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Are the postmortem concentration changes of the synthetic cannabinoid *cumyl*-5F-P7AICA and its *N*-pentanoic acid metabolite dependent on the environmental conditions? – A systematic study following pulmonary administration to pigs

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ABSTRACT

The number of fatal cases involving synthetic cannabinoids (SCs) is increasing. However, interpretation of postmortem (PM) toxicological findings is challenging, due to unknown PM intervals and possible redistribution processes or instabilities. Only sparse data on SCs are available. Therefore, a controlled pig study was performed to determine the PM stability of *cumyl*-5F-P7AICA under different environmental conditions. Ten pigs inhalatively received 200 µg/kg body weight *cumyl*-5F-P7AICA each. Six hours later, they were euthanized and biopsies of the main tissues and body fluids were taken. Animals were stored in air or water (n=5 each) and samples were repeatedly taken for three days. Quantification of *cumyl*-5F-P7AICA and its *N*-pentanoic acid metabolite (NPA) was performed using standard addition or a fully validated method (blood) followed by LC-MS/MS. Time-dependent concentration changes of *cumyl*-5F-P7AICA were observed in liver, bile fluid and muscle specimens at both conditions. Concentrations of NPA only changed considerably in duodenum content of animals stored in air. Environment-dependent concentrations changes were only observed for *cumyl*-5F-P7AICA in kidney and the NPA metabolite in duodenum content. Overall, *cumyl*-5F-P7AICA and its metabolite seem to be quite stable in PM specimens. Hence, also central blood might be analyzed, if no peripheral blood is available.

1. Introduction

In addition to ‘common‘ drugs, such as cocaine or heroin, numerous fatal intoxications related to the use of synthetic cannabinoids (SCs) have been reported in the literature worldwide (Boland et al., 2020; Doerr et al., 2022; Ferrari Júnior et al., 2022; Giorgetti et al., 2020; Schaefer et al., 2013; Walle et al., 2023). In the last years, several new initially legal SCs with slightly modified chemical structures, e.g., with a 7-azaindole core structure, emerged on the drug market to circumvent legal restrictions. However, such modifications can lead to higher potencies, resulting in life-threatening intoxications or even death (Oberhofer, 2018; Walle et al., 2023; Zawadzki et al., 2021). Accordingly, also these substances were covered by law in Germany. In this context knowledge of the toxicokinetic (TK) properties, especially the postmortem (PM) stability as well as a possible PM redistribution (PMR), is essential for the interpretation of toxicological PM findings, as a degradation or new formation of ingested substances can occur PM. Well-known examples of a PM formation are ethanol or gamma hydroxybutyrate as, on the contrary, a PM degradation was reported, e.g. for cocaine or diacetylmorphine (Skopp, 2010). Until now, only sparse data on PM tissue distribution of SCs as well as their PM stability are available in the literature. However, these data are usually based on case reports, where neither the dose and the time of consumption nor the PM interval (PMI) is known (Adamowicz, 2016; Boland et al., 2020; Doerr et al., 2022; Giorgetti et al., 2020; Kraemer et al., 2019; Walle et al., 2023). In addition, PM autolysis leading to a disintegration of membrane functions, resulting in a passive diffusion of substances into surrounding tissues has to be considered for example (Brockbals et al., 2018;

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Drummer and Gerostamoulos, 2002; Gerostamoulos et al., 2012; Skopp, 2010). Moreover, the extent of this redistribution is dependent on the physicochemical (e.g. lipophilicity) as well as the TK properties of the respective substances (Pélissier-Alicot et al., 2003; Skopp, 2010). Furthermore, due to a PM decrease of pH and a microbial colonization of the corpse, a further degradation of substances can occur, influencing the PM stability of drugs in tissue and body fluid samples (Skopp, 2010).

In order to prove a possible intoxication after ingestion of drugs (of abuse) as the cause of death in a putrefied corpse, all these processes must be considered when interpreting PM toxicological findings, as the measured analytical values are not necessarily reflecting the ante-mortem situation, which can quickly lead to a misinterpretation, if the PM properties of the respective substance are unknown.

Several systematically controlled pig studies on the perimortem tissue distribution as well as the PM stability, respectively tendency for PMR have been successfully performed on SCs with an indole core structure (Schaefer et al., 2017, 2019, 2020a) and structurally modified SCs with a 7-azaindole core (Doerr et al., 2024)after intravenous or inhalative administration.

Comparison of the perimortem and PM TK data of a SC with a 7-azaindole core structure reported by Doerr et al. (2024) with the data obtained by Schaefer et al. (2017), (2019), (2020a) for SCs with an indole core indicates that the tissue distribution, the PM stability and the tendency for PMR of SCs seem to be significantly affected by the changes of their physicochemical properties resulting from modifications in the chemical structure.

Hence, in the present study, already published data on the perimortem tissue distribution of SCs with a 7-azaindole core structure (Doerr et al., 2024) should initially be supplemented by data on the structurally modified SC *cumyl*-5F-P7AICA as well as its *N*-pentanoic acid (NPA) metabolite in the framework of a systematically controlled study after pulmonary administration using the pig model.

Additionally, as already mentioned above, putrefaction of a corpse can vary greatly depending on prevailing storage or environmental conditions, such as temperature, oxygen availability or humidity. This principle was already elucidated by Johann Ludwig Casper by means of the so-called ‚Casper-rule', which describes the degree of putrefaction and, thus, also of the autolytic processes as a function of the prevailing ambient conditions (Dettmeyer et al., 2014; Vennemann and Brinkmann, 2003). Accordingly, the degree of putrefaction of a corpse after 1-week in air corresponds approximately to that of a 2-week water storage or a 8-week storage in soil (Dettmeyer et al., 2014; Vennemann and Brinkmann, 2003). Therefore, a change of the PMR degree as well PM stability of SCs may also be expected due to a delayed onset of the autolytic processes, if the corpse is stored under oxygen-deficient conditions, e.g. in water. In a previous study, Schaefer et al. found PM time- and temperature-dependent concentration (conc.) changes especially of RCS-4 and HO-RCS-4 (Schaefer et al., 2020a). Data on the PM stability and PMR of SCs in tissues and body fluids under different environmental conditions, e.g. water storage, are not availableso far. Therefore, in a second step of the present study the time- and oxygen-dependent conc. changes of *cumyl*-5F-P7AICA and its main metabolite were compared under different storage conditions, at air and in water, for the first time. The PM data obtained should further be compared with those found in perimortem specimens.

2. Materials and methods

2.1. Chemicals, reagents and blank whole blood samples

Detailed information on the chemicals and reagents used for the study, their origin and the preparation of blank whole blood samples can be found in the Electronic Supplementary Material (ESM).

2.2. Preparation of stock solutions, quality control (QC) samples, calibration standards, and calibrators for standard addition

Standard stock solutions (1000 µg/mL) were prepared by dissolving the obtained solid substances in ethanol. To obtain working standard solutions (0.01 µg/mL, 0.1 µg/mL, 1 µg/mL, and 10 µg/mL), the stock solutions were diluted with ethanol, respectively. For preparation of the QC low (final conc. of 5 ng/mL *cumyl*-5F-P7AICA and 0.2 ng/mL NPA) and high (final conc. of 35 ng/mL *cumyl*-5F-P7AICA and 0.8 ng/mL NPA) samples, the respective working standard solution was diluted with ethanol. Furthermore, the spiking solutions for calibration standards were also prepared by diluting the working solutions with ethanol to obtain solutions for final blood conc. of 0.5, 10, 20, 30, 40, and 50 ng/mL as well as 0.1, 0.3, 0.5, 0.7, 0.9, and 1.1 ng/mL of *cumyl*-5F-P7AICA and NPA, respectively. Calibrators for standard addition were also prepared by dissolving working standard solutions with ethanol. The respective conc. of the calibrators used for the different specimens are depicted in Table 1. All prepared solutions were stored at −20 °C.

