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The psychoactive effects of "Light Cannabis". From Italian law to psychopharmacology

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INDEX

Introduction

European Regulations no. 1307/2013 allows the cultivation of hemp for varieties with a Δ9-tetrahydrocannabinol (Δ9-THC) content not exceeding 0.2%.

In Italy, law no. 242/2016 allowed the cultivation of hemp up to 0.6% of Δ9-THC, intended for the production of:

- food and cosmetics;
- semi-finished products such as fiber, wood chips, oils or fuels; material for the use in green manure;
- organic material for bioengineering work or products for green building;
- material for phytoremediation of polluted sites;
- horticulture.

Law no. 242/2016 aimed at discharging farmers from penal liability in the event that plants with a Δ9-THC percentage higher than 0.2% were present in their production chains. Human consumption for recreational purposes was not included among the activities permitted. This product is sold as an "environmental perfume" and "herbal incense", "not for human use" and, according to the label indications, cannot be consumed. However, web forums for cannabis consumers explain how to smoke this product, including its effects and contraindications.

In the absence of explicit laws banning the commercialization of flowering tops with a Δ9-THC content between 0.2 and 0.6%, these products gained popularity because they contain Cannabidiol (CBD), which has been reported to reduce anxiety and promote sleep through a sedative effect. Manufacturers are thus commercializing hemp with low content of Δ9-THC, which is referred to as "light cannabis". The increase in the number of light cannabis shops was very significant, starting with a few in early 2000 to more than 700 in March 2019. The commercialization of low Δ9-THC products and variable CBD concentration is proliferating, and cannabis farmers have been working to create new cannabis varieties, expressing up to a 25% total of CBD and less than 1% total Δ9-THC.

In this context, the Italian Supreme Court, established that hemp with a Δ9-THC content below 0.6% cannot be commercialized for human use, when the "psychotropic effect" of the product and its "offensiveness" can be demonstrated.

Several studies explored the relationship between cannabis products with a high content of Δ9-THC, blood levels and psychomotor functions, but few pharmacokinetic studies on limited population samples and no psychopharmacological studies have been performed on light cannabis consumption.

The first chapter of this work reports the European and Italian legislation on hemp cultivation, as well as the hemp production chain and commercial activities developed after the Law no. 242/2016. The influence of "light cannabis" commercialization and its effects on prescription trends in Italy will be also discussed.

The second chapter reports the pharmacological aspects and the psychoactive effects of light cannabis, along with pharmacokinetics of the main Cannabis compounds: Δ9-THC, CBD and Cannabinol (CBN). Pharmacodynamics studies are reported and discussed in order to better understand the psychopharmacological proprieties of cannabis compounds. Finally, the definition of the "minimum psychoactive dose", based on scientific evidence, is defined.

The third chapter reports the experimental study, aiming to assess Δ9-THC and CBD blood concentration following light cannabis smoking and its effects on young adults' vigilance, cognitive and motor skills. A "smoking session" under controlled experimental conditions was reproduced. Eighteen young adults were enrolled and consumed three light cannabis cigarettes containing 400 mg of inflorescences each, with a percentage of 0.41% of Δ9-THC and of 12.41% of CBD. Blood samples were collected before the experiment (t0), after each light cannabis cigarette (t1→t3), 60 (t4) and 120 (t5) minutes after the beginning of the experiment. Five performance tasks and a subjective scale were employed for measuring cognitive and psychomotor performances the day before the experiment (TT0) and after the third cigarette (TT1). The results and their possible repercussion will be discussed.

Chapter 1. Legal status of Cannabis products

1.1 The European Legislation

Industrial hemp has been grown in Europe for many hundreds of years. Through the Middle Ages and until the end of the sailing ship period, hemp was an important crop in many European countries including the UK, France, Netherlands, Germany, Spain and Italy. The most important applications for the strong fiber were canvas for sails and sacks, canvas water hoses and fabrics, as well as ropes. Hemp is a multi-purpose crop, delivering fibers, shives, seeds and pharmaceuticals. Currently the fiber is used for light weight papers, insulation material and bio-composites. The shives, the woody inner core of the stem, are used for animal bedding and construction. Hemp seeds, small nuts with a high nutritional value, can be consumed raw or pressed into hemp seed oil. Both seeds and oil are used for human food and animal feed [1].

The main issue regarding hemp cultivation and its related products is that hemp contains, in variable quantities, Δ9-tetrahydrocannabinol (Δ9-THC), which has a known psychopharmacological activity and is sold as an illicit drug on the black market. According to the EMCCDA, in the European Union 90.2 million adults aged 15-64 years (27.2 %) have tried cannabis during their lives. Cannabis products account for the largest share (39 %) of the European illicit drug retail market, with an estimated minimum value of 11.6 billion euros in 2017 [2] [3].Therefore, Europe and State Members permit hemp cultivation under strict conditions.

The European Commission (EC) Regulation No 1251/1999 and the following 2860/2000 included hemp grown for fiber in the support system for producers of certain arable crops. EC Regulation No 1672/2000 also added Article 5a[1], laying down that the tetrahydrocannabinol content of hemp vari-

[1] art.1, Paragraph 4, Regulation (EC) No 1672/2000

eties used must not exceed 0.2% and that and the Member States must establish a system for verifying said Δ9-THC content. On this point, Commission Regulation (EC) No 2860/2000 established that the labels contained on packaging should be transmitted to the competent authorities to ensure the use of certified seeds.[2] The commission also established a common practice for laboratories to quantify the Δ9-THC in hemp in annex XIII of the Commission Regulation (EC) No 1251/1999[3]. Furthermore, Member States must send a report on the Δ9-THC content findings to the Commission indicating, for each variety, how many samples collected exceed the Δ9-THC content of 0,1 %, for which further controls are expected for the following marketing year.[4]

EU Regulation 1307/2013, confirmed that it is legal to cultivate and supply cannabis plants for hemp fiber only if their Δ9-THC content does not exceed 0.2 %.[5] Payments under the Common Agricultural Policy are therefore granted only for areas sown with certified seeds of specified hemp varieties offering certain guarantees with regard to their psychotropic content[6]. Imports of hemp are also subject to certain conditions to ensure the above-mentioned Δ9-THC limit is respected[7] (EU Regulation 1308/2013).

1.2 The Italian situation and the law no. 242/2016

In Italy, the new regulation on cultivation of cannabis is provided by law no. 242/2016[8]. The law allows the cultivation of *cannabis sativa L.* and has as its main purpose the support and promotion of the cultivation and agro-industrial chain of hemp aimed at promoting this crop.[9] Paragraph 2 of art. 1 establishes that this discipline applies to hemp cultivations of admitted varieties, registered in

[2] art.1, Paragraph 2, Regulation (EC) No 2860/2000
[3] art.1, Paragraph 3.1 Regulation (EC) No 2860/2000
[4] art.1, Paragraph 3.1 Regulation (EC) No 2860/2000
[5] art.32, Paragraph 6, Regulation (EU) No 1307/2013
[6] paragraph 28, Regulation (EU) No 1307/2013
[7] paragraph 154, Regulation (EU) No 1308/2013
[8] law 242/2016, Provisions for the promotion of the cultivation and agro-industrial chain of hemp, published in the Official Gazette General Series n. 304 of 30 December 2016
[9] art. 1, paragraph 1, law 242/2016

the common catalog of varieties of agricultural plant species, pursuant to Article 17 of Council Directive 2002/53/EC of 13 June 2002. These crops do not fall within the scope of application of the Consolidated Law on Drug discipline referred to in the decree of the President of the Republic published on October 9, 1990, n. 309, and therefore remain subject only to law 242/2016.

With particular reference to cultivation, the legislator has established that if the total Δ9-THC content of the plants is greater than 0.2 percent and within the limit of 0.6 percent, no responsibility is placed on the farmer.[10] What the legislator has omitted to regulate, despite the listing referred to in art. 2, paragraph 2[11], is the marketing - also aimed at human use - of the products deriving from these crops, thus creating a regulatory vacuum that has given rise to conflicting interpretations, not only in jurisprudence but also among those who sell these products.

On the lawfulness of the marketing of legal hemp, commonly referred to as "light cannabis", the jurisprudence of legitimacy has offered conflicting interpretative guidelines, as detailed below.

- In December 2018, The Supreme Court of Cassation, VI section, [12] initially recognized the lawfulness of the sole cultivation of cannabis sativa L. The marketing of cultivation products and the consequent conduct of possession and sale of cannabis derivatives continued, according to the Supreme Court, to be subject to the discipline of the Presidential Decree 309/1990, provided that the substances nevertheless have a detectable drug effect. The Court stated that "the law 242/2016 does not provide in its scope of application that of the marketing of the products of this cultivation, or the inflorescences (marijuana) and the resin (hashish). Therefore, the lawfulness does not extend to the conduct of possession and sale of these derivatives".

[10] art. 4, paragraph 5, law 242/2016
[11] art. 2, paragraph 2, law 242/2016
[12] Penal Cassation Section VI, published 17 December 2018, n. 56737

- In January 2019 the same Court[13] recognized the lawfulness of marketing by stating that: "from the lawfulness of Cannabis Sativa L. cultivated in compliance with the requirements of the law 242/2016 derives the lawfulness of the retail marketing of the related products containing a percentage of the active compound Δ9-THC lower than 0.6%". The Court also underlined that "in the matter of narcotic substances, the possession or transfer of inflorescences from cannabis cultivations permitted pursuant to Law no. 242 of 2 December 2016 and integrates the crime referred to in art. 73, DPR 9 October 1990, n. 309, provided that (i) the threshold of 0.6% Δ9-THC is exceeded and (ii) the active compound is capable to produce a psychoactive effect*".* With this statement it seems possible to deduce that the Court defines the amount of Δ9-THC capable of producing a psychoactive effect exclusively for values higher than 0.6%.

On the question relating to the scope of the law 242/2016, a jurisprudential contrast was raised, due to the difficulties in coordinating the discipline referred to in law 242/2016 with the Consolidated Law on drugs (Presidential Decree 309/90).

Recently, with a sentence filed on 10 July 2019[14], the Supreme Court of Cassation, United Criminal Sections, addressed the sales of inflorescences falls within the applicability of the law or not, and is, therefore, criminally relevant. The Court stated that the marketing to the public of cannabis sativa L. and, in particular, of its leaves, inflorescences, oil and resin, obtained from its cultivation, does not fall within the scope of application of law no. 242 of 2016, which qualifies as lawful only the cultivation of hemp belonging to the varieties registered in the common catalog of agricultural plant species, pursuant to art. 17 of Council Directive 2002/53 / EC, of 13 June 2002, which exhaustively lists the derivatives of the cultivation that can be marketed.[15] As a consequence, the transfer, the sale

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[13] Cass. Pen., Sez. VI, 31 january 2019 n. 4920

[14] Cass pen. United Section 10 july 2019, n. 30475

[15] The sentence attributes a mandatory nature to the seven categories of products listed in art. 2, paragraph 2, law 242/2016, which can be obtained from the agro-industrial cultivation of cannabis sativa L.: (a) food and

and, in general, the marketing to the public of the derivatives of the cultivation of cannabis sativa L., such as leaves, inflorescences, oil, resin, integrates the crime referred to in art. 73, DPR n. 309/90, even with a Δ9-THC content lower than 0.6%. The Court reported that *"what needs to be checked is not the percentage of active compound contained in the substance sold, but rather the suitability of the substance to produce a psychoactive effect"*.

The illegality of uses of cannabis sativa L. that differ from those provided for by law 242/2016 was thus clarified, i.e. the transfer, sale and public marketing of products derived from cannabis sativa L. What emerges from the jurisprudence of the Supreme Court is the attention to the psychoactive effect or dose, and the abandonment of the Δ9-THC percentages as a unique criterion to determine lawfulness, which is identified with the concrete suitability of the substance to produce harmful effects in those who take it. This attention indicates that the limits, thus regulated by the legislator, do not fully convince or at least do not seem applicable to the product marketed. It therefore becomes necessary to first clarify what is meant by "psychoactive dose".

Starting from the assumption that art. 4, paragraph 5, of Law 242/2016 considers Δ9-THC levels lawful if they remain within 0.2 percent, while it considers levels within 0.6 percent not to be sanctioned, it is difficult to understand whether the legislator believes that within the first or the second threshold there is no psychoactive effect, and hence the inability of the substance to produce psychotropic effects in those who take it both in the medium and long term. However, considering the cumulative effect that these active compounds have in the body given their high degree of lipophilicity, the mere percentages cannot be accepted to determine the so-called " psychoactive effect ", which is clearly dose-dependent.

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cosmetics produced exclusively in compliance with the disciplines of the respective sectors; (b) semi-finished products, such as fiber, sheaves, powders, wood chips, oils or fuels, for supplies to industries and craft activities in various sectors, including energy; (c) material intended for the practice of green manure; (d) organic material intended for bioengineering works or useful products for green building; (e) material aimed at phytoremediation for the remediation of polluted sites; (f) crops dedicated to teaching and demonstration activities as well as research by public or private institutes; (g) crops intended for horticulture.

