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Cathinone metabolism and biliary excretion in an ex-vivo pig liver model: Example of 4-Cl-PVP and eutylone

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ABSTRACT

Objective: Recently, the pig liver model perfused *ex vivo* using a normothermic machine perfusion (NMP) has been proposed as a suitable model to study xenobiotic metabolism and biliary excretion. The aim of our study is to describe the metabolism of NPS such as cathinones (with a focus on 4-Cl-PVP and eutylone) in blood and bile, using a normothermic perfused pig liver model.

Methods: Livers (n = 4) from male large white pigs, 3–4 months of age and weighing approximately 75–80 kg, were harvested and reperfused onto an NMP (LiverAssist®, XVIVO) using autologous whole blood at 38 °C. 4-Cl-PVP and eutylone were administered as a bolus in the circulating blood at T0 with the aim of achieving a concentration of 1 µg/mL in the reperfusion system. The assays were carried out on plasma and bile between 0 and 120 min after cathinone administration using a targeted and untargeted approaches based on liquid chromatography coupled with high resolution mass spectrometry (Q-Exactive Thermo Scientific®).

Results: In plasma, the concentration of 4-Cl-PVP and eutylone decreased rapidly with elimination half-lives of 4 min and 0.25 min, respectively. Their phase I and phase II metabolites were detected in plasma as early as 1 min. In bile, 4-Cl-PVP and eutylone were detected with maximum intensity between 0 and 30 min post-administration, and the main metabolites found in plasma were found in bile. Phase II derivatives showed increasing biliary excretion over time up to 120 min.

Conclusion: The pig liver model perfused *ex vivo* using an NMP represent a promising model in pharmacotoxicology, particularly for toxicokinetic investigations of cathinones. This model may be of interest in the absence of authentic cases of cathinone consumption or other NPS consumption to identify relevant metabolites consumption markers. In addition, the possibility of collecting bile in this model represents an additional advantage for studying biliary excretion of NPS and their metabolites in forensic toxicology.

1. Introduction

New psychoactive substances (NPS) constitute a complex and diverse category of compounds, commonly referred to as designer drug, synthetic drugs, or “legal highs” (Luethi and Liechti, 2020; Peacock et al.,

2019). These substances are typically either analogues of existing scheduled drugs and pharmaceuticals or newly synthesized chemicals designed to replicate the actions and psychoactive effects of licensed medications and other regulated substances (Batisse et al., 2020; O’Hagan and McCormack, 2019; Soussan and Kjellgren, 2016). Due to

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their sheer number, various chemical structures, and compositions, NPS present substantial challenges for drug users, forensic toxicologists as well as for the healthcare system, and drug control policies worldwide (Shafi et al., 2020). For these reasons, they have been characterized as a growing worldwide epidemic (Griffiths et al., 2010; Zawilska and Andzejczak, 2015). Among NPS, synthetic cathinones (SCs) represent the second largest family of NPS and a significant threat to the health, security, and well-being of numerous individuals (European Monitoring Centre for Drugs and Drug Addiction, 2022; Weinstein et al., 2017). These substances are synthetic psychostimulants structurally analogous to cathinone, an alkaloid naturally produced in the khat plant (Capriola, 2013). SCs are often used recreationally in contexts such as parties, nightclubs, music festivals, and during chemsex (Karila et al., 2015). Their abuse results in pronounced sympathomimetic effects, as well as psychosis, aggression, and occasionally violent and erratic behaviors (Barrio et al., 2016; Schifano et al., 2020).

Two SCs, namely 4-Cl-PVP and eutylone have been reported to be widely used with adverse reactions like hypertension and tachycardia, heart failures and health risks due to their modalities of administration. SCs are well known to have a rapid elimination in human body, but while the metabolism of these two SCs has already been studied (Pelletier et al., 2022, 2023) the measurement of their metabolites can be used to extend their detection window and as surrogates for metabolism evaluation using experimental models.

Metabolism of SCs is essentially hepatic and the lack of specific markers of cathinone consumption hampers the ability of toxicology laboratories to detect cases of intoxication. Exploring the metabolism of these substances requires high-performance tools and models, with the aim of identifying relevant consumption markers. To date, various methodological approaches have been described in the literature to study cathinone metabolism, including *in vitro*, *in vivo* and/or *in silico* approaches (Manier et al., 2018; Meyer et al., 2012; Pedersen et al., 2013; Pelletier et al., 2022, 2023; Pozo et al., 2015; Schwelm et al., 2024).

However, these models have limitations and may not completely mimic the human liver metabolism or allow elimination pathways to be studied. Therefore, new more transposable models are required, allowing to accurately investigate the metabolism and to explore the elimination directly “out of” the liver (i.e. in hepatic vein’s blood and in bile), transporter-enzyme interactions, and potential drug-drug interactions (Stevens et al., 2020).

