



National Institute for Public Health  
and the Environment  
*Ministry of Health, Welfare and Sport*

**Risk assessment of (herbal preparations  
containing) *Salvia divinorum***

RIVM letter report 2021-0222  
A. Zwartzen





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and the Environment  
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## Colophon

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## Synopsis

### **Risk assessment of (herbal preparations containing) *Salvia divinorum***

In the Netherlands, (online) smart shops sell dried leaves and extracts of the herb *Salvia divinorum*. Products containing *Salvia divinorum* are used for their mind-altering and stimulant properties.

RIVM has assessed whether products containing *Salvia divinorum* are harmful to human health. This assessment has found that people using these products can suffer from hallucinations, restlessness, confusion, increased heart rate, increased blood pressure and psychosis. These health effects can even occur at the recommended quantity.

RIVM advises consumers not to use (herbal preparations containing) *Salvia divinorum*. The institute also advises the Ministry of Health, Welfare and Sport to consider restricting or banning the sale of (herbal preparations containing) *Salvia divinorum*.

Products containing *Salvia divinorum* offered for sale consist of (dried) leaves or herbal extracts. Most people smoke or vape these products. You can also chew the leaves or extract tea from them. The effects are caused by the active ingredient salvinorin A. It is not known how many people use these products.

Keywords: *Salvia divinorum*, salvinorin A, herbal preparation, psychoactive substance, hallucinogenic substance, mind-altering substance.



## Publiekssamenvatting

### **Risicobeoordeling van (kruidenpreparaten met) *Salvia divinorum***

In Nederland worden in (online) smartshops gedroogde bladeren en extracten van het kruid *Salvia divinorum* verkocht. *Salvia divinorum* wordt gebruikt als geestverruimend en stimulerend middel.

Het RIVM heeft gekeken of dit product schadelijk is voor de gezondheid. Hieruit blijkt dat mensen onder andere last kunnen krijgen van hallucinaties, onrust, verwardheid, verhoogde hartslag, verhoogde bloeddruk en psychoses. Deze gezondheidseffecten kunnen al ontstaan bij het aanbevolen gebruik.

Het RIVM adviseert consumenten daarom geen (kruidenpreparaten met) *Salvia divinorum* te gebruiken. Verder adviseert het instituut het ministerie van VWS om te overwegen de verkoop van (kruidenpreparaten met) *Salvia divinorum* te beperken of te verbieden.

*Salvia divinorum* is te koop als (gedroogde) blaadjes of als kruidenextract. De meeste mensen roken of vaperen dit. Je kunt ook op de blaadjes kauwen of er thee van zetten. De effecten worden veroorzaakt door de het actieve bestandsdeel salvinorine A. Het is niet bekend hoeveel mensen dit product gebruiken.

Kernwoorden: *Salvia divinorum*, salvinorine A, kruidenpreparaat, psychoactieve stof, hallucinogene stof, geestverruimende stof.



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## Summary

### Introduction

In December 2020, the Minister for Medical Care and Sport of the Ministry of Health, Welfare and Sport (VWS) announced actions to better regulate food supplements and herbal preparations in the Netherlands, thereby facilitating enforcement. One of those actions is to expand the list included in the Herbal Preparations Decree of the Dutch Commodities Act<sup>1</sup> with substances/botanicals that are either forbidden or restricted (i.e. subject to a maximum limit) in food supplements or herbal preparations (van Ark, 2020). In order to determine whether a substance or botanical needs to be included in this list, a risk assessment is warranted. The selection of substances and botanicals chosen for risk assessment was based on the prerequisite that the substances/botanicals were sold on the Dutch market and (widely) used in the Netherlands and that there were indications for possible health risks, e.g. Rapid Alert System for Food and Feed (RASFF) reports, from enforcement institutes. The current assessment is about (herbal preparations containing) *Salvia divinorum*.

Currently, there are no specific restrictions for the use of *S. divinorum* in herbal preparations included in the Herbal Preparations Decree of the Dutch Commodities Act<sup>1</sup>, the EU directive<sup>2</sup> on the manufacture, presentation and sale of tobacco and related products, or the Opium Act<sup>3</sup>.

### Use of *S. divinorum*

*S. divinorum* is sold as a natural herb for recreational use. *S. divinorum* is considered one of the most potent naturally occurring hallucinogens and induces powerful visual and auditory hallucination. The main active constituent of *S. divinorum* is salvinorin A (Brito-da-Costa et al., 2021). *S. divinorum* products can be smoked, vaporized, used to make tea (ingestion) and chewed upon without swallowing (sublingual). Of the users, 92.6% reported smoking or vaporizing *S. divinorum* products (Baggott, 2004). Salvinorin A can also be ingested when products are chewed without being swallowed, when juices are pressed from the leaves and mixed with water or alcohol, when leaves are ground and dissolved in water, or when leaves are used as to prepare tea.

### Previous evaluations

*S. divinorum* is listed in the EFSA Compendium of Botanicals for its psychoactive effects and ability to induce tachycardia<sup>4</sup>.

The Bundesinstitut für Risikobewertung (BfR) has published a risk assessment of *S. divinorum* in 2012 in which no quantitative risk assessment was performed (BfR, 2012). Due to its high acute hazard potential as a hallucinogen, the authors recommended including *S.*

<sup>1</sup> <https://wetten.overheid.nl/BWBR0012174/2020-07-01>. Accessed 26 august 2021.

