



**Изучение свойств адамантан-1-ил-1-(5-фторпентил)-1Н-индазол-3-карбоксилата (5F-АПИНАК) с целью химико-токсикологического и судебно-химического анализа**

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**Лаборатория судебно-химических**

**и химико-токсикологических исследований**

**отдела судебно-химических и химико-токсикологических экспертиз**

**ФГБУ «РЦСМЭ» Минздрава России (Москва)**

**Отделение судебной медицины департамента диагностики**

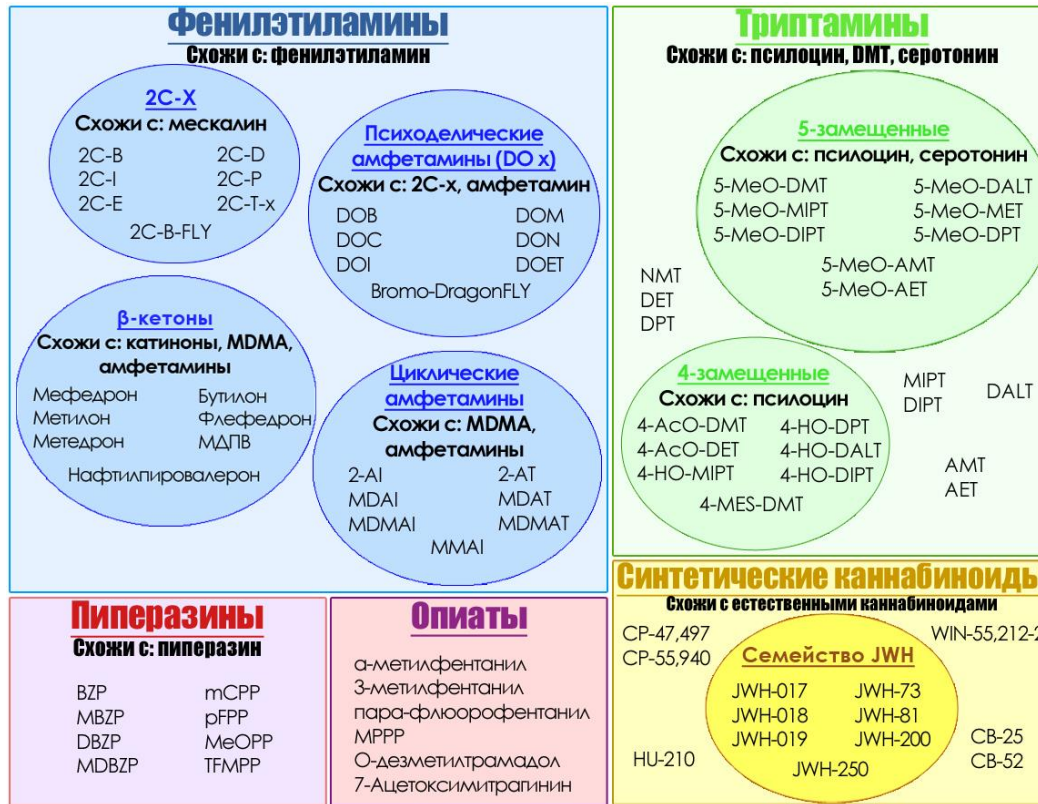
**и общественного здоровья Университета Вероны, Верона, Италия**

2021 год

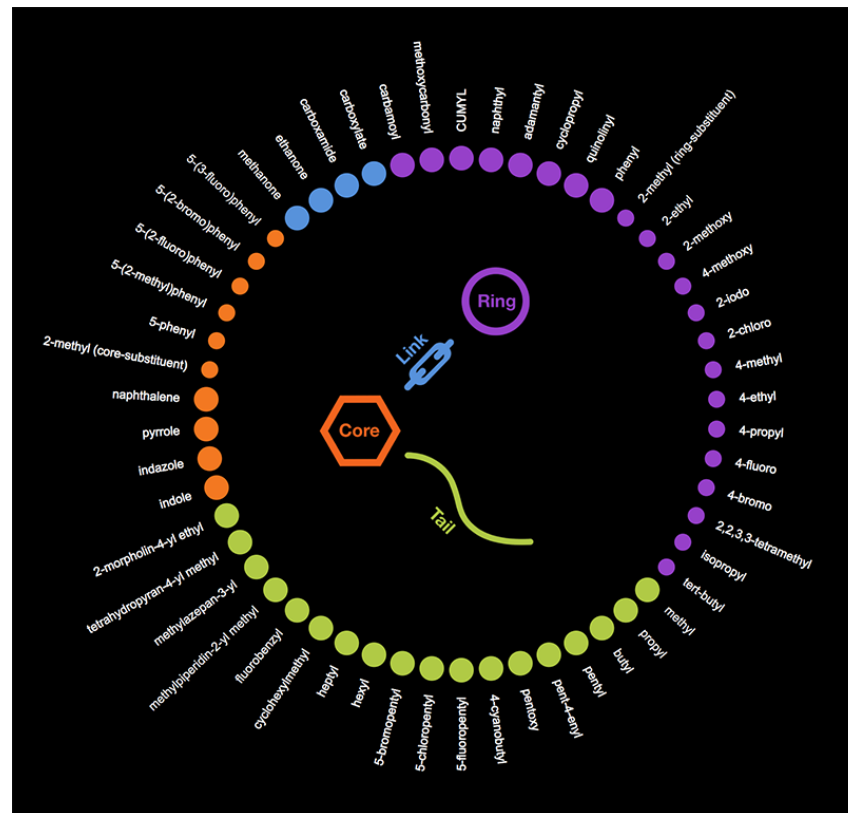
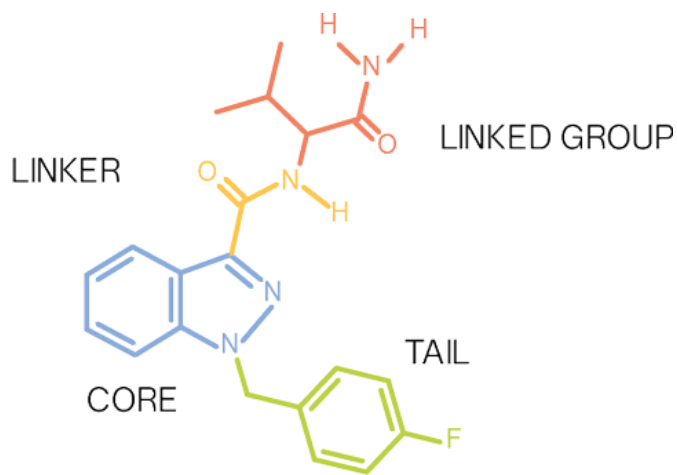
# Актуальность



## Новые психоактивные вещества («дизайнерские наркотики»)



# Актуальность



# Актуальность

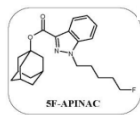
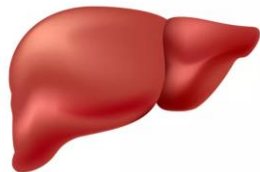


