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Exposure to Synthetic Psychoactive Substances: a potential cause for increased human hepatotoxicity markers

Running header title: Hepatotoxic Effects of Synthetic NPS

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Abbreviation list :

ALF: acute liver failure
DILI: drug-induced liver injury
NPS : New psychoactive substances
SC: Synthetic cannabinoids
SO: Synthetic opioids
CNS: Central nervous system
CYP: Cytochrome P450
NAC: N-acetylcysteine
ROS: reactive oxygen species
ALT: alanine aminotransferase
AST: aspartate aminotransferase
GGT: gamma-glutamyltransferase
ALP: alkaline phosphatase

ATP: Adenosine triphosphate

NBOMe: Dimethoxyphenyl-N-[(2-methoxyphenyl)methyl] ethanamine derivatives

5F-PB-22: 1-(5-Fluoropentyl)-1H-indole-3-carboxylic acid 8-quinolinyl ester

AB-CHMINACA: n-[(1S)-1-(aminocarbonyl)-2-methylpropyl]-1-(cyclohexylmethyl)-1h-indazole-3-carboxamide

MDPV: methylenedioxypyrovalerone

25B-NBOMe: 2-(4-bromo-2,5-dimethoxyphenyl)-N-(2-methoxybenzyl)ethan-1-amine

25C-NBOMe: N-(2-methoxybenzyl)-2-(4-chloro-2,5-dimethoxyphenyl)ethanamine

MDAI: 5,6-Methylenedioxy-2-aminoindane

AMT: Alpha-methyltryptamine

HLM: human liver microsomes

THC: Δ9-tetrahydrocannabinol

CB1: Cannabinoid receptor 1

CB2: Cannabinoid receptor 2

EMCDDA: European Monitoring Centre for Drugs and Drug Addiction

RNS: reactive nitrogen species

4F-iBF: 4-fluoro-isobutyrylfentanyl

4Cl-iBF: 4-chloro-isobutyrylfentanyl

iBF: isobutyrylfentanyl

GSH: reduced glutathione

4-MEC: 4'-methyl-N-ethylcathinone

MDMA: 3,4-methylenedioxymethamphetamine

4-FMA: 4-fluoromethamphetamine

25I-NBOMe: N-(2-methoxybenzyl)-2-(4-iodo-2,5-dimethoxyphenyl)ethanamine

MDA: 3,4-methylenedioxyamphetamine

5-APB: 5-(2-aminopropyl)benzofuran

6-APB: 6-(2-aminopropyl)benzofuran

5-MAPB: 1-(benzofuran-5-yl)-N-Methylpropan-2-aMine

5-MeO-MiPT: 5-Methoxy-N-methyl-N-isopropyltryptamine

5-MeO-DALT: N,N-Diallyl-5-methoxytryptamine

BZP: N-benzylpiperazine

MDBP: 1-(3,4-methylenedioxy-benzyl)piperazine

TFMPP: 1-(3-trifluoromethyl-phenyl)piperazine

MeOPP: 1-(4-methoxyphenyl)piperazine
NMDA: N-methyl-d-aspartate
MXP: methoxphenidine
α-PVP: α-pyrrolidinovalerophenone
PRH: Primary rat hepatocytes
3,4-DMMC: 3',4'-dimethylmethcathinone
3-MMC: 3-methylmethcathinone
GSSG: oxidized glutathione

Genes list:

MAP3K6, mitogen-activated protein kinase kinase kinase 6
ABCD4: ATP binding cassette subfamily D member 4
LSS: lanosterol synthase
SREBP: sterol regulatory element binding protein

Abstract

BACKGROUND: Approximately 30 million people worldwide consume New Psychoactive Substances (NPS), creating a serious public health issue due to their toxicity and potency. Drug-induced liver injury is the leading cause of liver disease, responsible for 4% of global deaths each year.

CONTENT: A systematic literature search revealed 64 case reports, *in vitro* and *in vivo* studies on NPS hepatotoxicity. Maximum elevated concentrations of aspartate aminotransferase (136-15632 U/L), alanine transaminase (121.5-9162 U/L), total bilirubin (0.7-702 mg/dL) (0.04-39.03 mmol/L), direct (0.2-15.1 mg/dL)(0.01-0.84 mmol/L) and indirect (5.3 mg/dL) (0.29 mmol/L) bilirubin, alkaline phosphatase (79-260 U/L), and gamma-glutamyltransferase (260 U/L) were observed as biochemical markers of liver damage, with acute and fulminant liver failure the major toxic effects described in the NPS case reports. Controlled *in vitro* laboratory studies and following *in vivo* rat and mice NPS exposure provide data on potential mechanisms of toxicity. Oxidative stress, plasma membrane stability, and cellular energy changes led to apoptosis and cell death. Experimental studies of human liver microsome incubation with synthetic NPS, with and without specific cytochrome P450 inhibitors, highlighted specific enzyme inhibitions and potential drug-drug interactions leading to hepatotoxicity.

SUMMARY: Mild to severe hepatotoxic effects following synthetic NPS exposure were described in case reports. In diagnosing the etiology of liver damage, synthetic NPS exposure should be considered as part of the differential diagnosis. Identification of NPS toxicity is important for educating patients on the dangers of NPS consumption and to suggest promising treatments for observed hepatotoxicity**.**

Keywords: Hepatotoxicity, clinical toxicology, new psychoactive substances, liver disease, NPS

Introduction

Liver disease is the 11th leading cause of death, annually accounting for two million deaths or 4% of all deaths worldwide (1). Cirrhosis and liver carcinoma are among the major causes. Although rare, acute liver failure (ALF) is associated with high morbidity and mortality and is the main reason for urgent liver transplantation. Drug-induced liver injury (DILI) is characterized by elevated serum liver enzymes, acute inflammation and a strong immune response. Long-term abuse of paracetamol, acetaminophen, and anti-tuberculosis drugs are the most frequent causes of DILI; however, there are new emerging classes of hepatotoxins that clinical chemists, pathologists and toxicologists must consider as potential causative agents.

New psychoactive substances (NPS) are a broad group of drugs of natural and synthetic origin that are not controlled by the 1961 and 1971 United Nations Drug Control Conventions (2,3). NPS are commonly identified as "designer drugs", "recreational drugs" and "smart drugs", and are advertised online as "legal highs," "bath salts," and "incense" to circumvent legal controls that restrict their distribution. In recent years, NPS availability increased steadily, with the United Nations Office on Drugs and Crime stating that more than 1100 NPS were reported worldwide for the first time, including 19 in 2022 alone (Figure 1) (4). There are many NPS drug classes including synthetic cannabinoids (the largest) (SC), synthetic opioids (SO), and synthetic cathinones.

NPS are a global problem because of serious human health risks and the rapid introduction of modified substances (4,5). NPS intoxication case reports describe effects on the central nervous system (CNS) and cardiovascular systems, including seizures, psychosis, aggression, tachycardia, hypertension, hyperthermia, and acute and sometimes fatal hepatic failure (6–8). The liver is the main organ for phase-I and phase-II biotransformation reactions (9). Cytochrome P450 (CYP) enzymes oxidize, reduce and/or cleave NPS to forms that may undergo further phase-II metabolism. Drugs enter the circulation following different ingestion routes, reach the liver, and exert their toxic potential before and/or after metabolism.

The World Health Organization reported more than 280 million people worldwide (about 5.5% of the population aged 15-64) have used psychoactive substances (10). In 2019, there were 366,000 deaths from DILI,

an increase of 10% from 2010 to 2019 (4). Among many drugs to consider in this clinical setting are NPS. An ever-expanding global market for NPS attests to the difficulty of controlling access to these potent and dangerous compounds. In 2021, 0.62% of the global population or 30 million people used NPS. This high prevalence creates a serious public health threat due to NPS potency, and additional toxins and adulterants introduced during synthesis in clandestine laboratories. The paucity of information regarding newly introduced NPS makes it urgent to inform relevant stakeholders of this emerging danger.

N-acetylcysteine (NAC) treatment is helpful for stabilization and recovery of liver function in many cases (11). NAC is a precursor of the amino acid L-cysteine, whose role in the synthesis and regeneration of reduced glutathione is well known (12). Glutathione is a potent antioxidant that protects tissues from reactive oxygen species (ROS) during oxidative stress. NAC plays a hepato-protective role, restoring glutathione and eliminating oxygen-derived free radicals; however, in severe cases liver transplantation is required (11). The diagnosis of hepatotoxicity is based on liver marker concentrations including alanine aminotransferase (ALT) and aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), direct and indirect bilirubin, and alkaline phosphatase (ALP). Abnormal concentrations suggest liver dysfunction/impairment. Clinical DILI generally refers to hepatotoxicity from prescription drugs, but there are multiple reports following ingestion of drugs of abuse. Amphetamines, cannabis, cannabidiol, and opioids increase serum liver enzyme concentrations and produce morphological and histological damage, and inflammation (13–15). Clinical data also showed an impairment in mitochondrial function with decreased cytoplasmic adenosine triphosphate (ATP) concentrations, and increased oxidative stress, apoptosis, and liver inflammation. Lack of knowledge of NPS hepatotoxic potential may prevent recognition of the causative agent and lead to a wrong diagnosis.

This review of NPS hepatotoxicity includes clinical case reports and *in vitro* and *in vivo* laboratory studies investigating mechanisms of toxicity to support clinical findings. Synthetic derivatives of cannabinoids, cathinones, opioids, amphetamines, phenylethylamines, aminoindanes, benzofurans, tryptamines, piperazines, piperidines, and benzodiazepines were considered. The goal is to provide guidance regarding symptomatology

for each NPS class to raise awareness of their hepatotoxic potential, to support diagnosis in hospital emergency departments and clinical practice, and to encourage further research on the topic.

Materials and Methods

Literature searches were conducted in parallel in PubMed, Scopus, Web of Science, and Google Scholar to investigate clinical hepatotoxicity data produced by synthetic NPS opioids, cathinones, cannabinoids, phenethylamines, phencyclidines, piperazines, benzodiazepines, tryptamines, and psychostimulants. Advanced database search functions included multiple keywords (using quotation marks) and truncated words (using asterisks). The search terms hepatotoxicity OR "liver toxicity" were employed in combination with the following terms for each compound class:

- Opioids: "Synthetic opioid*", "Novel synthetic opioid*", "Synthetic benzimidazole opioid*", "nitazene analogue*";
- Cathinones: "Synthetic cathinone*", "Bath salts"," Legal highs", "Research chemical*";
- Cannabinoids: "Synthetic cannabinoid*", "Herbal blends", Spice, K2, Kronic;
- Phenethylamines: Phenethylamine*;
- Phencyclidines: Phencyclidine*;
- Piperazines: Piperazine*;
- Benzodiazepines: "Novel benzodiazepine*", "Benzodiazepine analogue*", "Designer benzodiazepine*";
- Tryptamines: Tryptamine*;
- Psychostimulants: Psychostimulant*;
- New psychoactive substances : "New psychoactive substance*", NPS, "Novel psychoactive substance*", "Smart drug*"

Articles reporting *in vivo* and *in vitro* hepatotoxic effects in humans and animals were included. Each result was carefully screened by two authors independently to remove those unrelated to hepatotoxic effects and to eliminate duplicates. Articles on non-synthetic NPS were eliminated and the time range was restricted from 2000-2023.