<sup>2</sup> [DIRECTIVE 2014/40/EU of the European Parliament and of the Council of 3 April 2014](https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32014L0040).

<sup>3</sup> <https://wetten.overheid.nl/BWBR0001941/2021-10-28>. Accessed 08-04-2022.

<sup>4</sup> [https://combodb.ecomole.com/report/3472?botanic\\_page=22](https://combodb.ecomole.com/report/3472?botanic_page=22). Accessed 08-04-2022.

*divinorum* in list A of Appendix III (prohibited substances) of Regulation (EC) No. 1925/2006 on the addition of vitamins and minerals and of (ingredients containing) certain other substances to foods.

A monograph in the Natural Medicine Comprehensive Database states that usage of *S. divinorum* is possibly unsafe and has been shown to cause serious adverse effects (Natural Medicine, 2020).

### **Products on the Dutch market**

*S. divinorum* can be bought on the internet as dried leaves, extracts or herbal mixtures on the Dutch market. The concentration of salvinorin A in (dried) leaves found in literature ranges from 0.4-7.8 mg/g leaves (no Dutch products were analysed) (Medana et al., 2006; Wolowich et al., 2006). Several Dutch web shops also sell extracts with strengths 5-40 times the strength of dried leaves (Dutch-smart.nl). Literature data on these extracts showed that the concentrations advertised by the seller can differ greatly (in both directions) from the analytically determined concentrations (no Dutch products were analysed) (Hernández-Bello et al., 2015; Moreira et al., 2014; Wolowich et al., 2006). Sellers are most likely unaware of the actual concentrations of the products for sale. The dosage recommended by the sellers and on user platforms is dependent on the type of product and method of use.

### **Exposure assessment**

Based on the recommended use by the sellers and user platforms, and the measured concentrations of salvinorin A in leaves or the claimed concentrations of salvinorin A in extracts by web shops, the estimated exposure for consumers (for an individual of 70 kg) is 1.46-111 µg/kg body weight (bw) when smoking dried leaves and 19-77 µg/kg bw when smoking the 5X, 10X and 20X strength extracts (inhalation exposure). The estimated exposure through smoking dried leaves can be higher than following the use of extracts since the recommended use for the dried leaves is more than proportionally higher than for the extracts. Exposure to salvinorin A through chewing *S. divinorum* (extract) leaves or using a quid was calculated to be 12-891 µg/kg bw for dried leaves and 19-26 µg/kg bw for the 5X strength extract (sublingual exposure). When tea is made from dried leaves, users are exposed to 230-4300 µg/kg bw (oral exposure via ingestion). Additional exposure from other sources than herbal preparations sold for recreational purposes is highly unlikely.

### **Biological data**

- In humans, following sublingual exposure, bioavailability of salvinorin A is low (Mendelson et al., 2011), whilst salvinorin A is quickly absorbed following inhalation (Johnson et al., 2016; Maqueda et al., 2016; Ranganathan et al., 2012). Highest concentrations in plasma of humans was reached within minutes following inhalation, and a large volume of distribution was detected (Maqueda et al., 2016). In monkeys, salvinorin A was widely distributed throughout the brain following intravenous exposure, showing uptake over the blood-brain barrier (Hooker et al., 2008). In humans, a rapid decline in plasma concentrations was seen (Johnson et al., 2016; Maqueda et al.,

2016; Ranganathan et al., 2012), and the half-life ( $t_{1/2}$ ) was determined to be short ( $\sim 50$  min) (Maqueda et al., 2016).

- Using cell-based and animal experiments, it was determined that salvinorin A is metabolized fast by blood esterases, mainly carboxylesterases, cytochrome P 450 enzymes, namely 2D6, 1A1, 2C18 and 2E1, UGTB27, and lactonases to salvinorin B, and lactone-ring-open forms of salvinorin A and B (Teksin et al., 2009; Tsujikawa et al., 2009). In humans, salvinorin A is excreted via renal filtration (Pichini et al., 2005). In monkeys, collection in the gall bladder also took place, suggesting excretion via feces (Hooker et al., 2008).
- No acute toxicity data after inhalation, oral or sublingual exposure are available. After intraperitoneal exposure, acute toxicity is low in mice with median lethal doses of  $\geq 1000000$   $\mu\text{g}/\text{kg}$  bw extract (González-Trujano et al., 2016).
- No short-term toxicity data are available after inhalation, oral or sublingual exposure. Following sub-chronic intraperitoneal exposure to salvinorin A at max 6400  $\mu\text{g}/\text{kg}$  bw, no histological effects were seen (Mowry et al., 2003).
- No genotoxicity studies are available for *S. divinorum* or salvinorin A.
- No chronic toxicity and carcinogenicity studies are available for *S. divinorum* or salvinorin A.
- No reproduction and developmental toxicity studies are available for *S. divinorum* or salvinorin A.
- All other animal studies used routes of exposure not relevant for smoking, sublingual or oral use of *S. divinorum* or salvinorin A. Many studies using monkeys, rats and mice describe neurological effects like sedative effects, depressive-like effects, antinociceptive effects, decreased locomotor activity, decreased attention, decreased memory and decreased dopamine levels in the brain (see section 5.2.6 for references).
- Volunteer studies in which volunteers inhaled salvinorin A ( $\geq 1.5$   $\mu\text{g}/\text{kg}$  bw) showed that psychoactive effects (including agitation, confusion, dizziness, hypertension, tachycardia and symptomatic toxic psychosis) and acute psychotropic effects (including psychosis, paranoia, hallucination, disorientation) can occur. Physiological effects on hormone levels, blood pressure and connectivity in the brain were seen at higher concentrations ( $\geq 14$   $\mu\text{g}/\text{kg}$  bw) (see section 5.2.7 for references).
- One volunteer study determined effects of salvinorin A following sublingual exposure. At the highest dose tested (47  $\mu\text{g}/\text{kg}$  bw), no psychoactive or physiological effects were found (Mendelson et al., 2011).
- No human studies were found that studied effects after ingestion (e.g. via tea).