# Цель



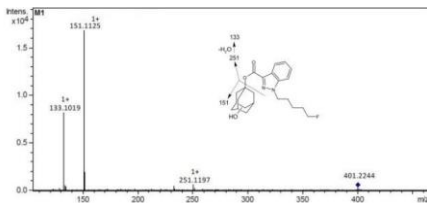
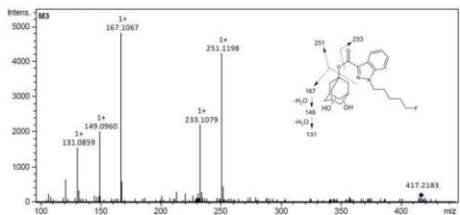
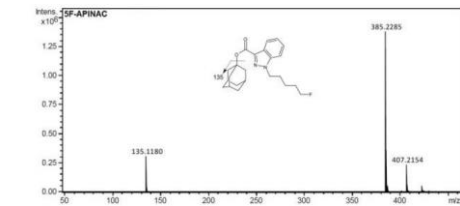
**Изучение свойств адамантан-1-ил-1-(5-фторпентил)-1Н-индазол-3-карбоксилата (5F-АПИНАК) для целей химико-токсикологического и судебно-химического анализа**

# Материалы и методы исследования (*in vitro*)

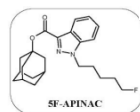


50 мкл НАДФН раствора  
700 мкл  
деионизированной воды  
200 мкл фосфатного  
буфера (pH 7.4, 05 M)

37 °C 1 час



# Материалы и методы исследования (*in vivo*)



3, 6, 24 часа

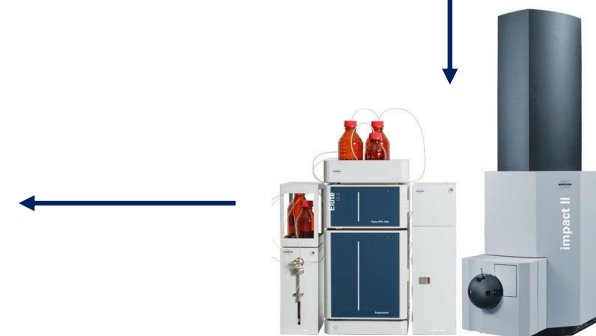
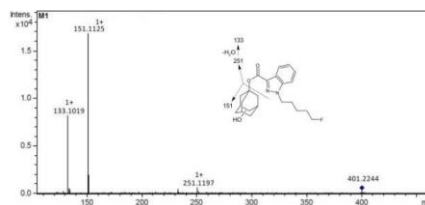
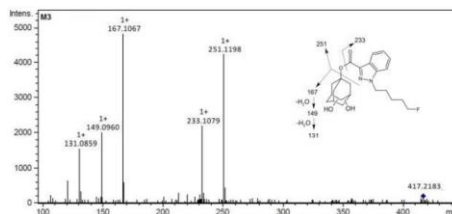
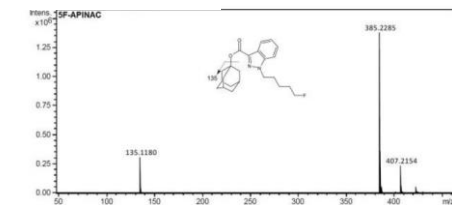


$\beta$ -глюкуронидаза



Этилацетат  
70 °C

Метанол:вода



# Материалы и методы исследования



Жидкостной хроматограф

Bruker Elute

Колонка

Intensity solo 2 1.8 мкм, 10 мм, 2.1 мм

Температура термостата 40°C

Объем вводимой пробы 2 мкл

Подвижная фаза А

1% раствор метанола в воде

Подвижная фаза В

5 мМ формиат аммония, 0.1% муравьиная

кислота в метаноле

Скорость потока

0,20 мл/мин

Масс-спектрометрический детектор

Maxis impact UHRQqTOF

Характеристика источника

ионизация в электроспрее

Полярность

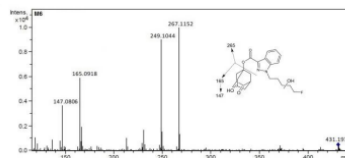
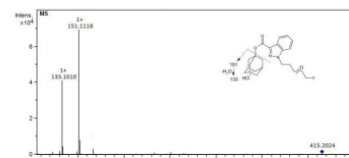
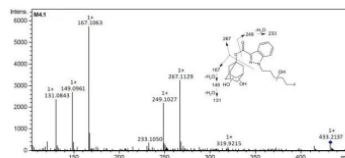
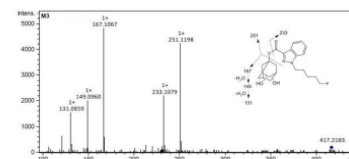
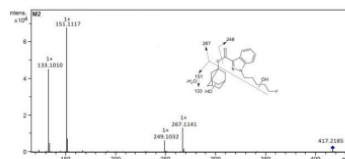
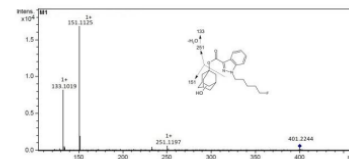
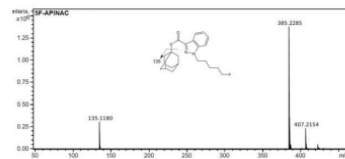
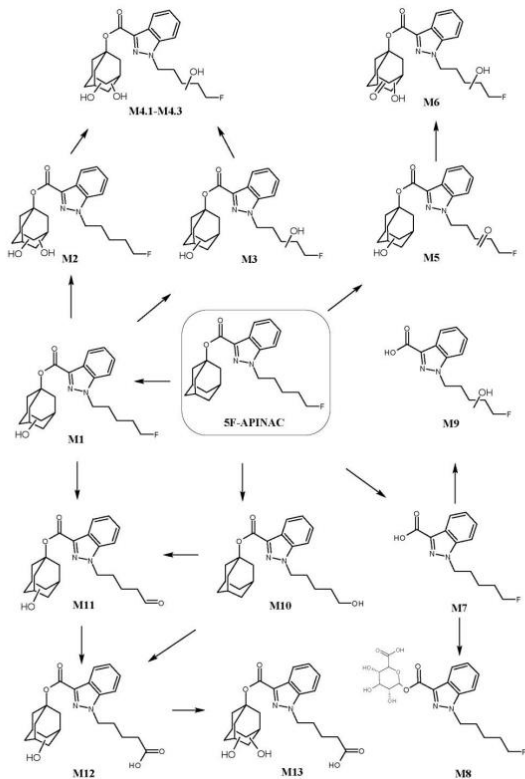
положительная

Время, мин	%А	%В	Скорость потока, мл/мин
0.0	96.0	4.0	0.2
1.0	96.0	4.0	0.2
6.0	50.0	50.0	0.2
10.0	0.1	99.9	0.2
10.05	96.0	4.0	0.2
14.0	96.0	4.0	0.2