Finally, the Circular of the Ministry of the Interior dated 31.07.2018, reports that hemp inflorescences with a content in Δ9-THC higher than 0.5% fall within the notion of narcotic substances. However, this calculation is formulated on the threshold of 5 mg of Δ9-THC, which "in percentage terms" (5 mg of active compound) are equivalent to 0.5% (at this concentration, 1 g of inflorescence contains about 5 mg of active compound). It follows that quantities of 5 mg of Δ9-THC per single dose would allow us to attribute - at least in theory - the characteristics of a psychoactive substance to the inflorescences. Moreover, as documented during the aforementioned issues, from inflorescences with Δ9-THC concentrations even lower than 0.5%, products with higher percentages could be obtained (such as hashish), thanks to the extraction of the active compound with mechanical tools and laboratory processes that are not particularly articulated. Although the Ministry of the Interior identifies 0.5% as a threshold above which cannabis should be considered psychoactive, this refers to a single dose of 1 g of inflorescences, without taking into account a possible intake of doses higher than 1 gram.

1.3 Hemp production chain in Italy and commercial activities

In the last ten years, and especially after the law 242/2016, the cultivation of cannabis for legal purposes in Italy rapidly increased.

Giupponi et al. [3] conducted a survey on a sample of 30 farms randomly scattered throughout the Italian territory in order to understand the characteristics of the hemp production chain and the purposes of crop production. Results showed that:

- 83% of the farms were set up directly for the cultivation of hemp, while the remaining 17% were converted from a different crop (mainly horticulture);
- almost all the hemp farms (97%) were set up within the last ten years, given that hemp cultivation in Italy was officially permitted only from the 1990s (**Figure 1.1**);
- the certified seeds come both from Italy, through hemp associations and cooperatives (Tecnocanapa, Assocanapa and Federcanapa) and from Europe, in particular Germany and

France, followed by North-East Europe. A direct connection between the choice of a variety of seeds and the final product was not found;

- almost all the farms use the crop for the production of more than one end-product. Some farms provide a product for transformation while others have a complete production chain as part of the farm or cooperate with local businesses for direct transformation. Only five farms declared selling inflorescences for technical use. Apart from one farm that indicates as the only purpose of the hemp cultivation the not well- defined "technical use", all the crops of the farms in the survey are destined for purposes specified clearly by Italian legislation.
- C. sativa has been recently classified among the low-input multipurpose crop for biomass production in marginal agricultural lands [4]. Hemp is associated with environmental benefits such as low pesticide and herbicide requirements (7% of farms declared use of pesticides and only one farm out of 30 uses chemical fertilization) and adaptability to a wide range of agronomic conditions (only 30% farms need irrigation).
- More than 20% of the farms declared promoting cultural, multifunctional and educational activities, in particular courses and public events, educational laboratories (for production of food, food oil or cosmetics), environmental conservation and remediation, gastronomy showrooms. This is in compliance with legislation 142/2016, that encourages the "*support and promotion of the cultivation and supply chain of hemp (Cannabis sativa L.)*".
- An anonymous web questionnaire on commercial activities associated with hemp ("Grow Shops") was conducted. A grow shop usually sells gardening material to grow hemp plants and also products such as food and dietary supplements, cosmetics, clothing and even green building items, but the main product are dried inflorescences for "technical use". Seven-hundred-seventy-six hemp shops were counted, mainly in Lombardy, Lazio and Campania and mostly set up in the last three years. The increase in the number of grow shops was very significant, starting from a few in early 2000 to more than 700 in March 2019 [3].

Dei Cais et al. applied a validated LC-UV method to more than nine hundred light cannabis samples in order to determine the total Δ9-THC content and assess their legality [5]. Based on the law 242/2016, only 18 % of the crops can be considered legal for the market (total Δ9-THC<0.2 %) and 10 % of the samples should be destroyed, because they have a concentration of Δ9-THC > 0.6 %. The 58 % of the samples containing a Δ9-THC level between 0.2 and 0.5 %, while the 14 % of samples had a Δ9-THC content between 0.5 and 0.6%

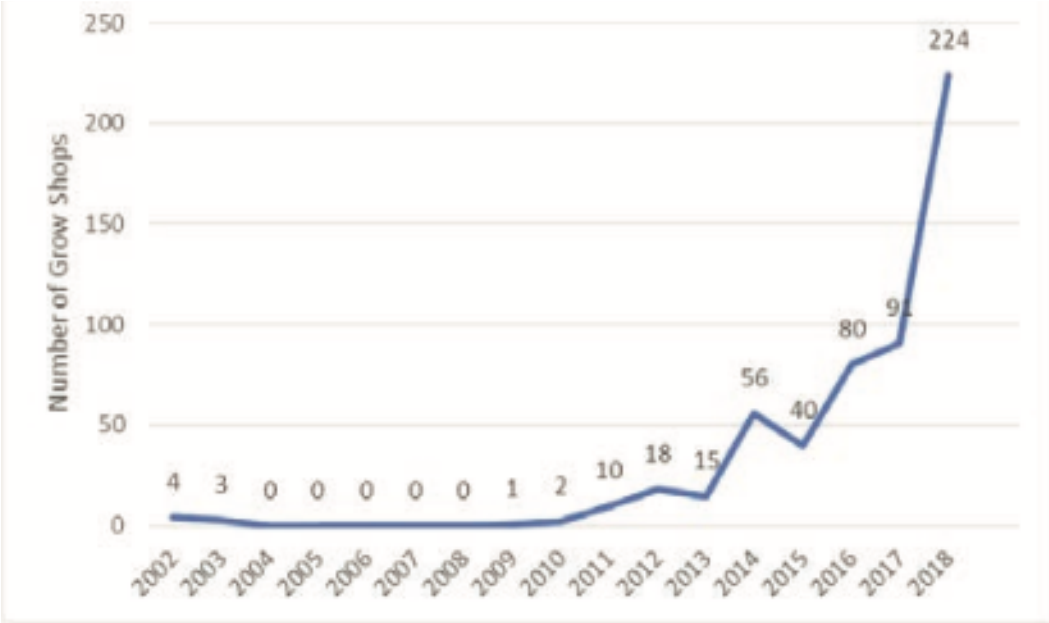

Figure 1.1 Number of grow shops set up from 2002 to 2019 [3]

Law 242/2016 is devoted to regulating and incentivizing the production and commercialization of industrial cannabis. However, the law does not regulate on the production of inflorescences, thus creating a legislative gap that some start-ups have exploited by beginning to sell, from May 2017, cannabis inflorescences with a low level of Δ9-THC and a naturally high level of cannabidiol (CBD), named *light cannabis*. These grow shops (retailers that already sold industrial cannabis-related products) were mostly located in the proximity of cannabis cultivations, in areas close to waterways and humid soil. Consequently, while this unintended liberalization occurred simultaneously in the entire

territory, in the short run, the level of intensity was not homogeneous. However, 1 year after post-liberalization, para-pharmacy, herbalists, and tobacco shops followed suit, exposing the Italian territory to more homogenous market coverage **(Figure 1.2)**.

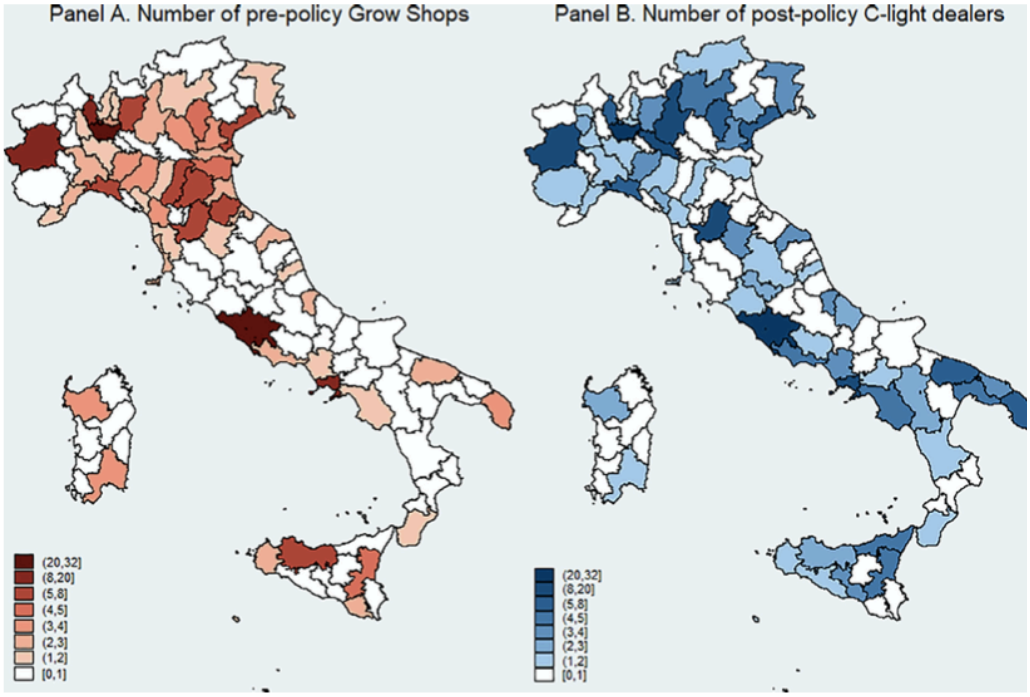

Figure 1.2 Distribution of pre-policy grow shops and post-policy Cannabis light (C-light) dealers [6].

This unintended liberalization has given the opportunity to study [6] changes in the equilibrium supply of street marijuana in a market, such as the Italian one, where illegal and legal retailers co-exist. Moreover, in Italy there is a well-known presence of strong criminal organizations that entirely control the market of illegal substances, often in partnership with international criminal organizations. The market of cannabis-derived drugs roughly represents 91.4% of the illegal drugs confiscated in Italy [7] and is estimated at around 3.5 billion euros. The study employed a differences-in-differences (DID) design with a unique dataset recording information on all 106 Italian provinces, running from 2016 to February 2018. Data **[6]** including the quantity of illicit substances confiscated in the Italian territory, as well as the monthly number of anti-drug operations conducted by police

forces and the number of people arrested for drug-related offences, were made available by the National Police at the province level. These data were then matched with provincial data on the pre-policy (October 2016) territorial diffusion of grow shops, collected from official retailers' websites. Finally, the data were linked to provincial demographic variables provided by the National Institute of Statistics (ISTAT). The availability of monthly data on confiscations and drug-Related offences, along with the unexpected nature of the liberalization, allowed the effect of interest to be estimated within a very short window of time relative to the policy, when law enforcement and police effort adaptations were extremely unlikely, thus ruling out any endogenous adjustment. For any grow shop serving a local market before the policy, liberalization led to a decrease of up to 14% in monthly confiscations of illegal marijuana. This corresponds to a reduction in elasticity of 3.3% in confiscations in response to a 10% increase in the number of grow shops per province. Liberalization also impacted the illegal supply of other cannabis-derived drugs, leading to an 8% reduction in the supply of hashish and a 32% decrease in the number of plants confiscated monthly per each grow shop. Moreover, the unintended liberalization also caused other indirect effects on organized crime. For instance, the total number of people arrested for drug-related offences fell by 3%; focusing on those categories, which are often used as street drug dealers, incarcerated foreigners were reduced by 3%, and minors by 15%. Compared to the entire illegal drug market and traffic, cannabis-derived substances account for more than 90% of the total amount of confiscated drugs. Carrieri et alt. calculated that lost revenue as a result of light cannabis liberalization ranges from 90 to 170 million euros per year, which does not seem very high when compared to revenue of the entire market of illegal cannabis-related drugs, estimated to be around 3.5 billion euros in Italy. Nonetheless, these results show that even a mild form of liberalization, concerning an imperfect substitute product of street marijuana such as light cannabis, which contains low level of Δ9-THC, could still harm organized crime.

1.4 Influence of "light cannabis" products on prescription trends in Italy

Emerging data suggest that use and abuse of prescription drugs may be decreasing in states where medical cannabis is legal [8]. Some authors hypothesize that this trend could also be repeated with light cannabis. Carrieri et al. in 2020 [6] explored medical substitution between light cannabis (the substitution effects of which are induced by a compound of cannabis, the CBD) and several different types of prescription drugs by exploiting territorial heterogeneity in product availability, given that the first retailers of light cannabis were the existing "grow shops". For all drug categories, for which medical marijuana can be considered as a substitute or adjuvant therapy, a significant and negative effect is documented. By analyzing the availability of light cannabis in the period of time surrounding the approval of the law (January 2016 to February 2018), the local availability of light cannabis led to a significant decrease in the number of dispensed drugs sales by approximately 1.6% on average. The boxes of anxiolytics prescribed and sold decreased by approximately 11.5%, there was a 10% reduction of dispensed sedatives and the number of dispensed anti-psychotics decreased by 4.8%. Interestingly, these are also the type of drugs for which CBD – but not light cannabis itself – is recognized or advertised as having a clinical effect [9]. More subtle but still significant effects are found for anti-epileptics (-1.5%), anti-depressants (-1.2%), opioids (-1.2%), anti-migraines (-1%). These are all drugs requiring a constant and consistent therapy, often prescribed by specialists, and for which the switching to an "alternative therapy" based on self-medication might be more problematic. Moreover, it is noteworthy that opioids, anti-depressants and anti-epileptics are all pharmaceuticals that show severe side effects and can be associated with social stigma. In Italy, opioids are also generally less prescribed. Indeed, some patients may have seen in this "light cannabis" a mostly accessible product which does not require any medical prescription. The pharmaceuticals considered have shown patterns of substitutability with medical marijuana [10], which however presents some differences with respect to the light one. For instance, medical marijuana (rich in Δ9-THC) is largely used to treat chronic pain, glaucoma, insomnia and anxiety. Instead, CBD is often associated with anti-psychotic, analgesic, anti-inflammatory, anti-arthritic, and anti-neoplastic properties and is used

to treat inflammations, migraines, depression, and anxiety [9]. An event study specification shows that the substitution between these pharmaceuticals had a larger effect starting from the third month after the introduction of the product in the local market and remained statistically significant also after six months post-liberalization. This is further corroborated by anecdotal evidence from Google Trends, which shows an increasing number of queries on the potential clinical effects of light cannabis after the introduction of the policy.