### No safe use level

Safety of a herbal preparation can be presumed when “available data would allow concluding that exposure to known levels of the botanical ingredient has occurred in large population groups for many years without reported adverse effects” (EFSA, 2009). *S. divinorum* is listed in the EFSA Compendium of Botanicals, in which botanicals and botanical preparations are listed with a potential to contain toxic, addictive,

psychotropic, or other substances that may be of concern, and therefore the presumption of safety does not apply.

It is not possible to establish a health-based guidance value (HBGV) for *S. divinorum* or for salvinorin A due to limited toxicological information. There are some studies available that look at the behavioural or psychoactive effects of salvinorin A. No information on genotoxicity, chronic toxicity, carcinogenicity or reproduction and developmental toxicity for either *S. divinorum* or salvinorin A was found.

Several case reports and human volunteer studies on *S. divinorum* or salvinorin A are available. Inhalation of *S. divinorum* generally led to acute psychotropic effects. In humans, an overall lowest LOEL of the various studies of 1.5 µg/kg bw salvinorin A could be derived for psychoactive effects after a single inhalatory dose. For sublingual exposure, a NOEL of 47 µg/kg bw salvinorin A (the highest dose tested) could be derived for the absence of psychoactive or physiological effects in a study in humans. No human data were available from which a LOEL or NOEL could be derived for adverse effects after oral ingestion of salvinorin A.

## **Risk assessment**

### *Inhalation*

The estimated exposure range for users smoking dried leaves of 1.46-111 µg/kg bw (for a 70 kg individual) is above the LOEL for psychoactive effects of 1.5 µg/kg bw salvinorin A. This means that acute psychoactive effects can be expected. The estimated exposure after smoking extracts with 5X-20X strength compared to leaves of 19-77 µg/kg bw (for a 70 kg individual) exceeds the LOEL greatly and therefore psychoactive effects are expected. This is supported by reported adverse effects in case reports, including agitation, confusion, dizziness, flushed sensation, hypertension, tachycardia, symptomatic toxic psychosis and acute psychotropic effects.

### *Sublingual uptake*

A margin of exposure (MOE) based on the obtained NOEL of 47 µg/kg bw and the estimated exposure of 12-891 µg/kg bw for dried leaves and 19-26 µg/kg bw for the 5X strength extract, MOEs of 0.053-3.92 and 1.81-2.47 were found for dried leaves and 5X strength extract, respectively. In this case, a MOE of at least 10 is deemed sufficient, based on a factor 10 for interindividual differences. Given this small margin, psychoactive effects cannot be excluded.

### *Ingestion*

No data were available to derive a NOEL or LOEL after oral exposure to (ingestion of) *S. divinorum* or salvinorin A. However, since tea is made and ingested for its effects, any adverse effects following ingestion of *S. divinorum* can be expected although a quantitative assessment is not possible.

### *Interactions and sensitive populations*

Interactions may occur when combined with other psychoactive compounds (including cannabis and (synthetic) hallucinogenic drugs), or medications affecting the mechanism of action, or the uptake and

metabolism of salvinorin A. In addition, genotypical differences in the kinetic processes (enzymatic degradation, transporter based efflux) of salvinorin A could prolong the psychoactive effects and increase the severity of the effects induced following salvinorin A exposure.

### **Conclusions and recommendations**

Acute adverse health effects may occur following inhalation or sublingual use of (herbal preparations containing) *S. divinorum* currently available on the Dutch market. The adverse effects are caused by the psychoactive compound salvinorin A and can already occur at the recommended quantity. Acute effects that may occur include affected attention, latency, sedation-like effects and visual and auditory hallucinogenic effects. Effects following oral exposure (ingestion) can be expected, although no quantitative risk assessment was possible for this route of exposure.

Toxicological data regarding long-term use, genotoxic or carcinogenic effects or effects on reproduction and development are lacking and no firm conclusions can be drawn on these aspects.

Based on the acute adverse health effects, RIVM advises consumers to not use (herbal preparations containing) *S. divinorum*. RIVM advises VWS to consider regulation of (herbal preparations containing) *S. divinorum*.



## 1 Introduction

### 1.1 Background

In December 2020, the Minister for Medical Care and Sport of the Ministry of Health, Welfare and Sport (VWS) announced the actions that would be taken to better regulate food supplements and herbal preparations in the Netherlands, thereby facilitating enforcement. One of those actions is to expand the list included in the Herbal Preparations Decree of the Dutch Commodities Act<sup>5</sup> with substances/botanicals that are either forbidden or restricted (i.e. subject to a maximum limit) in food supplements or herbal preparations (Van Ark, 2020). In order to determine whether a substance or botanical needs to be included in this list, a risk assessment is warranted. The selection of substances and botanicals chosen for risk assessment was based on the prerequisite that the substances/botanicals were sold on the Dutch market and (widely) used in the Netherlands and there were indications for possible health risks, e.g. Rapid Alert System for Food and Feed (RASFF) reports, from enforcement institutes. The current assessment is about the herbal preparation *Salvia divinorum*.