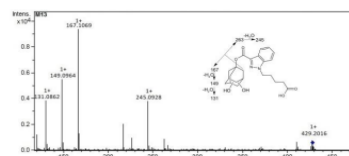
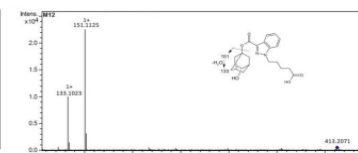
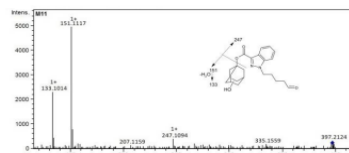
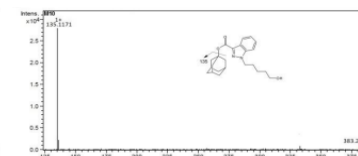
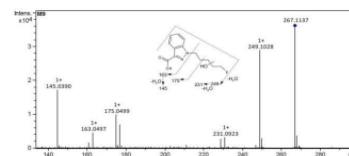
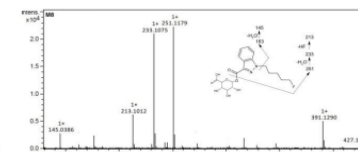
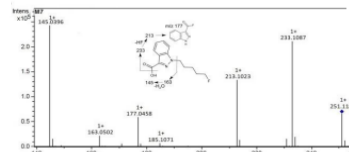
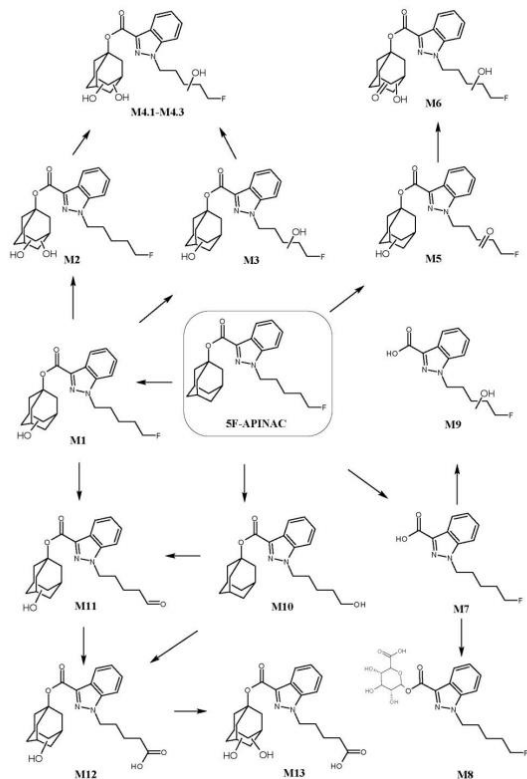
Отделение судебной медицины департамента диагностики и общественного здоровья Университета Вероны, Верона, Италия  
Appolonova, S. A., Palacio, C .... & Savchuk, S. A. In vivo and in vitro metabolism of the novel synthetic cannabinoid 5F-APINAC.



# Результаты исследования



# Результаты исследования



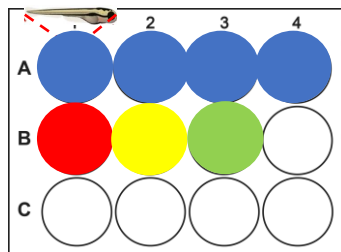
# Материалы и методы исследования

## Определение летальности 5F-АПИНАК



Мальки  
зебрафиш

Возраст: 0 дней после  
оплодотворения (дпо)



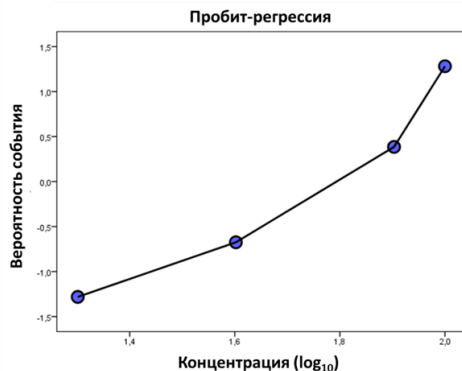
Опытные группы  
(20 мальков в лунке)

Контрольные группы:

- (20 мальков в лунке)
- + контроль: 3,4-ДХА
- контроль: среда E3
- контроль: 1% ДМСО

96-часовое  
воздействие

Определение  
летальности



Статистический анализ данных: SPSS 23.0

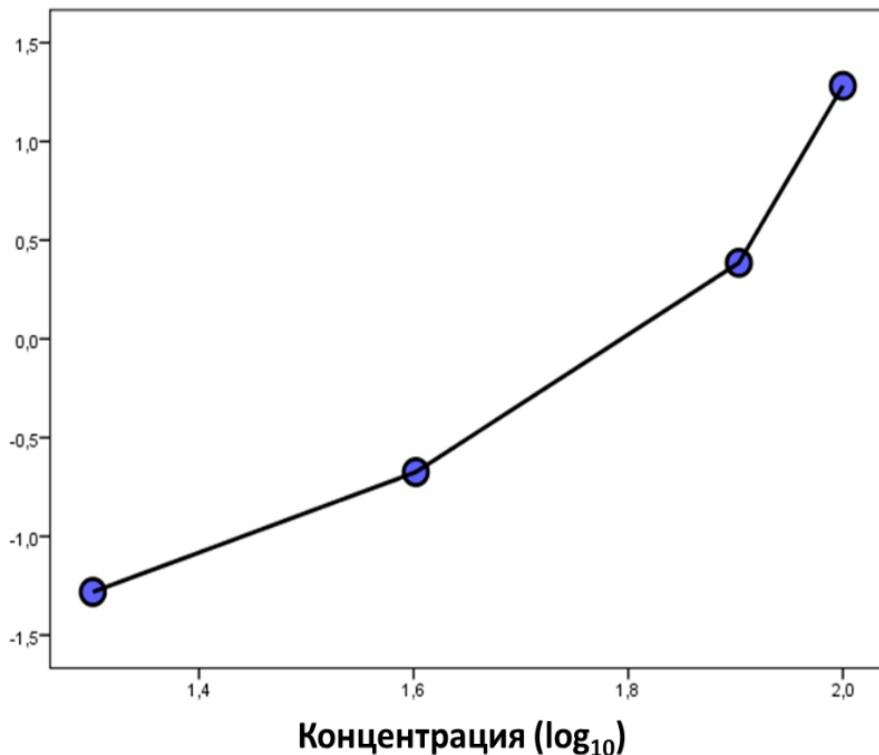


# Материалы и методы исследования

## Определение летальности 5F-АПИНАК



Пробит-регрессия



Опытная группа	Количество эмбрионов в группе	Количество погибших эмбрионов	Выживаемость в группе (%)
10 мкМ 5F-АПИНАК	20	0	100
20 мкМ 5F-АПИНАК	20	2	90
40 мкМ 5F-АПИНАК	20	5	75
80 мкМ 5F-АПИНАК	20	13	35
100 мкМ 5F-АПИНАК	20	18	10
Положительный контроль (4 мг/л 3,4-дихлоранилина в 1% растворе ДМСО в среде E3)	20	15	25
Отрицательный контроль (среда E3)	20	1	95
Контроль растворителя (1% раствор ДМСО в среде E3)	20	0	100