Results

Figure 2 shows the bibliographic flowchart performed according to the Prisma guidelines (16). A total of 6291 potentially relevant reports were identified, of which 5793 were rejected as duplicates or because hepatotoxic effects and/or liver damage were not described. The final review included 64 papers, 19 case reports, 1 multicenter study, and 44 research articles. Figure 3 illustrates the core NPS chemical structures and some derivatives for each NPS class.

Case reports of human hepatotoxicity following synthetic NPS intake are described in Table 1. Liver congestion and liver failure were the main hepatic abnormalities identified following SC, synthetic cathinones, Dimethoxyphenyl-N-[(2-methoxyphenyl)methyl] ethanamine derivatives (NBOMe), synthetic aminoindanes, and synthetic tryptamines intake. Men were overwhelmingly represented (90%) in the 35 case reports of synthetic NPS exposure. The table shows 13 ante-mortem case reports associated with synthetic cannabinoids intake; however, the specific synthetic cannabinoid was only identified in 6 cases, most likely because of the inability to identify urinary metabolites of newly emerging NPS. 1-(5-Fluoropentyl)-1H-indole-3-carboxylic acid 8-quinolinyl ester (5F-PB-22) and n-[(1S)-1-(aminocarbonyl)-2-methylpropyl]-1-(cyclohexylmethyl)-1h-indazole-3-carboxamide (AB-CHMINACA) were the primary analytes detected. In 3 co-exposure cases, ethanol may have contributed to the observed liver toxicity. Eight of 13 cases were reported from the USA and the remainder from Japan. There were 5 antemortem and 14 postmortem synthetic cathinone case reports, with the causative agent specifically identified in all, due to the ability to detect the parent compound in biological matrices. Methylenedioxypyrovalerone (MDPV), methylone, and butylone were the primary intoxicant in 15 cases. MDPV was identified in 12 cases, methylone in 3, and butylone in 1. The contribution of benzodiazepine

co-exposure in 5 hepatotoxicity cases is unknown and should be further explored. Sixteen of 19 cases were reported from the USA and the remaining from Poland, UK, and Germany. A single NBOMe antemortem case described an 2-(4-bromo-2,5-dimethoxyphenyl)-N-(2-methoxybenzyl)ethan-1-amine (25B-NBOMe) and N-(2-methoxybenzyl)-2-(4-chloro-2,5-dimethoxyphenyl)ethanamine (25C-NBOMe) intoxication with benzodiazepine co-exposure from China. 5,6-Methylenedioxy-2-aminoindane (MDAI), a synthetic aminoindane, alone was identified in a UK hepatotoxicity case. Similarly, only alpha-methyltryptamine (AMT), a synthetic tryptamine, was found in a postmortem liver intoxication and failure case from the USA.

Controlled *in vitro* and *in vivo* studies of the hepatotoxic effects of synthetic NPS exposure are shown in Table 2. These studies are included to provide data on potential mechanistic effects of synthetic NPS exposure to better understand the etiology of human hepatic NPS toxicity. These studies document changes in intracellular calcium, mitochondrial depolarization, DNA fragmentation, nuclear size, plasma membrane integrity, succinate dehydrogenase activity, cytotoxicity, cell death and apoptosis after synthetic NPS exposure. Other *in vitro* study designs utilized incubations of synthetic NPS with human liver microsomes (HLM) with and without specific inhibitors to understand NPS enzyme inhibition, potential drug-drug interactions and hepatotoxicity. Much of the hepatotoxicity relates to the production of oxidative stress increasing ROS, intracellular calcium concentrations, apoptosis and cell death, and decreasing ATP and glutathione. *In vivo* models quantified morphological, and histological liver damage, and serum liver enzymes (ALT, AST and GGT).

Discussion

SYNTHETIC CANNABINOIDS

SC are a class of designer drugs that mimic Δ9-tetrahydrocannabinol (THC) effects by binding with varying affinity to endogenous cannabinoid receptors 1 (CB1) and 2 (CB2) (17). Fifteen new SC were described by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) from 2022-2023, adding to more than 200 monitored since 2008, making this the most widely observed NPS class (18). Despite worldwide containment and surveillance measures, SC were linked to numerous intoxications and deaths in recent years. SC may be full CB1 and CB2 receptor agonists with greater potency and binding affinity than the partial

agonist, THC, inducing effects at low concentrations (17). Cannabinoid receptors are primarily localized in the CNS (CB1) and in peripheral tissues (CB1 and CB2), including red blood cells, spleen, and liver immune tissues. SC can trigger a wide variety of psychoactive effects including hallucinations, convulsions, nausea, vomiting, palpitations, tachycardia, chest pain, and shortness of breath. Many SC have longer half-lives than THC, resulting in prolonged toxicological effects over time and greater health consequences. Acute liver damage and fulminant liver failure were reported following SC abuse in subjects with no prior history of liver disease (Table 1). Marked agitation, seizure-like activity, rhabdomyolysis, lactic acidosis, abdominal pain, and vomiting were recorded as common symptoms during emergency department admission (6,19,20). Maximum concentrations of AST (15632 U/L, normal range 8-48 U/L), ALT (9162 U/L, normal range 7-55 U/L), total bilirubin (20.4 mg/dL [1.13 mmol/L], normal range 0.2-1 mg/dL [0.01-0.05 mmol/L]), direct bilirubin (15.1 mg/dL [0.84 mmol/L], normal range < 0.3 mg/dL [< 0.02 mmol/L]) and indirect bilirubin (5.3 mg/dL [0.29 mmol/L], normal range 0.2-0.8 mg/dL [0.01-0.04 mmol/L]), ALP (260 U/L, normal range 44-147 U/L), and GGT (260 U/L, normal range 5-40 U/L) were reported (see Table 1 case studies), demonstrating hepatotoxicity (19–23).

In five SC intoxications reported in Table 1, patients were successfully treated with NAC (6,19–22). The effectiveness of NAC treatment for SC hepatotoxicity suggests that hepatic glutathione reduction and increases in free radical production impaired liver function (6,22).

Multiple studies documented high SC concentrations and induction of oxidative stress, manifested primarily as impairment of mitochondrial and endoplasmic reticulum function (24–28). In *in vivo* Wistar rat studies, SC upregulated genes involved in the inflammatory response (MAP3K6, ABCD4, and LSS genes), documenting that gene activation pathways and their transcription factors contribute to ROS regulation and reactive nitrogen species (RNS) production (29). The toxicity mechanisms induced by SC and metabolites include DNA damage and apoptosis resulting in complex liver disease.

SYNTHETIC OPIOIDS

The opioid crisis began in the USA in 1990 with increased prescribing of natural and semisynthetic opioids and culminated in 2022 with more than 70,000 opioid deaths (15,18). The reasons behind the rapid spread of this new NPS class were high demand for opioids fueled by overprescribing of analgesics resulting in the development of opioid dependence in a large number of individuals. The response in the US was to change prescribing practices but this led to dynamic increases in heroin use when prescription opioids were less available. Clandestine laboratories took advantage of the low production cost and potent and persistent effects of SO and flooded the market with fake oxycodone, fentanyl, fentanyl derivatives and other prescription opioids. Even more problematic is that SO were also added as adulterants to methamphetamine, heroin, and cocaine and other illegal drugs producing liver toxicity and many overdose deaths of unsuspecting drug users.

In Europe, 73 new SO were registered on the black market in the last 15 years. Although SO are not the largest synthetic NPS group monitored by EMCDDA, they are under close observation due to their serious risks to human health. SO act as agonists, partial agonists, or antagonists at mu-opioid, kappa-opioid, delta-opioid, and nociceptine/orphanin FQ receptors, which are most prevalent in the CNS (30). Respiratory depression, miosis, sedation, euphoria, dyspnea, and decreased gastrointestinal motility are the major SO effects. Of particular interest are fentanyl derivatives that are synthetic phenylpiperidines 50 to 100 times more potent than morphine.

In recent decades, multiple structurally related fentanyl analogs were developed that mimic fentanyl effects with equivalent or greater potency. After butyryl fentanyl and acetyl fentanyl, the major abused SO in the US is currently fentanyl itself. Consumption of fentanyl and its analogs is increasing, representing a global public health problem. In Japan, the fentanyl analogs 4-fluoro-isobutyrylfentanyl (4F-iBF), 4-chloro-isobutyrylfentanyl (4Cl-iBF), and isobutyrylfentanyl (iBF) are classified as “designated substances for their health and sanitation hazards” (31). Proof of the toxicity of 4F-iBF was the induction of respiratory depression producing hepatic and renal ischemia in F344/Jcl rats. The ischemia reduced renal excretion and hepatic metabolism and was accompanied by infiltration of inflammatory cells, tissue degeneration and necrosis.

Another SO class are non-fentanyl analogs including tapentadol and tramadol, which combine μ receptor activation and inhibition of 5-hydroxytryptamine and noradrenaline reuptake (32,33). These two distinct

mechanisms act synergistically, increasing potency despite low μ opioid receptor binding affinity. Tramadol and tapentadol impaired metabolic liver function and oxidative capacity, as well as increased AST and ALT concentrations in Wistar rats. In addition, increases in oxidative stress accompanied by histological changes and necrotic phenomena were observed.

SYNTHETIC CATHINONES

Synthetic cathinones are β-keto phenethylamines are sold as "bath salts", "plant fertilizers" or "jewelry cleaners" and labeled "not for human consumption" to escape recognition by law enforcement (18). Synthetic cathinones are usually snorted or ingested in powder, crystal or tablet form and constitute the second largest group of NPS monitored by the EMCDDA.

Synthetic cathinones cross the blood-brain barrier and inhibit dopamine, norepinephrine, and serotonin transporters with varied selectivity (34). Considering their similarity in structure to amphetamines, synthetic cathinones are powerful psychostimulants that cause amphetamine-like psychoactive and sympathomimetic effects. Hyperthermia, hypertension, rhabdomyolysis, tachycardia, coagulopathy, hyperglycemia, metabolic acidosis, and multi-organ failure are symptoms common to synthetic cathinone poisonings. Body temperatures ranging from 39.5-41.8°C were noted in patient emergency room case reports following synthetic cathinone intoxications (35–42). Associated with hyperthermia were maximum elevated AST concentrations, 10105 U/L after MDPV, 366 U/L after methylone, and 5700 U/L after butylone, and ALT concentrations, 5132 U/L after MDPV, 138 U/L after methylone, and 2500 U/L after butylone. In addition, total bilirubin (0.7-2.4 mg/dL [0.04-0.13 mmol/L]), and ALP (33-212 U/L) were also elevated (35,37,38). In all of these cases, these liver enzyme abnormalities were followed by liver failure.