Pig liver is admitted as the closest human proxy regarding liver after primate liver due to its anatomical, physiological, and biochemical similarities (Helke and Swindle, 2013; Zuber et al., 2002). Additionally, a significant advantage of using porcine livers is the similarity in phase I and phase II biotransformation reactions to those in humans, notably shown by a quantitative proteomic analysis comparing the expression of transporters and metabolizing enzymes in human and pig livers (Elmorsi et al., 2020). Given that *in vivo* models are generally more effective than *in vitro* models such as human liver microsome or S9 liver fraction for generating phase I and II metabolites (Carlier et al., 2017; Gicquel et al., 2021; Swortwood et al., 2016), it is expected that the use of *ex vivo* models (derived from *in vivo*) is also highly relevant for metabolite generation. Consequently, *ex vivo* perfused pig liver using a normothermic machine perfusion (NMP) has been increasingly used as a surrogate model of human liver in the fields of: organ conservation, transplantation and also in pharmacological analysis (Stevens et al., 2023). Ultimately, pig liver is highly considered for xenotransplantation (Cooper et al., 2016) to answer to the worldwide organ shortage.

In metabolism studies, the *ex vivo* perfused pig liver model enables authentic metabolic activity to be maintained while providing a highly controlled experimental environment. This model offers optimal simulation of xenobiotic blood perfusion and hepatic biotransformation, with greater control over experimental conditions than in an *in vivo* model. In particular, frequent blood sampling can be performed directly from the hepatic veins, allowing precise analysis of metabolites exiting the liver.

This provides a highly accurate reflection of hepatic metabolism for the substances under investigation (Stevens et al., 2021). In addition, while determining biliary clearance of drugs in humans remains challenging due to the anatomical inaccessibility of bile within the hepatobiliary tract (Ghibellini et al., 2006), the *ex vivo* pig liver model facilitates bile sampling at various time points, enabling real-time qualitative and quantitative analysis of xenobiotic biliary excretion. These are clear advantages over other models.

The aim of our study is to describe the hepatic metabolism of NPS such as cathinones (with a focus on 4-Cl-PVP and eutylone) in blood and their biliary excretion in bile, using a normothermic perfused pig liver model.

2. Material and methods

2.1. Animals and liver procurement

All experiment were conducted at INRAE experimental facilities (UE1421 UEPR, Saint-Gilles, France; Agreement No. D35-275-32) in accordance with the current ethical standards of the European Community (Directive, 2010/63/EU), and the French legislation on animal experimentation and ethics. The French Ministry of National Education, Research and Innovation and the Regional Ethics Committee in Animal Experiment of Brittany validated and approved the entire procedure described in this work.

Four conventional large white male pigs of 75–80 kg (i.e. aged of approximately 4 months), which correspond to the usual weight of an adult human male, were sacrificed after a standardized procedure of electronarcosis followed by exsanguination allowing to collect 2 L of autologous blood, immediately stored with a 25,000 UI of heparin.

The liver was then procured from the rest of the abdominal viscera with ligation of the cystic duct and flushed through the portal vein with 500 ml of physiologic. Liver was then flushed with 2 L of cold (4 °C) University of Wisconsin preservation solution and then stored with crushed ice until perfusion.

Aorta and portal vein were cannulated in order to perfuse the liver while a 8French feeding tube was inserted on the main bile duct for collecting the bile and a 22French plastic tube fixed in the retro-hepatic vena cava to easily collect blood samples at the “exit” of the liver allowing to accurately evaluate the metabolism.

2.2. Perfusion procedure

Perfusion was performed using the liver assist® device at 38 °C using autologous whole blood supplemented with albumin and sodium bicarbonate, perfused through both the portal vein and the artery. The perfusion pressure was set at 10 mmHg on the portal vein and 60 mmHg on the artery. The oxygenation was made with a mixture of air (usually 1 L/min) and oxygen (usually 0.3 L/min) and adapted according to the blood gas value to reproduce physiological value.

Blood samples were collected from vena cava and analyzed using the ABL FLEX PLUS® (Radiometer, Copenhagen, Denmark) analyzer allowing to adjust the ionic level (kalium, bicarbonate, calcium). The blood glucose and lactate clearance level were also monitored to evaluate the liver metabolism.

Bile production was collected every 30 min.

After 2 h of normothermic perfusion, viability criteria (Groen et al., 2024) were checked. After validation, the experimental procedure was performed for a duration of 2 h.

2.3. Drug administration during perfusion

Eutylone and 4-Cl-PVP powders were provided from seizures in Rennes University Hospital laboratory. NMR analysis was performed as already described (Pelletier et al., 2023) to unambiguously confirm the chemical structure in the seized sample, to check its purity and exclude

the presence of other organic compounds (adulterants). Eutylone and 4-Cl-PVP, selected on the basis of their known metabolism, are shown respectively in Fig. 1, enabling us to assess the suitability of the *ex vivo* pig liver model for the study of SCs metabolism. Elimination half lives were estimated using eutylone and 4-Cl-PVP kinetics curves.