# Материалы и методы исследования

## Целевая метаболомная панель



Жидкостной хроматограф

Agilent 1290 Infinity II

Колонка

Discovery PFP HS F5 2.1 x 150

Температура термостата 45°C

Объём вводимой пробы 2 мкл

Подвижная фаза А

0,1% раствор муравьиной кислоты в воде

Подвижная фаза В

100% раствор ацетонитрила

Скорость потока

0,40 мл/мин

Градиент

0 мин – 1% В

4 мин – 10% В

9 – 10 мин – 90% В

10.1 – 12 мин – 1%В

Время анализа 12 мин

Масс-спектрометрический детектор

Agilent 6460 Triple Quad

Характеристика источника

ионизация в электроспрее

Полярность

положительная и отрицательная

Соединение	Внутренний стандарт	Т, мин	Родительский	Дочерний
			ион	ион
ГАМК	ГАМК-d6	1.0	104	69
Глутаминовая кислота	ГАМК-d6	1.0	148	84
Глутамин	ГАМК-d6	1.0	147	130
Триптофан	L-триптофан-d5	5.3	203	142; 116
5-гидрокситриптофан	Кинуренин-d4	3.3	221	204; 162
Серотонин	Серотонин-d4	3.5	177	160; 132 105
5-HIAA	5-HIAA-d5	5.9	192.1	146.1
Триптамин	Индол-3-уксусная кислота-d4	6.3	161.1	144.1; 115.1
Фенилаланин	ГАМК-d6	3.8	166	120
Тирозин	ГАМК-d6	2.5	182	165
Дофамин	Дофамин-d4	1.8	154	119; 91
Норэпинефрин	Норметанефрин-d3	1.1	152	107
Норметанефрин	Норметанефрин-d3	1.8	166	134
Эпинефрин	Норметанефрин-d3	1.4	184	166; 151
Метанефрин	Норметанефрин-d3	2.8	198	165; 148
Холин	Дофамин-d4	2.0	104	60
Кинуренин	Кинуренин-d4	3.3	209.1	146; 94
Кинуреновая кислота	Ксантуреновая кислота-d4	7.4	190.1	144.1
Антралиновая кислота	Индол-3-уксусная кислота-d4	4.0	138.1	120.1; 92
Ксантуреновая кислота	Ксантуреновая кислота-d4	5.3	206.1	160; 132.1
Хинолиновая кислота	Хинолиновая кислота-d3	2.7	168.1	106.1; 78.1
Индол-3-карбоксальдегид	Индол-3-карбоксальдегид-13C8	7.2	146.1	118.1; 91.1
Индол-3-уксусная кислота	Индол-3-уксусная кислота-d4	7.1	176.1	130.1
Индол-3-масляная кислота	Индол-3-масляная кислота-d4	7.7	204.2	144.2; 130.1
Индол-3-молочная кислота	Индол-3-молочная кислота-d5	6.8	206.1	118.1
Индол-3-акриловая кислота	Индол-3-уксусная кислота-d4	5.3	188.1	170; 115
Индол-3-припионовая кислота	Индол-3-пропионовая кислота-d2	7.4	190.1	130.1
Кортизол	Дофамин-d4	6.8	363	327; 121

# Материалы и методы исследования

## Фармакометаболомное исследование 5F-АПИНАК



Возраст: 2/6 дней после оплодотворения (дпо)

**Мальки зебрафиш**



**Группа сравнения**  
– 20 мальков в лунке (n=3)

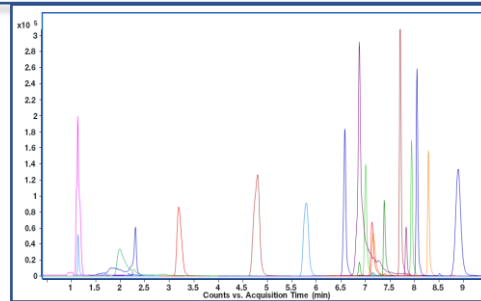
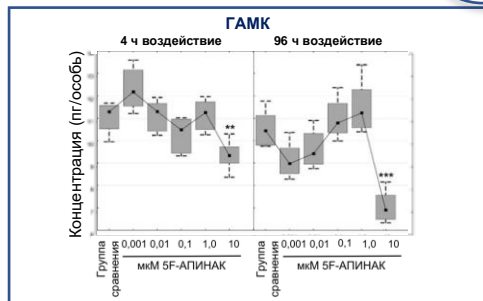
**Тестовая группа:**  
1) 0,001 мкМ; 2) 0,01 мкМ;  
3) 0.1 мкМ; 4) 1,0 мкМ;  
5) 10 мкМ  
по 20 мальков (n=3)

**Время воздействия:**

- 1) 4,0 часа
- 2) 96 часов

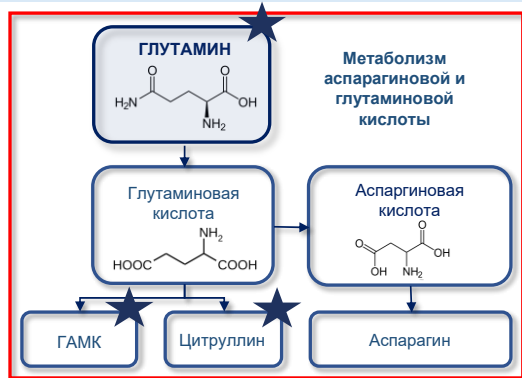
Пробоподготовка образцов

ЦЕЛЕВОЕ метаболомное профилирование  
ВЭЖХ-МС/МС

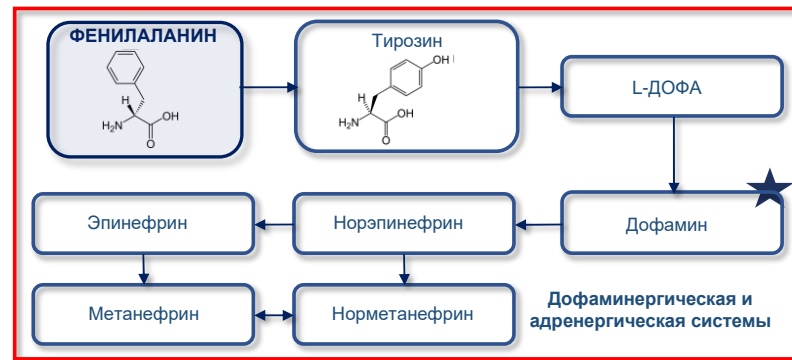
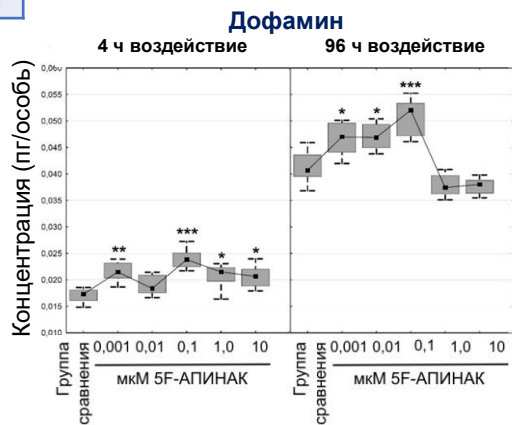
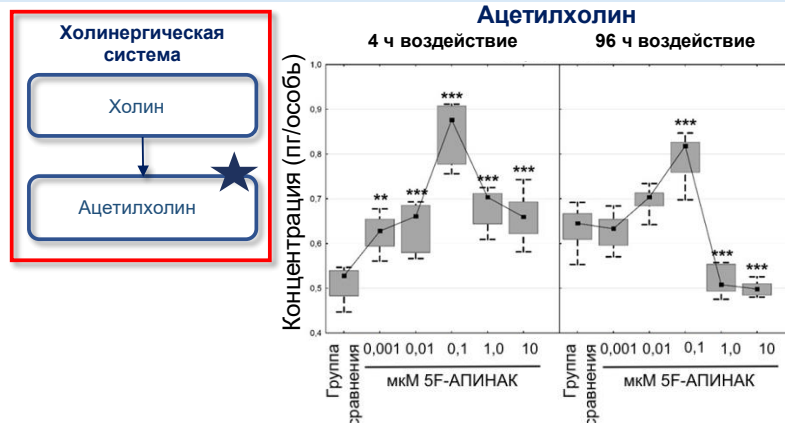
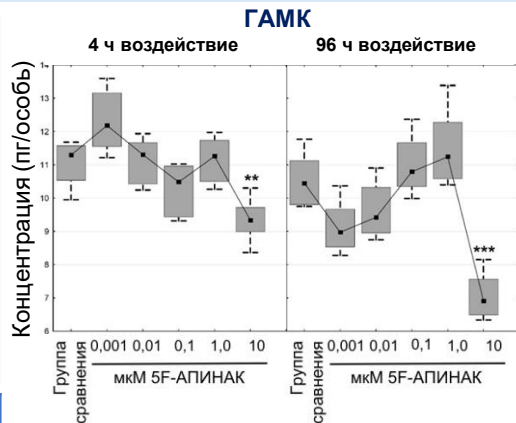
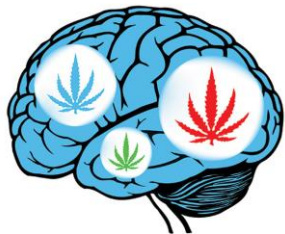