Chapter 2. The psychoactive effect of “light cannabis” products

2.1 Definition of psychoactive effect: the law and the science

The Supreme Court of Cassation, on 10 July 2019, specified that the sale of Cannabis Sativa L. products is not illicit if "*such derivatives are, in practice, devoid of any psychoactive or psychotropic efficacy, according to the principle of offensiveness ".*

On this point, the main psychoactive constituent of Cannabis Sativa L. is Δ9-THC. The legitimacy of the sale of the derivatives of Cannabis Sativa L. must therefore take into consideration the evaluation of any psychoactive or psychotropic effect based on the quantity of Δ9-THC contained in each product and capable of producing harmful effects on human health. It becomes imperative to find suitable technical reference points to reach this type of evaluation.

For the purpose of quantitative definition of the psychoactive dose, the limit of 0.2% -0.6% of Δ9-THC present in cultivated hemp established by law 242/2016 cannot be recalled. That is because these limits are imposed in order to distinguish those farmers deserving of incentives provided by the state for plantations with less than 0.2% of Δ9-THC, from those who are not, as they grow plants with quantities of Δ9-THC between 0.2% and 0.6%. And again, to point out those growers whose plants exceed that 0.6% of Δ9-THC which has been imposed as the maximum limit and, consequently, must be seized and destroyed. Therefore, these percentages do not have a legal for the Italian legislation on narcotics (Presidential Decree 309/1990), but only refer to the use of hemp for those purposes which are permitted by law.

On the other hand, identifying a psychoactive dose by taking as a reference the percentage of active compound present in the plant does not provide any information on the dose that is sufficient to obtain psychotropic effects in an individual. In this case it is necessary to refer to the actual quantity of active principle contained in the substance taken, which can reach the necessary quantity of active principle to obtain the psychoactive effect, regardless of the percentages in the raw product.

A "psychoactive dose" would already be provided for by the law in the "Maximum limits of psychotropic substances" by art. 73 paragraph 1-bis of the Consolidated Law approved with Presidential Decree 309/1990, as amended by Law no. 49 " *which refer to the average single dose (DMS), established by a commission of experts and toxicologists, as the quantity of active compound for each single intake which is able to produce a psychotropic effect in a tolerant and dependent subject*". However, the law, after having consulted with experts in the sector, defined that the average quantity used by Δ9-THC consumers to obtain a psychoactive effect was equal to 25 mg. This is an empirically calculated dose for individuals who are chronic Δ9-THC users, and therefore have already developed a tolerance towards the substance[16]. Furthermore, the purpose of this determination is not to define the minimum dose capable of producing psychoactive effects, but to consider the "average dose" contained in a "joint" for eminently legal purposes. Neither the accumulation effect nor the scientifically recorded minimum thresholds for the onset of effects on non-tolerant individuals after gradual intake of Δ9-THC have been legally established.

As regards the lawfulness of commercialization of light cannabis, the legislator should have as a priority the defense of the most vulnerable subjects - of adolescents, first of all, who are the greatest consumers of cannabis. This shifts the attention to the dose that can have narcotic effects harmful to health in non-tolerant subjects; otherwise the State (in contrast to scientific evidence) would find itself considering harmless a quantity of active principle capable of altering the physiological neuropsychic condition of an individual.

Both the quantity of active compound actually taken and the administration route have a medical-toxicological value, given that the effects on humans are quite different if the substance is inhaled or ingested, even in the case of Δ9-THC. The "minimum psychoactive dose" on the basis of the tolerability of the non-tolerant user ("naive" subject), should be defined according to scientific evidence.

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[16] Art.73, co,1 bis Presidential Decree 309/1990 309/1990, modified by law n. 49/2006

The “minimum psychoactive dose” will be defined as follows:

1. the "psychoactive" effects of Δ9-THC refer to the dose in mg, and not just to the simple percentage of this ingredient present in the products;
2. the psychoactive dose is the minimum one capable of producing psychoactive effects and it corresponds to the minimum quantity of the active compound able to negatively interfere with the various neuropsychic systems of the person, by altering their cognitive abilities, vigilance, alertness and perception systems along with various other functions;
3. It should also be taken into consideration that the effects of Δ9-THC vary from person to person, with differentiated harmful potential based on a series of coexisting conditions [11]:
    a. Age under 18 (related to brain development);
    b. Sex;
    c. Presence of several other active compounds in the plant taken (in particular cannabidiol or CBD);
    d. Previous degree of cannabis use (tolerance);
    e. Simultaneous use of alcohol and / or substances that depress the central nervous system (CNS);
    f. Conditions of genetic vulnerability for psychiatric disorders.
4. The "psychoactive" effect of Cannabis will be identified as the measure of offensiveness, as well as of the psychotropic action, deriving from the use of this substance in non-tolerant people, thus applying a precautionary principle in defining the minimum dose dangerous both to the health and the impairment of a person’s neuropsychic and coordination functions. Studies that correlate blood concentrations detected following the use of products containing cannabis with a low Δ9-THC content will also be evaluated in order to relate them with concentrations that are potentially suitable for causing a psychotropic effect.

2.2 Pharmacokinetics

Δ9-THC absorption in the blood strongly depends on the route of delivery: when consumed by smoking the substance is rapidly transferred from the lungs into the blood and from there to the brain, while oral administration may cause degradation in the acidic environment of the stomach and first pass metabolism in the liver [12]. As a lipophilic compound, Δ9-THC is rapidly absorbed by highly vascularized tissues such as the lung, heart, brain and liver. Secondly, Δ9-THC is distributed in adipose tissue, especially in chronic and frequent smokers who accumulate large quantities of Δ9-THC in their body, which are subsequently released over time while maintaining their effects [13].

The free compound binds to cannabinoid receptors in the brain, spinal cord and peripheral nervous system, thus producing the characteristic effects of cannabis. Other substances can bind to these receptors and have an effect that is either additive, synergistic or antagonistic to the effects of cannabis. Furthermore, the degree of addiction, age and concurrent diseases can also influence the intensity of the effect. Both subjective and acute effects are already measurable after the first inhalation of cigarette smoke containing cannabis, generally returning to the baseline within 3-6 hours of exposure. A single inhalation of cannabis can even reach plasma concentrations around 25μg/L, rising at 50 μg/L after two aspirations, and reaching 80 μg/L after three aspirations with the appearance of the so-called "psychoactive" effect right from the first aspiration [14, 15]. Although great variability has been found between smokers, immediate tachycardia and subjective effects begin to manifest from the first aspiration.

Oral absorption is slower, with lower and delayed peak concentrations. The time taken to reach maximum plasma Δ9-THC concentration after oral ingestion is approximately 2-4 hours, compared to the few minutes it takes after smoking. Δ9-THC plasma concentrations decrease rapidly due to rapid tissue distribution and hepatic metabolism. Occasional cannabis smokers report a greater psychotropic effect at lower Δ9-THC concentrations than chronic smokers with higher Δ9-THC amounts. This corresponds to the onset of the tolerance phenomenon [16].

The national forensic toxicological literature [17] , with regard to the amount of Δ9-THC above which a certain type of cannabis must be qualified as narcotic, limits the recognition of suitability to hemp whose Δ9-THC content is such as to "ensure" its absorption in an effective dose, identified in at least 5 mg of Δ9-THC when taken by inhalation. However, studies on the dose of Δ9-THC required to induce pharmacological effects in humans have documented the first effects as early as 2 mg of Δ9-THC in a cannabis cigarette. Starting from a bioavailability of 10-35% for smoked Δ9-THC, 0.2 mg of Δ9-THC is absorbed from the 2 mg. However, only 1% of this dose is found in the brain tissue at peak blood concentration. This indicates that of the 0.2 mg only 2 μg of Δ9-THC reach the brain and have neuro-pharmacological effects [18]. In a number of deceased cannabis users, consistently higher Δ9-THC brain levels than blood levels have been documented, and in some cases the concentrations were still measurable in the brain while no longer in the blood [19]. Recent studies have shown that acute administration by inhalation with a vaporizer of Δ9-THC, even at a low dose (8 mg), alters emotional responses, causes a motivational and “stoned/high“ state in users for up until 2-3 hours after the administration of Δ9-THC [20]. Δ9-THC at such a low dose (8 mg) is already able to induce the onset of some psychotic symptoms, including the increase in negative symptoms in the Brief Psychiatric Rating Scale (BPRS) test [21], cognitive disorganization and perceptual distortions in the PSI test [22] (Psychotomimetic States Inventory), and a significant alteration of the working memory of the individual. The working memory represents an interface between perception, long-term memory and actions that underlies thinking processes, and is therefore an important element for many other cognitive functions, including language, problem solving, reasoning and abstract thinking. A significant reduction in working memory and a clear deficit in attention and psychomotor control processes were also observed after oral administration of 10 mg of Δ9-THC or of standardized Cannabis extract with 10 mg Δ9-THC [23]. These studies show that even at low doses there is a risk of important neuropsychic reactions such as psychosis, perception distortions and an alteration of cognitive functions that can have a highly disabling impact on the predisposed / vulnerable individual.

Increasing the dose of Δ9-THC (~ 15 mg) by inhalation (inhaled from standardized cigarettes) there is an increase in heart rate and a significant reduction of the individual's working memory, accompanied by neurophysiological changes (EEG) in the cortical areas involved [24].

Furthermore, oral administration of Δ9-THC at 7.5 mg and 15 mg induces, depending on the dose, an increase in heart rate, dizziness, dry mouth, impairing the subject's memory and concentration and increasing the feeling of a "high" (understood as "high") in the consumer [25, 26]. To this extensive symptomatology should also be added a clear alteration of the memorization processes, in particular of those involved in the acquisition and maintenance of new information [25] and an increase in impulsive behaviors in humans [27].

Taking a higher dose of Δ9-THC (20 mg) by mouth causes drowsiness, fatigue and dizziness for up to 6 hours after assumption [28], and clear mood changes in the users [29]. Furthermore, this oral dose of Δ9-THC causes psychomotor alterations [28]. These concentrations, along with the significant sedation effect and the onset of dizziness, can clearly promote a state of "lethargy" with important implications for public health, such as work or road accidents.

Studies on the pharmacokinetics of cannabis containing a high percentage of Δ9-THC have observed that after the intake of inflorescences by inhalation (smoke, vaporization) the peak plasma concentration is detected on average in a period of time between 30 minutes and 1 hour. The elimination of Δ9-THC from the blood, as well as the correlated duration of drug-related cognitive impairment, is instead a complex process, since there is considerable individual variability depending on the metabolic (genetic) phenotype and the type of user (habitual consumer, occasional or naive). Moreover, the elimination speed inevitably depends on the dose of Δ9-THC taken. Pharmacokinetic studies [30] have shown that after inhaling 16 mg of the active compound (Δ9-THC), the latter persists in the blood for up to 7 hours, and up to 12.5 hours after doubling the dose, although significant differences can be observed depending on the consumer. The pharmacokinetic picture is further complicated by the study of repeated intakes over time. Repeated intakes of cannabis with a high percentage of Δ9-THC (studied in a population of subjects free to consume ad libitum) considerably

prolong the presence of Δ9-THC in the blood when compared to a single intake, thus prolonging the psychomotor effects. Figure 1 shows a comparison between blood curves following a single intake (a) and after multiple intakes (b).

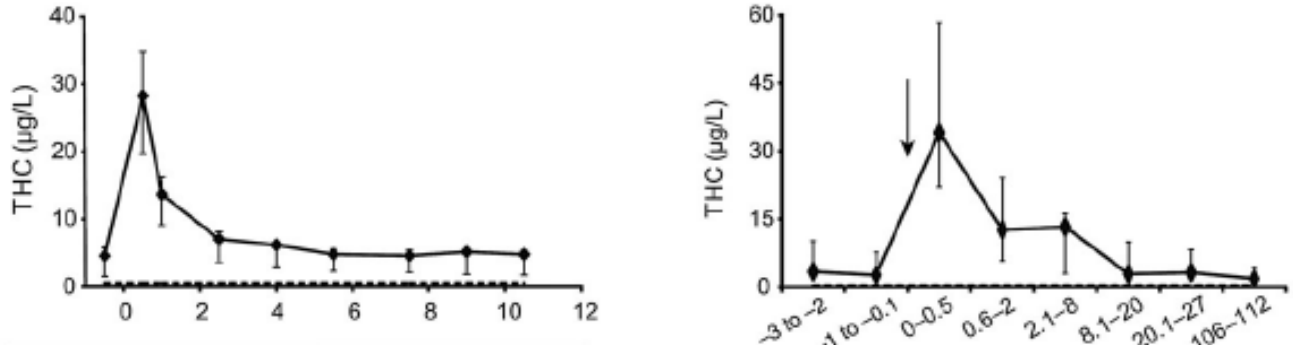

Figure 2.1. Plasma Cannabinoid Pharmacokinetics After Controlled Smoking and Ad libitum Cannabis Smoking in Chronic Frequent Users [30].