2.3. Preparation of buffer solutions

The preparation of the buffer solutions is described in detail in the ESM.

2.4. In vivo study

2.4.1. Animals

As described previously (Walle et al., 2021) the in vivo experiment performed in the present study was conducted in accordance with the German legislation on protection of animals and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (permission number: 44/2019).

Ten domestic male pigs (Swabian Hall strain) with a body weight (BW) between 44 and 64 kg were used. The pigs had free access to tap water, daily standard chow and were kept fasting for 12 h before the experiment, while they still had free access to water.

2.4.2. Surgical procedures and study design

The surgical procedures and the study design were in accordance to those already published elsewhere (Doerr, 2024). A detailed description

Table 1
Calibrator concentrations (conc.) of *cumyl*-5F-P7AICA and its *N*-pentanoic acid (NPA) metabolite used for the standard addition approach divided between the different approaches as well as the various specimens in ng/g tissue/ body fluid specimen. (./. = not added).

Perimortem specimens						
Specimen	**Calibrator conc. of *cumyl*-5F-P7AICA [ng/g]**			**Calibrator conc. of NPA [ng/g]**		
	1	**2**	**3**	**1**	**2**	**3**
Brain/ liver/ kidney/ muscle tissue	0.5	1	1.5	0.5	1	1.5
Lung	5	10	15	0.1	0.2	0.3
Bile fluid/ duodenum content	5	10	15	5	10	15
Adipose tissue	10	20	30	./.	./.	./.
Postmortem specimens						
Specimen	**Calibrator conc. of *cumyl*-5F-P7AICA [ng/g]**			**Calibrator conc. of NPA [ng/g]**		
	1	**2**	**3**	**1**	**2**	**3**
Brain/ liver/ kidney/ muscle tissue	1	2	3	0.2	0.4	0.6
Lung	3	6	9	0.2	0.4	0.6
Bile fluid	1.5	3	4.5	5	10	15
Duodenum content	5	10	15	2	4	6
Adipose tissue	10	20	30	./.	./.	./.

on the surgical procedures can be found in the ESM.

Regarding the study design, a stock solution of *cumyl*-5F-P7AICA (5000 μg/mL) was prepared by dissolving the solid substance in ethanol as already described in a previous study (Walle et al., 2021). In order to achieve the desired 200 μg/kg BW dose, the required volume of the stock solution was taken or diluted with ethanol, respectively. The dilution was subsequently administered inhalatively within 12 min by nebulization applying the inspiration-triggered mode (< 0.2 mL/min) of the M-neb flow+ ventilation ultrasonic nebulizer MN-300/7 (Nebutec, Elsenfeld, Germany) in accordance to the administration setup already published by Schaefer et al. (Schaefer et al., 2018).

After sampling of approximately 10 mL peripheral blood (PB) from the jugular vein (t=360 min),the pigs were euthanized by administering 0.12 mL/kg BW T61 (Intervet Deutschland GmbH, Unterschleißheim, Germany) six hours after drug administration. A small piece of muscle was collected from the inner thigh during the removal of the catheter from the left artery. Following, the abdominal cavity was opened and samples of different body fluids, organs, and tissues were drawn (PMI 0) as follows: subcutaneous (s.c.) adiposetissue (AT) was sampled from the inside of the incision. Depending on the amount contained, approximately 1–2 mL of bile fluid were removed from the gall bladder using a syringe. The puncture site was closed with a clamp to prevent leaking. A piece of liver (approximately 10 g) was then cut out while the cut surface was cauterized to stop bleeding. To obtain duodenum content, the upper part of the small intestine (duodenum) was divided into 4 sections of approximately 30 cm each using stitches. The first section, starting at the pylorus, was opened and the duodenum content squeezed out. The other parts were put back for PM sampling. Subsequently, the renal capsule was opened to take some perirenal AT as well as cut off a piece of kidney tissue (approximately 10 g). In order to access the lung tissue and central blood (CB), the diaphragm was first severed with a scalpel. Sampling of lung tissue and CB was carried out inside the chest cavity. Therefore, a piece of lung tissue was initially cut off. Following, CB was collected by puncturing the heart using a syringe and a 14 G cannula. A total of about 10 g was sampled from each tissue specimen. The respective organs and tissues were left in situ and the abdominal cavity was sutured. Subsequently, a small incision was made in the neck of the pig to obtain dorsal AT. Finally, the skull was opened using a head saw and some cerebrum tissue was sampled. The skullcap was put on again and the skin was sutured.

Subsequently, one group of the animals (n=5) was stored at room temperature (RT), whilst the other part (n=5) was kept in approximately 50 L water lying in a bathtub, with the water not being replaced over the entire duration of the experiment. Further specimens of the above-mentioned organs and body fluids were drawn after 24 h (PMI 1), 48 h (PMI 2), and 72 h (PMI 3) in analogy to the previously described procedure with a few modifications. In brief, PB was drawn from the femoral or the brachiocephalic vein at PMI 1–3, after the veins have been exposed with a scalpel. To obtain muscle specimens, the clamps at the inner thigh were removed. After sampling, the skin was sutured again using stitches. S.c. AT was also taken at the incision site during the opening of the abdominal cavity. Bile fluid was removed from the gall bladder in accordance to the procedure described above. A piece of liver was cut off, while a cauterization of the cut surface was no longer necessary. For duodenum content, the respective separated section of the small intestine was opened and the content was pressed out. Regarding kidney tissue, sampling was performed by cutting off small pieces of tissue specimens after the collection of perirenal AT. However, the sampling site was switched to the second kidney at PMI 2 or 3. Lung tissue and CB were sampled in accordance to the previously described procedure. However, at PMI 3, sampling of CB was performed after the heart has been removed from the chest and opened. Dorsal AT was collected as previously described. For sampling of brain tissue, the skullcap was initially removed. After the skull was closed again, the skin was sutured with stitches. However, brain specimens of the animals stored in water were only collected after 72 h. The respective organs and tissues were left in situ and the abdominal cavity was sutured after each time point. Analogously to the perimortem sampling procedure, a total of about 10 g was sampled from each PM tissue specimen.

Regarding the perimortem and PM sampling procedure of lung, liver and kidney tissue, no exact sampling location could be maintained. All specimens were stored at −20 °C until analysis.

2.5. Preparation of blood specimens

Since the volume of PM PB and CB samples was very low and no standard addition could be performed, *cumyl*-5F-P7AICA as well as its NPA were quantified using a fully validated method (unpublished data) based on a solid phase extraction (SPE) in analogy to the procedure already published by Schaefer et al. for the (synthetic) cannabinoids JWH-210, RCS-4, and THC (Schaefer et al., 2019, 2020a). The limit of detection was set at 0.06 ng/mL for *cumyl*-5F-P7AICA and 0.006 ng/mL for the NPA metabolite, the lower limits of quantification were defined as the lowest calibrator in blood (0.5 ng/mL for *cumyl*-5F-P7AICA, 0.1 ng/mL for the NPA). Analysis was performed by a liquid chromatography (LC) quadrupole time-of-flight (TOF)-mass spectrometer (MS) system. A detailed description of the blood sample preparation can be found in the ESM.