Regenerative liver treatment with NAC for 3 days was performed following MDPV and butylone intoxication (35). Total bilirubin decreased from 702 mg/dL (39.03 mmol/L) to normal (0.2-1.0 mg/dL [0.01-0.05 mmol/L]) results following treatment. Comparative studies of synthetic cathinone hepatotoxic effects under normothermic (37°C) and hyperthermic (40.5°C) conditions showed significantly increased cytotoxic potential at higher temperatures (34,43). Increased production of ROS and RNS, reduction of antioxidant reserves

(intracellular reduced glutathione [GSH]), dysregulation of pathways related to ascorbate and pyruvate metabolism, impairment of mitochondrial energy metabolism, and activation of intrinsic and extrinsic apoptotic pathways were found, demonstrating that synthetic cathinones possess significant hepatotoxic potential, especially at higher temperatures.

Potency of the different synthetic cathinones, pentedrone, MDPV, 4'-methyl-N-ethylcathinone (4-MEC), and methylone, and 3,4-methylenedioxymethamphetamine (MDMA) were compared showing concentration-dependent cytotoxic potential, but pentedrone, MDPV, and 4-MEC were much more potent than MDMA in terms of ROS and RNS production, decreased GSH concentrations, and increased lipid peroxidation (44). Other *in vitro* and *in vivo* synthetic cathinone studies (26,34,43–45), documented decreased antioxidant defense capabilities and a parallel increase in ROS and RNS that triggered apoptotic and necrotic processes, resulting in morphological and histological changes in the liver. These studies and others also demonstrated mitochondrial dysfunction including alterations in membrane potential, damage to the electron transport chain, and dysfunction of energy metabolism (46,47).

SYNTHETIC AMPHETAMINES

Amphetamine derivatives retain the stimulant properties of classical amphetamines (48). The main symptoms are psycho-stimulation, euphoria, increased arousal, hypertension, hyperthermia, and increased blood pressure. Like amphetamines, their derivatives act on monoamine transporters, inducing neurotransmitter release while simultaneously blocking their reuptake, increasing neurotransmitter concentrations in the synapse. Depending on the amphetamine ring substitution, the affinity and binding strength to monoamine transporters change considerably, inducing differing magnitudes and types of effects. Synthetic phenethylamines are marketed in powder form, are dissolved in a liquid or enclosed inside cigarette paper and swallowed, termed 'bombing' or 'parachuting' (18).

4-fluoromethamphetamine (4-FMA) has higher affinity for the serotonin transporter than amphetamine, producing hyperthermia, tachycardia, euphoria, and increased affection and empathy (49). After 24 h incubation of 37 μM-10 mM and 1-30 mM 4-FMA and rat HepG2 and HepaRG cells, respectively,

mitochondrial damage included alterations in membrane potential and energy metabolism impairment resulting in poor intracellular ATP production. Amphetamine-like stimulants such as 4-FMA induced oxidative stress through impairment of antioxidant defenses resulting in high ROS and RNS concentrations, loss of plasma membrane integrity and initiation of programmed and unprogrammed cell death.

SYNTHETIC PHENYLETHYLAMINES

NBOMe are a novel class of potent synthetic hallucinogens, structural analogs of 2C-compounds with an N-benzyl-methoxy group (50). NBOMe are available on the illicit market as "acids", "Holland films" and "N-bombs", sold in powder or tablet form. 25B-NBOMe, 25C-NBOMe, and N-(2-methoxybenzyl)-2-(4-iodo-2,5-dimethoxyphenyl)ethanamine (25I-NBOMe) were first detected in 2011. The N-benzyl-methoxy group endows high affinity for serotonin receptors where they act as potent agonists to produce hallucinations. In addition, NBOMe are also alpha-adrenergic receptor agonists generating stimulating effects including euphoria, increased empathy and sociability, confusion, convulsions and seizures, hypertension, hyperthermia, rhabdomyolysis, acidosis, and multiple organ failures. In the single published case of NBOMe-induced hepatotoxicity, increased AST and ALT, and plasma urea concentrations of 492 U/L, 463 U/L, and 111.71 mg/dL (6.21 mmol/L), respectively, were reported in a 31-year-old man following ingestion of 'Holland film' a few hours before hospitalization. To investigate toxic effects at the cellular level, Gampfer et al. exposed liver cells to 25C-NBOMe and 25I-NBOMe and monitored cell count, nuclear size and intensity, mitochondrial membrane potential, cytosolic calcium levels, and plasma membrane integrity (26). The only parameter significantly affected was cell count. No other changes were detected, requiring further investigation to clarify NBOMe hepatotoxic effects.

SYNTHETIC AMINOINDANES

Recently, aminoindanes, strong bronchodilators and analgesics, became popular recreational drugs due to their potent empathogenic and entactogenic effects similar to MDMA (51). The aminoindane chemical structure is

similar to amphetamine with different ring substitutions. Illicit aminoindanes are sold as powders, crystals, or pills, and commonly labelled as “research chemicals” or “plant food”.

MDAI is a serotonin-releasing agent and reuptake inhibitor, producing psychoactive effects of mild euphoria, empathy, and intense sensory experiences. However, high serotonin concentrations in synapses increases the risk of serotonin toxicity, including neurotoxicity and cardiotoxicity, manifested as tachycardia, hyperthermia, hypertension, and convulsions. Hospitalization following intake of 5 g MDAI was reported in the UK (52). The patient was in a state of agitation and confusion, accompanied by hyperthermia and tachycardia. Within hours, the clinical picture worsened, and the patient developed multi-organ failure including liver and kidney failure as well as disseminated intravascular coagulation. Maximum ALT (9541 U/L), ALP (42 mg/dL [2.33 mmol/L]), and total bilirubin (128 mg/dL [7.12 mmol/L]) concentrations were found prior to treatment in the liver intensive care unit. After 6 days of treatment with fluid resuscitation, vasopressin, high frequency oscillatory ventilation, high volume continuous veno-venous hemofiltration, noradrenaline for hypotension, NAC and vitamin K, his health improved.

To investigate MDAI’s hepatotoxic effects, liver cells were exposed to MDAI or MDMA and cell count, nuclear size and intensity, mitochondrial membrane potential, cytosolic calcium levels, and plasma membrane integrity evaluated (28). MDAI increased cytosolic calcium, reduced cell count, nuclear size, nuclear intensity and mitochondrial membrane potential.

SYNTHETIC BENZOFURANS

Benzofurans are phenethylamine derivatives that possess psychostimulant properties similar to the common drugs of abuse MDMA and 3,4-methylenedioxyamphetamine (MDA) (53). 5-(2-aminopropyl)benzofuran (5-APB) and 6-(2-aminopropyl)benzofuran (6-APB), the two main benzofurans taken recreationally, are sold on the illicit market as powders or tablets and inhaled and ingested by parachuting or bombing. Synthetic benzofurans have structural and pharmacological similarities to amphetamines and act as agonists of serotoninergic receptors and as inhibitors of monoamine transporters, producing entactogenic and stimulant

effects, including hypersensitivity to visual and auditory stimuli. At higher doses, cardiotoxicity and neurotoxicity including tachycardia, hypertension, hyperthermia, dizziness, and anxiety may occur. High ROS and RNS concentrations, low GSH concentrations, impaired mitochondria function and membrane potential, and decreased intracellular ATP concentrations documented oxidative stress (54). Decreased ATP concentrations followed disruption of the electron transport chain and hyperpolarization of the mitochondrial membrane induced caspase activation and apoptosis (55). 5-APB and 1-(benzofuran-5-yl)-N-Methylpropan-2-aMine (5-MAPB) produced greater toxicity than MDMA at the same concentrations.

SYNTHETIC TRYPTAMINES

Tryptamines are natural alkaloids with hallucinogenic properties due to their core indole ring structure, similar to serotonin (56). Illicit tryptamines are sold in tablet or powder form and labelled “not for human consumption” and “research chemicals”. Routes of administration include sniffing, smoking, swallowing, and intravenous or intramuscular injection. Synthetic tryptamines stimulate serotonin receptor agonists producing hallucinations, changes in visual and auditory perception, and behavioral and mood disorders. In addition, there are cardiac and neurological symptoms, tachycardia, hypertension, hyperthermia, anxiety, euphoria, confusion, and delirium (57).

A 22-year-old man found unconscious after consuming 1g AMT had 24.7 mg/kg AMT in his liver, suggesting severe apoptotic damage leading to necrosis, accompanied by severe inflammation and structural damage. Although 5-Methoxy-N-methyl-N-isopropyltryptamine (5-MeO-MiPT) and N,N-Diallyl-5-methoxytryptamine (5-MeO-DALT) induced an imbalance in membrane potential impairing organelle function, other parameters including cell count, nuclear size, and intracellular calcium levels were not altered (26). Intraperitoneal injection of 0.27 mg/kg 5-MeO-MiPT induced histological and structural changes in the liver of CD1 mice indicating inflammation, significant caspase 3 and 8 increases and apoptosis (58).

SYNTHETIC PIPERAZINES

Piperazine derivatives are sold as legal alternatives to amphetamine derivatives such as MDMA (59), and are marketed as ‘party pills’, ‘Legal X’, ‘Herbal ecstasy’ or ‘XXX’, sometimes in combination with each other. The

most common are the benzylpiperazines, such as N-benzylpiperazine (BZP) and its methylenedioxy-analogue 1-(3,4-methylenedioxy-benzyl)piperazine (MDBP), and the phenylpiperazines, such as 1-(3-trifluoromethyl-phenyl)piperazine (TFMPP) and 1-(4-methoxyphenyl)piperazine (MeOPP). Although the psychostimulant effects are of lesser magnitude and intensity than those of amphetamine derivatives, piperazine-derived drugs increase dopamine and serotonin producing a similar behavioral profile (60). At low doses, mild effects such as euphoria and wakefulness are produced, as well as anxiety, and confusion. At higher doses, there is sympathomimetic toxicity with tachycardia, hypertension, and palpitations and hepatic toxicity.

In vitro studies conducted on different liver cell lines, demonstrated common toxicity mechanisms. BZP, MDBP, TFMPP, and MeOPP increased ROS and RNS concentrations and simultaneously decreased GSH concentrations (61–63). Piperazine derivatives induced functional damage to mitochondria, altering the mitochondrial membrane potential that impaired functionality of the electron transport chain. The cell is no longer able to produce adequate ATP necessary for energy maintenance, increasing oxidative stress and compromising cell viability. These effects trigger initiation of apoptotic processes as evidenced by elevated caspase 3 and 8 concentrations.

Piperazine derivatives also affected cholesterol and lipid metabolism by altering transcriptional regulation of genes involved in cholesterol biosynthesis, specifically sterol regulatory element binding protein (SREBP). This transcription factor enters the nucleus, binds promoters, and induces transcriptional activation of several genes involved in sterol and lipid synthesis and transport. Alterations in cholesterol and lipid biosynthesis and transport most likely induce hepatic phospholipidosis and steatosis.

