doi:10.1016/j.euroneuro.2014.12.012.

51. Eshleman AJ, Wolfrum KM, Reed JF, Kim SO, Swanson T, Johnson RA et al. Structure-activity relationships of substituted cathinones, with transporter binding, uptake, and release. *J Pharmacol Exp Ther* 2017;360:33-47. doi:10.1124/jpet.116.236349.

52. Zwartsen A, Verboven AHA, van Kleef R, Wijnolts FMJ, Westerink RHS, Hondebrink L. Measuring inhibition of monoamine reuptake transporters by new psychoactive substances (NPS) in real-time using a high-throughput, fluorescence-based assay. *Toxicol In Vitro* 2017. doi:10.1016/j.tiv.2017.05.010.

53. Beregi LG, Hugon P, Le Douarec JC, Laubie M, Duhault J. Structure-activity relationships in CF_3-substituted phenethylamines. In: Costa E, Garattini S, editors. International Symposium on Amphetamines and Related Compounds Proceedings of the Mario Negri Institute for Pharmacological Research New York: Raven Press Books Ltd.; 1970. p. 21-61.

54. Fuller RW, Mills J, Marsh MM. Inhibition of phenethanolamine N-methyltransferase by ring-substituted α-methylphenethylamines (amphetamines). *J Med Chem* 1971;14:322-5. doi:10.1021/jm00286a012.

55. Santi DV, Danenberg PV. Phenylalanyl transfer ribonucleic acid synthetase from *Escherichia coli*. Analysis of the phenylalanine binding site. *Biochemistry* 1971;10:4813-20. doi:10.1021/bi00801a032.

56. Nowak TS, Munro HN. Inhibition of cell-free protein synthesis initiation by amphetamine: association with reduction in tRNA aminoacylation. *Biochem Biophys Res Commun* 1977;77:1280-5. doi:10.1016/S0006-291X(77)80118-6.

57. Fuller RW, Hemrick-Luecke SK. Influence of ring and side chain substituents on the selectivity of amphetamine as a monoamine oxidase inhibitor. *Res Commun Subst Abuse* 1982;3:159-64.

58. Nonaka R, Nagai F, Ogata A, Satoh K. In vitro screening of psychoactive drugs by [^{35}S]GTPγS binding in rat brain membranes. *Biol Pharm Bull* 2007;30:2328-33. doi:10.1248/bpb.30.2328.

59. den Hollander B, Sundström M, Pelander A, Ojanperä I, Mervaala E, Korpi ER et al. Keto amphetamine toxicity - Focus on the redox reactivity of the cathinone designer drug mephedrone. *Toxicol Sci* 2014;141:120-31. doi:10.1093/toxsci/kfu108.

60. Hondebrink L, Verboven AHA, Drega WS, Schmeink S, de Groot MWGDM, van Kleef RGDM et al. Neurotoxicity screening of (illicit) drugs using novel methods for analysis of microelectrode array (MEA) recordings. *NeuroToxicology* 2016;55:1-9. doi:10.1016/j.neuro.2016.04.020.

61. Simmler LD, Buchy D, Chaboz S, Hoener MC, Liechti ME. In vitro characterization of psychoactive substances at rat, mouse, and human trace amine-associated receptor. *J Pharmacol Exp Ther* 2016;357:134-44. doi:10.1124/jpet.115.229765.

62. Snyder SH, Coyle JT. Regional differences in H^3-norepinephrine and H^3-dopamine uptake into rat brain homogenates. *J Pharmacol Exp Ther* 1969;165:78-86.

63. Ferris RM, Tang FL, Maxwell RA. A comparison of the capacities of isomers of amphetamine, deoxypipradrol and methylphenidate to inhibit the uptake of tritiated catecholamines into rat cerebral cortex slices, synaptosomal preparations of rat cerebral cortex, hypothalamus and striatum and into adrenergic nerves of rabbit aorta. *J Pharmacol Exp Ther* 1972;181:407-16.

64. Fuller RW, Hines CW, Mills J. Lowering of brain serotonin level by chloroamphetamines. *Biochem Pharmacol* 1965;14:483-8. doi:10.1016/0006-2952(65)90221-2.

65. Beaton JM, Smythies JR, Benington F, Morin RD, Clark LC, Jr. Behavioral effects of some 4-substituted amphetamines. *Nature* 1968;220:800-1. doi:10.1038/220800a0.

66. Gál EM. The effects of intraventricularly administered *p*-chloroamphetamine and its analogs. *Psychopharmacol Bull* 1976;12:52-4. doi:10.1016/0028-3908(75)90098-2.

67. Sherman A, Gál EM, Fuller RW, Molloy BB. Effects of intraventricular p-chloroamphetamine and its analogs on cerebral 5-HT. *Neuropharmacology* 1975;14:733-7. doi:10.1016/0028-3908(75)90098-2.

68. Vial H, Guillemin G, Pacheco H. Effets de dérivés de l'amphétamine et de produits psychotropes sur le taux de tryptophane, sérotonine et d'acide hydroxyl-5-indolyl-3-acétique dans le cerveau du rat. *J Pharmacol* 1976;7:177-90.

69. Beregi SL, Duhault J. Structure-anorectic activity relations in substituted phenethylamines. *Arzneimittelforschung* 1977;27:116-8.

70. Harvey JA, McMaster SE, Fuller RW. Comparison between the neurotoxic and serotonin-depleting effects of various halogenated derivatives of amphetamine in the rat. *J Pharmacol Exp Ther* 1977;202:581-9.

71. McElroy JF, Miller JM, Meyer JS. Fenfluramine, p-chloroamphetamine and p-fluoroamphetamine stimulation of pituitary-adrenocortical activity in rat: evidence for differences in site and mechanism of action. *J Pharmacol Exp Ther* 1984;228:593-9.