2.6. Preparation of tissue and body fluid specimens

The preparation of perimortem as well as PM tissue and body fluid specimens was performed in accordance to previous published studies (Schaefer et al., 2020a, 2020b). In brief, 2 g of the solid tissue (lung, liver, kidney, brain, muscle tissue) or 1 g bile fluid and duodenum content was homogenized with water in a ratio of 1 amount tissue + 4 amounts water or 1 amount body fluid + 9 amounts water, respectively. For quantification of *cumyl*-5F-P7AICA and its NPA metabolite, a standard addition approach was performed. For this purpose, the homogenates were first divided into four 0.5 g portions. Subsequently, to create a stand addition curve, one of those aliquots was prepared without and the others with the addition of 25 μL of different analyte conc. Thereafter, 20 μL of an ethanolic stable-isotope-labeled internal standard mixture solution (SIL-IS;1 ng/20 μL RCS-4-d_9), 0.5 mL acetate buffer (pH 4), and 50 μL β-glucuronidase/arylsulfatase was added and after vortexing, incubation for enzymatic hydrolysis was performed at 60 °C for 2 h. The hydrolysis was stopped by the addition of 0.5 mL acetonitrile. After vortexing and centrifugation at 3500 *g* for 8 min the supernatants were fortified with 1.5 mL phosphate buffer (pH 9). After an additional vortexing step, the mixtures were loaded on conditioned solid phase cartridges. After thee washing steps with 3 mL phosphate buffer (pH 9), 3 mL acetic acid (0.25 M), and 3 mL water, 60 μL acetone were added and the cartridges were dried under a maximum vacuum of 10 inHg. Subsequently, 1.5 mL of a methanol-acetone mixture (1:1, *v/v*) was added for elution of the substances. After evaporation of the eluates at 60 °C under a gentle stream of nitrogen, the dry residues were dissolved by the addition of 50 μL of a mixture (50:50, *v/v*) of mobile phases A (0.1 % aqueous formic acid) and B (0.1 % formic acid in acetonitrile). For analysis, 5 μL of the solutions were injected onto a LC-triple quadrupole-MS system.

Regarding AT specimens, 2 g AT was homogenized with acetonitrile (1 amount AT + 5 amounts acetonitrile). After centrifugation at 3500 *g* for 8 min, four 0.5 mL portions were prepared with and without different conc. of *cumyl*-5F-P7AICA to perform a standard addition approach. Subsequently, 20 μL SIL-IS (1 ng/20 μL RCS-4-d_9) was added. After evaporation under nitrogen at 60 °C, the dry residues were dissolved in 100 μL of a mixture of mobile phases A and B (50:50, *v/v*). Finally, 20 μL of the respective extracts were injected onto a LC-triple-quadrupole-MS system.

2.6.1. Standard addition approach

In the present study, a standard addition approach was performed to

quantify the conc. of *cumyl*-5F-P7AICA and its NPA metabolite in different tissues and body fluids respectively, with the exception of blood specimens. For this purpose, four portions of the homogenates with and without the addition of different analyte conc. were prepared (Table 1). Evaluation of the conc. in tissues and body fluids was performed using Microsoft Office Excel 2003 (Redmond, WA, USA). Therefore, by plotting the respective calibrator conc. against the analyte/SIL-IS ratio, a linear regression was performed to create standard addition calibration equations as follows: $y = a\,x + b$. Calculation was performed depending on the slope (a) as well as the intercept (b), while the negative point of interception of the x-axis represents the unknown drug conc.

2.7. LC-MS/MS apparatus

Instrumentation, chromatographic, and mass spectrometric conditions of the LC-triple quadrupole-MS as well as the LC-quadrupole TOF-MS used for the analysis of the respective extracts are described in detail in the ESM. Furthermore, detailed information on the respective precursor and product ions, the retention time as well as the analysis conditions can be found in Supplementary Table 1.

2.8. Evaluation of drug conc. changes and statistical tests

Calculation of the PM median drug conc. changes of *cumyl*-5F-P7AICA and its NPA metabolite was performed in accordance to previous published studies (Nordmeier et al., 2022; Schaefer et al., 2020a). Analogously, the conc. ascertained at PMI 1–3 were compared with the

Table 2
Mean and median concentrations (conc.) ± standard deviations (SD) of *cumyl*-5F-P7AICA and its *N*-pentanoic acid metabolite in ng/mL or ng/g measured in different tissue and body fluid specimens collected six hours after drug administration defined as the postmortem interval (PMI) 0 as well as 24 h (PMI 1), 48 h (PMI 2), and 72 h (PMI 3) after euthanasia of five pigs and a following storage at room temperature (RT) or water, respectively. Brain specimens were only taken at PMI 3 when stored in water. Median conc. are depicted in square brackets. All stated concentrations are approximated. Deviations in the number of samples tested are indicated accordingly.