*Salvia divinorum* is available on the Dutch market as a natural herb for recreational use. *S. divinorum* is considered one of the most potent naturally occurring hallucinogens and induces powerful visual and auditory hallucinations (Valdés, 1994). The main active constituent of *S. divinorum* is salvinorin A.

### 1.2 Information on existing assessments

*S. divinorum* is listed in the EFSA Compendium of Botanicals for its psychoactive effects and ability to induce tachycardia.

The Bundesinstitut für Risikobewertung (BfR) has published a risk assessment of *S. divinorum* in 2012 (BfR, 2012). In this assessment, no quantitative risk assessment was performed. Due to its high acute hazard potential as a hallucinogen, the authors recommended including *S. divinorum* in list A of Appendix III (prohibited substances) of Regulation (EC) No. 1925/2006 on the addition of vitamins and minerals and of (ingredients containing) certain other substances to foods.

A monograph in the Natural Medicine Comprehensive Database states that usage of *S. divinorum* is possibly unsafe and has been shown to cause serious adverse effects, including slurred speech, confusion, paranoia, depersonalization, blunted affect, hallucinations and psychosis (Natural Medicine, 2020).

No other toxicological evaluations by international committees and institutes or national organizations were identified.

<sup>5</sup> <https://wetten.overheid.nl/BWBR0012174/2020-07-01>. Accessed 26 august 2021.

### 1.3 Information on existing legislations

Salvinorin A is classified by the European Chemicals Agency (ECHA) as Human Health hazards following Classification & Labelling criteria and is labelled 'harmful if swallowed' (Acute Tox. 4; H302)(ECHA).

In Belgium, *S. divinorum* is included in list 1 of the Appendix to the Royal Decree of 29 August 1997. This means that *S. divinorum* is considered as a dangerous plant and may not be used in or as food (Koninkrijk België, 1997).

In Italy, *S. divinorum* is included in a list of plant-based extracts not allowed in food supplements set up by the Ministry of Health (Ministry of Health Italy).

In Lithuania, *S. divinorum* is included in the list of ingredients of plant origin prohibited in food supplements of the Approval of Lithuanian Hygiene Standard HN 17:2016 (Minister of Health Lithuania).

In France, *S. divinorum* is listed as a poisonous drug, however the listing only applies when intended for human consumption or advertised as having psychotropic effects (Minister of Health and Sports France).

Most countries in Europe have added *S. divinorum* to their acts restricting controlled drugs and substances. Spain, Germany, Denmark, Belgium, Sweden, Poland, Italy, France and Croatia prohibited possession and/or sale by placing *S. divinorum*, salvinorin A, or both, on their lists of controlled substances. In Norway, Iceland, Finland and Estonia, *S. divinorum* can be legally used for medicinal purposes. In Portugal, the production, distribution, sale and possession of *S. divinorum* and salvinorin A/B is prohibited (Brito-da-Costa et al., 2021).

Currently, in the Netherlands, there are no specific restrictions for the use of *S. divinorum* in herbal preparations included in the Herbal Preparations Decree of the Dutch Commodities Act<sup>6</sup>, the EU directive<sup>7</sup> on the manufacture, presentation and sale of tobacco and related products, or the Opium Act (Brito-da-Costa et al., 2021; Raad van State).

<sup>6</sup> <https://wetten.overheid.nl/BWBR0012174/2020-07-01>. Accessed 26 august 2021.

<sup>7</sup> [DIRECTIVE 2014/40/EU of the European Parliament and of the Council of 3 April 2014.](#)

## 2 Literature search

The risk assessment for (herbal preparations containing) *S. divinorum* was conducted using the recently developed template for the safety assessment of plant food supplements as a basis (de Wit-Bos et al., 2019).

A search strategy was developed to capture relevant literature for the risk assessment of *S. divinorum* and salvinorin A. Search terms were formulated to describe the herb, including its main constituent, to identify references describing toxicity or adverse outcomes, and to include animal data as well as human data. The search terms included the Latin name '*Salvia divinorum*', the name and CAS number of its main constituent, e.g. 'Salvinorin (A)', 'CAS: 83729-01-5', and various common names, e.g. 'Divin\* sage', 'Seer's sage', 'Hierba/Yerba de Maria', 'Plant of the gods' and 'Hoja de la pastora'. The search strategy also included terms to obtain articles describing the toxic effects and kinetics of *S. divinorum* or salvinorin A (e.g. '\*toxic\*', 'risk\*', 'safe\*', '\*kinetic\*'). Three databases were searched on the 23<sup>th</sup> of September 2021, including Pubmed, Embase and Scopus. The obtained references were judged for their relevance based on title/abstract. Primary research articles were included in order to assess whether methods were well-performed and described. Articles were excluded when they did not describe the toxic effect of *S. divinorum* or salvinorin A or only described (possible) therapeutic effects. In addition to the databases, references in the selected articles were checked to identify possible additional references missed in the literature search. This led to the final inclusion of 21 additional articles describing the toxicological effects of *S. divinorum* or salvinorin A. Other references were included for the background information and kinetics of the herbal preparation and its main constituent.