Concerning cannabis with a low percentage of Δ9-THC, there are few pharmacokinetic studies in the literature, referring to very small series, which have observed a detectability up to about 4-6 hours, with the blood peak occurring after about 30 minutes to 1 hour, similarly to cannabis with a high Δ9-THC content. Unfortunately, there are no cognitive and pharmacological studies on smoking cannabis with a low Δ9-THC principle that would allow us to outline a pharmacokinetic profile following single and/or repeated intakes with the correlated psychoactive/psychotropic effect. With reference to actual cases, it can be assumed that repeated and close intakes (for example two or more cigarettes smoked within an hour or so) of quantities of Δ9-THC that are slightly lower than the minimum psychoactive dose identified in the following paragraphs can also reach the psychoactive threshold.

In the absence of specific scientific studies, it is not possible to understand if repeated intakes of cannabis preparations with low Δ9-THC content significantly increase the psychoactive effect, similarly to what happens after intake of cannabis with a high Δ9-THC percentage.

2.3 Receptors

Δ9-THC, like other psychoactive drugs, interacts with brain receptors to produce its effects. Cannabinoid (CB1, 2) receptors are G-protein-coupled receptors, present at a high density in the frontal cortex, basal ganglia, hippocampus, and cerebellum, and at a minor density in the hypothalamus, nucleus accumbens and amygdala [31, 32].

In 1988, the first cannabinoid receptor CB1 was identified and cloned [33]. CB1 cannabinoid receptors are mainly located on presynaptic terminals and are present in many areas of the brain. In the nucleus accumbens (fundamental structure of the gratification systems), Δ9-THC binds to CB1 receptors that are located next to dopamine neurons, thus increasing the amount of dopamine released in synapses [34]. Dopamine binds to its postsynaptic neurons, producing euphoria. In the hippocampus (fundamental structure for storage processes), Δ9-THC binds to CB1 receptors on the glutamate terminals, producing damaging effects on memory. Persistent use of cannabis makes the brain adapt to the continuous stimulation of the CB1 cannabinoid receptor, by reducing the density of the receptors (downregulation). Significant "downregulation" of CB1 cannabinoid receptors has been observed in frequent and chronic cannabis smokers. These receptors were restored after prolonged abstinence (brain plasticity) [35]. Furthermore, reduced CB1 cannabinoid receptor density and residual Δ9-THC concentrations were accompanied by significant psychomotor impairment, with critical conditions monitored for at least 3 weeks [36].

2.4 Not only Δ9-THC: Cannabidiol and Cannabinol

Beside Δ9-THC, other compounds that have been identified are: cannabidiol (CBD), cannabigerol (CBG), cannabinol (CBN, the oxidation product of Δ9-THC and a marker of drug deterioration status), cannabichromene (CBC) and olivetol, a cannabinoid precursor. Cannabidiol (CBD) possesses analgesic and anti-inflammatory activities which are mediated by inhibition of cyclooxygenase and lipoxygenase; it does not possess psychoactive properties. CBD is therefore a non-psychoactive agent with a range of interesting and potential therapeutic indications.

2.4.1 Cannabidiol

Cannabidiol (CBD) is the second most abundant cannabinoid present in Cannabis sativa L. and can constitute up to 40% of the extracts of the plant. However, CBD concentrations are highly variable and depend on the growing conditions, the different phenotypes of illicit cannabis and on the part of the plant analyzed [37]. Pharmacokinetics processes of CBD depend on the route of administration and the frequency or magnitude of exposure. CBD is metabolized in the liver and intestines by different isoforms of cytochrome P450. Like Δ9-THC, CBD is subjected to a significant first-pass effect; however, unlike Δ9-THC, a large proportion of the dose is excreted unchanged in the feces [38]. The bioavailability of CBD following the smoked route averaged 31% (range 11%– 45%) as compared to the intravenously administered drug. CBD's half-life following oromucosal spray is between 1.4 and 10.9 hours, between 2 and 5 days after chronic oral consumption, and up to 31 hours after smoking. CBD will achieve a maximum plasma concentration between 0 and 4 hours [39]. Cannabidiol is highly lipophilic, able to easily pass across biological barriers and rapidly absorbed into fatty tissues and highly perfused organs (brain, heart, lung and liver), quickly decreasing its blood concentration. Plasma protein binding of CBD is similar to that of Δ9-THC at around 97%, thus capable of causing concentration increase of co-administered drugs and subsequential possible adverse effects [40]. In a series of recent studies, cigarette smokers with some "light cannabis" experience were asked to smoke "light cannabis" containing 0.16% Δ9-THC and 5.8% CBD [41-43]. The highest CBD concentrations in oral fluid (OF), serum and blood were observed 0.5 h after the start of smoking and the compound was still measurable up to 4 hours after administration. After four "light cannabis" cigarettes were smoked with a one-hour interval between each cigarette, CBD concentrations in blood serum and OF overlapped with those obtained after smoking a single cigarette, suggesting that CBD is poorly absorbed after repeated smoking.

Because of its high lipophilicity, CBD crosses the blood-brain barrier, modulating the central nervous system. Unlike Δ9-THC, CBD does not activate CB1 and CB2 receptors, which likely accounts for its lack of psychotropic activity. Despite presenting low affinity for CB1 and CB2 receptors

though, CBD can interact with these receptors at doses equal to or lower than 1 μM, acting as a negative allosteric modulator of the CB1 receptor[20] or inverse agonist of the CB2 receptor [44]. Moreover, CBD can antagonize Δ9-THC effects via non-CB1/CB2 receptors, such as GPR55, which is activated by Δ9-THC and blocked by CBD. The dose, route of administration, time between the intake of CBD and Δ9-THC (whether as a pre-treatment, simultaneous or subsequent), as well as the CBD/Δ9-THC ratio, seem to play an important role in the interaction between these two cannabinoids. Both high and low doses of CBD have been shown to raise Δ9-THC concentrations in the blood and brain, prolonging Δ9-THC disposition in the central nervous system [45-48] and suggesting that CBD inhibits the metabolism of Δ9-THC [49, 50]. CBD may also potentiate some of Δ9-THC beneficial effects, as it seems to reduce Δ9-THC psycho-activity, enhancing its tolerability and widening its therapeutic window, if administrated before Δ9-THC. A pharmacodynamic interaction may occur when both cannabinoids are taken together, mainly at a high dose ratio of CBD/Δ9-THC. Most recently, a study performed by Morgan et al. [51] showed no attenuation by the 16 mg CBD of psychotomimetic or cognitively impairing effects of the 8 mg Δ9-THC, thus concluding that at a ratio of 2:1 CBD does not attenuate the acute psychotic and memory impairing effects of vaporized Δ9-THC. The study also reported a blunted antipsychotic response to CBD in frequent users, while infrequent users showed reduced scores on the Psychotomimetic States Inventory (PSI) following CBD alone.

The dampening of neuronal excitability through the reduction of glutamate release indirectly protects against the development of cannabis use disorder [20] and attenuates psychotomimetic and anxiogenic effects induced by high doses of Δ9-THC in humans. This may partly explain why users of cannabis preparations with high CBD:Δ9-THC ratios are less likely to develop psychotic symptoms than those who consume preparations with low CBD:Δ9-THC ratios [52]. In human studies, users of low CBD strains of cannabis perform significantly worse on cognitive tests [51] and show higher psychotic-like symptoms [53] and reduced grey matter concentration in the hippocampus [54] compared with users of higher CBD strains. Another study [55] examined the acute effects of CBD and

Δ9-THC alone and combined when administered by vaporization. A randomized placebo controlled trial was conducted, where 36 subjects, 18 frequent (must have used cannabis at least once per month for 2 years) and 18 infrequent (must have used at least once in the past 2 years with 5–10 lifetime uses) cannabis users, were asked to complete 5 drug conditions spaced one week apart: placebo vs CBD alone (400 mg); Δ9-THC alone (8 mg) vs Δ9-THC combined with low (4 mg) or high (400 mg) doses of CBD. Both objective (blind observer ratings non the Clinician Administered Dissociative States Scale, CADSS) and subjective (self-rated) measures of intoxication were the primary outcomes, administered at time 0, again ~ 55 min after main dose drug administration) and during the recovery period. CBD showed some intoxicating properties relative to placebo. Low doses of CBD when combined with Δ9-THC enhanced (particularly in infrequent cannabis users), while high doses of CBD reduced the intoxicating effects of Δ9-THC. Simultaneous but not sequential inhalation of Δ9-THC and CBD was shown to attenuate some effects of Δ9-THC. Most effects were significant at $p < .0001$[55].

The anxiolytic effect exerted by CBD has been mainly related to its agonist activity towards serotonin type 1A (5HT1A) receptors [56]. In some human studies, greater concentrations of CBD have been associated with better cognitive performance, especially memory [57]. This compound is also an allosteric modulator of opioid receptors, specifically mu and delta. Analgesic myorelaxant and antiepileptic actions of CBD are achieved through the increasing of inhibitory tone in cortical and striatal membranes, obtained from the inhibition of GABA reuptake and the positive allosteric modulation of GABA A receptor [58]. CBD has also been reported to be neuroprotective, sedating, antiemetic and anti-inflammatory; the latter, mediated by the inhibition of cyclooxygenase and lipoxygenase, being several hundred times higher than that of acetylsalicylic acid. Furthermore, it inhibits the synthesis of leukotriene TXB4 in polymorphonuclear cells. These neuroprotective actions could be exploited after hypoxic ischemic exposure [59] or to decrease the leukocyte infiltration in the brain in some autoimmune diseases, such as multiple sclerosis [60]. Regarding cancer, CBD has

exhibited antiproliferative and proapoptotic activities through the antagonism over G protein-coupled receptors (GPR) 55 [56] in different types of cancer, including breast, lung, colon, brain, and others [59]. The studies [43] on "light cannabis" smoking showed that after smoking up to 232 mg CBD within a four hour period caused sleepiness. The most recent evidence on cannabinoids effectiveness on sleep refers to the therapeutic potential of CBD for the treatment of insomnia [61], due to its anxiolytic, antipsychotic and neuroprotective properties.

In Italy there are no laws banning CBD, which is not yet registered as a medicinal agent. Due to this gap in the law, some hemp shops freely offer CBD products (oil, crystals, etc.) [40].

2.4.2 Cannabinol

Cannabinol (CBN) is a natural constituent of Cannabis sativa with approximately 10% of the activity of Δ9-THC. CBN metabolism is similar to that of Δ9-THC with the hydroxylation of C9 yielding the primary metabolite. Due to the fact that one additional ring is aromatic, CBN is metabolized less extensively and more slowly than Δ9-THC. The average bioavailability of a smoked CBN dose, as compared to intravenous CBN, was 41% with a range of 8% to 77% [38].

2.5 Definition of the "minimum psychoactive dose"

As already explained, we consider a "psychoactive dose" the minimum dose capable of producing psychoactive effects defined in scientific literature, that is, the minimum quantity of active compound able to negatively interfere with various neuropsychic systems of the person, by altering their cognitive abilities, alertness and perception systems along with many other functions.

The main neuropsychic effects of Δ9-THC include slowdown in reaction time, decreased motor coordination, specific short-term memory defects, difficulty in concentration and particular impairment in complex tasks requiring attention. A suppressive action on memory and learning has also been documented in experimental animals [62].

In defining the "minimum psychoactive dose" of a plant product containing Δ9-THC, it must be considered that the pharmacodynamics of this active compound is influenced by a series of variables including the different and simultaneous presence of cannabidiol (CBD), which is contained in various percentages according to the type of cannabis.

In plants, De Cas et al. [5] observed a linear correlation between the two compounds in the same sample in a population of n=922 light cannabis samples.

Low concentrations of CBD in the product lead to an increased duration of acute and psychoactive symptoms from Δ9-THC, whilst simultaneously creating a "mitigating" effect on the possible psychotic symptoms, since CBD is a negative allosteric modulator able to reduce the binding of Δ9-THC to CB1 receptors. CBD enhances the analgesic efficacy of Δ9-THC by prolonging its duration of action (it activates the serotonin pathway at the level of the dorsal raphe) and at the same time it seems to reduce the side-effects relative to heart rate, respiratory rate and body temperature [63].

In assessing the psychoactive capacity by applying the precautionary principle, it must be taken into consideration that Δ9-THC is often taken together with alcohol and other psychotropic substances capable of increasing those psychoactive effects even at low doses.

Cannabis effects are similar to those of alcohol and benzodiazepines and include slowing of reaction time, decreased motor coordination, specific short-term memory defects, difficulty in concentration and particular impairment in complex tasks requiring attention. The effects are dose related, but can already be demonstrated after taking relatively low doses (5-10 mg of Δ9-THC in a cigarette) even in regular cannabis users, and they have been observed in many studies through a wide range of neurocognitive and psychomotor tests [62]. The amplification of the effects of alcohol use in conjunction with these low doses was also observed [64, 65].

The effects of orally administered Δ9-THC were evaluated for five Δ9-THC dose levels (0, 5, 10, 15, 20 mg) on 16 volunteers [66]. Mood changes and performance measures were recorded four times before and after drug administration. Oral Δ9-THC produced a significant dose-dependent reduction in performance, starting from doses of 5 mg for a period of more than three hours. A

similar timeframe was observed for the Δ9-THC effects perceived by the person (on average from 5 mg upwards) based on the subjective evaluation of intoxication ("feeling of out of tune") [67]. The subjective evaluation often presented a temporally delayed appearance when compared to the real decrease in performance documented by the observers. This last datum documents the “late” perception of the effect in comparison to the real one. Some authors [62] report that the "high" effect perceived after ingesting plant products containing Δ9-THC can be induced with low doses of Δ9-THC, up to 2.5 mg in a Cannabis cigarette, which cause a feeling of intoxication along with perception of decreased anxiety, depression and tension and a simultaneous increase in sociability [68]. In a recent study Solowij et al. [55] observed that a dose of 8 mg when taken by vaporization causes an increase in heart rate.