***Cumyl*-5-P7AICA**								
	RT				**Water**			
	PMI 0	PMI 1	PMI 2	PMI 3	PMI 0	PMI 1	PMI 2	PMI 3
Lung	19 ± 9.1[15]	9.5 ± 8.4[9.3]	20 ± 11[9.3]	25 ± 7.0[12]	4.5 ± 3.8[3.1]	12 ± 17[2.4]	16 ± 9.1[15]	10 ± 7.4[8.6]
Liver	1.4 ± 0.95 [1.6]	2.2 ± 0.86[2.1]	3.5 ± 1.5*[3.5]	5.1 ± 6.4[3.3]	0.95 ± 0.78 [0.90]	1.6 ± 1.1[1.2]	2.3 ± 1.3[2.2]	4.6 ± 4.0[2.2]
Kidney	2.0 ± 2.1[1.4]	3.0 ± 1.5[3.1]	3.4 ± 1.9[2.6]	3.4 ± 1.4[2.3]	1.2 ± 0.69[1.2]	1.6 ± 0.73[1.5]	2.4 ± 1.4[1.7]	2.4 ± 1.3[2.0]
Brain	0.58 ± 0.12 [0.55]	0.97 ± 0.36 [0.91]	6.1 ± 7.8[1.2](n = 4)	1.7 ± 0.92[0.99]	0.54 ± 0.47 [0.41] (n = 4)			10 ± 11[6.4]
Muscle	1.8 ± 1.6 [0.87](n = 4)	1.1 ± 0.46[1.1]	1.0 ± 0.52[1.0]	1.7 ± 0.65[1.8]	0.42 ± 0.36 [0.34]	0.92 ± 0.59 [0.76]	0.90 ± 0.31 [0.80]	1.5 ± 0.96*[1.1]
AT dorsal	8.4 ± 4.8[7.4] (n = 4)	10 ± 10[8.5]	4.4 ± 2.5[3.6]	6.2 ± 1.4[5.9]	7.7 ± 4.1[7.7]	4.8 ± 3.0[5.3]	4.0 ± 3.4[3.7]	6.4 ± 1.1[6.4]
AT s.c.	11 ± 7.5[10]	10 ± 7.4[12]	12 ± 11[7.7]	8.9 ± 4.4[9.7]	11 ± 5.3[10]	8.3 ± 2.2[8.3]	9.3 ± 4.7[7.6]	9.2 ± 4.6[9.4]
AT perirenal	15 ± 7.8[12]	16 ± 12[13]	12 ± 6.6[10]	11 ± 8.8[6.6]	14 ± 5.8[13]	12 ± 3.2[12]	8.7 ± 1.6[9.0]	9.6 ± 2.5[10]
Bile fluid	6.7 ± 3.0[5.2]	5.1 ± 1.6[3.3]	2.4 ± 1.1*[2.2] (n = 3)	3.6 ± 1.1*[3.6] (n = 2)	6.7 ± 4.8[7.2]	4.5 ± 0.52[4.4]	3.2 ± 0.43[3.2]	2.8 ± 0.77[3.0]
Duodenum	2.5 ± 1.4[1.7] (n = 3)	3.1 ± 1.6[2.0](n = 3)	3.4 ± 2.5[2.3]	2.5 ± 1.4[1.9]	3.5 ± 3.3[0.0] (n = 2)	1.8 ± 1.1[1.4]	1.4 ± 0.77[1.4] (n = 4)	2.2 ± 0.70[2.0] (n = 4)
PB	0.47 ± 0.15 [0.47]	0.46 ± 0.12 [0.32](n = 3)	0.46 ± 0.21 [0.15](n = 2)	0.30(n = 1)	0.34 ± 0.11 [0.33]	0.49 ± 0.14 [0.35](n = 3)	0.40 ± 0.004 [0.20](n = 2)	0.46(n = 1)
CB	0.20 ± 0.11 [0.18]	0.21 ± 0.084 [0.17]	0.37 ± 0.31 [0.28](n = 4)	0.26 ± 0.059 [0.26]	0.22 ± 0.084 [0.21]	0.26 ± 0.14 [0.21]	0.25 ± 0.064 [0.27](n = 4)	0.28 ± 0.16 [0.28]
***Cumyl*−5-P7AICA N-pentanoic acid**								
	RT				**Water**			
	PMI 0	PMI 1	PMI 2	PMI 3	PMI 0	PMI 1	PMI 2	PMI 3
Lung	neg.	neg.	1.0 ± 0.042[0.0] (n = 2)	0.81(n = 1)	neg.	neg.	neg.	neg.
Liver	0.71 ± 0.70 [0.61]	1.0 ± 0.52[0.59] (n = 3)	1.5 ± 0.10[0.0] (n = 2)	1.9 ± 2.3[1.2]	0.61 ± 0.23 [0.67]	0.39(n = 1)	0.72 ± 0.87[0.0] (n = 2)	0.71 ± 0.64 [0.42](n = 4)
Kidney	0.84 ± 0.63 [0.69]	0.59 ± 0.15 [0.61](n = 4)	1.2 ± 0.52 [0.97](n = 3)	0.89 ± 0.41 [0.71](n = 4)	0.78 ± 0.50 [0.56]	1.3 ± 1.9[0.42] (n = 4)	0.87 ± 0.37 [0.76]	0.31 ± 0.30 [0.33](n = 3)
Brain	neg.	neg.	neg.	neg.	neg.			neg.
Muscle	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.
AT dorsal	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.
AT s.c.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.
AT perirenal	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.
Bile fluid	37 ± 17[23] (n = 4)	8.0 ± 3.7[9.0]	18 ± 13[13](n = 3)	22 ± 16[22](n = 2)	29 ± 46[7.1](n = 4)	16 ± 7.2[13](n = 4)	21 ± 17[11](n = 4)	6.7 ± 2.9[6.1](n = 4)
Duodenum	8.4 ± 3.2[8.7]	5.2 ± 3.3[4.1](n = 4)	3.6 ± 3.2[1.5]	2.4 ± 1.0*[2.5]	2.1 ± 2.3[1.4]	1.0 ± 1.0[0.36] (n = 3)	1.0 ± 0.25[0.77] (n = 3)	1.9 ± 1.3[0.54] (n = 3)
PB	0.10 ± 0.084 [0.060]	0.067 ± 0.008 [0.060](n = 3)	0.053 ± 0.023 [0.040](n = 3)	0.047 ± 0.003 [0.045](n = 3)	0.060 ± 0.012 [0.060]	0.055 ± 0.030 [0.040](n = 4)	0.050 ± 0.014 [0.020](n = 2)	0.041 ± 0.009 [0.040](n = 4)
CB	0.046 ± 0.048 [0.050]	0.035 ± 0.025 [0.037]	0.036 ± 0.026 [0.017](n = 4)	0.027 ± 0.015 [0.025]	0.038 ± 0.015 [0.030](n = 4)	0.026 ± 0.007 [0.029]	0.024 ± 0.015 [0.020]	0.019 ± 0.012 [0.010]

AT = adipose tissue; s.c. = subcutaneous; PB = peripheral blood; CB = central blood; neg.=negative
* = statistically significant concentration changes ($p<0.05$)).

respective conc. at PMI 0 (Table 2). For this purpose, the following equation was used:

$$\Delta c(\%) = \frac{c(PMI1-3)-c(PMI0)}{c(PMI0)} x100$$

$\Delta c > 0$: *increase*

$\Delta c < 0$: *decrease*

Thereby, a value of $\Delta c > 0$ means an increase and a value of $\Delta c < 0$ a decrease of the respective PM conc. at the sampling time compared to the perimortem conc. at PMI 0.

Statistical tests were performed using GraphPad Prism 5.00 (GraphPad Software, San Diego, CA, USA). Additionally, to examine the respective conc. changes of the analytes in the tissue and body fluid specimens at different points of time, a non-parametric Friedman-test ($p<0.05$) followed by the Dunn's multiple comparison post-hoc test was applied.

Furthermore, for comparison of the obtained conc. after storage at RT with those obtained after storage in water, a non-parametric Mann-Whitney U test ($p < 0.05$) was used.

3. Results

3.1. Standard addition approach

In the present study, a standard addition approach was successfully applied for quantification of *cumyl*-5F-P7AICA and its NPA metabolite in different tissue und body fluid specimens. The regression coefficients (r^2) ranged between 0.90 and 1.0 for *cumyl*-5F-P7AICA and between 0.90 and 0.99 for the NPA metabolite, respectively.

3.2. Perimortem distribution

To determine the perimortem distribution of *cumyl*-5F-P7AICA and its NPA metabolite, various tissue and body fluid samples were drawn six hours after inhalative drug administration (PMI 0). In general, the respective conc. of the analytes showed high interindividual variations in the different analyzed specimens. The mean conc. as well as the corresponding standard deviations of both substances at PMI 0 are listed in Table 2. As far as *cumyl*-5F-P7AICA is considered, the highest conc. was found in lung and bile fluid, followed by kidney and duodenum content, if AT is initially disregarded. On the other hand, lowest conc. were observed in PB and CB (Table 2).