SYNTHETIC PIPERIDINES

Piperidine derivatives belong to the category of dissociative drugs developed for anesthesia in the 1950s (64). The most common dissociative drugs are ketamine and phencyclidine that act directly on N-methyl-d-aspartate (NMDA) receptors, affecting glutamate neurotransmission, and producing psychotropic effects. Substitutions on the chemical structure give rise to synthetic derivatives such as methoxphenidine (MXP) that appeared on the illicit market in the early 2000s, sold as a “legal high” or “research chemicals” and consumed orally,

sublingually or via insufflation. MXP shares its chemical structure with ketamine, and therefore, possesses a high binding affinity for NMDA receptors (65). In addition, it interacts with the monoamine transporters of norepinephrine, dopamine, and serotonin, albeit with lower binding affinity than to NMDA. Common effects among users are anxiety, impaired cognitive function, euphoria, blurred vision, and confusion.

When the hepatotoxic potential of MXP from the illicit market was compared to MXP synthesized under good manufacturing procedures, the IC_{50} indicated greater toxicity for the illicit drug (66). Data derived from X-ray diffraction indicated the presence of impurities and anions that increased toxicity.

SYNTHETIC BENZODIAZEPINES

The literature search produced no results describing NPS benzodiazepine hepatotoxic effects.

GENERAL DISCUSSION

The use and abuse of NPS produced considerable liver damage as described in clinical reports, and *in vitro* and *in vivo* studies. Patients reported variable effects from mild liver damage, including transient increases in liver enzymes, bilirubin and ALP with subsequent disease resolution, to cases of fulminant hepatic failure, even in patients without any prior history of liver disease (8,37,57). Patients with toxic NPS liver concentrations of 0.19 ng/mg α-pyrrolidinovalerophenone (α-PVP) (8), 0.88 ng/mg methylone (37), and 24.7 ng/mg AMT (57) manifested liver failure and ALF. In the cases where NAC treatment was applied, it improved or resolved liver function in all cases. Maximum reported serum AST and ALT, total bilirubin, direct and indirect bilirubin, ALP, GGT, and glutathione concentrations provided clinical markers of liver damage (19–22,35). The lack of NPS-specific liver markers increases the difficulty in making a complete and accurate diagnosis.

Potential novel markers of NPS hepatotoxicity include glutamate dehydrogenase, osteopontin, and amphoterin (67). Glutamate dehydrogenase is a marker of mitochondrial toxicity in ALF and DILI cases, with impairment of mitochondrial structure and function and altered cytosolic calcium, intracellular ATP, and lactate dehydrogenase concentrations. ATP insufficiency reduced cell metabolism, causing irreversible damage and apoptosis. The ratio of glutamate dehydrogenase to ALT provided a correlation between mitochondrial and

liver toxicity, suggesting that glutamate dehydrogenase may be a useful clinical marker to detect NPS liver damage (68). Intravenous heroin and mephedrone users have a high incidence of hepatitis C viral infections (69). Diagnostic tests for hepatitis C virus infection are readily available, suggesting it could be useful to investigate the relationship between NPS or polydrug intake and incidence of infection.

Illicit NPS often contain a mixture of psychoactive substances such as 'Legal X', a mixture of BZP and TFMPP piperazine derivatives (70). In combination, these two substances have an additive effect on hepatotoxicity and inhibit CYP450 enzymes. Serious or lethal effects cannot be excluded even when doses are low (61). Clinical reports of multiple drug exposures frequently documented severe liver dysfunction (Table 1). Ethanol increases the metabolic rate of CYP2E1 involved in the hepatic metabolism of MDPV and 4-FMA (71). Increased CYP2E1 activity intensified the toxicity of MDPV and 4-FMA demonstrating an important drug-drug interaction.

At present, there are no established clinical guidelines for the management of acute NPS toxicity. One possible treatment to limit NPS liver toxicity is NAC, an established treatment for DILI. Its antioxidant potential restores normal liver function in patients with synthetic cannabinoid intoxication and could be extended to treatment of other NPS category DILI.

NPS incubation with HepG2, HepaRG, and primary rat hepatocytes (PRH) liver cell lines were conducted to understand the role of metabolism in drug-induced hepatotoxicity, as these cell lines have different CYP enzyme expression profiles (45). Cytochrome P450 inhibition assays are valuable for understanding drug metabolism, assessing toxicity, and providing useful information for understanding a drug's pharmacokinetics. Each drug has a specific metabolization pathway, characterized by CYP enzymes isoforms whose role in drug-induced toxicity changes considerably depending on the substance tested. CYP2D6, CYP2E1, and CYP3A4 affect the metabolism of 3',4'-dimethylmethcathinone (3,4-DMMC), buphedrone, butylone, *3-methylmethcathinone* (3-MMC), 5-APB, and 6-APB increasing their toxicity (45,49,62,72). In contrast, CYP enzymes have a detoxifying effect on 4-FMA and TFMPP, and in particular the CYP2E1 and CYP2D6 isoforms decrease the toxicity of buphedrone and 3,4-DMMC, respectively.. As shown in *in vitro* studies,

inhibition of CYP enzymes with a detoxifying role increased the extent of cytotoxic damage, bringing additional evidence that toxicity may occur from the parent drug and its metabolites.

Such studies are also important in the context of drug-drug interactions, as individual drug metabolism may be altered, and the risk of adverse effects may increase. Examples include interactions between NPS and prescription drugs. Antiretroviral drugs treating HIV infections interfere with the metabolism of amphetamine-like drugs by reducing CYP3A4-mediated metabolism and increasing NPS toxicity (73). Also of interest is a study on liver microsomes that were co-exposed to a mixture of prescription drugs and drugs belonging to different NPS classes (74) showing inhibition of specific CYP enzymes depending on the type of substance. In general, all NPS tested inhibited the CYP2D6 enzyme involved in their metabolism, increasing drug concentrations that may accumulate in the liver and induce greater toxic effects.

Conclusions

For all these classes of NPS except benzodiazepines, for which there are no published data, there is evidence of hepatotoxicity. Clinical data showed an alteration of liver function parameters, increasing AST, ALT, total bilirubin, direct and indirect bilirubin, alkaline phosphate and GGT. Hepatic failure, sometimes fulminating, was associated with high concentrations of single NPS or a combination of abused substances. Polydrug intake frequently altered CYP450 activity, intensifying hepatotoxic effects that could be lethal even at low doses. *In vitro* and *in vivo* studies strongly support the clinical data, documenting decreases in cell viability markers (cell count, nuclear size, and intensity), impairment of mitochondrial functions (mitochondrial membrane depolarization, high concentrations of cytoplasmic calcium, decreased cellular ATP), and a concomitant increase in oxidative stress (increased ROS and RNS species, decreased GSH/GSSG ratio). In addition, apoptosis was apparent in cells and liver sections, sometimes accompanied by local inflammation and necrosis. NAC is an effective treatment following SC, synthetic cathinones and synthetic aminoindanes intake and might be useful for other NPS exposures. When liver abnormalities are found, investigation of glutamate dehydrogenase concentrations may be valuable to assess mitochondrial impairment. In diagnosing the etiology of liver damage, synthetic NPS should be considered as causative agents. Testing may not be available for

many of these emerging drugs in most hospitals; however, reference laboratory testing is readily available, and identification can educate patients on the dangers of NPS ingestion and also suggest promising treatments.

CRediT authorship contribution statement

Aurora Balloni: Conceptualization, Methodology, Formal analysis, Investigation, Supervision, Writing – original draft, Writing – review & editing. **Anastasio Tini:** Formal analysis, Investigation, Writing – original draft. **Emilia Prospero:** Formal analysis, Investigation. **Francesco Paolo Busardò:** Conceptualization, Methodology, Formal analysis, Investigation, Supervision, Writing – review & editing**. Marilyn Ann Huestis** Conceptualization, Methodology, Formal analysis, Investigation, Supervision, Writing – original draft, Writing – review & editing. **Alfredo Fabrizio Lo Faro:** Conceptualization, Methodology, Formal analysis, Investigation, Supervision, Writing – original draft, Writing – review & editing.

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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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Table 1. Case reports of hepatotoxicity associated with new psychoactive substance (NPS) intake.

	Sex, age (years)	NPS	Concentrations	Co-Exposure (ng/mL, unless specified)	Hepatic symptoms and liver biomarker results	Date Country	Ref.
Synthetic cannabinoids	M 18 M 27	5F-PB-22 5F-PB-22	1.5 ng/mL (iliac blood) 1.3 ng/mL (serum)	Alcohol THC-COOH 246	Liver congestion Fulminant liver failure	2014 USA	(7)
	M 45	K2/Spice	-	None	AST 793 U/L, ALT 799 U/L, TB 0.7 mg/dL (0.04 mmol/L), DB 0.2 mg/dL (0.01 mmol/L), ALP 260 U/L & GGT 2260 U/L Toxic hepatitis	2014 USA	(22)
	5 patients	AB-CHMINACA, α-PHP	-	None	Liver injury	2013-2014 Japan	(23)
	M 56	K2/ Spice	-	Alcohol 104 mg/dL (blood)	AST 15632 U/L, ALT 9162 U/L, TB 2.0 mg/dL (0.11 mmol/L) & ALP 79 U/L Acute liver failure	2016 USA	(21)
	-	K2/Spice	-	None	AST 1031 U/L, ALT 1922 U/L, TB 20.4 mg/dL (1.13 mmol/L), DB 15.1 mg/dL (0.84 mmol/L), IB 5.3 mg/dL (0.29 mmol/L) & ALP 123 U/L Fulminant liver failure	2017 USA	(20)
	M, 22	K2/Spice	-	Alcohol	AST 1335 U/L, ALT 2436 U/L, TB 3.8 mg/dL (0.21 mmol/L) & DB 1 mg/dL (0.05 mmol/L) Acute liver injury	2018 USA	(19)
	M, 29 M, 28	K2/Spice K2/Spice	- -	MDMA None	Fulminant liver failure Fulminant liver failure	2014 USA	(6)

						2014 USA	
Synthetic cathinones	M 27	MPHP	100 ng/mL (serum)	None	Toxic liver damage	2010 Germany	(39)
	M 28	MDPV& Butylone	-	None	AST 5700 U/L, ALT 2500 U/L & TB 702 mg/dL (39.03 mmol/L) Acute liver failure	2010 UK	(35)
	F 24	Methylone, Butylone	-	None	AST 366 U/L & ALT 138 U/L Liver failure	2011 USA	(38)
	M 23	Methylone	840 ng/mL (iliac blood), 1000 ng/mL (heart blood), 1400 ng/mL (vitreous humor), 12000 ng/mL (gastric contents), 550 ng/mL (urine)	Dextromethorphan <0.02	AST 10105 U/L, ALT 6196 U/L, TB 1.4 mg/dL (0.08 mmol/L) & ALP 104 U/L Liver failure	2012 USA	(37)
	M 23	Methylone	560 ng/mL (peripheral blood), 580 ng/mL (heart blood), 920 ng/mL (vitreous humor), 4500 ng/mL (gastric contents), 0.88 ng/mg (liver), 230 mg/L (urine)	Ethanol 30 mg/dL (peripheral blood), 10 mg/dL (vitreous humor); benzodiazepines, cannabinoids, fentanyl	Liver failure		
	M40	MDPV	670 ng/mL (urine), 82 ng/mL (serum)	Lidocaine, trimethoprim, acetaminophen	AST 10873 U/L, ALT 6623 U/L Liver failure	2012 USA	(40)
	M 39	MDPV	700 ng/mL (heart blood) 1000 ng/mL (peripheral blood)	Diphenhydramine 100, promethazine 200, nordiazepam 100 (heart blood)	Liver failure	2011 USA	(36)