In addition, grey literature was searched, including Natural Medicine Comprehensive Database, EFSA Compendium of Botanicals, Commission E monographs, Martindale (38<sup>th</sup> edition), Hagers Enzyklopädie der Arzneistoffe und Drogen, European Union Herbal Monographs of the Committee on Herbal Medicinal Products (HMPC) of the European Medicines Agency (EMA), European Scientific Cooperative on Phytotherapy (ESCOP) monographs and the European Pharmacopoeia. No relevant information, other than the forementioned evaluation of BfR, the monograph in the Natural Medicine Comprehensive Database and the listing in the EFSA Compendium of Botanicals, was found. Information on the chemical properties of the main constituent of *S. divinorum* was obtained using databases like ChemIDplus, eChemPortal, and CompTox.



### 3 Description of the product

#### 3.1 Identity and nature of the source material

##### 3.1.1

##### *Botanical (preparation)*

*Salvia divinorum*, or divining sage, is a species belonging to the Lamiaceae family, which is cultivated for their attractive flowers, fragrant leaves and medicinal benefits. *Salvia rosmarinus* (rosemary), *Ocimum basilicum* (basil) and *Lavendula officinalis* (lavender) belong to the same family (WCSP, 2021).

The name *divinorum* was given to the plant since it is traditionally used in divination and spiritual healing by Mazateca Indians living in forests in Oaxaca, Mexico. Traditionally, leaves of this expected cultigen are shewed, or grounded before they are ingested with water. The plant is also used for its analgesic, anti-inflammatory and diuretic properties (Zawilska and Wojcieszak, 2013). It has various synonyms that refer to its use in divination, e.g., 'plant of the gods', 'seer's sage' and 'herba de Maria' (Table 1).

Table 1 Information related to the classification of *S. divinorum*.

<b>Scientific (Latin) name</b>	Family: <i>Lamiaceae</i> Species: <i>Salvia divinorum</i> Epling & Játvia, Bot. Mus. Lefl. 20: 75 (1962).
<b>Synonym(s)</b>	<i>Salvia divinorum</i> <i>S. divinorum</i>
<b>Common names</b>	Salvia Sally D Divining sage Diviner's sage Seer's sage Wahrsagesalbei Herb of the virgin Ska maria pastora Hierba de Maria Yerba de Maria Plant of the gods Hoja de la pastora Profetsalvia Aztekensalbei Magic mint Mexican mint
<b>Part used</b>	Leaves
<b>Geographical origin</b>	In the forest Sierra Mazateca, Oaxaca, Mexico. Can be grown in homes
<b>Growth and harvesting conditions</b>	No direct sunlight, light shade, most locations, prefers 15-27°C, large leaves are plucked from the plant

Sources: (Cambron, 2016; EMCDDA; Global Biodiversity Information Facility; Mansfeld's World Database of Agricultural and Horticultural Crops; Ott, 1996; Sociedad para la Preservación de las Plantas del Misterio; WCSP, 2021)

The plant can grow over one meter in height, has a hollow square stem and large ovate green and often dentate leaves with a yellow undertone (10-30 cm). The plant flowers rarely but can hold white curved flowers that are attached to a small violet calyx which grows in whorls (Cambron, 2016). The flowers produce very few seeds and no pollen tube is present. Since the naming of this plant in 1962, it is believed to be a cultigen which can only be cultivated using sprigs. Dried leaves are dark green, brownish or blackish due to the concentration of pigments (EMCDDA).

More recently, *S. divinorum* has gained interest by users trying to "expand consciousness" or to experiment by using natural hallucinogens. It is used recreationally as an alternative to marijuana and lysergic acid diethylamide (LSD). Reasons for its popularity are the accessibility, legality in some countries, perception of safety, and lack of detectability on drug screens (Zawilska and Wojcieszak, 2013). Contrary to traditional use, *S. divinorum* leaves are mainly smoked during recreational use.

## 3.2 Manufacturing process

### 3.2.1 Information on the method(s) of manufacture

Fresh leaves, dried leaves and extracts of *S. divinorum* are sold for recreational purposes. How extracts are produced is unknown, however several user sites include recipes for making extracts. Extracts are often made with acetone (Erowid.org, 2015a; c; Zamnesia.nl, 2020a), isopropanol (Erowid.org, 2015c) or (denatured) alcohol (Erowid.org, 2015b). The obtained solutions are sprayed over other *S. divinorum* leaves and solutions are let to evaporate (Erowid.org, 2015c; Reddit.com, 2020).

To dry home grown plants, several methods are mentioned on user guides. Options are to wait until the plant sheds leave, dry leaves at room temperature, using a food dehydrator, a conventional oven, or a microwave and to use calcium chloride (Sagewisdom.org, 2010).

### 3.2.2 Information on substances entering the manufacturing process

All solvents need to be evaporated before use of the extracts (Erowid.org, 2015a). When using calcium chloride to dry the leaves, it should not touch the leaves (Sagewisdom.org, 2010). No additional substances are thought to enter the herbal preparation during the manufacturing process. These additional substances are not included in this assessment.