72. Uchiyama N, Kikura-Hanajiri R, Goda Y, Wada M, Urade Y. Effects of new fluoro-substituted amphetamine analogs on electroencephalogram (EEG) power spectra in rats. *Int J Neuropsychopharmacol* 2008;11:235. doi:10.1017/S1461145708009462.

73. Nouveux produits anorexiants. Patent No. BE609630. Science Union et Cie, Société Francaise de Recherche Médicale, 1962.

74. Röhrich J, Becker J, Kaufmann T, Zörntlein S, Urban R. Detection of the synthetic drug 4-fluoroamphetamine (4-FA) in serum and urine. *Forensic Sci Int* 2012;215:3-7. doi:10.1016/j.forsciint.2011.04.004.

75. Nugteren-Van Lonkhuyzen JJ, Van Riel A, de Vries I, Rietjens SJ, Hondebrink L. Severe cardiovascular toxicity, cerebral hemorrhage and mortality after 4-fluoroamphetamine (4-FA). *Clin Toxicol* 2017;55:431.

76. Wijers CH, van Litsenburg RT, Hondebrink L, Niesink RJ, Croes EA. Acute toxic effects related to 4-fluoroamphetamine. *Lancet* 2017;389:600. doi:10.1016/s0140-6736(17)30281-7.

77. Johansen SS, Hansen TM. Isomers of fluoroamphetamines detected in forensic cases in Denmark. *Int J Legal Med* 2012;126:541-7. doi:10.1007/s00414-012-0671-0.

78. Rosano TG, Wood M, Ihenetu K, Swift TA. Drug screening in medical examiner casework by high-resolution mass spectrometry (UPLC-MSE-TOF). *J Anal Toxicol* 2013;37:580-93. doi:10.1093/jat/bkt071.

79. Rust KY, Baumgartner MR, Dally AM, Kraemer T. Prevalence of new psychoactive substances: A retrospective study in hair. *Drug Test Anal* 2012;4:402-8. doi:10.1002/dta.1338.

80. Al-Saffar Y, Stephanson NN, Beck O. Multicomponent LC-MS/MS screening method for detection of new psychoactive drugs, legal highs, in urine-Experience from the Swedish population. *Journal of Chromatography B* 2013;930:112-20. doi:10.1016/j.jchromb.2013.04.043.

81. Helander A, Beck O, Hägerkvist R, Hultén P. Identification of novel psychoactive drug use in Sweden based on laboratory analysis - initial experiences from the STRIDA project. *Scand J Clin Lab Invest* 2013;73:400-6. doi:10.3109/00365513.2013.793817.

82. Pedersen AJ, Dalsgaard PW, Rode AJ, Rasmussen BS, Mueller IB, Johansen SS et al. Screening for illicit and medicinal drugs in whole blood using fully automated SPE and ultra-high-performance liquid chromatography with TOF-MS with data-independent acquisition. *J Sep Sci* 2013;36:2081-9. doi:10.1002/jssc.201200921.

83. Madias JE. Takotsubo syndrome due to 4-fluoroamphetamine. *Clin Toxicol* 2015;53:136. doi:10.3109/15563650.2014.998767.

84. Al-Abri S, Meier KH, Colby JM, Smollin CG, Benowitz NL. Cardiogenic shock after use of fluoroamphetamine confirmed with serum and urine levels. *Clin Toxicol* 2014;52:1292-5. doi:10.3109/15563650.2014.974262.

85. Poklis JL, Wolf CE, Poklis A. 4-Fluoroamphetamine in serum and urine from an intoxicated patient with life-threatening hyperpyrexia. *J Anal Toxicol* 2016;40:171-2. doi:10.1093/jat/bkv139.

86. Laskowski LK, Landry A, Vassallo SU, Hoffman RS. Ice water submersion for rapid cooling in severe drug-induced hyperthermia. *Clin Toxicol* 2015;53:181-4. doi:10.3109/15563650.2015.1009994.

87. Maas A, Wippich C, Madea B, Hess C. Driving under the influence of synthetic phenethylamines: a case series. *Int J Legal Med* 2015;129:997-1003. doi:10.1007/s00414-015-1150-1.

88. Hondebrink L, Nugteren-van Lonkhuyzen JJ, Van Der Gouwe D, Brunt TM. Monitoring new psychoactive substances (NPS) in The Netherlands: data from the drug market and the Poisons Information Centre. *Drug Alcohol Depend* 2015;147:109-15. doi:10.1016/j.drugalcdep.2014.11.033.

89. Sundström M, Pelander A, Simojoki K, Ojanperä I. Patterns of drug abuse among drug users with regular and irregular attendance for treatment as detected by comprehensive UHPLC-HR-TOF-MS. *Drug Test Anal* 2016;8:39-45. doi:10.1002/dta.1818.

90. Knippels MCJ, Essers IMM, Magdelijns FJH, van Twist DJL. 'Ecstasy-light': niet zo onschuldig als het lijkt. Toxische effecten van 4-Fluoramfetamine. *Ned Tijdschr Geneeskd* 2017;161:D1356.

91. Wille SMR, Richeval C, Nachon-Phanithavong M, Gaulier JM, Di Fazio V, Humbert L et al. Prevalence of new psychoactive substances and prescription drugs in the Belgian driving under the influence of drugs population. *Drug Test Anal* 2017. doi:10.1002/dta.2232.

92. Wolf CE, Poklis JL, Cumpston K, Moss M, Poklis A. Acute dilated cardiomyopathy and myocardial injury after combined 4-fluoroamphetamine and modafinil ingestion. *Drug Test Anal* 2017;9:657-9. doi:10.1002/dta.1988.

93. Eve and Rave. Pillen mit 2C-B, 4-FA und mcpp getestet. Accessed http://www.eve-rave.ch/drugchecking-news/228-pillen-mit-2c-b-4-fa-und-mcpp-getestet (15.09.2015).