	M 46	MDPV Tramadol	10 ng/mL (heart blood) 0.016 ng/mg (brain) 0.012 ng/mg (liver) 17 ng/mL (vitreous humor) <50 ng/mL (blood)	Alprazolam 15 (blood)	Liver failure	2013 USA	(41)
	M 47	MDPV	162 ng/mL (peripheral blood) 280 ng/mL (heart blood) 3.72 ng/mg (liver) >750 ng/mL (bile) 13900 ng/mL (urine) 0.168 ng/mg (brain)	Oxymorphone 43 (blood), diazepam 313 (blood), nordiazepam 494 (blood), temazepam 33 (blood)	Liver failure		
	M 33	MDPV	> 4.8 ng/mg (liver)	Ethanol 44 mg/dL	Liver failure		
	F 43	MDPV Tramadol	18 ng/mL (peripheral blood) 28 ng/mL (heart blood) 0.052 ng/mg (liver) <50 ng/mL (peripheral blood) 14 ng/mL (vitreous humor)	Fentanyl 8 (blood), norfentanyl <1 (blood), trazodone 540 (blood), diazepam 301 (blood), nordiazepam 281 (blood)	Liver failure		
	M 51	MDPV Bupropion	129 ng/mL (femoral blood) 388 ng/mg (liver) 191 ng/mL (vitreous humor) 24 ng/mL (femoral blood)	Morphine 40 (blood), diazepam 303 (blood), nordiazepam 229 (blood)	Liver failure		

	M 32	MDPV	102 ng/mL (peripheral blood) 133 ng/mL (heart blood), 0.136 ng/mg (brain), 0.256 ng/mg (liver), 205 ng/mL (vitreous humor), >200 ng/mL (bile), 6100 ng/mL (urine)	Dextromethorphan 60 (blood)	Liver failure		
	M 32	MDPV	36 ng/mL (peripheral blood) 56 ng/mL (heart blood), 0.148 ng/mg (brain), 0.668 ng/mg (liver), 130 ng/mL (vitreous humor)	None	Liver failure		
		JWH-122, JWH-210	-				
	F 53	MDPV	20000 ng/mg (brain), 0.432 ng/mg (liver), 140 ng/mL (bile), 48 ng/mL (vitreous humor), 0.08 ng/mg (spleen)	Morphine 212 (blood), alprazolam 15 (blood)	Liver failure		
	M 33	Pyrovalerone	42 ng/mL (femoral blood), 59 ng/mL (heart blood), 0.048 ng/mg (brain), 0.124 ng/mg (liver), 70 ng/mL (bile), 24 ng/mL (vitreous	Amphetamine <50 (blood)	Liver failure		

		Pentylone	humor) -				
	M 24	MDPV	640 ng/mL (femoral blood), 0.896 ng/mg (brain), 6.080 ng/mg (liver), 1880 ng/mL (bile), 940 ng/mL (vitreous humor)	None	Liver failure		
	M 28	α-PVP	174 ng/mL (blood), 401 ng/mL (urine), 0.292 ng/mg (brain), 0.19 ng/mg (liver), 0.122 ng/mg (kidney), 0.606 ng/mL (gastric contents)	None	AST 136 U/L, ALT 121.5 U/L, TB 2.4 mg/dL (0.13 mmol/L) & ALP 212 U/L Liver failure	2014 Poland	(8)
	M 29	N-ethylpentylone	-	Total morphine 40 (blood), & lidocaine	AST 12246 U/L, ALT 5975 U/L Liver failure	2017 USA	(42)
Synthetic phenylethylamines	M 31	25B-NBOMe & 25C-NBOMe	-	Ketamine, midazolam, & lorazepam,	AST 492 U/L & ALT 463 U/L Liver damage	2014 China	(50)
Synthetic aminoindanes	M 21	MDAI	-	None	AST 9541 U/L, TB 128 mg/dL (7.12 mmol/L) & ALP 42 U/L Liver failure	1990 UK	(52)
Synthetic tryptamines	M 22	AMT	2000 ng/mL (blood), 9600 ng/mL (gastric content), 24.7 ng/mg (liver), 7.8 ng/mg (brain)	None	Liver failure	2003 USA	(57)

5F-PB-22=1-(5-Fluoropentyl)-1H-indole-3-carboxylic acid 8-quinolinyl ester; 25B-NBOMe= 2-(4-Bromo-2,5-dimethoxyphenyl)-N-(2-methoxybenzyl)ethanamine; 25C-NBOMe= N-(2-methoxybenzyl)-2-(4-chloro-2,5-dimethoxyphenyl)ethanamine; α-PHP= alpha-Pyrrolidinohexiophenone; α-PVP= α-pyrrolidinovalerophenone; AB-CHMINACA=n-[(1S)-1-(aminocarbonyl)-2-methylpropyl]-1-(cyclohexylmethyl)-1h-indazole-3-carboxamide;ALP=alkaline phosphatase; ALT= alanine transaminase; AMT= alpha-methyltryptamine; AST= aspartate aminotransferase; DB= direct bilirubin; GGT= gamma-glutamyl transferase; IB= indirect bilirubin; JWH-122= (4-Methyl-1-naphthyl)-(1-pentylindol-3-yl)methanone; JWH-210= 4-Ethylnaphthalen-1-yl-(1-pentylindol-3-yl)methanone; MDAI=5,6-Methylenedioxy-2-aminoindane; MDMA= 3,4-methylenedioxymethamphetamine; MDPV=

Methylenedioxypyrovalerone; MPHP= 4'-methyl-alpha-pyrrolidinohexanophenone; THC-COOH= 11-Nor-9-carboxy-Δ9-tetrahydrocannabinol; TB= total bilirubin.

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Table 2. *In vitro* & *in vivo* synthetic new psychoactive substances (NPS) hepatotoxicity studies

	NPS	*Co-exposure*	*Study design*	*Hepatotoxic effects*	*Ref.*
Synthetic cannabinoids	WIN	-	*In vitro* HepG2 cells exposed for 72 h to 10 μM	↑ apoptosis & mitochondria depolarization Upregulation Bax, Bcl-Xs, t-Bid & PPARγ genes; Downregulation survivin, phospho-AKT, Hsp72 & Bcl2 genes	(75)
	JWH-122	-	*In vitro* HepG2 cells exposed for 24 h to 10-100 μM each compound alone	<100 μM no effects 100 μM ↑ DNA fragmentation, ↓ succinate dehydrogenase activity	(S1)
	JWH-073, JWH-210	-	*In vitro* HepG2 cells exposed for 24 h to 10-100 μM	<100 μM no effects 100 μM ↑ DNA fragmentation	
	JWH-018	-	*In vitro* HepG2 cells exposed for 24 h to 10-100 μM	<100 μM no effects 100 μM ↓ succinate dehydrogenase activity	
	AM-694	-	*In vitro* HepG2 cells exposed for 24 h to 10-100 μM	No effects	
	AB-FUBINACA	-	Wistar rats injected with 5 mg/kg X 5d. Day 5 liver examined	Upregulation of Map3K6, Abcd4, & Lss genes, Downregulation of Ha02 gene	(29)
	5F-PB-22	-	*In vitro* HepG2 cells exposed for 72 h to 1.95-125 μM	10 μM ↓ cell count, nuclear size, nuclear intensity, & mitochondrial membrane potential, ↑cytosolic calcium	(28)

				100 µM ↓↓ cell count, nuclear size, nuclear intensity, mitochondrial membrane potential, & plasma membrane integrity, ↑↑cytosolic calcium	
	JWH-200	With/without 100 µM ABT (non-specific CYP450 inhibitor)	*In vitro* HepG2 cells exposed for 72 h to 1.95-125 µM	1.95 µM ↓ mitochondrial membrane potential 125 µM ↑↑ cytotoxicity, ↑ nuclear size & intensity, & cytosolic calcium, ↓↓ cell count ABT ↓ plasma membrane integrity	(25)
	A-796260		*In vitro* HepG2 cells exposed for 72 h to 1.95-125 µM	1.95 µM ↓ mitochondrial membrane potential & cytosolic calcium 3.91 µM ↓ nuclear intensity 7.81 µM ↑ cell count No additional ABT effects	
	5F-EMB-PINACA		*In vitro* HepG2 cells exposed for 72 h to 1.95-125 µM	1.95 µM ↓mitochondrial membrane potential 1.95-7.81 µM ↑ mitochondrial membrane potential 7.81 µM ↓ cell count & mitochondrial membrane potential 7.81-125 µM ↑ cell count 125 µM ↓ cell count No additional ABT effects	
	4F-MDMB-BINACA	-	4 dpf Zebrafish larvae injected with 4.19 nL 5 mM	↓ liver dimension	(S2)
	XLR-11	-	10 M Balb/c mice, 3 mg/kg ip X 5d, Day 6 blood collected	↑oxidative stress, inflammation, lipid peroxidation, serum AST & ALT & apoptosis	(27)
	CUMYL-CBMICA	With/without 100 µM ABT (non-specific CYP450 inhibitor)	*In vitro* HepG2 cells exposed for 48 h to 7.81 & 125 µM	7.81µM ↓ nuclear intensity, mitochondrial membrane potential & cytosolic calcium 125 µM ↓↓ nuclear intensity, mitochondrial membrane potential & cytosolic calcium No additional ABT effects	(26)
	4CN-CUMYL-BINACA	With/without 100 µM ABT (non-specific CYP450 inhibitor)	*In vitro* HepG2 cells exposed for 48 h to 7.81 & 125 µM	7.81µM ↓ mitochondrial membrane potential 125 µM ↓↓ mitochondrial membrane potential No additional ABT effects	