## 3.3 Chemical composition

Several studies have led to the isolation of various phytochemicals including salvinorins A-J, saldividins A-D, divinorins A-F and salvinicins A and B in the leaves of *S. divinorum* (Kutrzeba et al., 2007; Prisinzano, 2005; Tsujikawa et al., 2008). Salvinorin A is the main active compound present in *S. divinorum* and the only constituent known to induce psychotropic effects in humans. It is also present at much higher concentrations than the other isolated phytochemicals (Moreira, 2013). Many of the other salvinorins detected in *S. divinorum* are believed to be precursors or intermediates in the biosynthesis pathway of salvinorin A (salvinorin D, E, I and J). While salvinorin A is the only isolated chemical with a high affinity for the kappa opioid receptor (KOR), salvinorin G,

salvinicin A and divinorin D also had measurable affinities for the KOR (Harding et al., 2005; Lee et al., 2005). Salvinorin A is the most potent naturally occurring hallucinogen, the first known psychoactive diterpene and the first known non-nitrogenous hallucinogen (Vortherms and Roth, 2006).

A study by Siebert (2004) revealed that salvinorins are present in the external stem tissue, leaves, rachises, bracts, pedicles and calyces, but not in the roots, internal stem tissue, internal petiole tissue, cotyledons and corollas. Since the leaves are the only part used for recreational purposes, the determination of concentrations of the main constituents are performed in leaves. Salvinorins A, B and C are found in higher concentrations compared to the other salvinorins, however the composition varies depending on the geographic origin of the plant (Medana et al., 2006). One research group showed that 0.34% of the mass of dried *S. divinorum* leaves consists of salvinorin A. Other phytochemicals are present in the range of 0.0001-0.026% (Bigham et al., 2003; Munro and Rizzacasa, 2003). High variability in concentrations salvinorin A measured in the leaves is seen (Table 2) mostly depending on the geographic origin, and cultivation and extraction method (Barnes and Snow, 2012; Medana et al., 2006).

*Table 2 Salvinorin A concentrations determined in various S. divinorum samples.*

<b>Sample</b>	<b>Concentration (mg/g leaves)</b>
Leaves from private collections and endemic populations of Oaxaca, Mexico	0.89-3.7 <sup>1</sup>
Leaves from plants endemic to Sierra Mazatecan, Mexico	7.6 <sup>2</sup>
Leaves from Hawaiian plants, USA	7.8 <sup>2</sup>
Leaves purchased on the internet, USA	0.408 <sup>3</sup>
Leaves purchased on the drug market, Japan	3.2-5.0 <sup>4</sup>
Ground young leaves of plants purchased from the Vancouver Seed Bank	0.9 <sup>5</sup>

Sources: <sup>1</sup>: Gruber et al. (1999); <sup>2</sup>: Medana et al. (2006); <sup>3</sup>: Wolowich et al. (2006); <sup>4</sup>: Tsujikawa et al. (2008); <sup>5</sup>: Pelot et al. (2017)

Several smart shops note the concentration of salvinorin A in advertised products. One Dutch online smart shop advertises with a 5X, 10X, 15X and 20X strength extracts with respectively 13, 26, 39, 52 mg/g salvinorin A dried leaves (Sirius.nl). A different Dutch online smart shop advertises with 5X, 10X, 15X, 20X, 30X, 40X and 80X strength extracts with respectively 18, 36, 54, 72, 108, 144 and 288 mg/g salvinorin A dried leaves (Dutch-smart.nl). Research has shown that the concentrations listed often differ from the actual concentrations (Table 3), and that this strongly depends on the brand tested (Hernández-Bello et al., 2015). In most extracts, the concentration was lower than the claimed concentration. However, large differences between the studies are observed. Two of the sixteen samples contained concentrations close to or higher than the claimed concentration (109-114% and 136%, respectively). Sellers are most likely unaware of the actual concentrations of the products for sale.

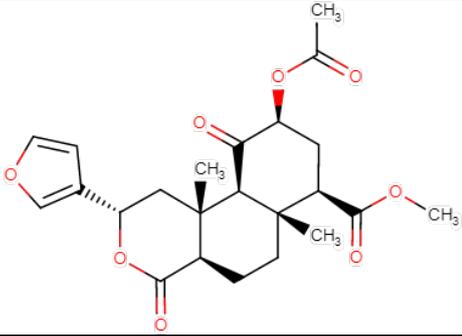
Table 3 Salvinorin A claimed and measured in various *S. divinorum* extracts.

Sample	Claimed concentration (mg/g)	Measured concentration (mg/g)	% of label claim
1x <sup>1</sup>	2.5	0.4	16%
5x <sup>1</sup>	12.5	0.1	1%
5x <sup>1</sup>	12.5	1.1	9%
5x <sup>2</sup>	16	8.3	52%
5x <sup>3</sup>	38-40	18	46-48%
10x <sup>1</sup>	25	1.0	4%
10x <sup>2</sup>	28	17	61%
10x <sup>3</sup>	76-80	2.6	3%
10x <sup>3</sup>	25	34	136%
20x <sup>1</sup>	50.0	0.5	1%
20x <sup>2</sup>	40	21	53%
40x <sup>2</sup>	80	29	36%
40x <sup>3</sup>	304-320	156	49-51%
60x <sup>3</sup>	456-480	521	109-114%
70x <sup>2</sup>	120	36	30%
100x <sup>2</sup>	160	55	34%

Sources: <sup>1</sup>: Wolowich et al. (2006); <sup>2</sup>: Hernández-Bello et al. (2015); <sup>3</sup>: Moreira et al. (2014)

Since salvinorin A is the main active ingredient of *S. divinorum*, the current risk assessment will focus on salvinorin A. An overview of the chemical structure and characteristics of salvinorin A can be found in Table 4.