**Обработка данных и статистический анализ:** XCMS;  
SIMCA P13.0; Metaboanalyst 5.0; Statistica 10.0 и SPSS 23.0

# Результаты (1-адамантил 1-(5-фторпентил)индазол-3-карбоксилат (5F-АПИНАК))

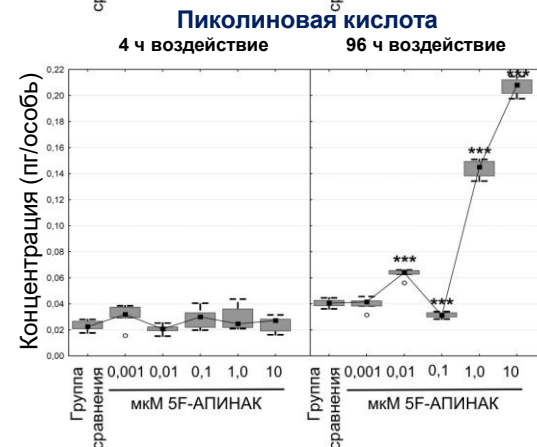
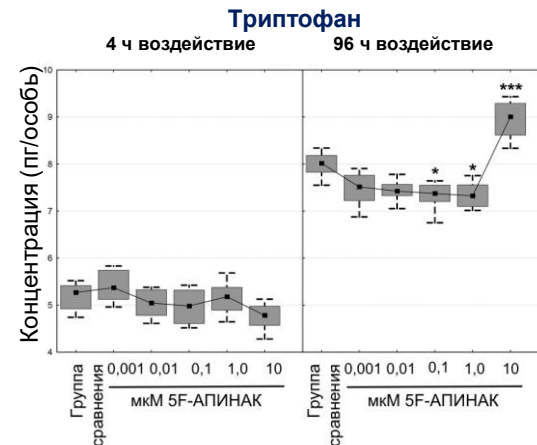
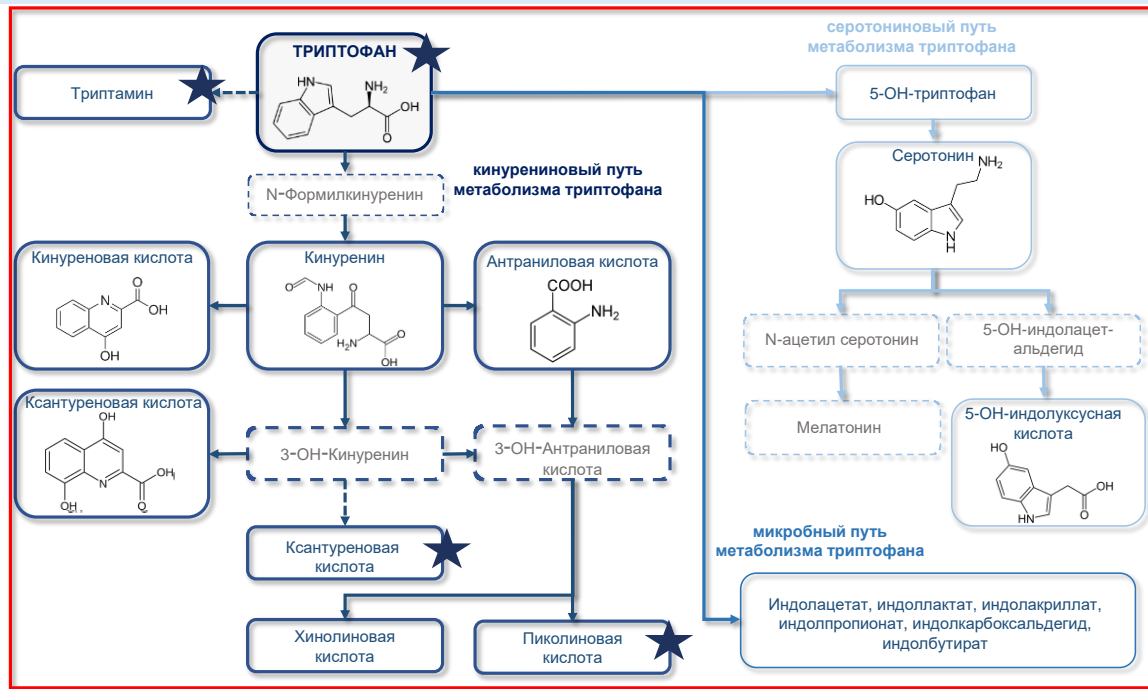