SCs (4-Cl-PVP and Eutylone) were administered as a bolus in the circulating blood (autologous porcine blood) at T0 with the aim of achieving a concentration of 1 µg/mL (20 mL diluted in isotonic NaCl) in the reperfusion system, corresponding to the order of magnitude of the concentrations found in cases of intoxication in humans (Pelletier et al., 2022, 2023).

2.4. Sample extraction

Blood samples were collected in heparinized tubes and bile samples were collected in falcon tubes. Both were centrifuged at 2,200g for 10 min at +4 °C.

Biological samples extraction method was already described (Le Daré et al., 2020a; Pelletier et al., 2021). Briefly, samples (200 µL) were transferred into 5 mL glass tubes and extracted with 300 µL of zinc sulphate solution at 0.1 M and 500 µL of methanol containing a deuterated internal standard (Ketamine-D4 at 1 ng/mL) were added as already reported (Allard et al., 2019). Supernatants were evaporated

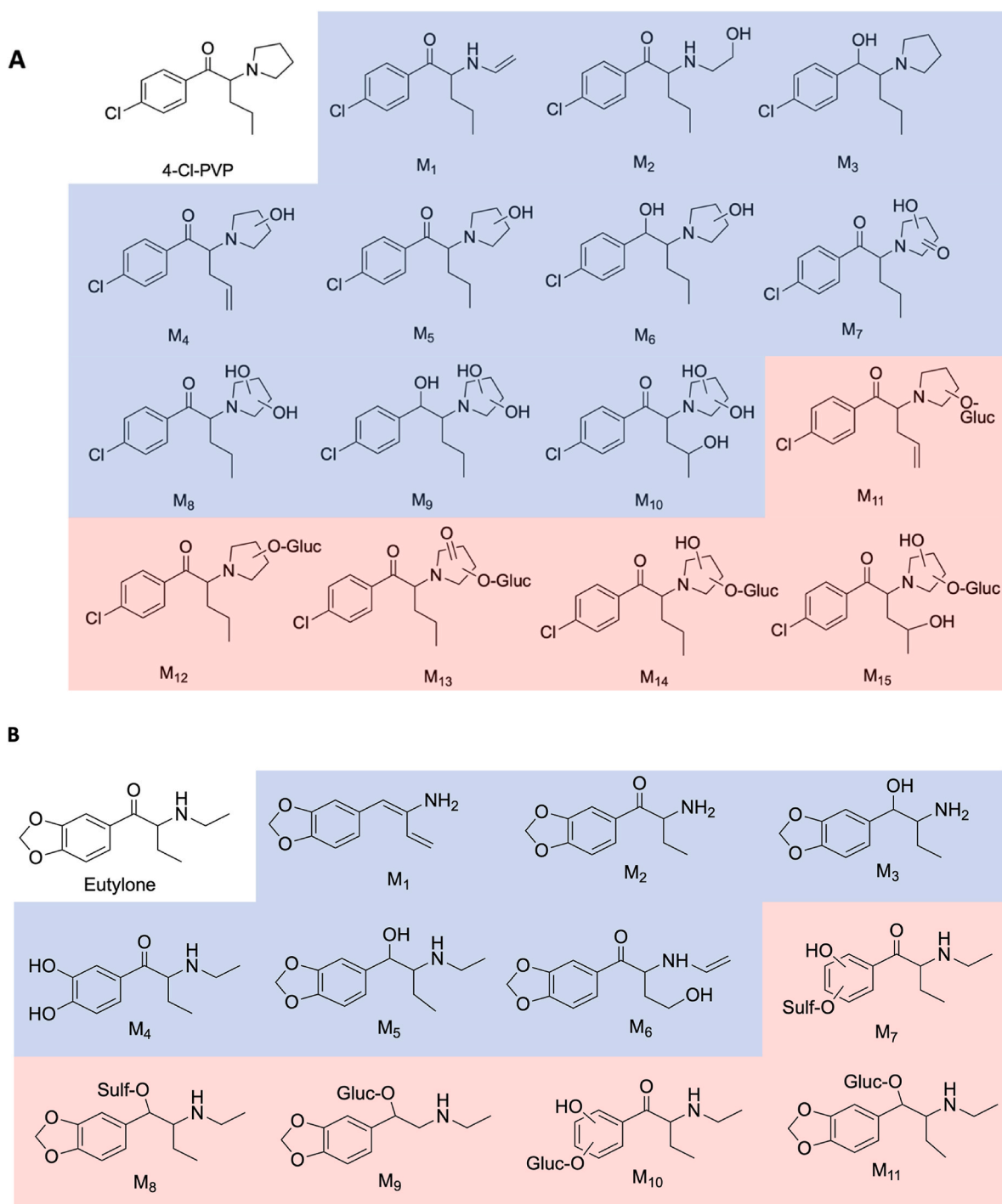


Fig. 1. 4-Cl-PVP (A) and Eutylone (B) phase I (in blue) and phase II (in red) metabolites in increasing order of *m/z* described according to the literature (Pelletier et al., 2022, 2023). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

and residues dissolved in 200 μL of water/acetonitrile/formic acid (90/10/0.09, v/v/v). The final extracts were transferred into vials for LC-HRMS/MS analysis.