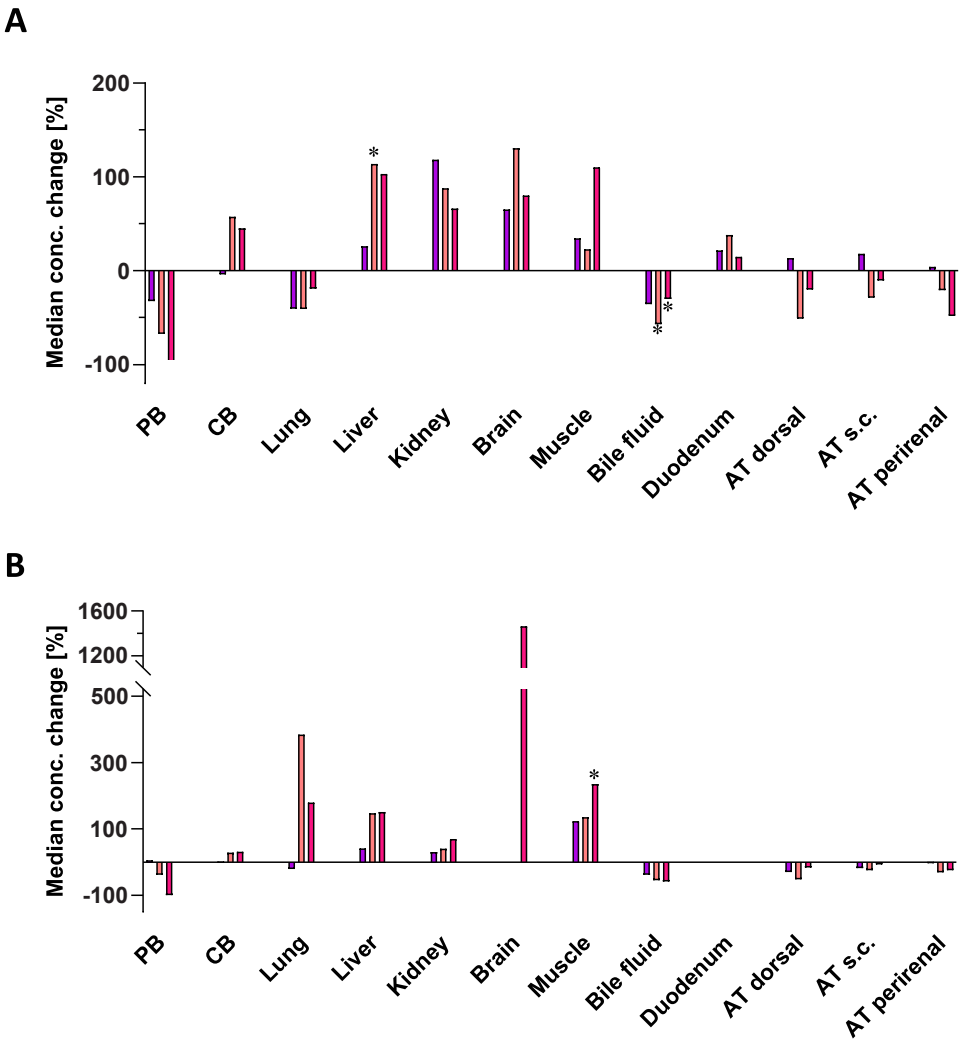

Fig. 1. Median time- and environmental-dependent postmortem concentration (conc.) changes [%] of *cumyl*-5F-P7AICA in different tissues and body fluids stored at air **(A)** and water **(B)** collected 24 h (postmortem interval (PMI) 1;), 48 h (PMI 2;), and 72 h (PMI 3;) after pulmonary administration of a 200 μg/kg body weight dose to pigs compared to PMI 0. (* = statistically significant concentration changes ($p<0.05$); PB = peripheral blood, CB = central blood, AT = adipose tissue, s.c. = subcutaneous).

Regarding the NPA metabolite, the highest conc. could primarily be assessed in bile fluid and duodenum content, followed by liver and kidney. In analogy to the parent compound *cumyl*-5F-P7AICA, lowest conc. of the metabolite were also detected in PB and CB (Table 2). Analysis of lung, brain, and muscle specimens yielded negative results.

Concerning AT specimens, highest conc. of *cumyl*-5F-P7AICA were found in perirenal AT, followed by subcutaneous AT, with lowest conc. detected in dorsal AT. The NPA metabolite could not be found in any AT specimen.

3.3. PM conc. changes

3.3.1. Storage in air

The mother substance *cumyl*-5F-P7AICA could be determined in every solid tissue specimen sampled at PMI 1 – 3 stored at RT, with the exception of brain at PMI 2 (n=4). As for the body fluids, the parent substance could only be detected in a few samples of bile fluid (n=3 at PMI 2 and n=2 at PMI 3), duodenum content (n=3 at PMI 1), CB (n=4 at PMI 2), and PB (n=3 at PMI 1, n=2 at PMI 2 and n=1 at PMI 3).

Overall, in the examined specimens only few to rarely conc. changes could be observed during the entire experiment (Fig. 1a; Table 2). For example, compared to PMI 0, the median conc. of *cumyl*-5F-P7AICA slightly increased in liver, kidney, muscle, and brain specimens. On the contrary in perirenal AT and bile fluid the median PM conc. slightly decreased, especially from PMI 2 to PMI 3. Furthermore, PM conc. in lung, duodenum content, CB and dorsal as well as s.c. AT were found to be rather stable over the observed period. For PB, a continuous decrease of the median conc. could be observed until PMI 3.

Overall, as depicted in Fig. 1a, statistically significant conc. changes ($p<0.05$) were observed for *cumyl*-5F-P7AICA in liver between PMI 0 and PMI 2 as well as in bile fluid between PMI 0 and PMI 2 or PMI 3.

Regarding the NPA metabolite, analysis of muscle, brain, s.c., dorsal as well as perirenal AT were negative over the observed period of time. Highest conc. of the metabolite could be found in bile fluid and duodenum content at each sampling time (Table 2). First of all, it has to be stated that in lung, compared to PMI 0, the NPA metabolite could only be detected in isolated samples at PMI 2 and 3 (n=2 at PMI 2; n=1 at PMI 3). Compared to the median conc. at PMI 0, the conc. of the NPA metabolite remained comparably stable in kidney tissue until 72 h (Fig. 2a, Table 2). Regarding bile fluid no clear tendency was recognizable. In liver specimens, a slight decrease of the conc. could be found from PMI 1 to PMI 2, increasing again from PMI 2 to PMI 3. However, in duodenum content, the conc. of the NPA metabolite declined over time. Regarding PB and CB, PM conc. remained comparably stable until PMI 3.