Table 4 Chemical structure and characteristics of salvinorin A, the main active component of *S. divinorum* (ACToR).

<b>Chemical structure</b>	
<b>IUPAC name</b>	Methyl (2S,4aR,6aR,7R,9S,10aS,10bR)-9-(acetyloxy)-2-(furan-3-yl)-6a,10b-dimethyl-4,10-dioxododecahydro-2H-naphtho[2,1-c]pyran-7-carboxylate
<b>CAS No.</b>	83729-01-5
<b>Molecular formula</b>	C <sub>23</sub> H <sub>28</sub> O <sub>8</sub>
<b>Molecular weight</b>	432.469 g/mol
<b>Log P (octanol/water)</b>	2.5

### 3.4 Stability

Although no systematic studies have been performed, dried leaves, leaf extracts and pure salvinorin A are believed to be stable for at least several months at ambient temperature in the absence of light or air (EMCDDA). During storage of fresh leaves in the refrigerator, leaves lose potency after a few days (Turner, 2004).

### 3.5 Use and use levels

An overview of the type of *S. divinorum* containing herbal preparations or related products can be found in Table 5. *S. divinorum* is sold as dried leaves, extracts of the dried leaves and as mixed herbal preparations. Fresh leaves were not found on the Dutch market. However, it is possible to cultivate these at home after plants or seeds are bought. Dried leaves and extracts can be bought online or in local smart shops. Extracts contain dried leaves with claimed 5 to 80 times (5X-80X strength) higher concentrations of salvinorin A than in non-extract dried leaves (Dutch-smart.nl; Sirius.nl).

According to a survey under 500 *S. divinorum* users, 92.6% reported to typically smoke or vaporize *S. divinorum* products. Of them, 37.3% smoked dried leaves, while 61.4% smoked extracts (Baggott, 2004). Extracts can be smoked using a pipe or a water bong at temperatures above 240-250°C or can be placed under the tongue for absorption of the active ingredient. Smoking using a bong or pipe is the most common way of administration for recreational use since the uptake is high and fast (psychoactive effects within a minute for the duration of 15-20 min (EMCDDA)). Using a vaporizer is not recommended since it cannot reach high enough temperatures to release the active ingredient salvinorin A. Both fresh and dried leaves can be chewed on for 30 min by making a 'quid' or cigar shaped bundle (Salvia-divinorum-info.com). The effects come on more slowly (10-20 min) and will continue for around 30 min (Salvia.net). The leaves are rarely ingested since the active ingredient is absorbed through the mucus layers into the blood stream. In addition, since degradation of salvinorin A in the gastrointestinal tract is high (EMCDDA), and salvinorin A is a substrate of an efflux transporter in the intestine (Teksin et al., 2009), a higher quantity of leaves would be needed (EMCDDA).

Fresh and dried leaves can also be soaked in alcohol or water overnight before juices are pressed from leaves and can be consumed with alcohol or water (Salvia.net). Dried leaves can be ground and the obtained powder can be dissolved in water. It is also possible to extract tea from dried leaves (Zamnesia.nl, 2020b). However, since salvinorin A is readily degraded in the gastrointestinal tract, these methods are less effective to induce psychoactive effects (EMCDDA). The recommended dosage per use is dependent on the type of product and method of use. Recommended uses by online smartshops are listed in Table 5.

Table 5 Recommended use by user platforms and online smartshops.

	<b>Oral (chew/quid)</b>	<b>Oral (ingestion, tea)</b>	<b>Oral (sublingual)</b>	<b>Inhale (smoking)</b>
<b>Fresh leaves</b>	6-30 leaves <sup>1,2,3</sup> , 10-50 g <sup>3</sup>	-	-	-
<b>Dried leaves</b>	8-20 leaves <sup>4,5</sup> , 2-10 g <sup>1,2,3,6</sup>	28 g/ 360 mL <sup>7</sup>	-	1 big leaf <sup>3</sup> , 0.25-1 g <sup>1,2,3,8</sup>
<b>5X strength</b>	-	-	0.1 g <sup>6</sup>	0.1-0.3 g <sup>1,2,7,9</sup>
<b>10X strength</b>	-	-	-	0.05-0.15 g <sup>1</sup>
<b>20X strength</b>	-	-	-	0.025-0.075 g <sup>1</sup>
<b>40X strength</b>	-	-	-	0.012-0.037 g <sup>1</sup>

Sources: <sup>1</sup>: Salvia-divinorum-info.com; <sup>2</sup>: Salvia.net; <sup>3</sup>: Erowid.org (2000); <sup>4</sup>: Dutch-smart.nl; <sup>5</sup>: Zamnesia.nl; <sup>6</sup>: Dutch-headshop.nl; <sup>7</sup>: Zamnesia.nl (2020b); <sup>8</sup>: Salvia-divinorum.nl (2006); <sup>9</sup>: Sirius.nl

In addition to products solely containing *S. divinorum*, several multi-ingredient herbal preparations containing *S. divinorum* can be found in the Dutch online smart shops. Examples of other herbs with which *S. divinorum* is combined are catmint, skullcap, wormwood, passion flower, damiana, poison lettuce, *Leonuru sibiricus* extract, *Canavalia maritima*, *Heima salicifolia*, hemp oil, kratom (extract) and *Artemisia absinthium* extract (Dutch-smart.nl).