	4 ч	96 ч
Глутамин	p<0.001 ↓	—
Цитруллин	p<0.05 ↓	—
ГАМК	p<0.01 ↓	p<0.001 ↓



	4 ч воздействие	96 ч воздействие
Дофамин	p<0.05 ↑	p<0.05 ↑

# Результаты (1-адамантил 1-(5-фторпентил)индазол-3-карбоксилат (5F-АПИНАК))

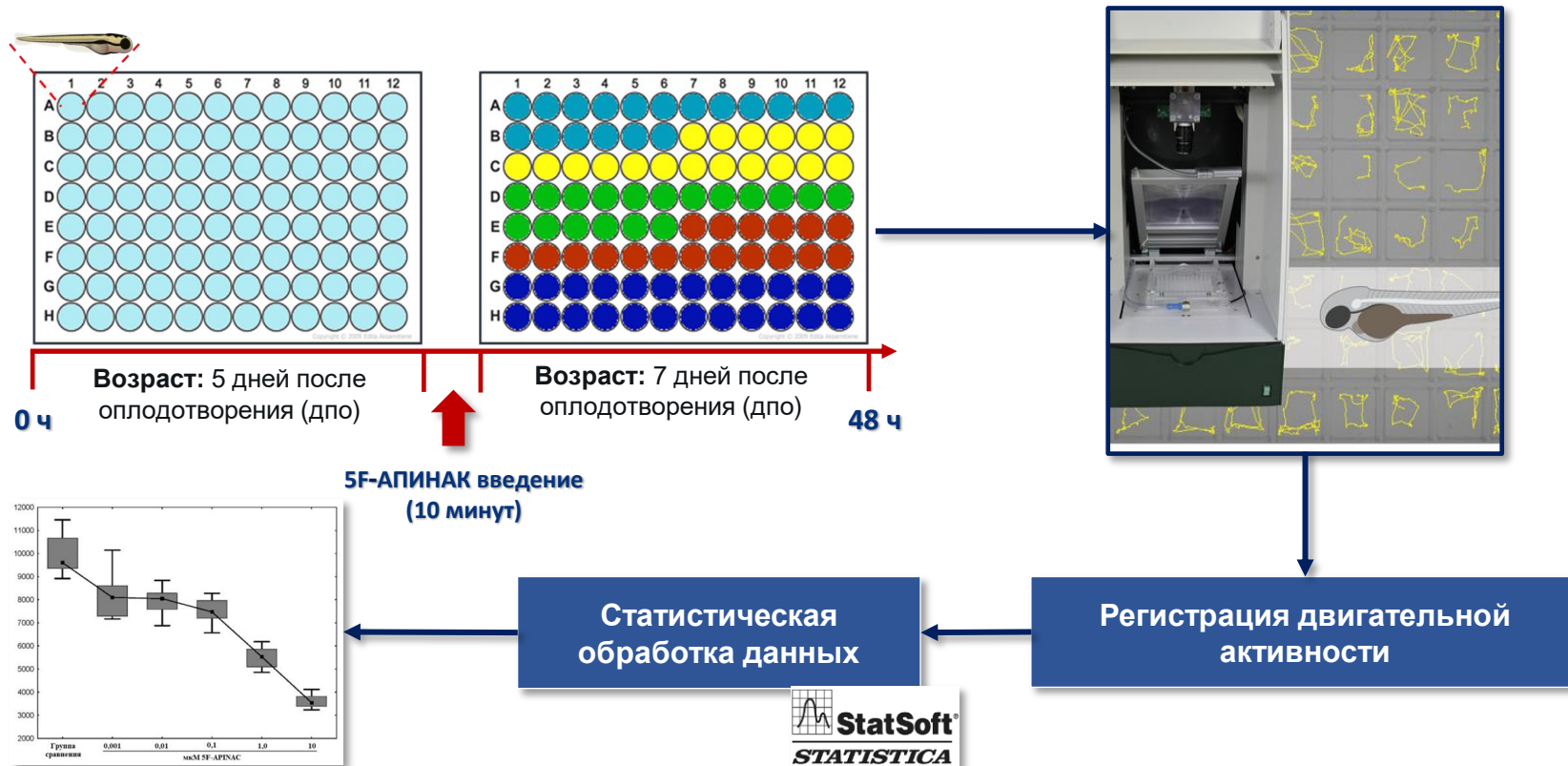


	4 ч воздействие		96 ч воздействие	
Триптофан	—		p<0.05	↓
Триптамин	p<0.05	↓	—	
Ксантуреновая кислота	—		p<0.05	↓
Пиколиновая кислота	—		p<0.001	↑

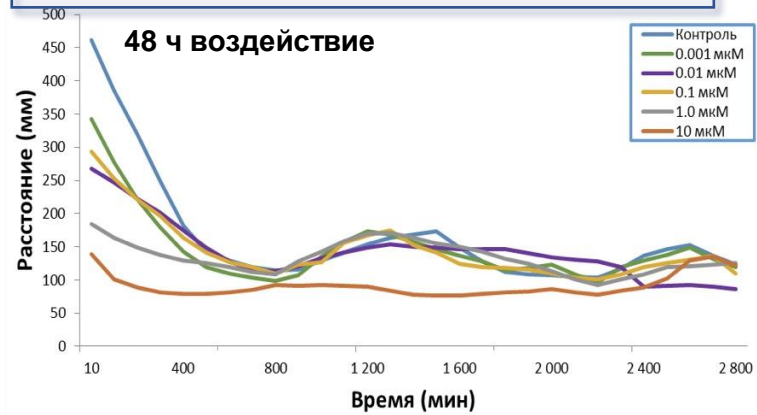
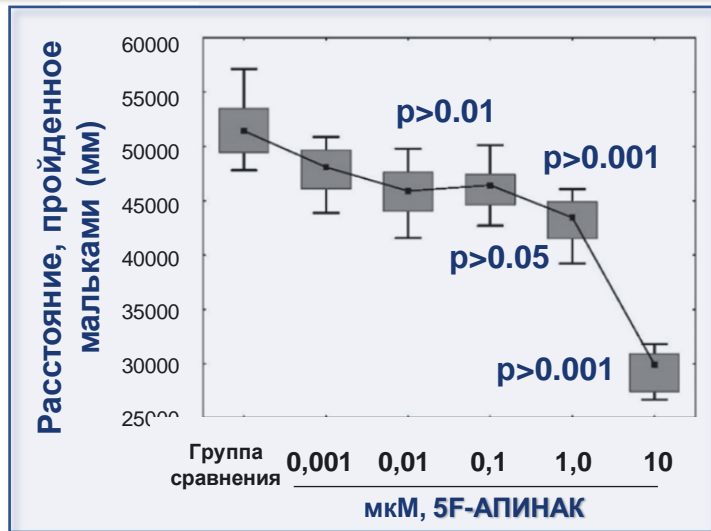
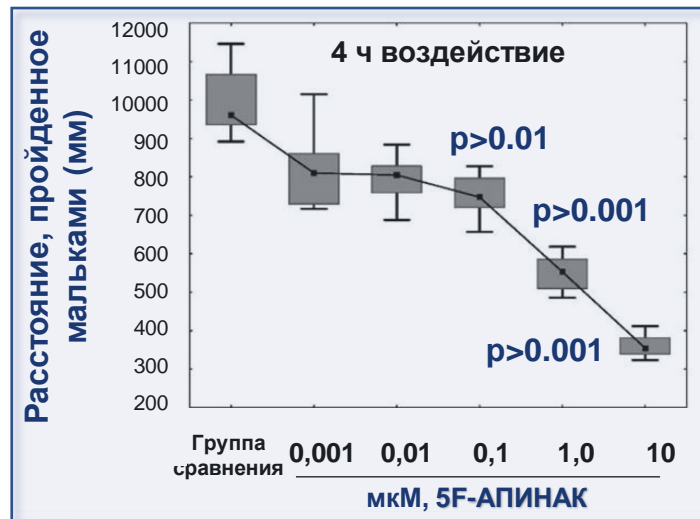
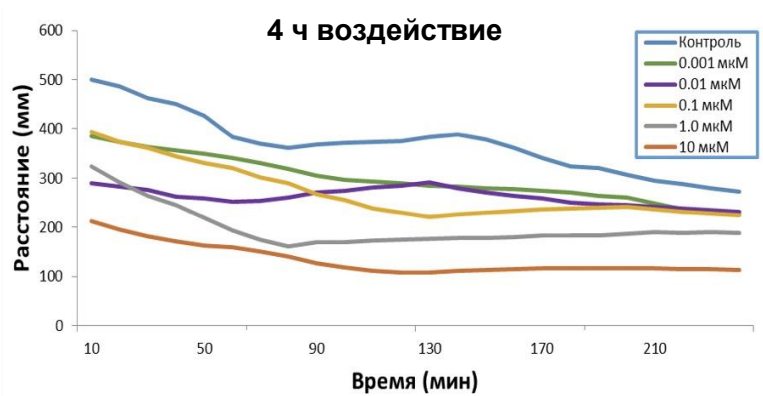