Statistically significant conc. changes ($p<0.05$) were only found for

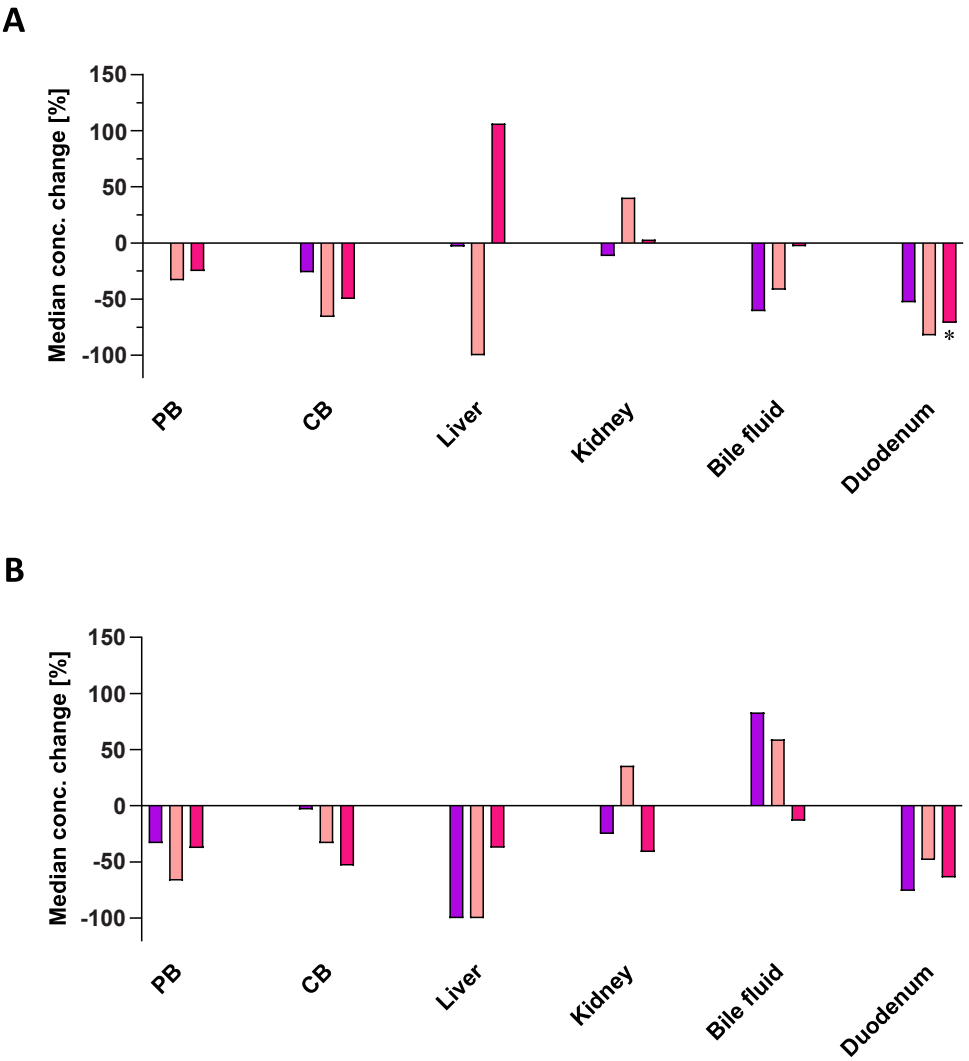

Fig. 2. Median time- and environmental-dependent postmortem concentration (conc.) changes [%] of *cumyl*-5F-P7AICA *N*-pentanoic acid in different tissues and body fluids stored at air **(A)** and water **(B)** collected 24 h (postmortem interval (PMI) 1;), 48 h (PMI 2;), and 72 h (PMI 3;) after pulmonary administration of a 200 μg/kg body weight dose to pigs compared to PMI 0 (PB = peripheral blood, CB = central blood).

duodenum content between PMI 0 and PMI 3 (Fig. 2a).

3.3.2. Storage in water

In tissues and body fluids stored in water (group 2), *cumyl*-5F-P7AICA could be quantified in every specimen, with the exception of PB (n=3 at PMI 1; n=2 at PMI 2 and n=1 at PMI 3), CB (n=4 at PMI 2), and duodenum content (n=4 at PMI 2 and 3 each). However, in accordance to a storage at air, only occasional and minor conc. changes were found when stored in water (Fig. 1b; Table 2).

Compared to PMI 0 (Fig. 1b, Table 2) a strong increase of the conc. could be noted especially in brain. However, brain specimens were only taken at PMI 3. In lung specimens, an increase of the conc. could be observed from PMI 1 to PMI 2 with a following slight decrease at PMI 3. Regarding PB and CB specimens, conc. of *cumyl*-5F-P7AICA remained relatively stable until PMI 3.

Statistically significant conc. changes ($p<0.05$) of *cumyl*-5F-P7AICA were found in muscle between PMI 0 and PMI 3 when stored in water.

Regarding the NPA metabolite, analysis of muscle, lung, brain and every AT specimen yielded negative results. In the samples, the NPA metabolite could only be detected in about half of all analyzed specimens. In terms of duodenum content, no clear trend could be observed over 72 h. In liver, kidney, bile fluid, CB as well as PB specimens, the PM median conc. of the NPA remained comparably stable for the entire period of the experiment (Fig. 2b, Table 2).

All in all, no significant conc. changes of the NPA metabolite could be found in tissues and body fluids stored in water.

4. Discussion

4.1. Dosage

In the present study, a dose of 200 μg/kg BW *cumyl*-5F-P7AICA was administered inhalatively to the pigs, resulting in total doses of 8.8 – 12.8 mg. This dosage of *cumyl*-5F-P7AICA was chosen, as, on the one hand, it has already been proven to be well tolerated by the animals in a previous metabolism study (Walle et al., 2021). On the other hand, comparable doses were successfully administered to the pigs in the context of previous TK studies on SC (Doerr et al., 2021, 2024; Schaefer et al., 2019, 2020a). Furthermore, depending on the administration route, the dosage used in the current work is a recommended dose of SC reported by drug addicts in drug fora.

4.2. Standard addition approach

In the current study, the standard addition approach was used for the qualitative and quantitative determination of *cumyl*-5F-P7AICA as well as its NPA metabolite in body fluids and tissue specimens. Analyzing PM tissue specimens, quantification based on a calibration curve using blood as matrix is not recommended, as this might considerably increase the variability. Hence, the standard addition approach was chosen deliberately, as it has the advantage that matrix effects are minimized, since each calibration curve is adapted on the respective matrix. Especially in PM toxicology, matrix effects can complicate or even falsify the interpretation of analytical results, in particular in the case of putrefied specimens. However, in the context of a common method validation procedure, different parameters must be evaluated using different blank matrices. However, PM tissues are prone to a high interindividual biological variance that leads to the question, if representative results can be obtained. Therefore, for quantification of drugs (of abuse) in PM tissue and body fluid specimens, national and international guidelines recommend the use of the standard addition approach (GTFCh, 2018; Jickells and Negrusz, 2008; Peters et al., 2007; Skopp, 2010; SOFT/AAFS, 2006).

4.3. Perimortem distribution pattern

First of all, it has to be noted that the perimortem body fluid and tissue specimens were drawn shortly after death and thus before separation into the different environmental conditions. Nevertheless, measurements of the respective specimens drawn at PMI 0 revealed partially different median conc. (Table 2) of *cumyl*-5F-P7AICA, especially in lung (15 ng/g vs. 3.1 ng/g), liver (1.6 ng/g vs. 0. 90 ng/g) and duodenum (1.7 ng/g vs. 0.0 ng/g), as well as its NPA metabolite in bile fluid (23 ng/g vs. 7.1 ng/g) and duodenum (8.4 ng/g vs. 1.5 ng/g). However, several issues have to be taken into account. Overall, it should first be noted that there are high inter-individual TK differences between the individual animals. Therefore, reduced or increased absorption, metabolism, distribution or excretion of the substances may have already occurred during the in vivo study, whereby the extent of the individual steps depends on the respective animal. This may therefore result in higher or lower conc. of the respective substances at PMI 0. Differences in the measured conc. may also be due to an inhomogeneous distribution of the substances. However, it was not possible to always ensure the same collection site. These issues should be considered, when interpreting the respective conc. at PMI 0.

However, as already described above, with few exceptions *cumyl*-5F-P7AICA was found in every tissue or body fluid specimen sampled 6 h after drug administration. On the contrary, the NPA metabolite could only be detected in liver, kidney, duodenum content, bile fluid, CB, and PB. The mean as well as median conc. of the examined substances at PMI 0 are shown in Table 2. As easily can be seen, besides AT, the highest mean conc. of *cumyl*-5F-P7AICA could be found in lung and bile fluid, whereas lowest conc. were detected in CB and PB specimens, respectively. For interpretation, the route of administration must be considered. As *cumyl*-5F-P7AICA was inhalatively administered, it is not surprising, that high conc. were found in the lung. Furthermore, the findings of the present study are in line with those already reported by Schaefer et al. in the framework of a perimortem TK study regarding the SCs JWH-210 and RCS-4, which display more or less pronounced lipophilic properties, as also highest conc. of the SC were found among other locations in lung specimens after inhalative administration (Schaefer et al., 2019). This observation of sequestration of drugs in lung tissue was already described for some liphophilic drugs a few years ago and is called ‘pulmonary first-pass uptake and metabolism’ (Bakhle, 1990; Bend et al., 1985; Boer, 2003). However, compared to the studies by Schaefer et al. (Schaefer et al., 2019, 2020a), generally lower conc. of *cumyl*-5F-P7AICA were found in the different perimortem specimens in the present study. For explanation, on the one hand, *cumyl*-5F-P7AICA contains a carboxamide unit in its chemical structure, as, compared to JWH-210 and RCS-4, possibly leading to a certain instability and, thus, a faster in vivo degradation. Furthermore, the lower lipophilicity of *cumyl*-5F-P7AICA (LogP = 4.20) compared to e.g. JWH-210 (LogP = 7.5) or RCS-4 (LogP = 5.6) (Schaefer et al., 2019) might also contribute to the comparably lower drug conc. in e.g. lung. Additionally, Doerr et al. published data on the perimortem and PM distribution of 5F-MDMB-P7AICA, a SC also containing a 7-azaindole core structure, after inhalative administration to pigs (Doerr et al., 2024). Here, the authors reported on highest perimortem concentrations of the parent substance in AT, duodenum content and bile fluid, while in lung specimens only a low amount of 5F-MDMB-P7AICA was found. Regarding the PM specimens, highest conc. of 5F-MDMB-P7AICA were found in AT followed by bile fluid. While the conc. in PB, CB and bile fluid measured by Doerr et al. were approximately in the same range as those found for *cumyl*-5F-P7AICA in the present study, lower perimortem and PM values were found for 5F-MDMB-P7AICA in the remaining tissue and body fluids (AT excluded) as compared to those found for *cumyl*-5F-P7AICA. In this context, it should be considered that in contrast to *cumyl*-5F--P7AICA, 5F-MDMB-P7AICA additionally contains an ester structure in the linked group of its chemical structure, which may lead to a fast degradation to its corresponding metabolite, as it was also reported by