Synthetic opioids	Tramadol, Tapentadol	-	10, 25 or 50 mg/kg tramadol or tapentadol ip Wistar rats. Urine, blood & liver collected after 24 h	↓ GFR, urine urea, & BuChE activity, serum urea, & liver glycogen ↑ urine proteins, & ALT, serum lipids, & liver steatosis, liver inflammation &liver vacuolization	(32)
	Tramadol, Tapentadol	-	10, 25 or 50 mg/kg tramadol or tapentadol ip Wistar rats X 14d for each drug, Day 14 urine, serum & liver collected	↑ ALT, AST, ALP, GGT & serum lipids, serum iron, ferritin, haptoglobin & HO-1, & liver steatosis, liver inflammation & liver vacuolization ↓ BuChE activity, complement C3 &C4, serum albumin & serum urea	(33)
	4F-iBF, iBF 4Cl-iBF	- -	M F344/Jcl rats, 5 mg/kg, qd X2d, Day 2 blood & liver collected M F344/Jcl rats, 5 mg/kg, q.d. X2d, Day 2 blood & liver collected	↑ ALT, AST, UN, creatinine, & inflammation & necrosis ↑ UN, no histopathological effects	(31)
Synthetic cathinones	Methylone Butylone Pentylone 3,4-MD-N-Benzylcathinone MDPPP MDPBP MDPV	Cocktail A & B	Each drug incubated separately with 100 µM HLM, & with cocktail A (12 µM phenacetin (CYP 1A2 inhibitor), 1.1 µM coumarin (CYP 2A6 inhibitor), 3.5 µM diclofenac (CYP 2C9 inhibitor), 8.5 µM dextromethorphan (CYP 2D6 inhibitor), 86 µM testosterone (CYP 3A4/5)) & cocktail B (30 µM bupropion (CYP 2B6 inhibitor), 2 µM amodiaquine (CYP 2C8 inhibitor), 21 µM omeprazole (CYP 2C19 inhibitor), & 20 µM chlorzoxazone (CYP 2E1 inhibitor))	↓ CYP2D6 & CYP2B6 activities ↓ CYP2D6, CYP1A2 & CYP2B6 activities ↓ CYP2D6, CYP2B6, CYP2C19 & CYP3A activities ↓ CYP2D6 & CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2E1 & CYP3A activities ↓ CYP2D6 & CYP2B6 activities ↓ CYP2D6, CYP2B6 & CYP1A2 activities ↓ CYP2D6, CYP1A2 & CYP2B6 activities	(74)

	Pentedrone, MDPV	-	*In vitro* PRH exposed for 48h to 0.05, 0.1, 0.2, 0.5,0.8, 1.0, 1.2, 1.6, 2.0, 2.5, 3.0, 4.0, 5.0 mM each drug	↑↑ cell death	(S3)
	Methylone, 4-MEC Bloom (Methylone, 4-MEC, Pentedrone, Dimethocaine & Isopentedrone), Blow (4-MEC, MDPV, & 3-MEC)	-	*In vitro* PRH exposed for 48h to 0.05, 0.1, 0.2, 0.5,0.8, 1.0, 1.2, 1.6, 2.0, 2.5, 3.0, 4.0, 5.0 mM each mix	↑ cell death	
	MDPV	-	*In vitro* PRH exposed for 48 h to 0.2-1.6 mM at 37°C & 40.5°C	37°C ↑ cytotoxicity, cell death, ROS & RNS, cytosolic calcium & caspases 3, 8 & 9 activities ↓ intracellular ATP & GSH 40.5°C ↑↑ cytotoxicity, cell death, ROS & RNS, cytosolic calcium & caspases 3, 8, & 9 activities ↓↓ intracellular ATP & GSH	(43)
	Pentedrone, MDPV, 4-MEC	PRH with/without 10 µM quinidine (CYP2D6 inhibitor), 1mM AA, 1mM NAC, or 100 mM Ac-LETD-CHO	*In vitro* HepaRG & PRH exposed for 24 h to 0.05-10 mM or 20 mM	↑↑ ROS & RNS, & apoptosis, ↓↓ GSH, & intracellular ATP CYP2D6 inhibitor ↓ cell death AA ↓ cell death NAC ↓ cell death Ac-LETD-CHO ↓ cell death	(44)
	Methylone	PRH with/without 10 µM quinidine (CYP2D6 inhibitor), 1mM AA, 1mM NAC, or 100 mM Ac-LETD-CHO	*In vitro* HepaRG & PRH exposed for 24 h to 0.05-10 mM or 20 mM	↑ ROS & RNS, & apoptosis, ↓ GSH, & intracellular ATP CYP2D6 inhibitor ↓ cell death AA ↓ cell death NAC ↓ cell death Ac-LETD-CHO ↓ cell death	

	MDPV	-	*In vitro* PRH exposed for 24 h & 48 h to 0.2-1.6 mM racemic solution & each enantiomer	↑ cell death, ↓ LDH No enantioselectivity effects	(S4)
	Mephedrone	Morphine hydrochloride 5.0 mg/kg	Day P30 Wistar rats 10 mg/kg ip X 7d. Day P60, morphine ip for 3d & liver collected	↑ adipose degeneration & steatosis	(S5)
	Bupropion, MDPV, Mephedrone, Naphyrone	-	*In vitro* HepaRG & HepG2 exposed for 24 h to 0.01 mM, 0.1 mM, 1 mM, & 2 mM each drug	0.2-1 Mm ↑↑ cytotoxicity, ROS & RNS, & lactate, ↓↓ GSH, & mitochondrial membrane potential, intracellular ATP, complex I & II activity	(S6)
	Methedrone, Methylone	-	*In vitro* HepaRG & HepG2 exposed for 24 h to 0.01 mM, 0.1 mM, 1 mM, & 2 mM each drug	≥2 mM ↑ cytotoxicity, ↓ intracellular ATP	
	Mephedrone	-	*In vivo* Naïve Swiss M mice 1.0, 2.5, 5.0, & 10.0 mg/kg ip. Liver collected after 1 h	↑ oxidative stress, & malondialdehyde, ↓ total antioxidant capacity & ascorbic acid	(S7)
	Mephedrone, Methylone, 4-MEC, MDPV	-	*In vitro* HepG2 cells exposed for 72 h to 1.95-125 µM	10 µM ↓ nuclear size & intensity, cell count, & mitochondrial membrane potential, ↑ cytosolic calcium 100 µM ↓↓ nuclear size & intensity, cell count, mitochondrial membrane potential, & plasma membrane integrity, ↑↑ cytosolic calcium	(28)
	DMB, DMP, Mephedrone, 3,4-DMMC, 4-MDMC, NEB	-	*In vitro* HepG2 exposed for 24 h to 0.05-18 mM each drug	0.8-1.28 mM ↑cytotoxicity & cell death	(46)
	Methcathinone, Buphedrone, Pentedrone, DMC, NEC, 4-MEC, NEP, DEC, 4-MDEC,	-	*In vitro* HepG2 exposed for 24 h to 0.05-18 mM each drug	≥2 mM ↑cytotoxicity & cell death	

	DEB, DEP, PPP, MPPP, PBP, α-PVP				
	3-MMC	With/without 500 μM metyrapone (CYP2E1 inhibitor), 10 μM quinidine (CYP2D6 inhibitor), 1 μM ketoconazole (CYP3A4 inhibitor), & 1 mM ABT (non-specific CYP450 inhibitor)	*In vitro* PRH exposed with 31 nM-10 mM for 24 h	10 μM ↑ caspases 3, 8 & 9 activities ≥10 μM ↑ ROS & RNS, apoptosis, necrosis & autophagy, ↓ GSH, intracellular ATP, & caspases 3, 8 & 9 activities CYP2E1 & CYP2D6 inhibitors ↓ cytotoxicity No additional CYP3A4 inhibitor & ABT effects	(72)
	MDPV	-	M CD-1 mice 2.5 or 5 mg/kg ip every 2 h times 3 After 24 h blood, urine & liver collected	↑ AST, ALT, LDH, total CK, creatinine, uric acid, BUN, GSSG, linoleic acid, hypoxanthine & xanthine, micro- & macrovesicular steatosis, sinusoidal dilatation & necrosis ↓ALP, &ATP & GSH, pyruvic, lactic, citric, malic, ascorbic & arachidonic acids, glycine & cysteine, 5'methyl-adenosine & adenosine-5'-monophosphate	(S8)
	MDPV	-	*In vitro* PMH exposed for 24 h to 70, 240 & 477 μM at 37°C & 40.5°C	37°C ↑ cell death, aspartate, glutamate & butanoate metabolism dysregulation, phenylalanine & tyrosine biosynthesis dysregulation, aminoacyl-tRNA biosynthesis dysregulation, ↓ LDH 40.5°C ↑↑ cell death, aspartate, glutamate & butanoate metabolism dysregulation, phenylalanine & tyrosine biosynthesis dysregulation, aminoacyl-tRNA biosynthesis dysregulation, ↓↓ LDH	(34)

	α-PEP	-	*In vitro* HepaRG exposed for 24 h to 12.5 or 25 μM each drug	↑cholesterol sulfate & 25-hydroxy-cholesterol	(S9)
	α-PBP		*In vitro* HepaRG exposed for 24 h to 12.5 or 25 μM each drug	↑ cytotoxicity & N-methylnicotinamide	
	3,4-DMMC	With/without 500 μM metyrapone (CYP2E1 inhibitor), 10 μM quinidine (CYP2D6 inhibitor), 1 μM ketoconazole (CYP3A4 inhibitor), 1 mM ABT (non-specific CYP450 inhibitor)	PRH exposed for 24 h to 1.10 μM–0.7 mM, HepG2 exposed for 24 h to 58.4 μM–2 mM, & HepaRG exposed for 24 h to 87.8 μM–2 mM	↑ cytotoxicity, cell death, ROS &RNS, GSSG, apoptosis, & caspase-8, -9, & -3 activities, autophagy ↓ GSH, LDH, ATP, mitochondrial membrane potential, & plasma membrane integrity CYP inhibitors ↑↑ cytotoxicity	(45)
	Butylone		PRH exposed for 24 h to 38 μM–10 mM, HepG2 exposed for 24 h to 1.59–20 mM, & HepaRG exposed for 24 h to 9.60 μM–18.2 mM	↑ cytotoxicity, cell death, ROS &RNS, GSSG, apoptosis, & caspase-8, -9, & -3 activities, autophagy ↓ GSH, LDH, ATP, mitochondrial membrane potential, & plasma membrane integrity CYP inhibitors ↑↑ cytotoxicity	
	Buphedrone		PRH exposed for 24 h to 37.7 μM–10 mM, HepG2 exposed for 24 h to 625 μM–32 mM, & HepaRG exposed for 24 h to 882 μM–32 mM	↑ cytotoxicity, cell death, ROS &RNS, GSSG, apoptosis, & caspase-8, -9, & -3 activities, autophagy ↓ GSH, LDH, ATP, mitochondrial membrane potential, & plasma membrane integrity CYP inhibitors ↑↑ cytotoxicity	
	Dibutylone	With/without 100 μM ABT (non-specific CYP450 inhibitor)	*In vitro* HepG2 exposed for 48 h to 7.81 & 125 μM each drug	7.81 μM ↓mitochondrial membrane potential, & nuclear size No additional ABT effects	(26)
	Ephylone (N-ethylpentylone)	With/without 100 μM ABT (non-specific CYP450 inhibitor)	*In vitro* HepG2 exposed for 48 h to 7.81 & 125 μM each drug	7.81 μM ↓ nuclear size, & cytosolic calcium 125 μM ↓↓ nuclear size No additional ABT effects	