On the basis of these considerations it is once again clear that it is necessary to introduce a precautionary criterion when evaluating the psychoactive dose, taking into account the many variables (sex, simultaneous intake of alcohol or drugs, addiction to Δ9-THC, routes of intake) which can lower the response threshold to Δ9-THC and which are often not altogether ponderable.

The role of Δ9-THC in drivers’ neuromotor impairment and the resulting occurrence of motor vehicle accidents has been traditionally established in experimental and epidemiological studies, [69] which have shown that Δ9-THC impairs cognition, psychomotor function and actual performance-driving, in a dose-related effect. The degree of impairment observed after doses up to 300 µg/kg of Δ9-THC (approximately 21 mg for a 70 kg man) was equivalent to the detrimental effect of a dose of alcohol which produces a blood alcohol concentration greater than or equal to 0.5 g/L (the legal limit for driving under the influence in most European countries).

Psychomotor effects start at Δ9-THC doses of around 6 mg (90 micrograms of Δ9-THC/kg for a person weighing 70 kg). A study [70] regarding the correlation between serum Δ9-THC concentrations and relevant psychoactive effects showed that the lowest effective dose of Δ9-THC capable of creating such effects corresponds to that of 2 mg taken orally. On this subject, Grotenhermen et al. [70] found that serum Δ9-THC concentrations up to 12-13 ng / mL can be produced by 2 mg of Δ9-

THC, and are related to an increased risk of road accidents. Subsequently, some authors [16, 71] have redefined these aspects, determining that the Δ9-THC blood concentration generating driving problems varies from 1-2 to 5 ng / mL, and depends on whether the user is a regular or occasional consumer of cannabis.

It is noteworthy that the median plasma Δ9-THC concentration in drivers who had already exhibited the effects was 2.5-5 ng/mL, thus significantly higher than in those who did not [15, 16].

Some authors [41] have studied Δ9-THC and CBD excretion profiles in blood, oral fluid and urine after smoking either one or four low-Δ9-THC cannabis cigarettes. Blood, oral fluid and urine samples were collected from six healthy light-cannabis users after smoking a 1 g cigarette containing 0.16% Δ9-THC and 5.8% CBD and from six subjects who smoked four cigarettes from 1 g within 4 hours. At the first collection (30 minutes after dosing), the highest concentrations of Δ9-THC and CBD were found in the blood (Δ9-THC 7.0-10.8 ng / mL; CBD 30.2-56.1 ng / mL) and the oral fluid (Δ9-THC 5.1–15.5 ng / mL; CBD 14.2-28.1 ng / mL). It follows that "Light cannabis" cigarette smoke produces a plasma concentration up to twice that necessary to cause alterations in driving.

A Swiss study [72] investigated Δ9-THC and CBD blood and urine concentrations of a naive user and modeled chronic user (2 joints per day for 10 days) after smoking a single CBD joint. Joints contained 200 mg of cannabis with Δ9-THC concentrations of 0.94% and 0.8% and CBD concentrations of 23.5% and 17% in the naive-smoker and chronic-smoker experiment, respectively. Samples were collected for 4 and 20 h after smoking start. Δ9-THC blood concentrations reached 2.7 ng/ml in the naive and 4.5 ng/mL in the chronic user. In both cases, the results were significantly above the Swiss road traffic threshold of 1.5 ng/mL, designating the user as legally unfit to drive directly after smoking. CBD blood concentrations of 45.7 and 82.6 ng/mL were reached for the naive and chronic user, respectively. Blood and urine samples were regularly collected during the 10-day smoking period, and while no accumulation of any cannabinoid was found in the blood during this time, urinary 11-nor-9-carboxy-Δ9-THC concentrations seemed to increase, which is important in abstinence testing (**Figure 2.1**) [72].

Most recently, some authors [43] studied Δ9-THC and CBD time courses in serum and physiological and behavioral effects associated with smoking 1 or 4 "light cannabis" cigarettes, as well as biomarkers to differentiate light cannabis versus illegal and medical cannabis use. Sera were obtained from 6 healthy light cannabis consumers and 6 individuals at different times (0.5, 1, 2, 3, 4, and 5 hours) after smoking 1 (1.6 mg Δ9-THC and 58 mg CBD) and 4 (6.4 mg Δ9-THC and 232 mg CBD) cigarettes in 4 hours, and analyzed by liquid chromatography–tandem mass spectrometry. In serum, minimal Δ9-THC concentration was observed after a single cigarette smoke, while repeated smoking increased it. CBD concentrations were higher, but did not increase linearly. The highest Δ9-THC and CBD concentrations were observed 0.5 hours after the start of the smoking of 1 cigarette and, similarly, 0.5 hours after the smoking of 4 cigarettes. Serum Δ9-THC ranged from 2.7 to 5.9 ng/mL for 1 cigarette and 11.0– 21.8 ng/mL for 4 cigarettes, while serum CBD varied from 5.7 to 48.2 ng/mL for 1 cigarette and 19.4–35.3 ng/mL for 4 cigarettes. In both cases, the mean Δ9-THC/CBD concentration ratio ranged from 0.2 to 0.9. There were no significant changes in blood pressure, heart rate, and body temperature, but participants who smoked 4 cigarettes experienced severe drowsiness. The authors concluded that serum Δ9-THC/CBD concentration ratio not higher than the mean value of 0.9 might be a useful biomarker to identify use of light cannabis versus that of illegal Δ9-THC cannabis (Δ9-THC/CBD concentration ratios generally greater than 10) or medical cannabis (where ratios are greater than 1) (**Figure 2.2**) [43].

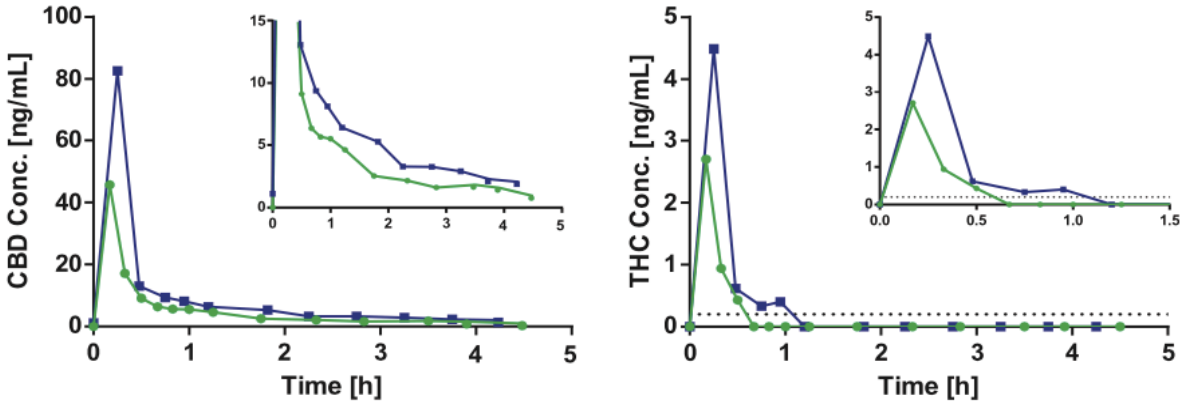

Figure 2.1 CBD (left) and Δ9-THC (right) blood concentrations obtained for the naive-smoker (circles) and chronic-smoker (squares) experiments. The dotted line shows the limit of detection (0.2 ng/mL). The smaller panels within the graphs show enlarged areas of interest. **[72]**

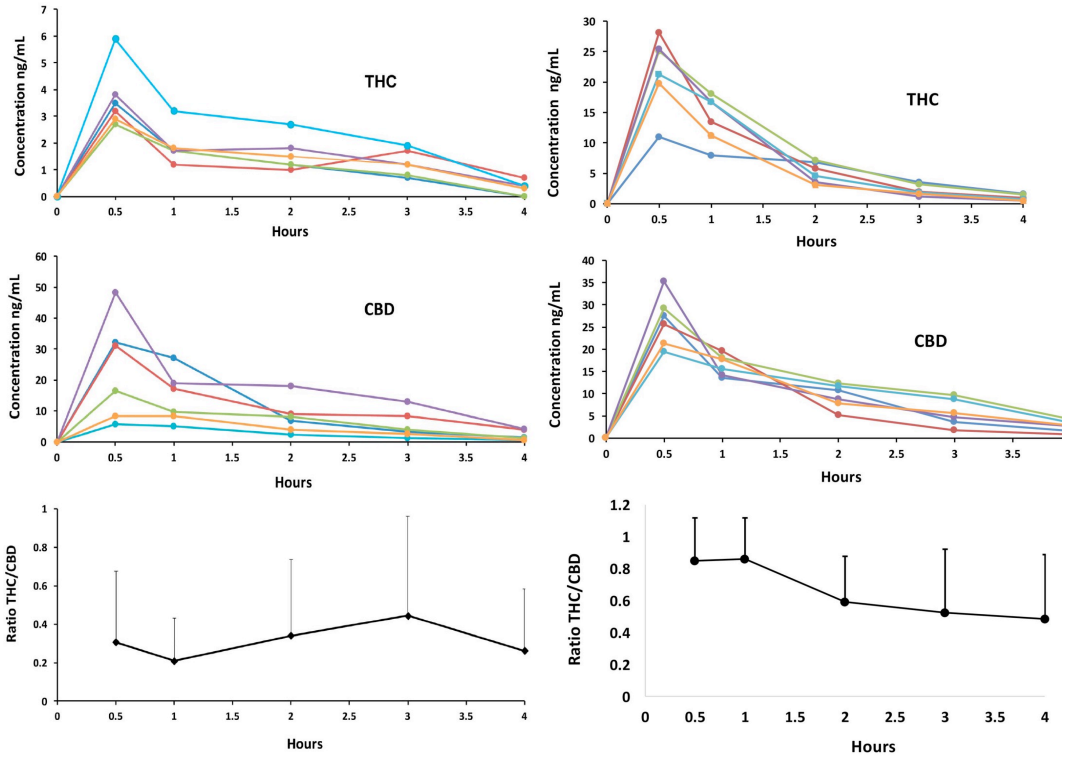

Figure 2.2. Time course of serum concentrations and concentration ratio of Δ9-THC and CBD after the smoking of 1 light cannabis cigarette (left) and time course of serum concentrations and concentration ratio of Δ9-THC and CBD after the smoking of 4 subsequent light cannabis cigarettes in 4 hours (right) [43].

Many well-known commercial sites related to Cannabis that are regularly visited by consumers *(https://www.practicalpainmanagement.com/)* have introduced a notice for the initial use of low doses of Δ9-THC, in order to avoid unwelcome reactions for the consumer who is at the beginning of his/her experience. This marketing technique recommends the minimum effective doses to produce perceptible and therefore appreciable effects on the subject. The dosage recommended by these organizations ranges from 1.5 to 5 mg for "beginners" with low Δ9-THC tolerance due to the absence of previous experience. The quantity of raw product that is necessary to connote a "psychoactive dose" therefore depends on the Δ9-THC quantity (in milligrams) that is actually contained in it. The higher the percentage of active compound contained in the vegetable, the lower the quantity of the vegetable product needed to reach the so-called "psychoactive dose" (Table 1).

	% of Δ9-THC										
	0.1	**0.25**	**0.5**	**1**	**2**	**3**	**4**	**5**	**10**	**20**	**30**
g. of product	**mg of Δ9-THC**										
0.5	0.5	1.25	2.5	5	10	15	20	25	50	100	150
1	1	2.5	5	10	20	30	40	50	100	200	300
2	2	5	10	20	40	60	80	100	200	400	600
3	3	7.5	15	30	60	90	120	150	300	600	900
4	4	10	20	40	80	120	160	200	400	800	1200
5	5	12.5	25	50	100	150	200	250	500	1000	1500
10	10	25	50	100	200	300	400	500	1000	2000	3000

Table 2.1: delta9-Δ9-THC dose in milligrams based on the percentage of active compound and the amount of vegetable. From the table it is clear that to reach 5 mg of active compound (Δ9-THC) the following are necessary (combinations underlined in the table):

a) 0.5 grams of material (oil, inflorescences) with a Δ9-THC percentage of 1 %%;

b) 1 gram of material (oil, inflorescences) with a Δ9-THC percentage of 0.5 %%;

b) 2 grams of material (oil, inflorescences) with a Δ9-THC percentage of 0.25%;

c) 5 grams of material (oil, inflorescences) with a Δ9-THC percentage of 0.1%.

Δ9-THC Dose = (% Δ9-THC x mg V) / 100

The evaluation of the psychoactive dose must consider both the total amount of active compound taken and the route of administration, on which the bioavailability of the substance depends, and not only the percentage. While inhalation gives a more immediate bioavailability of the substance, the oral route determines a later one. Moreover, the effects appear to be indirectly proportional to the habituation to the substance, with more precocious and evident clinical reactions in naive subjects who are not accustomed to the consumption of Δ9-THC. It is noteworthy that the subjective perception of the effects is not aligned with the objective significance of the same: this accounts for the decreased perception of risk in these subjects [70].

Given the wide range of variants that intervene in each individual and having to find a "numerical value" that can contain all the parameters contributing to the effects of the active compound Δ9-THC, the quantity of the substance necessary and sufficient to produce a clinically detectable alteration can be identified as 5 mg of Δ9-THC. Although some studies identify effects even with lower doses (up to 1-2 mg inhaled), all studies converge or contain this value. It follows that this can be identified as a threshold value for the application of the law, in analogy with the concept of cut-off already used in other areas.