Krotulski et al. in a stability study for such classes of SCs (Krotulski et al., 2021).

Herein, euthanasia of the animals and sample collection was already performed 6 h after drug administration, whereas, in the aforementioned studies (Schaefer et al. 2019, 2020a; Doerr et al. 2024), the pigs were put to death only after 8 h. This longer period of metabolic degredation should not be neglected, and might also be another explanatory reason for the comparatively lower tissue and body fluid conc. reported by Doerr et al. (2024).

Two markers are described in the literature to predict a possible PMR. On the one hand a central-to-peripheral blood (*C/P*) conc. ratio > 1, and on the other hand a liver-to-peripheral blood (*L/P*) ratio > 5 or 20–30 indicate the occurrence of PMR (Han et al., 2012; McIntyre, 2014). Considering the respective mean conc. detected in the present work for *cumyl*-5F-P7AICA (Table 2), *C/P* ratios lower than 1 could be calculated, speaking rather less for a redistribution from the peripheral to the central compartment. In contrast, for both storage conditions *L/P* ratios> 5 were especially found at PMI 2 and 3, suggesting that *cumyl*-5F-P7AICA may underlie a slight time-dependent PMR into surrounding tissues. This should be considered, when interpreting PM toxicological findings.

Regarding the NPA metabolite, besides in PB and CB specimens, it could especially be detected in organs involved in metabolism and elimination (liver, kidney, bile fluid, and duodenum content), with highest conc. in bile fluid followed by duodenum content (Table 2). This is not surprising and was also reported in previous TK studies conducted by Schaefer et al. for the SCs JWH-210 and RCS-4 (Schaefer et al., 2019). The higher conc. in bile fluid and duodenum content as compared to liver and kidney specimens suggest an enterohepatic circulation, as it has already been reported for other SCs (Schaefer et al., 2017, 2019). Analogously to the parent substance, substantially lower conc. of the NPA were detected in bile fluid and duodenum as compared to those reported by Schaefer et al. for e.g. RCS-4-COOH (Schaefer et al., 2019). On the other hand, comparing the NPA conc. of the present work with those reported by Doerr et al. for the dimethyl butanoic acid (DBA) metabolite of the SC 5F-MDMB-P7AICA, higher DBA conc. were found in bile fluid and duodenum content (Doerr et al., 2024). This might also be caused by the high instability of 5F-MDMB-P7AICA due to its ester structure, leading to higher conc. of the DBA metabolite. In addition, as previously mentioned, a longer duration of the in vivo experiment (8 h vs. 6 h in the present study) may be another reason for the higher DBA conc. measured by Doerr et al. (2024).

Comparison of the tissue distribution pattern of *cumyl*-5F-P7AICA and its NPA metabolite obtained in the present work with data from authentic fatal cases is not possible, as such data are lacking in the literature. Until now, only one fatal case with a contribution of *cumyl*-5F-P7AICA to the occurrence of death was published by Zawadzki et al. (2021). Indeed, in this fatal case, only PM blood and urine conc. of *cumyl*-5F-P7AICA were reported with conc. of 2.8 ng/mL in blood and 3.1 ng/mL in urine, respectively. Although the PMI is unknown in the case reported by Zawadzki et al., comparison with the PB conc. obtained in the present study at PMI 1–3 shows that the reported conc. in the fatal case are much higher. However, it should be borne in mind that such single case reports are subject to strong uncertainties, as usually neither the consumed dose and time of consumption nor the PMI are known. Accordingly, these data are difficult to compare with those from systematically controlled studies. Furthermore, comparison of the PM TK properties of different SCs also represents a difficulty due to their structural modifications, as shown by the aforementioned comparison of the SCs *cumyl*-5F-P7AICA and 5F-MDMB-P7AICA, both containing a 7-azaindole core structure but different linked groups, possibly resulting in a fast in vivo degradation and metabolism.

To sum up, the perimortem distribution pattern of JWH-210 and RCS-4 as well as of its metabolites is comparable to that of ‚newer' SCs with a 7-azaindole core structure. However, considerably lower conc. were detected in tissues and body fluids, possibly resulting from unstable units within the chemical structure, as also seen for the SC 5F-MDMB-P7AICA which contains a 7-azaindole core. In order to investigate a possible impact of *cumyl*-5F-P7AICA on the occurrence of fatal intoxications, lung samples as well as bile fluid and duodenum content should included in the standard specimens collected during autopsy in addition to the common matrices CB and PB, especially in the case of an extended PMI.

4.4. Comparison of the PM conc. changes at different storage conditions

In the present study, the PM time-dependent conc. changes of *cumyl*-5F-P7AICA and its NPA metabolite were investigated in various tissue and body fluid specimens stored at two different ambient conditions. Comparing the results obtained after storage at air and in water, *cumyl*-5F-P7AICA and its metabolite consistently showed only slight conc. changes throughout the observed time period.

As already described by Johann Ludwig Casper by means of the ‚Casper-rule' (Dettmeyer et al., 2014; Vennemann and Brinkmann, 2003), the extent of putrefaction varies largely depending on the prevailing storage or environmental conditions (e.g. temperature, availability of oxygen). Accordingly, the degree of putrefaction of a corpse after 1-week in air corresponds approximately to that of a 2-week storage in water or an 8-week storage in soil (Dettmeyer et al., 2014; Vennemann and Brinkmann, 2003). Hence, due to a delayed onset of the autolytic processes when stored under oxygen-deficient conditions (e.g. water), less PMR would be expected as compared in air. Contrary to the expectations, this difference could not be confirmed in the present work, as the conc. changes were comparable and statistical tests showed only two significant conc. changes. Regarding *cumyl*-5F-P7AICA, a statistically significant conc. change was only observed at PMI 1 in kidney samples and concerning the NPA metabolite a respective change was only found at PMI 2 in duodenum content.

However, it should be considered that the specimens examined were sampled postmortem and the extent of putrefaction does not only depend on the ambient temperature and can vary widely between different individuals. For example, some animals showed a strong green putrefaction already after 48 h, while others were still in a good condition even after 72 h. This difference can lead to strong interindividual variations of the conc. of the substances measured in tissues and body fluids. Additionally, in particular PM specimens also pose a great challenge to the analytical methods. High interindividual fluctuations have been observed when determining drug conc. in PM tissues and blood specimens, even if the method was fully validated (Brunet et al., 2010; Gleba and Kim, 2020; Nagasawa et al., 2016; Saar et al., 2012). Taken together, all these factors can lead to a high standard deviation, as also found in the present study (Table 2). Therefore, the results of statistical tests, which are generally based on the mean conc., should be regarded with great caution.