	4-MEAP	With/without 100 µM ABT (non-specific CYP450 inhibitor)	*In vitro* HepG2 exposed for 48 h to 7.81 & 125 µM	7.81 µM ↓ mitochondrial membrane potential No additional ABT effects	
Synthetic amphetamines	2,3-MDA 3,4-BDB 2,3-MMBDB 3,4-BDB-N-Isopropyl 3,4-MDA,3,4-MDMA, 3,4-MDEA, 2,3-BDB, 2,3-MBDB, 3,4-MBDB, 2,3-EBDB, M-Alpha	Cocktail A & B	Each drug incubated separately with 100 µM HLM, & with cocktail A (12 µM phenacetin (CYP 1A2 inhibitor), 1.1 µM coumarin (CYP 2A6 inhibitor), 3.5 µM diclofenac (CYP 2C9 inhibitor), 8.5 µM dextromethorphan (CYP 2D6 inhibitor), 86 µM testosterone (CYP 3A4/5)) & cocktail B (30 µM bupropion (CYP 2B6 inhibitor), 2 µM amodiaquine (CYP 2C8 inhibitor), 21 µM omeprazole (CYP 2C19 inhibitor), & 20 µM chlorzoxazone (CYP 2E1 inhibitor))	↓ CYP2A6, CYP2B6, CYP2D6, CYP2C19, CYP3A activities ↓ CYP2B6, CYP2D6, CYP2C19, CYP3A activities ↓ CYP2B6, CYP2D6, CYP2C19 activities ↓ CYP1A2, CYP2D6, CYP2B6 activities ↓ CYP2D6 activities	(74)
	4-FMA	With/without 10 µM quinidine (CYP2D6 inhibitor), 1 µM ketoconazole (CYP3A4 inhibitor), 500 µM, metyrapone (CYP2E1 inhibitor), & 1 mM ABT (non-specific CYP450 inhibitor)	*In vitro* PRH exposed to 37 µM-10 mM, HepaRG & HepG2 exposed to 1-30 mM for 24 h	↑ ROS & RNS, GSSG, & apoptosis & necrosis ↓ GSH, ATP, mitochondrial membrane potential, & plasma membrane integrity; CYP2D6 & CYP3A4 inhibitors↑ cytotoxicity CYP2E1 inhibitor↓ cytotoxicity No additional ABT effects	(49)

Synthetic phenylethylamines	25C-NBOMe, 25I-NBOMe	With/without 100 µM ABT (non-specific CYP450 inhibitor)	*In vitro* HepG2 exposed for 48 h to 7.81 & 125 µM each drug	7.81 µM ↓ cell count 125 µM ↓↓ cell count No additional ABT effects	(26)
Synthetic aminoindanes	MDAI	Cocktail A & B	Each drug incubated separately with 100 µM HLM, & with cocktail A (12 µM phenacetin (CYP 1A2 inhibitor), 1.1 µM coumarin (CYP 2A6 inhibitor), 3.5 µM diclofenac (CYP 2C9 inhibitor), 8.5 µM dextromethorphan (CYP 2D6 inhibitor), 86 µM testosterone (CYP 3A4/5)) & cocktail B (30 µM bupropion (CYP 2B6 inhibitor), 2 µM amodiaquine (CYP 2C8 inhibitor), 21 µM omeprazole (CYP 2C19 inhibitor), & 20 µM chlorzoxazone (CYP 2E1 inhibitor))	↓ CYP2D6, CYP1A2 & CYP2A6 activities	(74)
	MDAI	-	*In vitro* HepG2 exposed for 72 h to 10 & 100 µM	10 µM ↑ cytosolic calcium, ↓mitochondrial membrane potential 100 µM ↑ cytosolic calcium, ↓↓ cell count, nuclear size, nuclear intensity, & mitochondrial membrane potential	(28)
Synthetic benzofurans	5-APB 5-MAPB, 6-APB, 6-MAPB	Cocktail A & B	Each drug incubated separately with 100 µM HLM, & with cocktail A (12 µM phenacetin (CYP 1A2 inhibitor), 1.1 µM coumarin (CYP 2A6 inhibitor), 3.5 µM diclofenac (CYP 2C9 inhibitor), 8.5 µM dextromethorphan (CYP 2D6 inhibitor), 86 µM testosterone	↓ CYP2D6 & CYP3A activities ↓ CYP2D6 activities	(74)

			(CYP 3A4/5)) & cocktail B (30 μM bupropion (CYP 2B6 inhibitor), 2 μM amodiaquine (CYP 2C8 inhibitor), 21 μM omeprazole (CYP 2C19 inhibitor), & 20 μM chlorzoxazone (CYP 2E1 inhibitor))		
	5-APB, 5-MAPB	-	*In vitro* PRH exposed for 3 h to 4 mM	↑cell death, & ROS & RNS, GSSG, ↓ ATP, GSH, & mitochondrial membrane potential	(53)
	5-APB 6-APB	PRH with/without 500 μM metyrapone (CYP2E1 inhibitor), 10 μM quinidine (CYP2D6 inhibitor), 1 μM ketoconazole (CYP3A4 inhibitor), & 1 mM ABT (non-specific CYP450 inhibitor)	PRH exposed for 24 h to 37.7 μM-10 mM HepaRG exposed for 24 h to 110 μM-6.50 mM HepG2 exposed for 24 h to 293 μM-20 mM PRH exposed for 24 h to 37.7 μM-23 mM HepaRG exposed for 24 h to 1.17 μM-14 mM HepG2 exposed for 24 h to 192 μM-30 mM	↑ ROS & RNS, autophagy, apoptosis, & caspases 3 & 9 activities ↓ tGSH, LDH, mitochondrial membrane potential, intracellular ATP, & plasma membrane integrity; CYP3A4 & ABT inhibitors ↑↑ cytotoxicity CYP2D6 & CYP2E1 inhibitors ↑ cytotoxicity ↑ ROS & RNS, autophagy, apoptosis, & caspases 3 & 9 activities ↓ tGSH, LDH, mitochondrial membrane potential, intracellular ATP, & plasma membrane integrity; CYP3A4 & ABT inhibitors ↑↑ cytotoxicity CYP2D6 & CYP2E1 inhibitors ↑ cytotoxicity	(S10)
Synthetic tryptamines	5,6-MD-DALT	Cocktail A & B	Each drug incubated separately with 100 μM HLM, & with cocktail A (12 μM phenacetin (CYP 1A2 inhibitor), 1.1 μM coumarin (CYP 2A6 inhibitor), 3.5 μM diclofenac (CYP 2C9 inhibitor), 8.5 μM dextromethorphan (CYP 2D6 inhibitor), 86 μM testosterone (CYP 3A4/5)) & cocktail B (30 μM bupropion (CYP 2B6 inhibitor), 2 μM amodiaquine	↓ CYP2D6, CYP1A2, CYP2B6, CYP2C9, CYP2C19 & CYP3A activities	(74)

			(CYP 2C8 inhibitor), 21 µM omeprazole (CYP 2C19 inhibitor), & 20 µM chlorzoxazone (CYP 2E1 inhibitor))		
	5-MeO-MiPT	-	F CD1 mice 0.27 or 2.7 mg/kg ip. After 1 h liver collected	↑ caspase 3 & 8 activities No morphological changes	(58)
	5-MeO-MiPT	With/without 100 µM ABT (non-specific CYP450 inhibitor)	*In vitro* HepG2 exposed for 48 h to 7.81 & 125 µM	7.81 µM ↓ mitochondrial membrane potential & plasma membrane integrity 125 µM ↑ nuclear size No additional ABT effects	(26)
	5-MeO-DALT	With/without 100 µM ABT (non-specific CYP450 inhibitor)	*In vitro* HepG2 exposed for 48 h to 7.81 & 125 µM	125 µM ↓ mitochondrial membrane potential, ↑ nuclear size No additional ABT effects	
Synthetic piperazines	MDBZP	Cocktail A & B	Each drug incubated separately with 100 µM HLM, & with cocktail A (12 µM phenacetin (CYP 1A2 inhibitor), 1.1 µM coumarin (CYP 2A6 inhibitor), 3.5 µM diclofenac (CYP 2C9 inhibitor), 8.5 µM dextromethorphan (CYP 2D6 inhibitor), 86 µM testosterone (CYP 3A4/5)) & cocktail B (30 µM bupropion (CYP 2B6 inhibitor), 2 µM amodiaquine (CYP 2C8 inhibitor), 21 µM omeprazole (CYP 2C19 inhibitor), & 20 µM chlorzoxazone (CYP 2E1 inhibitor))	↓ CYP2D6 & CYP2B6 activities	(74)

	TFMPP	PRH with/without 500 µM metyrapone (non-selective CYP450 inhibitor) or 100 µM quinidine (CYP2D6 inhibitor)	PRH exposed for 24 h to 0.4 µM-2.5 mM, HepaRG exposed for 24 h to 1 µM-3 mM, HepG2 exposed for 24 h to 4.6 µM-3 mM	↑ cytotoxicity, ROS & RNS, GSSG, apoptosis, & caspase 3 activity, ↓ GSH, ATP, mitochondrial membrane potential; CYP450 & CYP2D6 inhibitor s↑ cytotoxicity	(62)
	MeOPP		PRH exposed for 24 h to 2.22 µM-20 mM, HepaRG exposed for 24 h to 3 µM-20 mM, HepG2 exposed for 24 h to 51.4 µM-16.36 mM	↑ cytotoxicity, ROS & RNS, GSSG, apoptosis, & caspase 3 activity, ↓ GSH, ATP, mitochondrial membrane potential; CYP2D6 inhibitors ↑ cytotoxicity	
	BZP		PRH exposed for 24 h to 45 µM-40 mM, HepaRG exposed for 24 h to 14 µM-40 mM, HepG2 exposed for 24 h to 39 µM-35 mM	↑ cytotoxicity, ROS & RNS, GSSG, apoptosis, & caspase 3 activity, ↓ GSH, ATP, mitochondrial membrane potential; CYP2E1 & CYP2D6 inhibitors ↑ cytotoxicity	
	MDBP		PRH exposed for 24 h to 2.22 µM-20 mM, HepaRG exposed for 24 h to 3 µM-20 mM, HepG2 exposed for 24 h to 68.5 µM-30 mM	↑ cytotoxicity, ROS & RNS, GSSG, apoptosis, & caspase 3 activity, ↓ GSH, ATP, mitochondrial membrane potential; CYP2E1 & CYP2D6 inhibitors ↑ cytotoxicity	
	BZP & TFMPP mixtures	-	*In vitro* PRH & HepaRG exposed for 24 h to Mix A (94% + 6%), or Mix B (93% + 7%), or Mix C (67% + 33%)	Mix A & C↑ RNS & ROS, GSSG ↓ GSH, ATP, & mitochondrial membrane potential Mix B ↑↑ RNS & ROS, GSSG ↓↓ GSH, ATP, & mitochondrial membrane potential	(61)
	BZP, MDBP, TFMPP, MeOPP	-	*In vitro* PRH exposed for 72 h to 0-20 mM each drug	Upregulation sterol C4-methyloxidase, isopentyl-diphosphate-Δ-isomerase, Cyp51A1, squalene epoxidase & farnesyl diphosphate synthase, glycoprotein transmembrane nmb, & fatty acid desaturase 1, SREBP-1 genes Downregulation betaine-homocysteine-S-methyltransferase 2 genes	(63)