2.6 Cannabis and minors

Among many public health and safety concerns, there is the effect on brain development in cannabis users younger than 17 [73]. Connections between different areas of the brain may not develop normally in these young regular cannabis smokers, and some of these changes in brain development may not be reversible. It is important to point out that in individuals predisposed to developing

schizophrenia or psychosis, their conditions might be unmasked by cannabis use and often their first schizophrenic episode occurs after the first intake of cannabis. Many scientific literature reviews have unequivocally clarified the dangers and damage deriving from the consumption, even occasional, of cannabis and its derivatives [73-76]. The 2019 European School Survey Project on Alcohol and Other Drugs (ESPAD) [77] is a report based on information provided by 99.647 students from 35 European countries, 25 of them being Member States of the European Union. It represents the most extensive data collection on substance use and risk behaviors in Europe. Results show that cannabis is perceived as the easiest illicit substance to get hold of, with around one third of students (32 %) rating it as easily obtainable. Cannabis was the most widely used illicit drug in all countries. About 16 % of students declared having used cannabis at least once in their lifetime, and Italy figures among the countries with the highest prevalence of cannabis use (27 %). Among students who had used cannabis in the last 12 months (13%), the Italian ones reported consuming it once a month on average (12 or more occasions). Overall, 7.1 % of the students had used cannabis in the last 30 days. A high variability was found among ESPAD countries, with the maximum rate observed in Italy (15 %). Trends in cannabis use indicate a general increase in both lifetime (from 11 % to 16 %) and last-30-day (from 4.1 % to 7.4 %) use between 1995 and 2019. On average, 2.4 % of the ESPAD students reported having used cannabis for the first time at age 13 or younger, with Italy being one of the countries with the highest rates (4.4 %). To estimate the risk of cannabis-related problems, the ESPAD questionnaire included the CAST (Cannabis Abuse Screening Test) scale. The results suggest that around 4.0 % of students in the total ESPAD population are at risk of developing cannabis-related problems, corresponding to an average proportion of 35 % among students who reported cannabis use in the last year. Overall, boys rate higher than girls on every aspect: lifetime cannabis use, frequency of use in the last 12 months, cannabis use in the past 30 days and prevalence of high-risk cannabis users. It is important to keep in mind that there is not a simple and direct relation between cannabis use and risky use, since other factors (quantities actually used, as well as social and cultural elements) might play a role in it. The 2019 EMCDDA also points out that in recent

years, new forms of cannabis have been developed as a result of advances in production techniques, and cannabis products tend to be much stronger than in the past, meaning that the potential health risks for adolescents might have changed [78].

2.7 Extraction with butane gas

The speed of onset of pharmacological effects and their intensity strictly depends on both the route of delivery and the individual metabolism of Δ9-THC [79]. In particular, the administration by inhalation, smoking or inhalation with a vaporizer, allows a faster absorption of the substance and a more rapid onset of effects.

The use of marijuana concentrates has escalated in recent years with butane extracts appearing particularly popular. The administration of butane hash oil, colloquially referred to as "dabbing," is distinct from traditional flower cannabis usage due to the Δ9-THC content of samples and the presence of impurities such as unpurged butane [80, 81]. Today's amateur extracts are often created using a process that involves butane, hence the term "butane hash oil" (BHO), but regardless of the solvent, the result is a product potentially far more potent than flower cannabis. Therefore, products with low concentrations of Δ9-THC and marketed for "oral" use, when extracted with the butane technique, can lead to more concentrated Δ9-THC products which, if taken by inhalation, can cause psychoactive and behavioral effects far greater than those that would be obtained after ingesting the same product.

Butane is a non-polar, Class 2 flammable liquefied gas that has a low boiling point (-0.5°C), which is helpful when cold-boiling the residual solvent from the concentrate solution. This process leaves behind the temperature-sensitive terpenes. Hydrocarbons are arguably the most efficient solvent for cannabis extraction. One advantage of hydrocarbon extraction is the sheer number of products that can be obtained from a single standard extraction without further refinement. Currently, the preferred method is to separate the crystalline high-cannabinoid extract (HCE) from the aqueous, high-

terpene extract (HTE). These fractions can be sold as separate stock keeping unit or recombined at a ratio of the processor's choosing to create a full-spectrum extract (FSE).

It is important to note that the starting material's quality has a direct effect on the finished concentrate's quality, regardless of extraction methodology.

The Butane Process include the following steps [82]:

1. The Wash: Cold butane is released from the solvent tank into the column, where it slowly washes over the plant material, dissolving the cannabinoids and terpenes from the cannabis. After that, the solution can be collected directly, or it can be processed through an in-line de-waxing column.
2. Winterization/Filtration: Butane extractions are not typically winterized and filtered because the low extraction temperatures dissolve almost no chlorophyll and because the low temperature limits the amount of dissolved lipids/waxes. Additionally, many closed-loop hydrocarbon extraction machines come equipped with in-line de-waxing systems. As with winterization, in-line de-waxing requires a minimum (-30°C) environment, but it is a single-solvent system, where winterization uses a secondary solvent.
3. Collecting Concentrate: Once the concentrate solution enters the collection pot, the residual butane is purged off passively by heating the vessel, which pushes the butane out of the concentrate solution back to the colder solvent tank. Then the extraction technician collects the concentrate solution and places it on a parchment sheet or into a glass media bottle for separation.
4. Removing Residual Solvent: Purge methods and durations are dependent on the desired finished product. If the desired end product is wax, the concentrate solution can be whipped for a couple of hours to remove all residual butane. If shatter is the desired product, the concentrate solution is spread thin across Teflon sheets and purged inside a vacuum oven for a minimum of 48 hours.

Despite the potential consequences of illicit production of butane hash oil, at-home production rates appear to be increasing and can be also performed with cannabis with low Δ9-THC content. However, there continues to be a lack of detailed literature for researchers and professionals to utilize when creating a response to this issue [80].

Chapter 3. Psychopharmacological experimental study

As reported in previous chapters, in Italy law no. 242/2016 allows the cultivation of hemp up to 0.6% of Δ9-tetrahydrocannabinol (Δ9-THC) for specific purposes. Human consumption for recreational purposes was not included among the activities permitted, but, in the absence of explicit laws banning the commercialization of flowering tops with a Δ9-THC content between 0.2 and 0.6%, these products gained popularity because they contain Cannabidiol (CBD), which has been reported to reduce anxiety and promote sleep through a sedative effect [9, 20, 37]. Manufacturers are thus commercializing hemp with low content of Δ9-THC, which is referred to as "light cannabis". The increase in the number of light cannabis shops was very significant, starting with a few in early 2000 to more than 700 in March 2019 [3]. The commercialization of low Δ9-THC products and variable CBD concentration is proliferating, and cannabis farmers have been working to create new cannabis varieties, expressing up to a 25% total of CBD and less than 1% total Δ9-THC.

In this context, the Supreme Court [83], established that hemp with a Δ9-THC content below 0.6% cannot be commercialized for human use, when the "psychotropic effect" of the product and its "offensiveness" can be demonstrated [84].

Several studies explored the relationship between cannabis products with a high content of Δ9-THC, blood levels and psychomotor functions [51, 55, 85], but few pharmacokinetic studies on limited population samples [41-43, 72, 86, 87] and no psychopharmacological studies have been performed on light cannabis consumption.

The aim of the present study was to assess Δ9-THC and CBD blood concentration following light cannabis smoking, and its effects on young adults' vigilance, cognitive and motor skills. A "smoking session" under controlled experimental conditions was reproduced. Seriate blood samples were collected in order to determine the pharmacokinetic profile of Δ9-THC and CBD. Psychomotor and cognitive performance were assessed by using laboratory tests measuring attention, cognitive functions, visual-spatial skills and vigilance.

3.1 Materials and methods

3.1.1 Experimental study

Eighteen healthy young adult volunteers (11 males and 7 females) participated in the study. Mean±SD age was 31.3±3.2 y.o. (males 32.4±3.2 y.o.; females 29.3±2.1 y.o.); mean weight was 72.3±13.7 Kg (males 79.1±8.3 Kg; females 56.8±7.1 Kg). Exclusion criteria were neurological, cardiovascular, respiratory and endocrine disease, a history of psychiatric disorders, a history of alcohol or substance use disorder (including frequent use of cannabis), renal failure, pregnancy or lactation, coagulopathies, anatomic mutilations and visual deficits, Δ9-THC or other drugs detection before the experiment. Inclusion criteria were past use of light cannabis products on at least 5 occasions during life and a previous history of tobacco smoking for at least 1 year. The study was conducted according to the code of ethics on human experimentation, as established in the declaration of Helsinki (1964) and amended in Edinburgh (2000) and was approved by the Ethical Committee of the University of Bologna. All subjects were fully informed on study procedures and aims and gave their written informed consent.

Subjects received three cannabis light cigarettes containing 400 mg of light cannabis with a percentage of 0.41% of Δ9-THC corresponding to 1.64 mg for each cigarette and of 12.41% of CBD corresponding to 49.64 mg for each cigarette. Subjects were asked to consume each cigarette according to their smoking habits, simulating as much as possible a recreational setting. The weight of the cigarette (400 mg) was based on the mean weight of cannabis cigarettes seized in the area of Bologna, and analyzed in the past 3 years by the Forensic Toxicology Laboratory of the University of Bologna. Participants were free to stop the experiment if they felt uncomfortable with the experimental setting, and could report any sensations felt during the experimental session for up to 24 hours. The smoking time varied for each participant, and was calculated as the “end time” of each cigarette from the beginning of the experiment, in seconds. Six blood samples were collected from each participant: before the beginning of the experiment (t0), after each light cannabis cigarette

(t1→t3) and 60 (t4) and 120 (t5) minutes after the beginning of the experiment. Six milliliters of whole blood were collected in glass tubes with EDTA for the analysis. Blood samples were conserved at a temperature of -20°C until analysis.

3.1.2 Determination of Δ9-THC and CBD in blood

Frozen blood was thawed at room temperature. Identification and quantification of cannabinoids in biological fluids were determined by a validated gas chromatography-mass spectrometry (GC-MS) method, following the international criteria [88, 89]. Two mL of whole blood were extracted with 3 mL hexane/ethyl acetate (9:1). After the extraction, the samples were evaporated to dryness, reconstituted in 30 µL N,O-Bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane and 1 µL injected onto a Shimadzu GC-2010 Plus gas chromatograph equipped with a model AOC-6000 autosampler system (Shimadzu) and interfaced with a QP 2010 Ultra mass spectrometer (Shimadzu). Analytes separation was achieved in 15 min on a Restek Rxi-5Sil MS column, 30 m length x 0.25 mm internal diameter x 0.25 mm film thickness. The oven temperature was programmed at 80 °C for 1 min, increased to 220°C at 40°C/ min (held 0.5 min), increased to 300°C at 10°C/min and maintained for 8 min. Samples were analyzed in splitless injection mode. Helium (purity 99,9999 %) was the carrier gas at a flow rate of 1,5 mL/min. The injection port, ion source and interface temperatures were: 300 °C, 230 °C, 150 °C, and 280 °C, respectively. The dwell time was 0.1 ms. Ions m/z 386, 371 (quantifier) and 303 were monitored for Δ9-THC-trimethylsilyl(TMS), m/z 458, 390 (quantifier); 337 and 301 for CBD-2TMS; m/z 389, 374 (quantifier) and 306 for Δ9-THC-d3-TMS in selected-ion-monitoring (SIM) mode. Linearity was tested in the interval (0.15 – 10 ng/ml) with a coefficient of determination of the curve greater than 0.998 for Δ9-THC (y=0.020583x+0.002676) and 0.995 for CBD (y=0.053377-0.005574). LLOQ was 0.15 ng/ml. Intra- and inter-assay precision and accuracy were always less than 20%.

3.1.3 Evaluation of psychomotor and cognitive performance task

Five performance tasks and a subjective scale were employed for measuring cognitive and psychomotor performances. Tests were performed the day before (TT0) the experimental session and, due to the very low half-life of Δ9-THC in blood and the duration of the tests, after collecting the blood sample in t3 (TT1). The following tests were performed in this order:

1. *Reaction Time* (RT) is one of the core methodological paradigms of human experimental and cognitive psychology, but is also commonly analyzed in psychophysiology, cognitive neuroscience, and behavioral neuroscience to help elucidate the biological mechanisms underlying perception, attention and also in the assessment of fitness to drive [90, 91]. The reaction time was assessed through a mobile device measuring the time interval between the appearance of a circle and the touch of the screen. The subject sits with finger poised over a button which s/he is required to press as quickly as possible when the stimulus is presented. The stimuli used were a yellow light. The reaction times (millisec) were recorded. The test was repeated 15 times each session. Execution time: 1 minute.

1. *Digit Symbol Substitution Test* (DSST) [92] is a standard neuropsychological test to understand human associative learning. Its clinical utility, owing to its brevity and high discriminant validity, was first recognized in the 1940s. The DSST is sensitive to the presence of cognitive dysfunction as well as to changes in cognitive function across a wide range of clinical populations, but has low specificity to determine exactly which cognitive domain has been affected. However, the DSST offers a practical and effective method for monitoring cognitive functions over time in clinical practice. The task requires the subject to fill boxes with symbols which are located at the top of the page and linked to the box number. The test lasts 90 seconds and the results are given by the number of correct symbols drawn by the participant. The order of symbols was changed in the two sessions to avoid performance bias. The results of the test are given in seconds. Execution time: 1 minute.