2.5. Analytical parameters

LC-HRMS was performed with an UltiMate 3000 pump coupled to a Q Exactive mass spectrometer (Thermo Scientific, San Jose, CA, USA) with a heated ESI source. The non-targeted LC-HRMS/MS screening method has been described elsewhere (Le Daré et al., 2020b). Briefly, LC was performed on an Accucore Phenyl Hexyl (100×2.1 mm, $2.6 \mu\text{m}$) column (Thermo Scientific, San Jose, CA, USA) at 40°C , using an injection volume of $10 \mu\text{L}$, an elution gradient, and a flow rate of $500 \mu\text{L}/\text{min}$ for 15 min. The orbitrap mass spectrometer operated in positive ESI mode, with the acquisition range of 100–700 m/z. Ion precursor selection was performed in "data-dependent" mode: the five most intense ions from the previous scan were selected for fragmentation ("top N of 5"). The exact masses, mass fragments and retention times corresponding to the already identified metabolites of 4-Cl-PVP and eutylone are available in supplementary data (Suppl. Tables 1 and 2).

2.6. Cathinones quantitation

Seized eutylone and 4-Cl-PVP powders have been tested at high purity levels. Eutylone dihydrogenated derivative (M5 metabolite) was

synthesized to permit its quantification in biological samples (Pelletier et al., 2023). Standard solution of eutylone, eutylone-M5 and 4-Cl-PVP were prepared at 1.0 g/L in methanol and were appropriately diluted to obtain working standard solutions. NPS concentrations were measured by LC-HRMS in a positive full scan mode analysis based on retention times and exact masses for eutylone, eutylone-M5 and 4-Cl-PVP. Calibration was performed using standard solutions spiked plasma specimens at concentrations ranging from 1 to 500 ng/mL .

2.7. Accurate integration of identified and annotated markers

Data processing of all metabolites described in flowed publications (Pelletier et al., 2022, 2023) was performed using the XCalibur 4.3. (ThermoScientific®) to ensure accuracy in the study of the excretion rates of metabolites. Prism® 9.5 was used to generate curves and plots.

3. Results

3.1. 4-Cl-PVP metabolism

To study 4-Cl-PVP metabolism kinetics, we administered 4-Cl-PVP ($1 \mu\text{g/mL}$) in the liver perfusate and monitored its concentration as a function of time (Fig. 3A). We note that the concentration measured at T_0 min corresponded to the expected concentration in the system (around 1000 ng/mL). We found that 4-Cl-PVP plasma concentration