94. Saferparty.ch. Achtung! 4 F-A verkauft als Ecstasy!. Accessed from http://www.saferparty.ch/download/file/4-Fluoramphetamin_Juni_2009.pdf (15 September 2015).

95. Giné CV, Espinosa IF, Vilamala MV. New psychoactive substances as adulterants of controlled drugs. A worrying phenomenon? *Drug Test Anal* 2014;6:819-24. doi:10.1002/dta.1610.

96. van der Gouwe D, Brunt TM, van Laar M, van der Pol P. Purity, adulteration and price of drugs bought on-line versus off-line in the Netherlands. *Addiction* 2017;112:640-8. doi:10.1111/add.13720.

97. Europol Drugs Newsletter July 2009. ALERT 2009-001 (SYNERGY) 4-Fluoroamphetamine. File No. EDOC #403292. The Hague, Netherland. Acessed https://ewsd.wiv-isp.be/Publications on new psychoactive substances/4-Fluoramphetamine/Europol Drugs Unit_Newsletter 2009-001.pdf (13 September 2015).

Annex 1: Report on WHO Questionnaire for Review of Psychoactive Substances for the 39th ECDD: Evaluation of 4-FA

Please refer to separate Annex 1 document published on ECDD website

Annex 2: Studies associated with the detection and chemical analysis of 4-fluoroamphetamine (amongst other substances) published in the scientific literature.

Techniques [a]	Comment	Reference
Thermal methods	Characterization of 4-FA HCl polymorphism.	Marthi et al.[1]
^{1}H-NMR	Characterizations of compound including use chiral Eu-shift reagents.	Podányi[2]
XRD	Characterization of crystal structure properties of compound.	Simon et al.[3]
GC-MS	Metabolism study in rats following administration of *para*-fluoro-deprenyl.	Lajtha et al.[4]
GC-MS, IR, NMR	Characterization of seized compounds.	Rösner et al.[5]
GC-MS, LC, LC-MS	Characterization of collected compounds.	Kikura-Hanajiri et al.[6]
LC-FL, LC-TOF-MS	Characterization of collected compounds.	Min et al.[7, 8]
GC-MS, LC-DAD	Characterization of collected compounds.	Takahashi et al.[9]
GC-(EI/CI)-MS(/MS)	Characterization of seized compounds.	Westphal et al.[10]
LC-FL, LC-ESI-TOF-MS	Characterization of collected compounds.	Inagaki et al.[11]
PS-MS, CE-MS, CZE-UV	Method development.	Jhang et al.[12]
LC-TOF-MS	Detection in whole blood samples related to casework.	Johansen and Hansen[13]
Various approaches to ionization and MS	Method development.	Lee et al.[14]
^{1}H-NMR and UV	4-FA obtained from synthesis.	Lichtenberger [15]
GC-MS	Employment of chiral derivatization reagent.	Mohr et al.[16]
Immunoassays	Evaluation of cross-reactivities to EMIT assays.	Nakanishi et al.[17]
GC-MS, LC-Q-TOF-MS, NMR	Characterization of seized compounds.	Reitzel et al.[18]
Immunoanalysis, GC-MS	Detection biofluids.	Röhrich et al.[19]
LC-Q-trap-MS/MS	Detection in hair samples.	Rust et al.[20]
LC-TOF-MS	Characterization of collected compounds.	Shanks et al.[21]
UHPLC-QqQ-MS/MS	Detection in oral fluid.	Strano-Rossi et al.[22]
PTR-MS	Method development.	Sulzer et al.[23]
LC-QqQ-MS/MS	Detection in authentic urine samples.	Al-Saffar et al.[24]
CE-LIF, CE-UV, MEKC-UV	Method development for saliva analysis.	Chen et al.[25]
LC-QqQ-MS/MS	Analyses of biofluids.	Helander et al.[26]
GC-MS	Method development and application to waste water analysis.	Mwenesongole et al.[27]
GC-MS, LC-MS, NMR	Synthesis and characterization of regioisomers.	Nakazono et al.[28]
LC-Q-TOF-MS	Method development in whole blood and application to casework.	Pedersen et al.[29]
Immunoanalysis	Cross-reactivity studies.	Petrie et al.[30]