2. The *Tower of London* (TOL) [93, 94] is a decision-making task that measures executive function and planning. The task consists in arranging three colored cylinders on three sticks. The subject's task is to determine as quickly as possible whether the end-arrangement can be accomplished using a computer software using the mouse to pick and release the cylinders from the beginning arrangement to the final arrangement requested by the program. The main performance measure is given by the total number of correct decisions and the time to complete 24 stages with increasing difficulty.
3. The *Trail Making Test* (TMT) [95] is one of the most popular neuropsychological tests and is included in most test batteries. It provides information on visual-spatial skills including visual search, scanning, speed of processing, mental flexibility, and executive functions. Both parts of the Trail Making Test consist of 25 circles distributed over a paper sheet. In Part A, the circles are numbered 1 to 25, and the subject should draw lines to connect the numbers in ascending order. In Part B, the circles include both numbers (1 – 13) and letters (A – L); as seen in Part A, the patient has to draw lines to connect the circles in an ascending pattern, but this time with the added task of alternating between the numbers and letters (i.e., 1-A-2-B-3-C, etc.). The results of the test are given in seconds. Execution time: 1 minute for both parts.
4. *The compensatory tracking task* (CTT) [96] studies the fluctuation in vigilance and requires continuous user input at near-one second intervals. It measures the subject's ability to control a displayed error signal in a first-order compensatory tracking task. The test requires a subject to use a trackball to keep a circular disk centred on a bullseye ring with an inner radius equal to the disk radius. The screen background is black, the disk light grey. The velocity of the radial disk continuously changes in magnitude and direction. The output is measured in mean radial distance of the target disk from the screen centre and the mouse velocity, which is the compensatory user input. The test was repeated three times for each session. Execution time: 3 minutes.
5. *Subjective "high"*. Subjects were required to rate their feeling of "high" as a percentage (0–100) of the maximum they ever experienced on a 100 visual analog scale [97].

3.1.4 Data analyses

Mean (SD) concentrations of Δ9-THC and CBD were calculated for each point (t0→t5) and graphically reported, to assess variation over time and inter-individual variability. The Δ9-THC/CBD ratio was obtained and reported through a graphical superimposition, after a spline curve smoothing. The mean concentrations of each point were compared between male and female subjects through a non-parametric unpaired t-test. The overall effect of Δ9-THC on psychomotor performances was studied by comparing the results obtained the day before the test (TT0) with the results obtained immediately after the collection of the third blood sample (TT1) through a paired nonparametric t-test (Wilcoxon test). A two-sided $p<0.05$ was considered to indicate statistical significance for all analyses. Statistical analyses were conducted using GraphPad Prism (8.00, GraphPad Software, La Jolla California USA).

3.2 Results

3.2.1 Experimental study

All subjects completed the experiment by smoking the three cigarettes according to their smoking habits.

Minimum, maximum, mean and median smoking time are reported below:

- first cigarette (sec): min 5.5; max 11.2; mean 8.6; median 8.0;
- second cigarette (sec): min 20.5; max 37.2; mean 27.6; median 27.5;
- third cigarette (sec): min 41.2; max 63.0; mean 53.6; median 53.5.

Minimum, maximum, mean and median collection-time are reported below:

- t0 (minutes): 0;
- t1 (minutes): min 7.8; max 14.0; mean 11.5; median 11.6;
- t2 (minutes): min 24.2; max 41.5; mean 31.9; median 30.8;
- t3 (minutes): min 44.5; max 66.0; mean 57.5; median 58.0;
- t4 (minutes): min 85.0; max 98.4; mean 91.4; median 90.5;

- t5 (minutes): min 115.0; max 130.0; mean 119.9; median 120.0;

At the end of the experiment, all subjects reported that, in a recreational setting, they would not smoke any more cigarettes.

3.2.2 Determination of Δ9-THC and CBD in blood

No cannabinoids were detected in the blood samples taken prior to smoking in all participants (t0). **Table 1** reports mean Δ9-THC and CBD concentrations with standard deviations (SD) for each collection time (t0→t5). All collection times are given relative to smoking start. **Figure 3.1** displays the mean time-course of Δ9-THC and CBD concentrations in blood for each collection time (t0→t5). The CBD/Δ9-THC superimposition, reported in **Figure 2**, shows that both Δ9-THC and CBD concentrations rapidly increased after the first intake, reached a steady state during the three doses, and decreased after the third. Δ9-THC decreases more rapidly than CBD. The higher point concentrations observed in single (different) subjects were 3.4 ng/ml in t1, 3.5 ng/ml in t2 and 3.0 ng/ml in t3 for Δ9-THC and 41.4 ng/ml in t1; 42.2 ng/ml in t2 and 44.7 ng/ml in t3 for CBD. The participant who showed average higher concentrations (t1= 3.37 ng/ml; t2= 2.91 ng/ml, t3=2.38 ng/ml, t4=1.48 ng/ml, t5=0.70 ng/ml) was a 55 Kg 30 y.o. female. In all subjects concentration detected in t1, t2 and t3 were higher than those detected in t4 and in t5. No significant differences were observed between average concentrations observed in males and in females.

3.2.3 Cognitive and motor skills

Complete data sets were collected for all tests. The results are showed and compared in **Figure 3**. No significant differences were observed between TT0 and TT1 for all performed tests ($p>0.05$). None of the subjects declared to feel "high" after consuming the third dose and at the end of the experiment nor reported adverse reactions in the following 24 hours.

	THC (ng/ml)				CBD (ng/ml)			
Blood collection	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**
t0	0.00	0.0	0.0	0.0	0.0	0.0	0.0	0.0
t1	1.0	0.8	0.3	3.4	10.6	10.3	1.6	41.4
t2	1.2	0.9	0.5	3.5	10.3	13.2	1.2	42.2
t3	1.0	0.8	0.3	3.0	15.1	14.8	0.8	44.7
t4	0.6	0.4	0.1	1.5	9.9	9.2	0.8	28.9
t5	0.3	0.3	0.0	0.8	6.2	5.7	5.7	0.0

Table 3.1 Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD) concentration. Mean=mean inter-individual concentration for each collection time; SD=Standard deviation; Min=minimum concentration detected among participants; Max=maximum detected among participant.

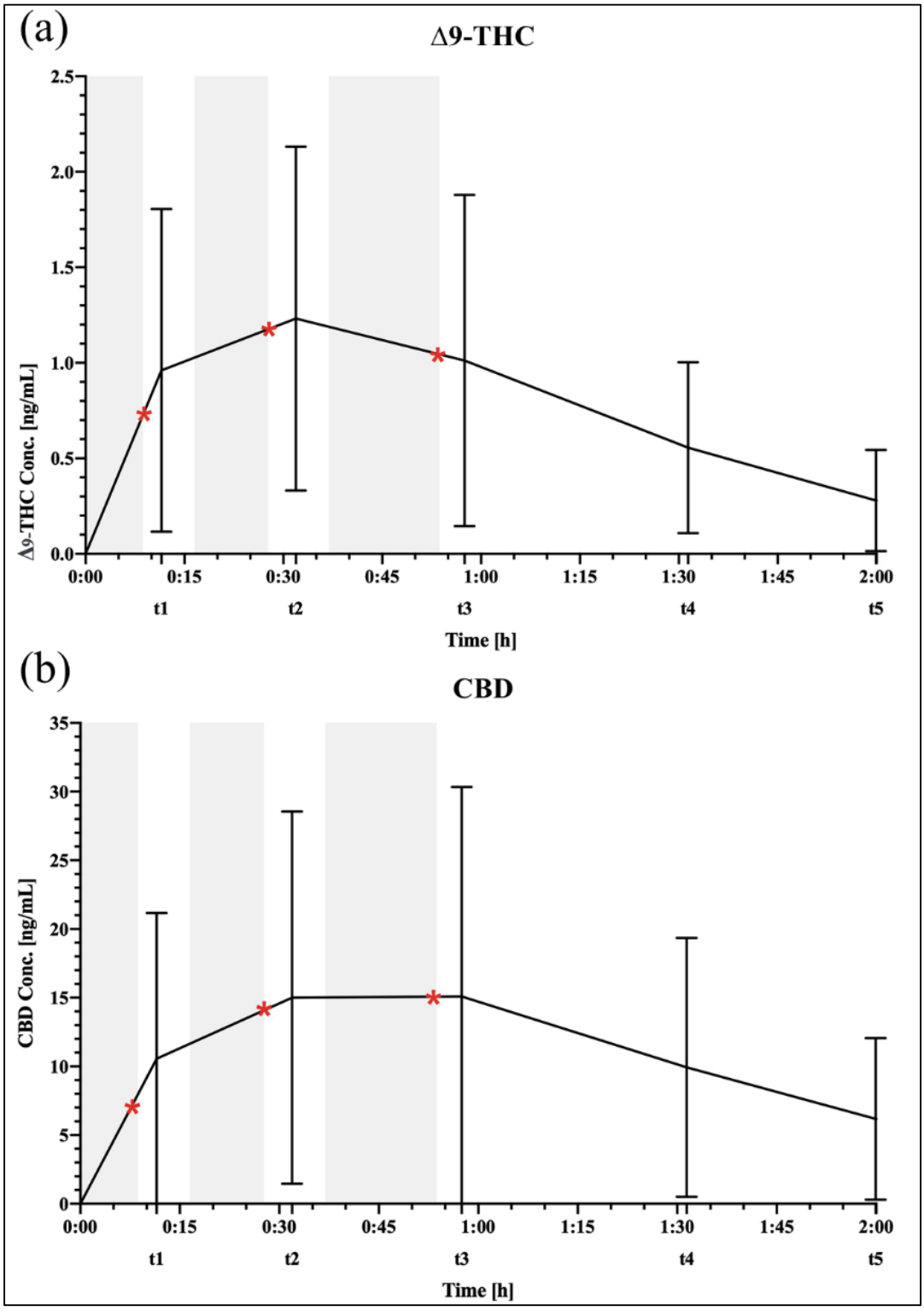

Figure 3.1. Mean time-course (t1→t5) of Δ9-tetrahydrocannabinol (Δ9-THC) (a) and Cannabidiol (CBD) (b) and Standard Deviation (SD) expressed in ng/ml. The light cannabis intakes are represented with the red asterisks. The grey zone represents the mean time taken to complete the light cannabis cigarettes. h: hours

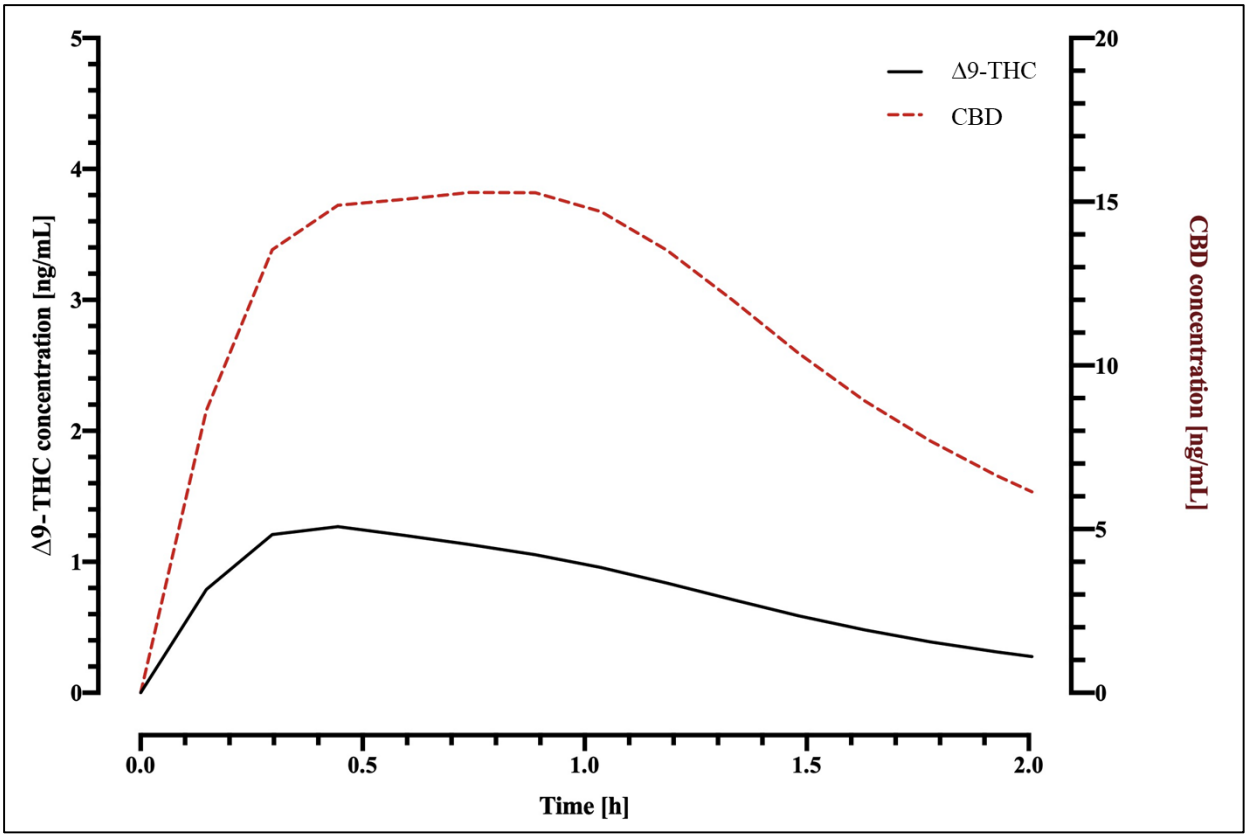

Figure 3.2 Superimposition of Δ9-tetrahydrocannabinol (Δ9-THC, black line, left y axis) and cannabidiol (CBD, red dotted line, right y axis) concentration over time, after spline smoothing. h: hours.