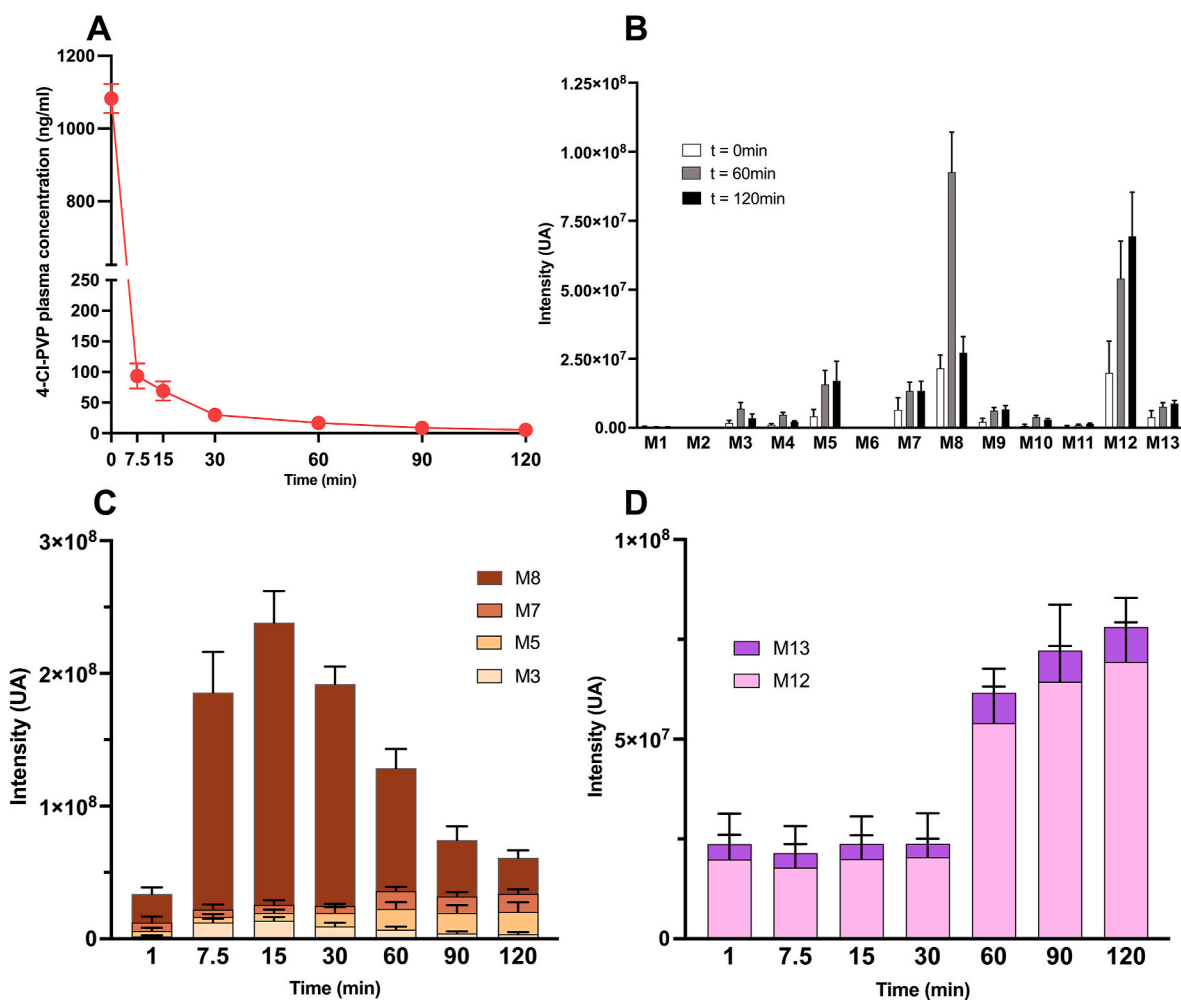


Fig. 2. Metabolic kinetics of 4-Cl-PVP in plasma after administration of a 4-Cl-PVP bolus ($1 \mu\text{g/mL}$) in the reperfusion system. (A) 4-Cl-PVP plasma concentration (ng/ml), (B) 4-Cl-PVP metabolites intensities measured in plasma at 1, 60 and 120 min after 4-Cl-PVP administration, and details of (C) Phase I and (D) phase II 4-Cl-PVP metabolite intensities.

decreased rapidly between 0 and 7.5 min and was no longer detected after 120 min. This allowed us to estimate graphically an half-life of 4 min for 4-Cl-PVP in this model (Fig. 2A).

In addition to 4-Cl-PVP, we simultaneously measured the response intensity of each of the described metabolites of 4-Cl-PVP (Fig. 3B). Eight metabolites, already described in Pelletier et al. (2022), were mainly detected between 1 min and 120 min, namely 6 phase I metabolites (M3, M5, M7, M8, M9 and M10) and two phase II metabolites (M12 and M13).

To further investigate the metabolic kinetics of the most intensively detected metabolites of 4-Cl-PVP (M3, M5, M7, M8, M12 and M13), their complete kinetic profiles are shown in Fig. 2C and D. We found that the maximum intensity of metabolites M3 and M8 (corresponding to dehydrogenated and dehydroxylated metabolite respectively) was detected after 15 min of incubation, before decreasing. In contrast, metabolites M5 and M7 (corresponding to hydroxylated metabolite and dehydrogenated + hydroxylated respectively) appeared to be more intensively detected after 120 min (Fig. 2C). Phase II metabolites M12 and M13 (corresponding to glucuronated metabolites) were detected with increasing intensity over time, peaking between 60 and 120 min of incubation (Fig. 2D).

In bile, 4-Cl-PVP was detected as early between 0 and 30 min. Its quantification, reported as a quantity (ng) in relation to the volume of bile collected during the respective periods, showed a progressive decrease in its excretion up to 120 min (Fig. 3A). The metabolites mainly found in bile were the same as in plasma, namely phase I metabolites (M3, M5, M7, M8) and phase II metabolites (M12 and M13) (Fig. 3B and C). In contrast to the bile concentration of 4-Cl-PVP, all these metabolites tend to accumulate over time (Fig. 3B and C).

3.2. Eutylone metabolism

To study eutylone metabolism kinetics, we incubated eutylone (1000 ng/mL) in the liver perfusate and monitored eutylone and its metabolite M5 concentration as a function of time (Fig. 4A). As observed for 4-Cl-PVP, eutylone concentration was near to 1000 ng/mL at T0 and metabolites were absent. We found that eutylone plasma concentration decreased rapidly between 0 and 7.5 min, with complete disappearance after 7 min. This allowed us to estimate a half-life of 0.25 min for eutylone in this model. Quantification of the M5 metabolite also revealed very early production of this metabolite, as early as 1 min (Fig. 4A). From 7.5 min, the M5 metabolite concentration was stable over time at around 150 mg/L.

The main metabolites found in plasma appeared within the first minute and were phase I (M2, M5) and phase II (M11) metabolites

(Fig. 4B). We found that the maximum intensity of metabolites was detected after 7.5 min of incubation, for both phase I and phase II metabolites. Other already described eutylone metabolites (namely M1, M3, M6, M7, M8, M9, M10) were not detected.