# Материалы и методы исследования

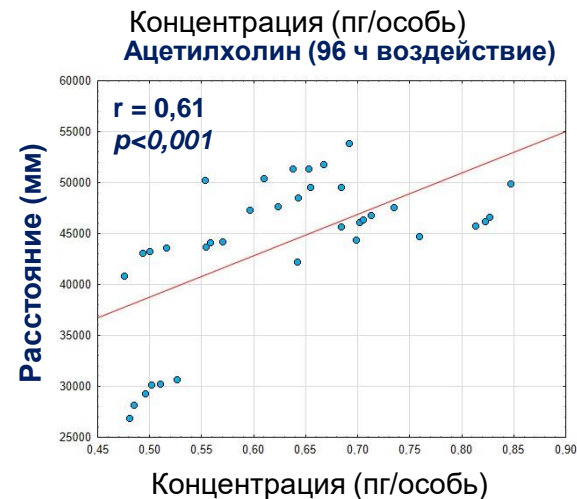
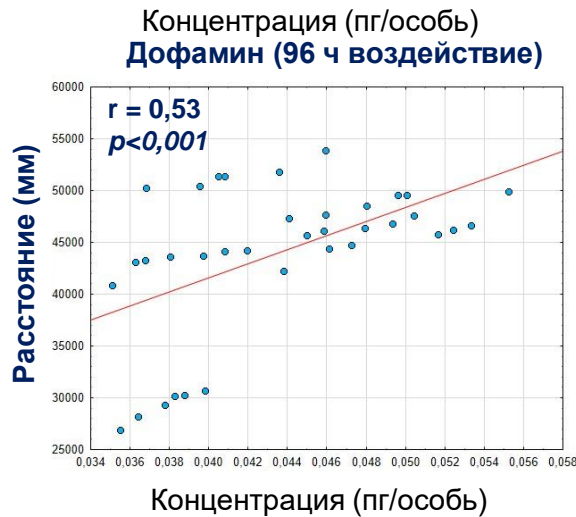
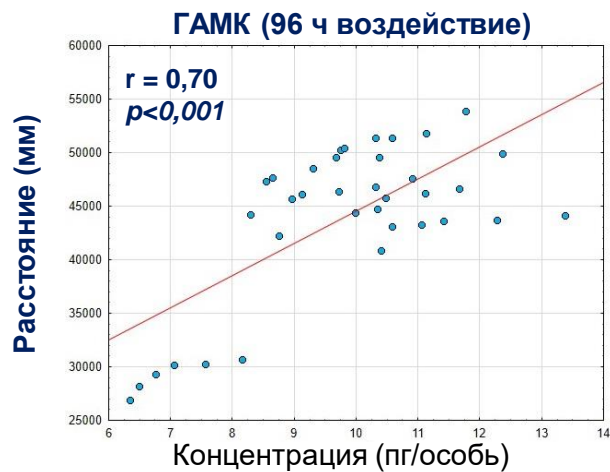
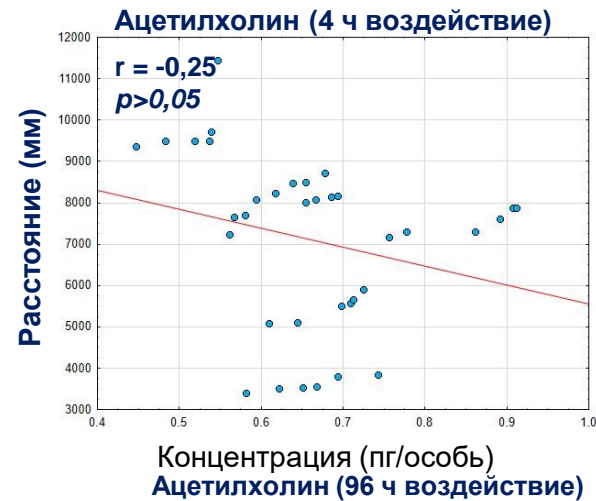
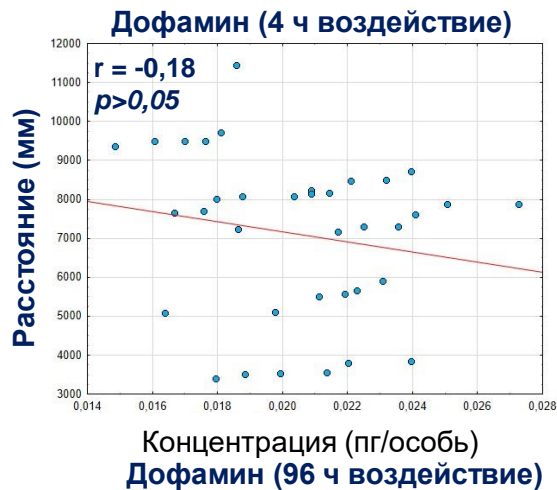
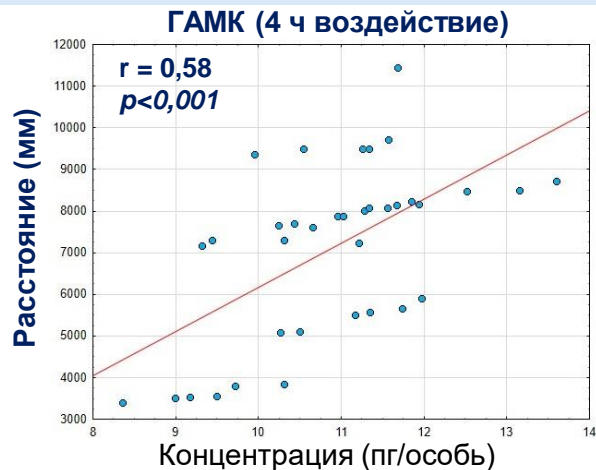
## Поведенческие эффекты 5F-АПИНАК на зебрафиш



# Результаты (1-адамантил 1-(5-фторпентил)индазол-3-карбоксилат (5F-АПИНАК))



# Результаты (1-адамантил 1-(5-фторпентил)индазол-3-карбоксилат (5F-АПИНАК))



# Выводы



- Проведено *in vitro* и *in vivo* исследование метаболизма 5F-АПИНАК. Показано, что 5F-АПИНАК подвергается метаболизму с образованием большого количества метаболитов. Основными метаболитами являлись гидроксипроизводные 5F-АПИНАК (моно-, ди- и тригидроксипроизводные), а также метаболиты 1-фторпентил-1H-индазол-3-карбоксилата (отщепление адамантанового цикла).
- Полулетальная концентрация 5F-АПИНАК для мальков рыб вида *Danio rerio* составила 55,0 мкМ (95% CI: 44,3-68,7 мкМ). Значение ЛК<sub>50</sub>, полученное в данном эксперименте, демонстрирует уровень токсичности 5F-АПИНАК на уровне других каннабиноидов.

# Выводы



- Воздействие 5F-АПИНАК вызвало изменения в концентрациях метаболитов ГАМКергической, серотонинергической, дофаминергической и холинергической систем, а также кинуренинового пути метаболизма триптофана.
- Выявлены корреляции концентрационных уровней нейромедиаторов с компонентом поведенческих эффектов, вызываемыми 5F-АПИНАК, у зебрафиш. Угнетение двигательной активности зебрафиш может быть связано с изменением концентраций гамма-аминомасляной кислоты, дофамина и ацетилхолина. Полученные данные согласуются с описанными эффектами каннабимиметиков на организм.



ORIGINAL ARTICLE

## In vivo and in vitro metabolism of the novel synthetic cannabinoid 5F-APINAC

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Received: 13 July 2019 / Accepted: 18 September 2019  
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**Abstract**

**Purpose** New generations of synthetic cannabinoids (SCs) have recognized strong psychoactive effects, rapidly becoming potent drugs of abuse. Characterization of metabolites associated with SCs is useful for their subsequent detection in biological samples. We aimed to investigate the metabolism of 5F-APINAC, a novel SC, in human liver microsomes (HLMs) (in vitro) and in a rat model (in vivo).

