

# Untangling the Intracellular Mechanisms Underlying Toxicity of Drugs of Abuse: A Review of the Detrimental Effects of New Psychoactive Substances (NPS)



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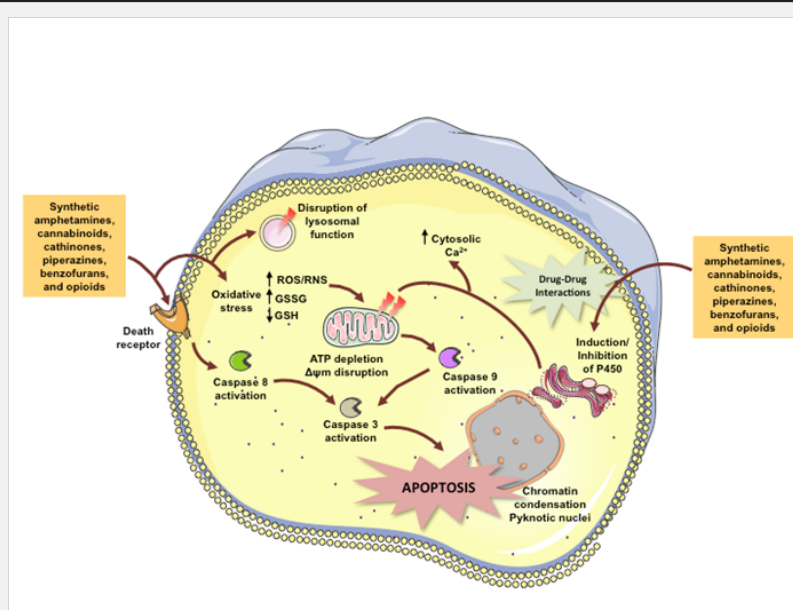
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## Mini Review

The recreational drug world is continuously evolving. Of note, the number of new psychoactive substances (NPS) reported for the first time has spread year on year. In 2016, 66 NPS were notified to the European early-warning system [1], reflecting the fast pace at which new drugs are introduced onto the market (over one per week). By the end of 2016, the European authorities were monitoring more than 620 NPS, compared with 350 in 2013 [1]. This fast-paced surge embodies a major challenge for (inter)national policy-makers due to the inefficient monitoring techniques, relevant social and life-threatening impact, and inadequate response by health care providers.

NPS are mainly of synthetic origin and comprise distinct drug classes, cannabinoids, cathinones, piperazines, benzofurans,

and opioids, being the most prevalent [1-14]. Many NPS are analogues of existing controlled drugs or pharmaceuticals initially developed as pharmacological research tools or potential therapeutic agents; but often designer molecules displaying completely distinct structures have also emerged [1-17]. Most NPS are often used in combination [1,3-6,8,9,12,13,15-18]. Although NPS are intended to mimic psychoactive effects of classic drugs (ecstasy, cannabis, heroin, etc.), they pose especially serious concerns for public health as they are endowed with noticeably higher potency. Clinical signs of intoxication are consistent with the pharmacological mechanisms of the corresponding classic congeners, but often NPS more strongly bind and activate biological targets, widening the severity and assortment of deleterious effects [1,3,4,6,8,9,11-13,15-17].



**Figure 1:** Schematic representation of putative intracellular mechanistic pathways sparked by New Psychoactive Substances (NPS).

While the number of NPS-related poisonings and deaths peaked at unparalleled alarming levels [1,3,9,11-13,15-17], scarce information is available on toxicological mechanisms and toxicokinetic profiles of most novel drugs. Recent *in vitro* works have shed some light on the molecular pathways implicit in NPS detrimental effects, for which much contributed my team's work over the last years [19-27]. Our research provided compelling evidence that a plethora of NPS related to piperazine [20,21,23-25,27], cathinone [19,22,26] and benzofuran [22] disrupt oxidative and energetic intracellular homeostasis and mitochondrial functioning, culminating on the activation of cell death pathways (Figure 1). Studies on the metabolism of some NPS displaying a methylenedioxy ring revealed analogies to those of respective classic drugs, implicating metabolic bioactivation as a source of ROS and other reactive compounds, including metabolites that exhibit higher toxicity than the parent drug [28]. Related toxicity mechanisms are therefore putatively expected for those NPS exhibiting similar structural backbones.

In agreement with several reports of clinical complications following co-abuse of NPS [4-6,8,9,13,15-18], another relevant toxicokinetic expectation stems from the occurrence of drug-drug interactions, as several classic drugs [29-35] and some NPS [19,23,24] have already proved to drastically exacerbate each others' toxicity when combined, even at non-toxic single doses.

A few years ago, most readily accessible information regarding the effects of NPS was originated from anecdotal reports from users posted to web-based drug discussion forums, and from clinical and forensic reports of deaths or severe injury. In spite of the significant recent advances on knowledge to the field, the toxicological profile of the majority of NPS remains far from being fully elucidated and studies are constantly required to assist interventions by law enforcement authorities, forensic laboratories, and medical providers. In this line, novel information on NPS pharmacokinetic properties and detection methods is imperative for the early detection of these drugs, clinical diagnosis of their intoxications, as well as to obtain and interpret forensic results. Also, the disclosure of the toxicological mechanisms involved in the detrimental NPS effects might provide relevant insights into the reasons behind occurrence of such extreme toxicity, potentiating the identification of therapeutic targets. All together, these aspects may embody an outstanding social impact by allowing estimations on the risk of drug threats, devising plans for minimizing such risks, and influencing (inter)national drug prevention policies.

Common mechanisms of cytotoxicity for synthetic amphetamines, cannabinoids, cathinones, piperazines, benzofurans, and opioids include pro-oxidative burst, with increased generation of reactive oxygen and nitrogen species (ROS/RNS), depletion of reduced glutathione (GSH) and concurrent formation of glutathione disulfide (GSSG). Oxidative stress is associated with abnormal function of intracellular organelles, such as the Endoplasmic Reticulum (ER), mitochondria, and lysosome. Disruption of mitochondrial

functioning is accompanied by dissipation of mitochondrial membrane potential ( $\Delta\Psi_m$ ) and depletion of energetic stores (ATP). Since both the ER and mitochondria play vital roles in the homeostasis of intracellular  $Ca^{2+}$ , mitochondrial and ER stress induced by ROS/RNS overload may result in  $Ca^{2+}$  dumping from these organelles into the cytoplasm, impelling activation of intracellular signaling pathways that evolve to cell death. Lysosomal impairment, by turn, may result in accumulation of material taken up from outside the cell to be digested or obsolete components of the cell itself, which may eventually affect the overall cell functioning and also culminate in cell death. All together, these mechanisms compel cell to suicide, as evidenced by chromatin condensation, pyknotic nuclei, and activation of both extrinsic (cleavage of caspase 8) and intrinsic (cleavage of caspase 9) apoptotic pathways that ultimately converge in activation of the execution phase (demonstrated by cleavage of caspase 3). Also of relevance, the interaction of NPS with the cytochrome P450 metabolizing enzymes may represent serious clinical consequences, as NPS are often co-consumed with other drugs. Accordingly, the inhibition of several isoforms had already proven to alter the toxicity of these drugs.

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