In bile, eutylone was detected as early as 30 min and decreased thereafter. Its quantification, relative to the volume of bile collected over the respective periods, showed a progressive decrease in excretion up to 120 min (Fig. 5A). Conversely, the quantity of metabolite M5 measured remained relatively stable throughout the kinetics in bile. The other main metabolites were detectable after 30 min, and their biliary excretion increased over time, with peak intensity found between 60 and 90 min for phase I metabolites (M2 and M5), and 90–120 min for the phase II metabolite (M11) (Fig. 5B and C).

4. Discussion

The aim of our study was to study the metabolism of 2 SCs (4-Cl-PVP and eutylone) in blood and bile, using an *ex-vivo* model of perfused pig liver.

First, metabolites found in this study were relevant in comparison with the literature for both 4-Cl-PVP and eutylone, at least for the main metabolites identified by molecular networking in previous studies (M3, M5, M7, M8, M12, M13 for 4-Cl-PVP and M2, M4, M5, M11 for eutylone) (Pelletier et al., 2022, 2023). This suggests that cathinone metabolism in pig liver is very similar to that in humans, requiring mainly phase I enzymes such as CYP2D6, CYP3A4, CYP2B6, CYP1A2, CYP2C9 and CYP2C19 (Lim et al., 2022; Springer et al., 2003) and phase II enzymes. Consistently, literature data on the proteomic characterization of xenobiotic-metabolizing enzymes reported a high degree of similarity to humans (Elmorsi et al., 2020; Stevens et al., 2021), also corroborated in metabolism studies identifying phase I and phase II metabolites (Lundahl et al., 2014). In addition, the large quantity of glucuronated metabolites of 4-Cl-PVP shows that this model has a good phase II metabolism. However, we did not find any eutylone sulfoconjugate derivatives in this model, in contrast to human studies performed in blood (Pelletier et al., 2023). These data raise the question of the relative expression of the enzymes responsible for sulfoconjugation (SULTs) in pigs, currently poorly studied. While Langsch and Bader (2001) showed that basal UGT activity was 5 times higher than that of SULTs in porcine liver cells in flat membrane bioreactors (Langsch and Bader, 2001), data in the literature nevertheless report that porcine models are capable of producing sulfoconjugated metabolites (Diaz and Squires, 2003; Schmitt et al., 2019; Sinclair et al., 2005).

The use of living pigs has also been reported for NPS metabolism studies and supports the interest of this model in this context. Nordmeier

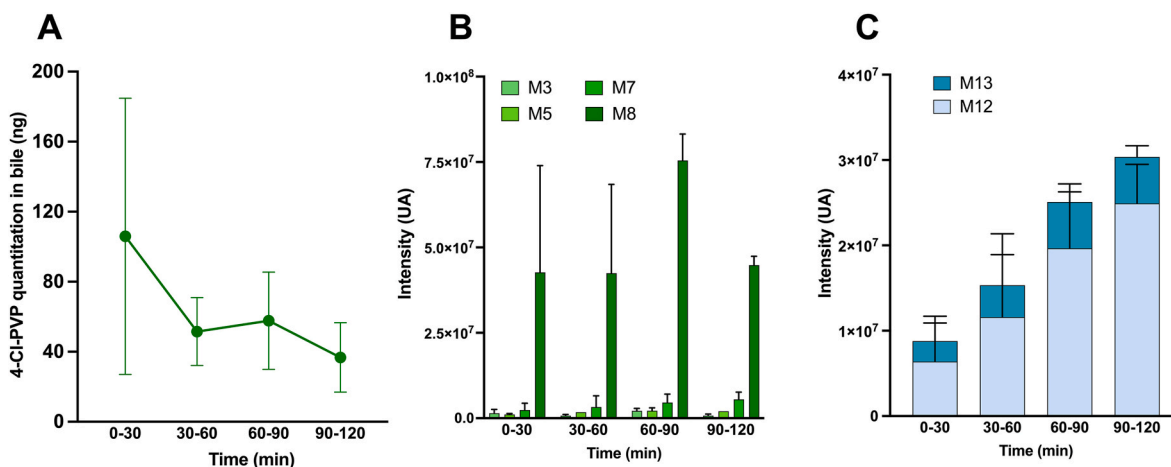


Fig. 3. Metabolic kinetics of 4-Cl-PVP in bile after administration of a 4-Cl-PVP bolus (1 $\mu\text{g/mL}$) in the reperfusion system. (A) 4-Cl-PVP quantitation, 4-Cl-PVP phase I (B) and phase II (B) metabolites intensities measured in bile between 0 and 120 min after 4-Cl-PVP bolus administration.