**Methods** For the in vitro study, the standard solution of 5F-APINAC was incubated with HLMs for 1 h at 37 °C. In the in vivo study, rats received 15 mg/kg of 5F-APINAC (dissolved in ethanol or in dimethyl sulfoxide, while a vehicle control group received placebo. Urine was collected 3, 6, and 24 h after administration. The metabolic characterization was performed using liquid chromatography–ion trap mass spectrometry and liquid chromatography–quadrupole time-of-flight mass spectrometry.

**Results** In total, 15 metabolites associated with 5F-APINAC were tentatively identified. The metabolites were classified as: 1-*adamantanol* derivatives, oxidative defluorination metabolites, and *N*-fluorenylindazole-3-carboxylic acid metabolites. The modifications included ester hydrolysis, mono-, di-, and trihydroxylation of adamantyl ring and *N*-fluorenylindazole moiety, oxidation (carbonyl formation) of the *N*-fluorenyl side chain, oxidative loss of fluorine, and glucuronidation, as well as combinations thereof. The predominant metabolic reaction was ester hydrolysis in both in vitro and in vivo experiments and formation of M9 (5-fluorenylindazole-3-carboxylic acid). However, the most recommendable metabolites for proving 5F-APINAC consumption in urine were M4, M7, and M13. No metabolites were detected in rat's urine 24 h after drug administration.

**Conclusions** The discovered metabolites are proposed to be incorporated into routine screening analytical methods as the urine markers of 5F-APINAC consumption. This is the first report to demonstrate the metabolism of 5F-APINAC to our knowledge.

**Keywords** 5F-APINAC metabolites · APINAC · New synthetic cannabinoid · Human liver microsomes (HLMs) · Rat urine · LC–QTOF–MS/MS

**Electronic supplementary material** The online version of this article (<https://doi.org/10.1007/s11419-019-00503-z>) contains supplementary material, which is available to authorized users.

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## Comparative Biochemistry and Physiology, Part C

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## Short- and long-term exposures of the synthetic cannabinoid 5F-APINAC induce metabolomic alterations associated with neurotransmitter systems and embryotoxicity confirmed by teratogenicity in zebrafish

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ARTICLE INFO

**Keywords:**

Novel psychoactive substances  
Synthetic cannabinoids  
Metabolomics  
Neurotransmission  
Metabolites  
5F-APINAC

ABSTRACT

**Introduction:** Synthetic cannabinoids are abused substances with strong psychoactive effects. Little is known about the effects on neurotransmission and the toxicity of the second-generation cannabinoid 5F-APINAC. The objective was to assess the influence of short- and long-term exposures of 5F-APINAC on metabolites associated with neurotransmission on zebrafish.

**Methods:** Short-term ("acute", 4 h) and long-term ("chronic", 96 h) exposures to 5F-APINAC were performed at 0.001, 0.01, 0.1, 1.0 and 10 mg/kg. Intervention groups were compared with a vehicle control. Each group  $n = 20$  zebrafish eggs/larvae. Metabolites related to neurotransmission were determined.

**Results:** In chronic exposure, larvae exposed to 10 mg 5F-APINAC presented morphological and developmental alterations. GABA had the lowest concentrations in higher exposure in acute ( $p < 0.01$ ) and chronic ( $p < 0.001$ ) experiments. Glutamine showed a decreasing trend in the acute experiment, but an ascending trend in the chronic exposure ( $p < 0.05$ ). In chronic exposure, tryptophan presented an overall decreasing trend, but with a neat increase at 10 mg 5F-APINAC ( $p < 0.001$ ). Tryptamine in acute exposure presented lower ( $p < 0.05$ ) concentrations at higher doses. Dopamine and acetylcholine presented higher ( $p < 0.05$ ) concentrations in the acute and chronic exposures, but with a drop at the highest doses in the chronic experiments. In chronic exposure, norepinephrine acid decreased, except for the highest dose. Picolinic acid was increased at the highest doses in the chronic experiment ( $p < 0.001$ ).

**Conclusions:** Short- and long-term exposures induced metabolomic alterations associated with the gamma-aminobutyric acid/glutamic acid, dopaminergic/norepinephrine, cholinergic neurotransmission systems, and the kynurenine pathway. Chronic exposure at 10 mg 5F-APINAC was associated with embryotoxicity confirmed by teratogenicity.

**Abbreviations:** Dayp(d) (day) post fertilization; UPLC-MS/MS, ultra performance liquid chromatography – tandem mass-spectrometry; SC, synthetic cannabinoids; NPS, novel psychoactive substances.

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<https://doi.org/10.1016/j.cbpc.2021.109000>

Received 12 October 2020; Received in revised form 21 December 2020; Accepted 1 February 2021

Available online 6 February 2021

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Article

## Pharmacokinetic Properties of the Novel Synthetic Cannabinoid 5F-APINAC and Its Influence on Metabolites Associated with Neurotransmission in Rabbit Plasma

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Citation: Shestakova, K.M.; Mesonzhnik, N.V.; Markin, P.A.; Moskaleva, N.E.; Nedorubov, A.A.; Brito, A.; Appolonova, E.G.; Kuznetsov, R.M.; Bochkareva, N.L.; Kukharevko, A., et al. Pharmacokinetic Properties of the Novel Synthetic Cannabinoid 5F-APINAC and Its Influence on Metabolites Associated with Neurotransmission in Rabbit Plasma. *Pharmaceuticals* 2021, 14, 668. <https://doi.org/10.3390/ph14070668>

Academic Editor: Riccardo

Jorge Otáñez-Oviedo

Received: 9 June 2021

Accepted: 8 July 2021

Published: 13 July 2021

**Abstract:** The strong psychoactive effects of synthetic cannabinoids raise the need for the deeper studying of their neurometabolic effects. The pharmacokinetic properties of 5F-APINAC and its influence on metabolites profiles associated with neurotransmission were investigated in rabbit plasma. Twelve rabbits divided into three groups received 1 mL 5F-APINAC at 0.1, 1 and 2 mg/kg. The intervention groups were compared with the controls. Sampling was performed at nine time points (0–24 h). Ultra-high-performance liquid chromatography–tandem mass spectrometry was used. The pharmacokinetics were dose-dependent (higher curve at a higher dose) with a rapid biotransformation, followed by gradual elimination within 24 h. The tryptophan concentrations rapidly decreased ( $p < 0.05$ ) in all tested groups, returning to the basal levels after 6 h. 5-Hydroxyindole acetic acid increased ( $p < 0.05$ ) in the controls, but this trend was absent in the treated groups. The aspartic acid concentrations were elevated ( $p < 0.001$ ) in the treated groups. L-Kynurenine was elevated ( $p < 0.01$ ) in the intervention groups receiving 1 mg/kg to 2 mg/kg. Dose-dependent elevations ( $p < 0.01$ ) were found for kynurenic acid, xanthurenic acid and quinolinic acid ( $p < 0.01$ ), whereas the anthranilic acid trends were decreased ( $p < 0.01$ ). The indole-3-propionic acid and indole-3-carboxaldehyde trends were elevated ( $p < 0.05$ ), whereas the indole-3-lactic acid trajectories were decreased ( $p < 0.01$ ) in the intervention groups. 5F-APINAC administration had a rapid biotransformation and gradual elimination. The metabolites related to the kynurenine and serotonergic system/serotonin pathways, aspartic acid intervention system and microbial tryptophan catabolism were altered.



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