Colour spot test	Method development using a spectrophotometric reagent.	Philp et al.[31]
LC-TOF-MS	Method development and application to postmortem blood samples.	Roman et al.[32]
UPLC-QqQ-MS, LC-Q-TOF-MS	Method development in blood matrix for application to casework.	Rosano et al.[33]
LC-DAD, LC-Q-MS	Method development and application to collected samples combined with *in vitro* pharmacology assays.	Rosenauer et al.[34]
Melting point, ^{1}H-NMR, GC-MS, ATR-FTIR	Characterization of reference material.	SWGDRUG[35]
GC-MS, LC-DAD, LC-Q-TOF-MS, NMR	Detection of seized compounds.	Zuba et al.[36]
HS-SRI-TOF-MS	Method development.	Acton et al.[37]
LC-Q-TOF-MS/MS, LC-Q-ion trap-MS/MS	Detection in serum and urine.	Al-Abri et al.[38]
LC-UV	Biocatalytic synthesis of chiral species.	Au et al.[39]
LC-DAD	Method development for chiral separation.	Geryk et al.[40]
TLC, GC-MS	Analysis of submitted samples to drug checking service.	Giné et al.[41]
GC-FID, NMR	Biocatalytic synthesis of chiral species.	Green et al.[42]
Immunoanalysis, GC-MS	Method development and chemical derivatizations.	Holler et al.[43]
LC-Q-TOF-MS	Method development for urine analysis.	Paul et al.[44]
LC-QqQ-MS/MS	Method development for hair analysis and application to authentic specimens.	Strano-Rossi et al.[45]
LC-QqQ-MS/MS	Method development for urine analysis and application to authentic specimens.	Tang et al.[46]
LC-UV	Chiral analysis of products obtained from Internet retailers.	Taschwer et al.[47]
LC-DAD	Method development for chiral separation.	Geryk et al.[48]
TLC, GC-NPD, GC-MS	Identification of submitted samples.	Hondebrink et al.[49]
LC-DAD	Method development for chiral separation.	Kalíková et al.[50]
EMIT, LC-QqQ-MS/MS	Detection in serum and urine.	Laskowski et al.[51]
GC-MS, LC-QqQ-MS/MS	Detection in serum.	Maas et al.[52]
GC-FID	Biocatalytic synthesis of chiral species.	Mutti et al. [53]
LC-QqQ-MS/MS	Method development in whole blood.	Odoardi et al.[54]
LC-QqQ-MS/MS	Method development for wastewater and river water analysis.	Senta et al.[55]
LC-QqQ-MS/MS	Method development for blood analysis.	Adamowicz et al.[56]
LC, SFC, CEC,	Method development for chiral separation.	Albals et al.[57]
Raman	Differentiation between regioisomers.	Chen et al.[58]
Presumptive colour tests	Use of commercially available reagents.	Cuypers et al.[59]
SFC	Method development for chiral separation	Geryk et al.[60]
GC-FID	Biocatalytic synthesis of chiral species.	Gomm et al.[61]
LC-Q-TOF-MS	Detection in pooled urine sample.	Kinyua et al.[62]
GC-MS, NMR	Analysis of seized material.	Ladroue et al.[63]
GC-FID	Biocatalytic synthesis of chiral species.	Martínez-Montero et al.[64]
GC-MS, LC-Orbitrap-MS	Detection in seized samples.	Odoardi et al.[65]

LC-MS/MS	Detection in hair.	Palamar et al.[66]
GC-MS	Detection in biofluids.	Poklis et al.[67]
LC-MS/MS	Detection in hair.	Salomone et al.[68]
LC-TOF-MS	Application to analyses of authentic urine samples.	Sundström et al.[69] and Heikman et al.[70]
LC-QqQ-MS/MS	Method development for blood analysis.	Vaiano et al.[71]
LC-TOF-MS	Method development for blood analysis.	Woldegebriel et al.[72]
GC-MS, LC-DAD	Analysis of submitted samples to drug checking service.	Brunt et al.[73]
LC-Q-TOF-MS, LC-Orbitrap-MS	Analysis of wastewater samples.	Causanilles et al.[74]
Not reported	Detection in biofluids.	Nugteren-Van Lonkhuyzen et al.[75]
LC-Orbitrap-MS	Method development for hair analysis.	Odoardi et al.[76]
GC-FID	Biocatalytic synthesis of chiral species.	Pushpanath et al.[77]
SFC, Q-TOF-MS, UV	Differentiation between regioisomers.	Segawa et al.[78]
LC-UV	Method development for chiral separation.	Taschwer et al.[79]
PALME, LC-MS	Method development for plasma and blood analysis.	Vårdal et al.[80]
GC-MS, LC-UV	Differentiation between regioisomers.	Weiß et al.[81]
Immunoanalysis, LC-MS (various)	Blood and oral fluid analysis of Belgian driving under the influence of drugs population.	Wille et al.[82]
GC-MS	Detection in urine sample.	Wolf et al.[83]

[a] XRD: X-ray diffraction; GC: gas chromatography; MS: mass spectrometry; IR: infrared; NMR: nuclear magnetic resonance spectroscopy; LC: liquid chromatography (various forms); FL: fluorescence; TOF: time-of-flight; EI: electron ionization; CI: chemical ionization; DAD: diode array detector; ESI: electrospray ionization; PS: paper spray; CE: capillary electrophoresis; CZE: capillary zone electrophoresis; Q: quadrupole; QqQ: triple quadrupole; PTR: proton-transfer-reaction; LIF: laser induced fluorescence; MEKC: micellar electrokinetic capillary chromatography; IT: linear ion trap; HS: head space; SRI: selective reagent ionization; TLC: thin-layer chromatography; NPD: nitrogen-phosphorus detector; EMIT: enzyme multiplied immunoassay technique; FID: flame ionization detector; SFC: supercritical fluid chromatography; CEC: capillary electrochromatography; PALME: Parallel artificial liquid membrane extraction.

References

1. Marthi K, Ács M, Pokol G, Tomor K, Eröss-Kiss K. DSC studies on the polymorphism and pseudopolymorphism of pharmaceutical substances. A complex system for studying physico-chemical behavior of binary mixtures. *J Therm Anal* 1992;38:1017-25. doi:10.1007/BF01979435.

2. Podányi B. Selegilin és rokon vegyületeinek vizsgálata NMR spektroszkópiával. *Acta Pharm Hung* 1992;62:218-24.

3. Simon K, Böcskei Z, Török Z. Selegilin és rokon vegyületek vizsgálata röntgendiffrakcióval. *Acta Pharm Hung* 1992;62:225-30.

4. Lajtha A, Sershen H, Cooper T, Hashim A, Gaal J. Metabolism of (-)-deprenyl and PF-(-)-deprenyl in brain after central and peripheral administration. *Neurochem Res* 1996;21:1155-60. doi:10.1007/BF02532389.

5. Rösner P, Quednow B, Girreser U, Junge T. Isomeric fluoro-methoxy-phenylalkylamines: a new series of controlled-substance analogues (designer drugs). *Forensic Sci Int* 2005;148:143-56. doi:10.1016/j.forsciint.2004.05.003.

6. Kikura-Hanajiri R, Kawamura M, Uchiyama N, Ogata J, Kamakura H, Saisho K et al. Analytical data of designated substances (Shitei-Yakubutsu) controlled by the pharmaceutical affairs law in Japan, part I: GC-MS and LC-MS. *Yakugaku Zasshi* 2008;128:971-9. doi:10.1248/yakushi.128.971.