In terms of *cumyl*-5F-P7AICA, only minor conc. changes (less than 200 % conc. change) were observed in most specimens at both storage conditions (Fig. 1a and b). This finding might also be attributable to analytical or interindividual deviations. In general, a PMR is essentially characterized by a redistribution of a substance from an organ with high conc. (drug reservoir) into the surrounding tissues with lower conc. (Skopp, 2010). This process depends on various factors, including the physicochemical properties of the respective substance, but also interindividual factors, such as the degree of putrefaction. For example, a PM increase of the pH value or a microbial colonization can lead to a degradation of the respective substances, a release from a protein bound form or a possible cleavage of glucuronides (Butzbach, 2010).

However, when interpreting the PM conc. changes observed for *cumyl*-5F-P7AICA in brain specimens, especially when stored in water, with regard to a possible PMR it should first be noted that brain specimens were only sampled at PMI 3, as a repeated opening of the skull during storage should be avoided. Since the brain represents a closed compartment, similar to e.g. vitreous fluid, a redistribution from other

organs containing higher conc. of the substance seems to be rather unlikely. Attempting to explain the increase of conc., it should be noted that it was not possible to take samples exactly from the same region of the brain, because among other things, the tissue had also already begun to liquefy. As a large amount of cannabinoid receptors 1 are located in the cerebellum (Pertwee, 1997; Svízenská et al., 2008), another plausible explanation for the comparably high PM conc. at PMI 3 in brain when stored in water might be a PMR of *cumyl*-5F-P7AICA from the cerebellum into the cerebrum during the period from PMI 0 to PMI 3. In terms of the blood specimens, PM conc. of CB specimens remained relatively stable in a low conc. range, whereas the median conc. of *cumyl*-5F-P7AICA in PB decreased over 72 h at both storage conditions. Interpreting this difference, it should be kept in mind that the parent substance could only be detected in single PB samples, resulting in low median conc., especially at PMI 3. Regarding this issue, it should furthermore be noted that PB specimens were collected from different veins (femoral or brachiocephalic vein), so that the sampling procedure was not standardized. Finally, due to the PM coagulation of blood and the increasing putrefaction of the corpses, only small amounts of PB could be obtained. In the context of the further analytical process, the blood specimens therefore had to be strongly diluted with blank whole blood. This procedure may have led to very low conc. of the analyte, also possibly below the LOD. Taken together, conc. changes in PB and CB specimens should be regarded with caution, as in most of the cases, the values were lower than the LLOQ (0.5 ng/mL) and sometimes nearby the LOD (0.06 ng/mL), possibly resulting in high analytical deviations. Therefore, in cases of PM toxicology, determined conc. of *cumyl*-5F--P7AICA in blood specimens should be regarded with caution, especially if there has been a prolonged PMI.

In summary, only a slight tendency for PMR was shown for *cumyl*-5F-P7AICA under both storage conditions. Due to the comparatively stable conc. of *cumyl*-5F-P7AICA during the whole experimental period of 72 h, CB also appears to be suitable for PM quantification, if no PB can be obtained. Regarding the NPA metabolite, only minor conc. changes (less than 100 %) in tissue and body fluid specimens were determined at both storage conditions, if detectable at all. This observation might potentially also be attributable to interindividual or analytical deviations. Compared to PMI 0, only a slight decrease in conc. of the NPA metabolite was observed in duodenum content at both storage conditions, with statistically significant changes of the conc. at PMI 2. A tendency for a PMR of the metabolite could not be shown in the present study, regardless of the respective storage condition.

To sum up, bile fluid and duodenum content as well as lung tissue appear to be suitable specimens for a qualitative PM detection of a previous consumption of *cumyl*-5F-P7AICA of the decedent, independent of the storage conditions. If PB is not available for quantification, e.g. due to a PM alteration of the corpse, CB appears to be a useful alternative specimen, as no relevant PMR was observed for *cumyl*-5F-P7AICA in the present study.

Taken together, the data obtained in the present work support the interpretation of PM toxicological cases as knowledge on the PM stability as well as the tendency on a PMR of *cumyl*-5F-P7AICA under different environmental conditions is gained. In addition, specific instructions are given for a correct sampling procedure over a PMI of 3 days in order to be able to detect potentially fatal intoxications after consumption of the SC *cumyl*-5F-P7AICA even in putrefied corpses.

4.5. Limitations

One limitation of the study is posed by the permanent re-opening of the abdominal cavity of the animals to take PM specimens, because this procedure may have led to a stronger contamination with microorganisms as well as a more aerobic environment inside the body, possibly resulting in a faster advanced putrefaction. Due to the storage in water and the associated changes of the skin condition, an absolutely tight seal of the abdominal cavity was not always possible. Thus, it should be considered that a small amount of the water may have entered the abdominal cavity, inducing e.g. a further contamination with microorganisms.

Furthermore, the lack of insect activity represents a further limitation of the present study, as the presence of insects has a significant influence on the extent of decomposition of a corpse. However, even without insect activity, a clear difference in the degree of putrefaction could be observed macroscopically during storage at different environmental conditions. So, an influence on the PM stability of exogenous substances might be expected. In practice, corpses are always colonized to varying degrees with insects. Whilst some corpses are not exposed to insect activity, others can be very heavily infested, e.g. found at home vs. in the forest or in the bathtub vs. in the river). A varying degree of decomposition is therefore expected depending on the environmental conditions, resulting in a different PM stability of substances. However, as no insect activity was tested in the context of the current study, the data obtained are only transferable to authentic human cases with no relevant insect activity to ensure comparability.

In addition, due to repetitive sampling, tissue and body fluid specimens were collected from different veins or sites of the organs, respectively. As the substances may have been inhomogenously distributed within the tissues, the difference may have led to an adulteration of the respective conc.

Last not least it has to be considered that the process of putrefaction was accompanied by the production of putrefactive liquid was produced during the examined time period of 72 h. In particular, this liquid was also located inside of the corpse, so that an impact on PMR cannot completely be excluded.

5. Conclusions

In a first part of the present study, the perimortem distribution patterns of *cumyl*-5F-P7AICA and its NPA metabolite after inhalative administration to pigs was investigated. In a second part of the study, the PM distribution patterns as well as the time- and oxygen-dependent conc. changes were determined. In general, both substances could primarily be detected in tissues involved in TK, such as absorption, metabolism and excretion (lung, liver, kidney, bile fluid/ duodenum content), even though in very low conc. If no standard specimens, such as PB and CB, are available due to an extended PMI, bile fluid and duodenum content and lung are suitable for PM toxicological analysis of the SC *cumyl*-5F-P7AICA. Statistically significant conc. changes associated with the storage conditions (in air vs water) were only observed for *cumyl*-5F-P7AICA in kidney at PMI 1 and the NPA metabolite in duodenum content at PMI 2.

Ethical approval

All experiments were performed in accordance with the German legislation on protection of animals and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (permission number: 44/2019).

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CRediT authorship contribution statement

Nadja Walle: Writing – original draft, Project administration, Methodology, Investigation, Formal analysis. **Benjamin Peters:** Project administration, Methodology, Investigation. **Adrian Doerr:** Software, Resources, Project administration. **Michael Menger:** Resources, Conceptualization. **Matthias Laschke:** Resources, Project administration, Conceptualization. **Peter Schmidt:** Writing – review & editing,

Supervision, Conceptualization. **Nadine Schaefer:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Markus Meyer:** Writing – review & editing, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.toxlet.2024.10.006.

Data Availability

Data will be made available on request.

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