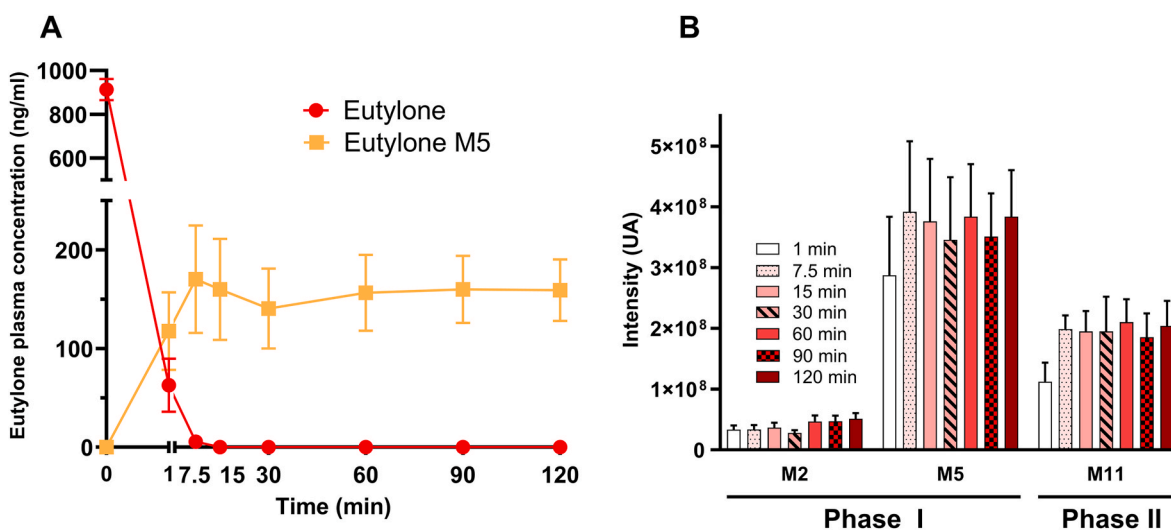


Fig. 4. Metabolic kinetics of eutylone in plasma after administration of an eutylone bolus (1 $\mu\text{g}/\text{mL}$) in the reperfusion system. (A) Eutylone plasma kinetics quantification and (B) detection of phase I and phase II plasma metabolites of eutylone.

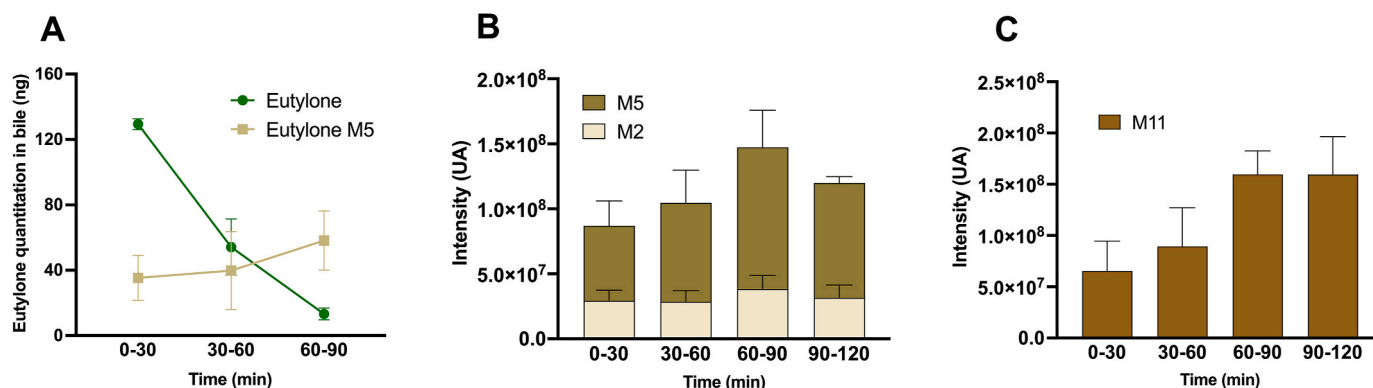


Fig. 5. Metabolic kinetics of eutylone in bile after administration of an eutylone bolus (1 $\mu\text{g}/\text{mL}$) in the reperfusion system. (A) Eutylone and eutylone-M5 quantitation in bile and eutylone phase I (B) and phase II (C) metabolites identification in bile measured between 0 and 120 min after eutylone bolus administration.

et al. (2020) elucidated the metabolic fate of U-47700 after intravenous administration to one pig *in vivo* and reported that the metabolic pattern was comparable to human *in vivo* data, including the production of sulfoconjugated metabolites (Nordmeier et al., 2020). After pulmonary administration of the synthetic cannabinoid Cumyl-5F-P7AICA, Walle et al. (2020) reported that the metabolic pattern of the porcine model *in vivo* was consistent with the metabolites already identified in humans (Walle et al., 2021). Similar findings were also described after pulmonary administration of the synthetic cannabinoid 5F-MDMB-P7AICA in pigs, in comparison with human data (Doerr et al., 2021, 2024).