7. Min JZ, Shimizu Y, Toyo'oka T, Inagaki S, Kikura-Hanajiri R, Goda Y. Simultaneous determination of 11 designated hallucinogenic phenethylamines by ultra-fast liquid chromatography with fluorescence detection. *J Chromatogr B* 2008;873:187-94. doi:10.1016/j.jchromb.2008.08.020.

8. Min JZ, Hatanaka S, Toyo'oka T, Inagaki S, Kikura-Hanajiri R, Goda Y. Rapid, sensitive and simultaneous determination of fluorescence-labeled designated substances controlled by the Pharmaceutical Affairs Law in Japan by ultra-performance liquid chromatography coupled with electrospray-ionization time-of-flight mass spectrometry. *Anal Bioanal Chem* 2009;395:1411-22. doi:10.1007/s00216-009-3046-8.

9. Takahashi M, Nagashima M, Suzuki J, Seto T, Yasuda I, Yoshida T. Creation and application of psychoactive designer drugs data library using liquid chromatography with photodiode array spectrophotometry detector and gas chromatography-mass spectrometry. *Talanta* 2009;77:1245-72. doi:10.1016/j.talanta.2008.07.062.

10. Westphal F, Roesner P, Junge T. Differentiation of regioisomeric ring-substituted fluorophenethylamines with product ion spectrometry. *Forensic Sci Int* 2010;194:53-9. doi:10.1016/j.forsciint.2009.10.007.

11. Inagaki S, Hirashima H, Taniguchi S, Higashi T, Min JZ, Kikura-Hanajiri R et al. Rapid enantiomeric separation and simultaneous determination of phenethylamines by ultra high performance liquid chromatography with fluorescence and mass spectrometric detection: application to the analysis of illicit drugs distributed in the Japanese market and biological samples. *Drug Test Anal* 2012;4:1001-8. doi:10.1002/dta.1327.

12. Jhang C-S, Lee H, He Y-S, Liu J-T, Lin C-H. Rapid screening and determination of 4-chloroamphetamine in saliva by paper spray-mass spectrometry and capillary electrophoresis-mass spectrometry. *Electrophoresis* 2012;33:3073-8. doi:10.1002/elps.201200270.

13. Johansen SS, Hansen TM. Isomers of fluoroamphetamines detected in forensic cases in Denmark. *Int J Legal Med* 2012;126:541-7. doi:10.1007/s00414-012-0671-0.

14. Lee H, Jhang C-S, Liu J-T, Lin C-H. Rapid screening and determination of designer drugs in saliva by a nib-assisted paper spray-mass spectrometry and separation technique. *J Sep Sci* 2012;35:2822-5. doi:10.1002/jssc.201200480.

15. Lichtenberger O. Fluorphenyl- und Fluormethoxyphenylalkylamine und deren Anwendung. Patent No. DE102011015842A1, 2012.

16. Mohr S, Weiß JA, Spreitz J, Schmid MG. Chiral separation of new cathinone- and amphetamine-related designer drugs by gas chromatography-mass spectrometry using trifluoroacetyl-L-prolyl chloride as chiral derivatization reagent. *J Chromatogr A* 2012;1269:352-9. doi:10.1016/j.chroma.2012.09.079.

17. Nakanishi K, Miki A, Zaitsu K, Kamata H, Shima N, Kamata T et al. Cross-reactivities of various phenethylamine-type designer drugs to immunoassays for amphetamines, with special attention to the evaluation of the one-step urine drug test Instant-View, and the Emit assays for use in drug enforcement. *Forensic Sci Int* 2012;217:174-81. doi:10.1016/j.forsciint.2011.11.003.

18. Reitzel LA, Dalsgaard PW, Müller IB, Cornett C. Identification of ten new designer drugs by GC-MS, UPLC-QTOF-MS, and NMR as part of a police investigation of a Danish Internet company. *Drug Test Anal* 2012;4:342-54. doi:10.1002/dta.358.

19. Röhrich J, Becker J, Kaufmann T, Zörntlein S, Urban R. Detection of the synthetic drug 4-fluoroamphetamine (4-FA) in serum and urine. *Forensic Sci Int* 2012;215:3-7. doi:10.1016/j.forsciint.2011.04.004.

20. Rust KY, Baumgartner MR, Dally AM, Kraemer T. Prevalence of new psychoactive substances: A retrospective study in hair. *Drug Test Anal* 2012;4:402-8. doi:10.1002/dta.1338.

21. Shanks KG, Dahn T, Behonick G, Terrell A. Analysis of first and second generation legal highs for synthetic cannabinoids and synthetic stimulants by ultra-performance liquid chromatography and time of flight mass spectrometry. *J Anal Toxicol* 2012;36:360-71. doi:10.1093/jat/bks047.

22. Strano-Rossi S, Anzillotti L, Castrignano E, Romolo FS, Chiarotti M. Ultra high performance liquid chromatography-electrospray ionization-tandem mass spectrometry screening method for direct analysis of designer drugs, "spice" and stimulants in oral fluid. *J Chromatogr A* 2012;1258:37-42. doi:10.1016/j.chroma.2012.07.098.

23. Sulzer P, Jürschik S, Agarwal B, Kassebacher T, Hartungen E, Edtbauer A et al. Designer drugs and trace explosives detection with the help of very recent advancements in proton-transfer-reaction mass spectrometry (PTR-MS). In: Aschenbruck N, Martini P, Meier M, Tölle J, editors. Future Security 7th Security Research Conference, Future Security 2012, Bonn, Germany, September 4-6, 2012. Heidelberg: Springer; 2012. p. 366-75.