Synthetic piperidines	Street-MXP Synthesized MXP	-	*In vitro* HepG2 exposed for 72 h to 0-1 mM each drug	↑↑ cytotoxicity, cell death, & apoptosis ↑ cytotoxicity, cell death, & apoptosis	(66)

Incubation temperature 37°C where not specified; AA: ascorbic acid; Abcd4: ATP-binding cassette sub-family D member 4; ABT: 1-Aminobenzotriazole; Ac-LETD-CHO: N-acetyl-Leu-Glu-Thr-Asp-aldehyde; AKT:serine/threonine protein kinase; ALP: Alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; Bax: Bcl-2-associated X protein; Bcl2: B-cell lymphoma 2; Bcl-xs:B-cell lymphoma-extra small; BuChE: butyrylcholinesterase; BUN: blood urea nitrogen; CK: creatine kinase; d: day; Day Px: postnatal day; dpf: days post fertilization; GFR: glomerular filtration rate; GGT: ϒ-glutamyl-transferase; Glycoprotein transmembrane nmb: Glycoprotein nonmetastatic melanoma protein B; GSH: L-glutathione reduced; GSSG: L-glutathione oxidized disodium salt; Hao2: Hydroxyacid oxidase 2; HO-1: heme oxygenase 1; HLM: human liver microsomes; ip: intraperitoneal injection; LDH:lactate dehydrogenase; Lss: Lanosterol Synthase; t-Bid:truncated interacting domain death agonist; Map3k6:Mitogen-activated protein kinase kinase kinase 6; NAC: N-acetyl-L-cysteine; Phospho-Hsp72: phosphorylated heat shock protein 72; PPARγ: Peroxisome proliferator-activated receptor gamma; PRH: primary rat hepatocytes; qd: once a day; SREBP-1: sterol regulatory element binding protein; UN: urea nitrogen.
2,3-BDB: 1-(2,3-methylendioxyphenyl)-2-butamine; 2,3-EBDB: 2-ethylamino-1-(2,3-methyledioxyphenyl)butane; 2,3-MBDB: 2-methylamino-1-(2,3-methyledioxyphenyl)butane; 2,3-MDA: 2,3-Methylenedioxyamphetamine; 2,3-MMBDB: 2-dimethylamino-1-(2,3-methyledioxyphenyl)butane; 3-MMC: 3-Methylmethcathinone; 3,4-BDB: 3,4-methylenedioxy-α-ethylphenethylamine;3,4-DMMC: 3,4-Dimethylmethcathinone; 3,4-MBDB: 2-methylamino-1-(3,4-methyledioxyphenyl)butane; 3,4-MDA: N-Ethyl-alpha-methyl-1,3-benzodioxole-5-ethanamine hydrochloride; 3,4-MDEA: 3,4-Methylenedioxy-N-ethylamphetamine; 3,4-MDMA: 3,4-Methylenedioxymethamphetamine; 3,4-MD-N-Benzylcathinone: 3,4-Methylenedioxy-N-benzylcathinone; 4Cl-iBF: 4-chloroisobutyrylfentanyl; 4CN-CUMYL-BINACA: 1-(4-cyanobutyl)-N-(1-methyl-1-phenylethyl)-1H-indazole-3-carboxamide; 4F-iBF: 4-fluoro-isobutyrylfentanyl; 4-FMA: 4-Fluoromethamphetamine; 4F-MDMB-BINACA: N-[[1-(4-fluorobutyl)-1H-indazol-3-yl]carbonyl]-3-methyl-L-valine, methyl ester; 4-MDEC: 4-methyl-N,N-diethylcathinone; 4-MDMC: 4'-methyl- N,N-dimethylcathinone; 4-MEAP: 4-Methyl-alpha-ethylaminopentiophenone; 4-MEC: 4'-methyl-N-ethylcathinone; 5F-EMB-PINACA: ethyl(1-(5-fluoropentyl)-1H-indazole-3-carbonyl)-L-valinate; 5F-PB-22: 1-(5-Fluoropentyl)-1H-indole-3-carboxylic acid 8-quinolinyl ester; 5-MeO-DALT: N,N-Diallyl-5-methoxytryptamine; 5-MeO-MiPT: 5-Methoxy-N-methyl-N-isopropyltryptamine; 5-APB: 5-(2-aminopropyl)benzofuran; 5-MAPB: 1-(benzofuran-5-yl)-N-Methylpropan-2-aMine; 5,6-MD-DALT: 5,6-methylenedioxy-DALT; 6-APB: 6-(2-aminopropyl)benzofuran; 6-MAPB: 6-[2-(Methylamino)propyl]benzofuran; 7'N-5F-ADB: methyl 2-(1-(5-fluoropentyl)-1H-pyrrolo[2,3-b]pyridine-3-carboxamido)-3,3-dimethylbutanoate; 25C-NBOMe: N-(2-methoxybenzyl)-2-(4-chloro-2,5-dimethoxyphenyl)ethanamine; 25I-NBOMe: 2-(4-Iodo-2,5-dimethoxyphenyl)-N-((2-methoxyphenyl)methyl)ethanamine; α-PBP: α-pyrrolidinobutiophenone; α-PEP: α-pyrrolidinoheptaphenone; α-PVP: α-pyrrolidinovalerophenone; A-796260: (1-(2-morpholinoethyl)-1H-indol-3-yl)(2,2,3,3-tetramethylcyclopropyl)methanone; AB-FUBINACA: N-[(1S)-1-(aminocarbonyl)-2-methylpropyl]-1-[(4-fluorophenyl)methyl]-1H-indazole-3-carboxamide; AM-694: 1-(5-Fluoropentyl)-3-(2-iodobenzoyl)indole; Buphedrone: (R,S)-2-(methylamino)-1-phenylbutan-1-one; BZP: N-benzylpiperazine; CUMYL-CBMICA: 1H-Indole-3-carboxamide; DEB: N,N-diethylbuphedrone; DEC: N,N-diethylcathinone; DEP: N,N-diethylpentedrone;DMB: N,N-dimethylbuphedrone; DMC: N,N-dimethylcathinone; DMP: N,N-dimethylpentedrone; iBF: isobutyrylfentanyl; JWH-018: 1-Pentyl-3-(1-naphthoyl)indole; JWH-073: 1-Butyl-3-(1-naphthoyl)indole; JWH-122: (4-Methyl-1-naphthyl)-(1-pentylindol-3-yl)methanone; JWH-200: 1-(2-(4-Morpholinyl)ethyl)-3-(1-naphthoyl) indole; JWH-210: 4-Ethylnaphthalen-1-yl-(1-pentylindol-3-yl)methanone; MDAI:5,6-Methylenedioxy-2-aminoindane; M-Alpha: 1-Methylamino-1-(3,4-methylenedioxyphenyl)propane; MDBP: 1-(3,4-

methylenedioxybenzyl)piperazine; MDBZP: 1-Piperonylpiperazine; MDPBP: 3,4-methylenedioxy-alpha-pyrrolidinobutiophenone; MDPPP: 3,4-Methylenedioxy-alpha-Pyrrolidinopropiophenone; MDPV: Methylenedioxypyrovalerone; MeOPP: 1-(4-methoxyphenyl)piperazine; Methcathinone: (R,S)-2-(methylamino)-1-phenylpropan-1-one; MPPP: 4'-methyl-α-pyrrolidinopropiophenone; MXP: Methoxphenidine; NEB: N-ethylbuphedrone; NEC: N-ethylcathinone; NEP: N-ethylpentedrone; PBP: α-pyrrolidinobutiophenone; Pentedrone: (R,S)-2-(methylamino)-1-phenylpentan-1-one; PPP: α-pyrrolidinopropiophenone; TFMPP: 1-(3-trifluoromethylphenyl)piperazine; WIN: (R-(þ)-(2,3-dihydro-5-methyl-3-[(4-morpholinyl)methyl]pyrol[1,2,3-de]-1,4-benzoxazin-6-yl)-(1-naphthalenyl) methanone mesylate); XLR-11: (1-(5-Fluoropentyl)-1H-indol-3-yl)-(2,2,3,3-tetramethylcyclopropyl)methanone.

Figure Legend

Figure 1. Number of internationally controlled drugs in 2022 & new psychoactive substances (NPS) identified by Member States at the global level, 2005-2022
Source: UNODC World Drug Report 2023 https://www.unodc.org/unodc/en/data-and-analysis/wdr-2023-online-segment.html. Access August 2023. The figure was adapted from the original source

Figure 2 Prisma literature search flowchart.

Figure 3. Basic chemical structure and examples of derivatives for each NPS class. a, cannabinoid; a_1, a_2, synthetic cannabinoids; b, opioid, b_1, b_2, synthetic opioids; c, cathinone, c_1, c_2, synthetic cathinones; d, amphetamine, d_1, d_2, synthetic amphetamines; e, phenylethylamine, e_1, e_2, synthetic phenylethylamines; f, aminoindane, f_1, f_2, synthetic aminoindane; g, tryptamine, g_1, g_2, synthetic tryptamines; h, piperazine, h_1, h_2, synthetic piperazines; i, piperidine, i_1, i_2, synthetic piperidine; j, benzofuran, j_1, j_1, synthetic benzofurans.

AB-FUBINACA: N-[(1S)-1-(aminocarbonyl)-2-methylpropyl]-1-[(4-fluorophenyl)methyl]-1H-indazole-3-carboxamide; CUMYL-CBMICA: 1H-Indole-3-carboxamide; iBF: isobutyrylfentanyl; 4-MEC: 4'-methyl-N-ethylcathinone; 4-FMA: 4-Fluoromethamphetamine; 3,4-MDEA: 3,4-Methylenedioxy-N-ethylamphetamine; 25C-NBOMe: N-(2-methoxybenzyl)-2-(4-chloro-2,5-dimethoxyphenyl)ethanamine; 25I-NBOMe: 2-(4-Iodo-2,5-dimethoxyphenyl)-N-((2-methoxyphenyl)methyl)ethanamine; MDAI:5,6-Methylenedioxy-2-aminoindane; NM2AI: N-Methyl-2-Aminoindane; 5-MeO-DALT: N,N-Diallyl-5-methoxytryptamine; 5-MeO-MiPT: 5-Methoxy-N-methyl-N-isopropyltryptamine; BZP: N-benzylpiperazine; MeOPP: 1-(4-methoxyphenyl)piperazine; MXP: Methoxphenidine; 5-APB: 5-(2-aminopropyl)benzofuran; 5-MAPB: 1-(benzofuran-5-yl)-N-Methylpropan-2-aMine