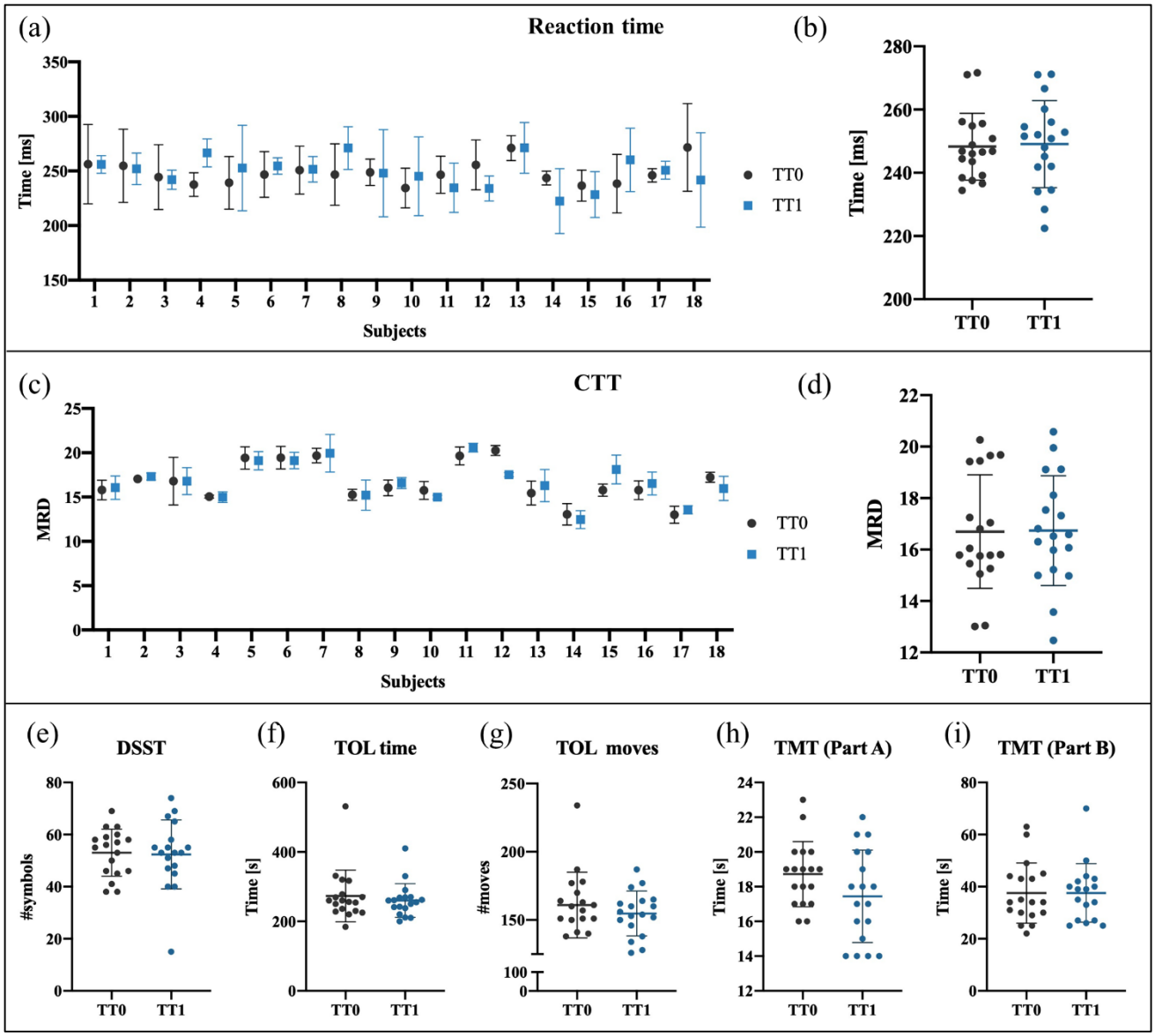

Figure 3.3. Results of psychomotor tests. Reaction time=Individual (a) and overall (b) Reaction Time expressed in milliseconds (mean and SD). CTT= Individual (c) and overall (d) Compensatory Tracking Task expressed in mean radial distance of the target disk from the screen center. Individual values (a) and (c) are expressed as mean and SD of single repetitions. Overall values (b) and (d) are expressed as mean and SD among participants. DSST = Digit Symbol Substitution Test expressed in number of symbols completed (mean and SD) (e). TOL= Tower of London expressed in total time in seconds (f) and number of moves (g) (mean, SD) TMT= Trail Making Test, Part A (h) and Part B (i) expressed in seconds (mean and SD).

3.3 Discussion

In the present study, we simulated a "smoking session" of light cannabis with low percentage of Δ9-THC (0.41%) and high percentage of CBD (12.4%), in a population of young adults. The total amount of product and the number of cannabis cigarettes were defined in order to simulate a recreational intake of light cannabis as much as possible. During the experiment, all subjects smoked three light cannabis cigarettes with 400 mg of flowering tops, which is the medium weight of a cannabis cigarette seized in the area of Bologna and analyzed in the Laboratory of Forensic Toxicology of the University of Bologna, in the last three years.

All study participants reported that a higher number of cigarettes, corresponding in this study to 1200 mg of herbal product, could hardly be consumed by smoking in a recreational setting.

The blood concentrations of both Δ9-THC and CBD rapidly increased after smoking the first light cannabis cigarette, followed by a steady state (second and third cigarettes) and decreased after consuming the third cigarette. Both Δ9-THC and CBD did not show a sharp accumulation in the blood, as one would expect after repeated intakes of equal doses, since single concentrations in t1, t2 and t3 were similar and higher than t4 and t5 in all participants. Toxicological results showed a decrease from the blood stream after the third dose, which seemed sharper for Δ9-THC, due to its shorter half-life compared to CBD. The Δ9-THC/CBD ratio never exceeded the unit, and maximum value observed in a single subject was 0.91 (obtained in t3). Since illegal cannabis rich in Δ9-THC contains higher Δ9-THC compared with CBD, and ratios in blood and serum are normally greater than 10 [98], Δ9-THC/CBD <1 might be useful in discriminating light cannabis versus illegal or medical cannabis with a high percentage of Δ9-THC, as previously proposed [43].

The pharmacokinetic profile of Δ9-THC and CBD were similar to those reported in previous studies on light cannabis [41, 43, 72]. Interestingly, the average concentrations of Δ9-THC were much lower than those previously observed in a population sample of 6 older adults smoking 1.6 mg and 6.4 mg of 0.16% Δ9-THC, while CBD blood levels (58 mg and 232 mg respectively) were similar [41, 43].

The blood concentrations of some of our participants were close to those observed in a 37-y.o. female smoking about 2 mg of 0.94% Δ9-THC (peak concentrations of 2.7 ng/ml of Δ9-THC and 45.7 ng/ml of CBD) [72], but average concentration are much lower. The differences observed reflects one of the strengths of our experiment, which is the larger population sample size compared to previous studies on light cannabis. Actually, among 18 participants we observed a very high SD, reflecting a high inter-individual variability that was not previously reported. This variability, that seems not influenced by gender differences, could be due to different smoking participant's habits, as participants were asked to smoke according to their habits without forcing the intake, and some subjects may have held the smoke in their lungs for a longer duration, leading to a higher absorption.

Another main finding of the present study is that no significant differences were observed between the results of psychomotor tests in TT0 (the day before the experiment), and in TT1 (after smoking the third light cannabis cigarette).

Previous psychopharmacological studies observed that, in some subjects, low doses of Δ9-THC as 2.5 mg [62], 5 mg [67] or 8 mg [55] at higher percentages were capable of producing subjective and/or objective impaired performance on psychomotor tasks or symptoms of intoxication. These observations are only apparently in contrast with our results. In fact, even if the total intake of Δ9-THC is similar with the cited studies, in our experiment participants smoked 4.92 mg of Δ9-THC contained in a higher volume of herbal product (1200 mg in total). The longer time interval needed for its complete consumption did not permit the accumulation of Δ9-THC in the blood stream, as a consequence of phase I metabolism and the known rapid uptake of cannabinoids by fat-containing tissues [62].

It is well known that the psychopharmacological effect of Δ9-THC progressively increases as a function of blood Δ9-THC [85]. After inhalation, Δ9-THC is absorbed through the lungs, rapidly enters in the bloodstream, reaches the brain within minutes and exert their effect by interaction with specific endogenous cannabinoid receptors. High concentrations are reached in neocortical, limbic, sensory and motor areas, and effects are perceptible within seconds and fully apparent in a few minutes [62].

After administering cannabis with high percentage of Δ9-THC to 20 study participants, Raemakers et al. [85] observed significantly impaired cognitive and motor performance in tracking and cognitive tasks at increasing serum concentrations: 71% impaired observations were obtained between 2 and 5 ng/ml and 100% impaired observations were obtained for concentrations > 30 ng/ml.

In our experiment, blood point concentrations between 2 and 3.5 ng/ml were detected in only 4 subjects, and blood concentrations between 1 and 2 ng/ml were detected in 7 subjects. Other participant's blood concentrations were lower than 1 ng/ml in all blood collections. Therefore, the lack of impairment observed in our participants can be interpreted as a consequence of the very low concentrations detectable in the blood after light cannabis consumption.

Moreover, the proprieties of CBD could have influenced the impairing effect of the product. In a recent randomized controlled trial, high doses of CBD (400 mg) taken together with Δ9-THC (12 mg) were observed to reduce the subjective and objective intoxicating effect of Δ9-THC [55], even if the mechanisms of interaction are still to be elucidated.

Since no studies investigated psychomotor effects of cannabis with a low percentage of Δ9-THC and high percentage of CBD, our results can be used for future comparisons.

The main limitation of the study is the short total monitoring period of 2 hours after the beginning of the experiment. Not all participants in fact reached the 0 concentration for both compounds, which is normally reached about 4 hours after the experiment [43]. However, middle- and long-term blood/serum concentration have already been studied, but little is known in the short time after smoking, when higher Δ9-THC and CBD concentrations and a higher psychotropic impairment are expected.

Another limitation of the study is that, due to the very short half-life of Δ9-THC expected, we decided to perform exclusively psychomotor tasks, according to previous literature [51, 55, 85]. Finally, the highly polymorphic enzymes involved in Δ9-THC and CBD phase I metabolism [38, 99] should be analyzed, in order to better explain the high inter-individual variability observed in Δ9-THC and CBD blood concentrations.

Conclusion

The present thesis paper aimed at defining the psychoactive effect of light cannabis, a product that is gaining popularity in Italy.

Currently, there is not a specific law in Italy that punishes the commercialization for human recreational use of cannabis products up to 0.6%. In fact, Italian Supreme Court in 2019 established that hemp with a Δ9-THC content below 0.6% cannot be commercialized for human use, only if the "psychotropic effect" of the product and its "offensiveness" can be demonstrated.

The "psychoactive dose" defined by the Supreme Court can be identified, from a scientific point of view, as the minimum dose capable of negatively interfering with neuropsychic systems of the person, by altering their cognitive abilities, alertness and perception systems, along with many other functions. For the wide range of variants that occur in each individual, finding a "numerical value" that can contain all the parameters contributing to the effects of Δ9-THC is a difficult task. The scientific literature reviewed in this work defines the quantity of 5 mg Δ9-THC as sufficient to produce clinically detectable alterations. However, some studies identify effects even with lower doses (up to 1-2 mg inhaled) in some individuals.

In the third part of this work, we tested this amount of Δ9-THC on a population of healthy volunteers, who smoked about 5 mg of 0.41% THC contained in a total amount of herbal product of 1200 mg divided into three cannabis light cigarettes. All study participants reported that a higher number of cigarettes could hardly be consumed by smoking in a recreational setting. Δ9-THC and CBD concentrations showed a high inter-subject variability, and the average concentrations were lower than those previously reported. Toxicological results showed a decrease of Δ9-THC and CBD after the third light cannabis cigarette, and a Δ9-THC /CBD ratio always < 1 was observed. This value might be useful in discriminating light cannabis versus illegal/medical cannabis consumption. Finally, the lack of impairment observed in our participants can be interpreted as a consequence of the very low concentrations detectable in the blood.

Even if the total intake of Δ9-THC could hypothetically have a psycotropic effect, in our experiment participants smoked 4.92 mg of Δ9-THC contained in a higher volume of herbal product. The time interval of about 1 hour needed for its complete consumption did not permit the accumulation of Δ9-THC in the blood stream, as a consequence of phase I metabolism and the known rapid uptake of cannabinoids by fat-containing tissues.

Our data showed that light cannabis with 0.41% of Δ9-THC and 12.41% of CBD is not capable of producing impairment of attention, cognitive function, vigilance and decision-making, as well as the subjective feeling of impairment in our selected population of young healthy adults. Further studies are needed on different population samples with different way of consumptions, analyzing also the pharmacogenetics on study participants.

The rapid diffusion of light cannabis consumption, as experienced in our country, could soon involve other European countries, and should be promptly addressed by the scientific community.

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