24. Al-Saffar Y, Stephanson NN, Beck O. Multicomponent LC-MS/MS screening method for detection of new psychoactive drugs, legal highs, in urine-Experience from the Swedish population. *Journal of Chromatography B* 2013;930:112-20. doi:10.1016/j.jchromb.2013.04.043.

25. Chen K-F, Lee H, Liu J-T, Lee H-A, Lin C-H. A microwave-assisted fluorescent labeling method for the separation and detection of amphetamine-like designer drugs by capillary electrophoresis. *Forensic Sci Int* 2013;228:95-9. doi:10.1016/j.forsciint.2013.02.045.

26. Helander A, Beck O, Hägerkvist R, Hultén P. Identification of novel psychoactive drug use in Sweden based on laboratory analysis - initial experiences from the STRIDA project. *Scand J Clin Lab Invest* 2013;73:400-6. doi:10.3109/00365513.2013.793817.

27. Mwenesongole EM, Gautam L, Hall SW, Waterhouse JW, Cole MD. Simultaneous detection of controlled substances in waste water. *Anal Methods* 2013;5:3248-54. doi:10.1039/c3ay40655e.

28. Nakazono Y, Tsujikawa K, Kuwayama K, Kanamori T, Iwata YT, Miyamoto K et al. Differentiation of regioisomeric fluoroamphetamine analogs by gas chromatography-mass spectrometry and liquid chromatography-tandem mass spectrometry. *Forensic Toxicol* 2013;31:241-50. doi:10.1007/s11419-013-0184-7.

29. Pedersen AJ, Dalsgaard PW, Rode AJ, Rasmussen BS, Mueller IB, Johansen SS et al. Screening for illicit and medicinal drugs in whole blood using fully automated SPE and ultra-high-performance liquid chromatography with TOF-MS with data-independent acquisition. *J Sep Sci* 2013;36:2081-9. doi:10.1002/jssc.201200921.

30. Petrie M, Lynch KL, Ekins S, Chang JS, Goetz RJ, Wu AHB et al. Cross-reactivity studies and predictive modeling of "Bath Salts" and other amphetamine-type stimulants with amphetamine screening immunoassays. *Clin Toxicol* 2013;51:83-91. doi:10.3109/15563650.2013.768344.

31. Philp M, Shimmon R, Stojanovska N, Tahtouh M, Fu S. Development and validation of a presumptive colour spot test method for the detection of piperazine analogues in seized illicit materials. *Anal Methods* 2013;5:5402-10. doi:10.1039/c3ay40511g.

32. Roman M, Ström L, Tell H, Josefsson M. Liquid chromatography/time-of-flight mass spectrometry analysis of postmortem blood samples for targeted toxicological screening. *Anal Bioanal Chem* 2013;405:4107-25. doi:10.1007/s00216-013-6798-0.

33. Rosano TG, Wood M, Ihenetu K, Swift TA. Drug screening in medical examiner casework by high-resolution mass spectrometry (UPLC-MSE-TOF). *J Anal Toxicol* 2013;37:580-93. doi:10.1093/jat/bkt071.

34. Rosenauer R, Luf A, Holy M, Freissmuth M, Schmid R, Sitte HH. A combined approach using transporter-flux assays and mass spectrometry to examine psychostimulant street drugs of unknown content. *ACS Chem Neurosci* 2013;4:182-90. doi:10.1021/cn3001763.

35. SWGDRUG. 4-Fluoroamphetamine Monograph. Latest revision 14 June 2013. Available at: http://www.swgdrug.org/Monographs/4-FA.pdf.

36. Zuba D, Byrska B. Prevalence and co-existence of active components of 'legal highs'. *Drug Test Anal* 2013;5:420-9. doi:10.1002/dta.1365.

37. Acton WJ, Lanza M, Agarwal B, Jürschik S, Sulzer P, Breiev K et al. Headspace analysis of new psychoactive substances using a selective reagent Ionisation-time of flight-mass spectrometer. *Int J Mass Spectrom* 2014;360:28-38. doi:10.1016/j.ijms.2013.12.009.

38. Al-Abri S, Meier KH, Colby JM, Smollin CG, Benowitz NL. Cardiogenic shock after use of fluoroamphetamine confirmed with serum and urine levels. *Clin Toxicol* 2014;52:1292-5. doi:10.3109/15563650.2014.974262.

39. Au SK, Bommarius BR, Bommarius AS. Biphasic reaction system allows for conversion of hydrophobic substrates by amine dehydrogenases. *ACS Catal* 2014;4:4021-6. doi:10.1021/cs4012167.

40. Geryk R, Kalíková K, Vozka J, Plecitá D, Schmid MG, Tesařová E. Enantioselective potential of chiral stationary phases based on immobilized polysaccharides in reversed phase mode. *J Chromatogr A* 2014;1363:155-61. doi:10.1016/j.chroma.2014.06.040.

41. Giné CV, Espinosa IF, Vilamala MV. New psychoactive substances as adulterants of controlled drugs. A worrying phenomenon? *Drug Test Anal* 2014;6:819-24. doi:10.1002/dta.1610.

42. Green AP, Turner NJ, O'Reilly E. Chiral amine synthesis using ω-transaminases: an amine donor that displaces equilibria and enables high-throughput screening. *Angew Chem, Int Ed* 2014;53:10714-7. doi:10.1002/anie.201406571.

43. Holler JM, Vorce SP, Knittel JL, Malik-Wolf B, Levine B, Bosy TZ. Evaluation of designer amphetamine interference in GC-MS amine confirmation procedures. *J Anal Toxicol* 2014;38:295-303. doi:10.1093/jat/bku017.

44. Paul M, Ippisch J, Herrmann C, Guber S, Schultis W. Analysis of new designer drugs and common drugs of abuse in urine by a combined targeted and untargeted LC-HR-QTOFMS approach. *Anal Bioanal Chem* 2014;406:4425-41. doi:10.1007/s00216-014-7825-5.