Second, cathinone metabolism was very rapid in this model for both 4-Cl-PVP and eutylone, as illustrated by their estimated elimination half-lives (4 min and 0.25 min, respectively). These data should be compared with the elimination half-life of SCs usually reported in humans, ranging from 2.15 to 7 h (Papaseit et al., 2016; Poyatos et al., 2022; Quesada et al., 2016; Toennes et al., 2003). This rapid disappearance of the parent molecule was associated with a rapid appearance of cathinone metabolites in blood, as early as 1 min for both phase I and phase II metabolites. This very high clearance of these SCs may be explained by two main hypotheses, namely (i) the use of a reduced blood volume (2L) compared with real-life conditions associated with the absence of other distribution compartments which rapidly drives the parent molecule through the liver and (ii) the presence of a greater quantity of xenobiotic-metabolizing enzymes in the porcine model than in human. However, the ability to find the same metabolites as in human in a

considerably shorter time is a serious advantage, making the *ex situ* NMP pig liver model an equivalent of an accelerated human liver model.

In the case of 4-Cl-PVP, phase I metabolites were found mainly in plasma during the first hour, in contrast to phase II metabolites which mainly accumulate at the second hour of incubation (Fig. 3). This suggests that phase II metabolites are probably produced from phase I metabolites, and that they might be better candidates as consumption markers in the event of late management following intoxication. The kinetics of phase I and II metabolites in the case of eutylone did not lead to similar observations, due to the very early production of all metabolites, as early as 1 min post-administration (Fig. 5). Taken together, these results suggest that the *ex vivo* pig liver model is a relevant tool for identifying markers of NPS consumption, whether they are phase I or phase II metabolites.

Third, we analyzed the blood directly collected in the supra hepatic veins and bile compartments in this model, providing valuable data on metabolite appearance kinetics and biliary excretion. Bile analysis was particularly valuable in this study, as this matrix is known to accumulate many metabolites. We found that some 4-Cl-PVP and eutylone are eliminated unchanged in this matrix, with a more rapid elimination for eutylone, which is no longer detected 90 min after administration. Phase I metabolites were intensely detected, and associated with an accumulation of phase II metabolites over time, whether for 4-Cl-PVP or eutylone. These considerations are particularly useful in forensic toxicology, since bile is a matrix of choice for *post-mortem* toxicological analysis. As

no other relevant model capable of producing bile exists, the *ex vivo* pig liver model on NMP might be the most valuable model to guide the search for consumption markers in potentially fatal cases of NPS intoxication.

In terms of perspectives, the *ex vivo* pig liver model could help reduce the number of animals needed for toxicity studies. Using an isolated liver allows for simultaneous study of metabolite production kinetics and histological sampling at different time points, whereas a sacrificed animal typically limits biological sampling to a single point in time. Lastly, this model enables modulation of liver perfusion rates and hypoxic conditions to study their impact on hepatic metabolism and toxicity.

This study should be interpreted with consideration of several limitations. First, this model does not allow for the study of absorption phases outside the intravenous route, nor for non-hepatic distribution and excretion phases. It does not account for the metabolism of other organs or the microbiota. Consequently, it is not designed for comprehensive pharmacokinetic studies but serves as a valuable model for investigating hepatic metabolism and biliary excretion. Second, the reperfused liver has a limited lifespan *ex vivo*, typically lasting from several hours to days (Lau et al., 2022), which may restrict the study duration and the ability to observe the long-term effects of xenobiotics or the potential appearance of late metabolites. Third, establishing and maintaining a hepatic reperfusion system is technically demanding and costly, requiring surgical expertise, specialized equipment, and specific reagents to preserve liver integrity *ex vivo*. Lastly, this model lacks many biological interactions inherent in a living organism, such as immune responses and hormonal regulation, which can influence xenobiotic metabolism in an *in vivo* context (Christensen and Hermann, 2012; Jeong, 2010; Kennedy, 2008; Morgan, 2017; Ruiz et al., 2013).

5. Conclusion

The pig liver model perfused *ex vivo* using an NMP represent a promising model in toxicology. In this model, we identified the same metabolites identified in humans exposed to these 4-Cl-PVP and eutylone, with an accelerated toxicokinetics. This model may be of interest in the absence of authentic cases of cathinone consumption or other NPS consumption to identify relevant metabolites consumption markers. In addition, the possibility of collecting bile in this model represents an additional advantage for studying not only the metabolism and the kinetics but also biliary excretion of xenobiotics, such as NPS and its metabolites in forensic toxicology.

CRedit authorship contribution statement

Romain Pelletier: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Brendan Le Daré:** Writing – review & editing, Writing – original draft, Validation, Supervision, Investigation, Conceptualization. **Thomas Kerforne:** Writing – review & editing, Supervision, Conceptualization. **Nicolas Patou Parvedy:** Writing – review & editing, Investigation, Investigation, Methodology, Supervision. **Florian Lemaitre:** Writing – review & editing, Conceptualization. **Camille Tron:** Writing – review & editing, Conceptualization. **Kevin Maunand:** Writing – original draft, Investigation, Conceptualization. **Anne Corlu:** Writing – review & editing, Conceptualization. **Isabelle Morel:** Writing – review & editing, Supervision. **Michel Rayar:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Thomas Gicquel:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Formal analysis, 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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fct.2024.115217>.

Data availability

Data will be made available on request.

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