45. Strano-Rossi S, Odoardi S, Fisichella M, Anzillotti L, Gottardo R, Tagliaro F. Screening for new psychoactive substances in hair by ultrahigh performance liquid chromatography-electrospray ionization tandem mass spectrometry. *J Chromatogr A* 2014;1372:145-56. doi:10.1016/j.chroma.2014.10.106.

46. Tang MHY, Ching CK, Lee CYW, Lam Y-H, Mak TWL. Simultaneous detection of 93 conventional and emerging drugs of abuse and their metabolites in urine by UHPLC-MS/MS. *Journal of Chromatography B* 2014;969:272-84. doi:10.1016/j.jchromb.2014.08.033.

47. Taschwer M, Seidl Y, Mohr S, Schmid MG. Chiral separation of cathinone and amphetamine derivatives by HPLC/UV using sulfated ß-cyclodextrin as chiral mobile phase additive. *Chirality* 2014;26:411-8. doi:10.1002/chir.22341.

48. Geryk R, Kalíková K, Vozka J, Tesařová E. Immobilized polysaccharide-based stationary phases for enantioseparation in normal versus reversed phase HPLC. *Chromatographia* 2015;78:909-15. doi:10.1007/s10337-014-2804-8.

49. Hondebrink L, Nugteren-van Lonkhuyzen JJ, Van Der Gouwe D, Brunt TM. Monitoring new psychoactive substances (NPS) in The Netherlands: data from the drug market and the Poisons Information Centre. *Drug Alcohol Depend* 2015;147:109-15. doi:10.1016/j.drugalcdep.2014.11.033.

50. Kalíková K, Geryk R, Vozka J, Tesařová E. Evaluation of differences between Chiralpak IA and Chiralpak AD-RH amylose-based chiral stationary phases in reversed-phase high-performance liquid chromatography. *J Sep Sci* 2015;38:711-9. doi:10.1002/jssc.201401002.

51. Laskowski LK, Landry A, Vassallo SU, Hoffman RS. Ice water submersion for rapid cooling in severe drug-induced hyperthermia. *Clin Toxicol* 2015;53:181-4. doi:10.3109/15563650.2015.1009994.

52. Maas A, Wippich C, Madea B, Hess C. Driving under the influence of synthetic phenethylamines: a case series. *Int J Legal Med* 2015;129:997-1003. doi:10.1007/s00414-015-1150-1.

53. Mutti FG, Knaus T, Scrutton NS, Breuer M, Turner NJ. Conversion of alcohols to enantiopure amines through dual-enzyme hydrogen-borrowing cascades. *Science* 2015;349:1525-9. doi:10.1126/science.aac9283.

54. Odoardi S, Fisichella M, Romolo FS, Strano-Rossi S. High-throughput screening for new psychoactive substances (NPS) in whole blood by DLLME extraction and UHPLC-MS/MS analysis. *Journal of Chromatography B* 2015;1000:57-68. doi:10.1016/j.jchromb.2015.07.007.

55. Senta I, Krizman I, Ahel M, Terzic S. Multiresidual analysis of emerging amphetamine-like psychoactive substances in wastewater and river water. *J Chromatogr A* 2015;1425:204-12. doi:10.1016/j.chroma.2015.11.043.

56. Adamowicz P, Tokarczyk B. Simple and rapid screening procedure for 143 new psychoactive substances by liquid chromatography-tandem mass spectrometry. *Drug Test Anal* 2016;8:652-67. doi:10.1002/dta.1815.

57. Albals D, Heyden YV, Schmid MG, Chankvetadze B, Mangelings D. Chiral separations of cathinone and amphetamine-derivatives: comparative study between capillary electrochromatography, supercritical fluid chromatography and three liquid chromatographic modes. *J Pharm Biomed Anal* 2016;121:232-43. doi:10.1016/j.jpba.2015.12.007.

58. Chen Y-F, Liu J-T, Pan D-S. Discrimination of fluoroamphetamine regioisomers by Raman spectroscopy. *J Chin Chem Soc* 2016;63:219-21. doi:10.1002/jccs.201500211.

59. Cuypers E, Bonneure AJ, Tytgat J. The use of presumptive color tests for new psychoactive substances. *Drug Test Anal* 2016;8:136-40. doi:10.1002/dta.1847.

60. Geryk R, Kalíková K, Schmid MG, Tesařová E. Enantioselective separation of biologically active basic compounds in ultra-performance supercritical fluid chromatography. *Anal Chim Acta* 2016;932:98-105. doi:10.1016/j.aca.2016.04.044.

61. Gomm A, Lewis W, Green AP, O'Reilly E. A new generation of smart amine donors for transaminase-mediated biotransformations. *Chem - Eur J* 2016;22:12692-5. doi:10.1002/chem.201603188.

62. Kinyua J, Negreira N, Miserez B, Causanilles A, Emke E, Gremeaux L et al. Qualitative screening of new psychoactive substances in pooled urine samples from Belgium and United Kingdom. *Sci Total Environ* 2016;573:1527-35. doi:10.1016/j.scitotenv.2016.08.124.

63. Ladroue V, Stemmelen A, Besacier F, Hologne M. Les NPS en vogue en région Rhône-Alpes: rapport de cas. *Toxicologie Analytique Et Clinique* 2016;28:311-22. doi:10.1016/j.toxac.2016.03.006.

64. Martínez-Montero L, Gotor V, Gotor-Fernández V, Lavandera I. But-2-ene-1,4-diamine and but-2-ene-1,4-diol as donors for thermodynamically favored transaminase- and alcohol dehydrogenase-catalyzed processes. *Adv Synth Catal* 2016;358:1618-24. doi:10.1002/adsc.201501066.

65. Odoardi S, Romolo FS, Strano-Rossi S. A snapshot on NPS in Italy: distribution of drugs in seized materials analysed in an Italian forensic laboratory in the period 2013-2015. *Forensic Sci Int* 2016;265:116-20. doi:10.1016/j.forsciint.2016.01.037.

66. Palamar JJ, Salomone A, Vincenti M, Cleland CM. Detection of "bath salts" and other novel psychoactive substances in hair samples of ecstasy/MDMA/"Molly" users. *Drug Alcohol Depend* 2016;161:200-5. doi:10.1016/j.drugalcdep.2016.02.001.

67. Poklis JL, Wolf CE, Poklis A. 4-Fluoroamphetamine in serum and urine from an intoxicated patient with life-threatening hyperpyrexia. *J Anal Toxicol* 2016;40:171-2. doi:10.1093/jat/bkv139.

68. Salomone A, Gazzilli G, Di Corcia D, Gerace E, Vincenti M. Determination of cathinones and other stimulant, psychedelic, and dissociative designer drugs in real hair samples. *Anal Bioanal Chem* 2016;408:2035-42. doi:10.1007/s00216-015-9247-4.

69. Sundström M, Pelander A, Simojoki K, Ojanperä I. Patterns of drug abuse among drug users with regular and irregular attendance for treatment as detected by comprehensive UHPLC-HR-TOF-MS. *Drug Test Anal* 2016;8:39-45. doi:10.1002/dta.1818.

70. Heikman P, Sundström M, Pelander A, Ojanperä I. New psychoactive substances as part of polydrug abuse within opioid maintenance treatment revealed by comprehensive high-resolution mass spectrometric urine drug screening. *Human Psychopharmacology Clinical and Experimental* 2016;31:44-52. doi:10.1002/hup.2512.

71. Vaiano F, Busardò FP, Palumbo D, Kyriakou C, Fioravanti A, Catalani V et al. A novel screening method for 64 new psychoactive substances and 5 amphetamines in blood by LC-MS/MS and application to real cases. *J Pharm Biomed Anal* 2016;129:441-9. doi:10.1016/j.jpba.2016.07.009.

72. Woldegebriel M, Gonsalves J, van Asten A, Vivo-Truyols G. Robust Bayesian algorithm for targeted compound screening in forensic toxicology. *Analytical Chemistry* 2016;88:2421-30. doi:10.1021/acs.analchem.5b04484.

73. Brunt TM, Nagy C, Buecheli A, Martins D, Ugarte M, Beduwe C et al. Drug testing in Europe: monitoring results of the Trans European Drug Information (TEDI) project. *Drug Test Anal* 2017;9:188-98. doi:10.1002/dta.1954.

74. Causanilles A, Kinyua J, Ruttkies C, van Nuijs ALN, Emke E, Covaci A et al. Qualitative screening for new psychoactive substances in wastewater collected during a city festival using liquid chromatography coupled to high-resolution mass spectrometry. *Chemosphere* 2017;184:1186-93. doi:10.1016/j.chemosphere.2017.06.101.

75. Nugteren-Van Lonkhuyzen JJ, Van Riel A, de Vries I, Rietjens SJ, Hondebrink L. Severe cardiovascular toxicity, cerebral hemorrhage and mortality after 4-fluoroamphetamine (4-FA). *Clin Toxicol* 2017;55:431.

76. Odoardi S, Valentini V, De Giovanni N, Pascali VL, Strano-Rossi S. High-throughput screening for drugs of abuse and pharmaceutical drugs in hair by liquid-chromatography-high resolution mass spectrometry (LC-HRMS). *Microchem J* 2017;133:302-10. doi:10.1016/j.microc.2017.03.050.

77. Pushpanath A, Siirola E, Bornadel A, Woodlock D, Schell U. Understanding and overcoming the limitations of *Bacillus badius* and *Caldalkalibacillus thermarum* amine dehydrogenases for biocatalytic reductive amination. *ACS Catal* 2017;7:3204-9. doi:10.1021/acscatal.7b00516.

78. Segawa H, Iwata YT, Yamamuro T, Kuwayama K, Tsujikawa K, Kanamori T et al. Differentiation of ring-substituted regioisomers of amphetamine and methamphetamine by supercritical fluid chromatography. *Drug Test Anal* 2017;9:389-98. doi:10.1002/dta.2040.

79. Taschwer M, Grascher J, Schmid MG. Development of an enantioseparation method for novel psychoactive drugs by HPLC using a Lux® Cellulose-2 column in polar organic phase mode. *Forensic Sci Int* 2017;270:232-40. doi:10.1016/j.forsciint.2016.10.011.

80. Vårdal L, Askildsen H-M, Gjelstad A, Øiestad EL, Edvardsen HME, Pedersen-Bjergaard S. Parallel artificial liquid membrane extraction of new psychoactive substances in plasma and whole blood. *J Chromatogr B: Anal Technol Biomed Life Sci* 2017;1048:77-84. doi:10.1016/j.jchromb.2017.02.010.

81. Weiß JA, Kadkhodaei K, Schmid MG. Indirect chiral separation of 8 novel amphetamine derivatives as potential new psychoactive compounds by GC-MS and HPLC. *Science & Justice* 2017;57:6-12.

82. Wille SMR, Richeval C, Nachon-Phanithavong M, Gaulier JM, Di Fazio V, Humbert L et al. Prevalence of new psychoactive substances and prescription drugs in the Belgian driving under the influence of drugs population. *Drug Test Anal* 2017. doi:10.1002/dta.2232.

83. Wolf CE, Poklis JL, Cumpston K, Moss M, Poklis A. Acute dilated cardiomyopathy and myocardial injury after combined 4-fluoroamphetamine and modafinil ingestion. *Drug Test Anal* 2017;9:657-9. doi:10.1002/dta.1988.