Most of the web shops mention warnings about the effects of *S. divinorum*. It is stated that this drug should only be used by experienced psychotropic drug users and that it should be used in the presence of a sober 'drug/trip sitter'. In addition, it is recommended that first time *S. divinorum* users should start with low doses since effects can be strong and vary between individuals. Also, usage is discouraged when pregnant or lactating, suffering from depressions, heart and vascular diseases and liver disorders. People using medications or monoamine oxidase (MAO) inhibitors are advised to consult the general practitioner or doctor before use.

User reports show that *S. divinorum* is also used in combination with cannabis, alcohol, mushrooms and hallucinogenic drugs e.g. *N,N*-dimethyltryptamine (DMT), lysergic acid diethylamide (LSD) and 2C-family (Erowid.org; Vohra et al., 2011). A survey under *S. divinorum* users stated that a third of the respondents combined *S. divinorum* with alcohol (13.7%), cannabis (33.2%) and other hallucinogens (6.6%) (Sumnall et al., 2010).

## 4 Exposure: extent and duration

### 4.1 Exposure from herbal preparation use

The exposure to *S. divinorum* and its main constituent salvinorin A may greatly differ because of the use of different products with varying strengths and possible repeated usage within one day. In addition, since the use of *S. divinorum* is thought to induce sensitization to the product, doses may vary (decrease) over time. User reports state some users only use *S. divinorum* once a year due to the intense experience. Others stated smoking 20-40 times a day, daily for 8 months (Reddit.com, 2018). Since the effects subside quickly following exposure through inhalation, it is possible to use it multiple times a day. According to the results of an online survey, users generally use it twice a month during a period of regular use (Sumnall et al., 2010).

Since extracts with >20X strength are less used, the estimation of the exposure to salvinorin A is based on leaves and extracts with a 5-20X strength. In Table 3, it was depicted that the concentrations salvinorin A in various products tested were not as labeled. In most cases, the concentrations found were lower. Since herbal preparations on the Dutch market were not tested, the estimated exposure is based on the claimed concentrations salvinorin A in the products on the Dutch market and the recommended use (worst-case; Table 3). Concentrations of salvinorin A in dried leaves on the Dutch market are not labelled/claimed. Therefore, the concentration salvinorin A measured in (dried) leaves, as listed in Table 2, were used to estimate the exposure using this product. A rough estimation of exposure can be found in Table 6. Since the effects occur following the inhalation of *S. divinorum* products over a very short time period, the exposure is expressed as µg/kg bodyweight per usage. This leads to an estimated exposure range following smoking of 0.10-7.8 mg salvinorin A per usage (i.e. 1.46-111 µg/kg bw for a 70 kg person). Following chewing of leaves or a quid, users are exposed to 0.8-78 mg salvinorin A (i.e. 12-891 µg/kg bw for a 70 kg person). When tea is made by extracting salvinorin A from dried leaves using hot water, users are exposed to 16-303 mg salvinorin A (i.e. 230-4300 µg/kg bw for a 70 kg person) when two cups of tea are consumed (Table 6). The estimated exposure through smoking dried leaves can be higher than following the use of extracts since the recommended use for the dried leaves is more than proportionally higher than for the extracts.

Table 6 Estimated exposure to salvinorin A.

Product	Recommended use	Measured/claimed concentrations	Estimated exposure	
<b>Inhalation</b>				
Dried leaves	0.25-1 g	0.408-7.8 mg/g <sup>1</sup>	0.10-7.8 mg	1.46-111 µg/kg bw <sup>3</sup>
5X strength	0.1-0.3 g	13-18 mg/g <sup>2</sup>	1.3-5.4 mg	19-77 µg/kg bw <sup>3</sup>
10X strength	0.05-0.15 g	26-36 mg/g <sup>2</sup>	1.3-5.4 mg	19-77 µg/kg bw <sup>3</sup>
20X strength	0.025-0.075 g	52-72 mg/g <sup>2</sup>	1.3-5.4 mg	19-77 µg/kg bw <sup>3</sup>

Product	Recommended use	Measured/claimed concentrations	Estimated exposure	
<b>Oral (chew/quid/sublingual)</b>				
Dried leaves	2-10 g	0.408-7.8 mg/g <sup>1</sup>	0.8-78 mg	12-891 µg/kg bw <sup>3</sup>
5X strength	0.1 g	13-18 mg/g <sup>2</sup>	1.3-1.8 mg	19-26 µg/kg bw <sup>3</sup>

Product	Recommended use	Measured/claimed concentrations	Estimated exposure	
<b>Oral (ingestion, tea)</b>				
Dried leaves	28 g/ 360 mL	0.408-7.8 mg/g <sup>1</sup>	16-303 mg <sup>2,3</sup>	230-4300 µg/kg bw <sup>3,4,5</sup>

The estimation is based on the recommended use and the claimed concentrations by the sellers of these products. Since the concentration of salvinorin A was not claimed in dried leaves, the measured concentration in products sampled outside of the Netherlands was used (Table 3).

<sup>1</sup> Measured concentration

<sup>2</sup> Claimed concentration

<sup>3</sup> Calculated for a 70 kg person

<sup>4</sup> Based on 2 cups of 250 mL tea consumption at 32-606 mg/L salvinorin A

<sup>5</sup> Under the assumption that all salvinorin A will be extracted from the leaves using hot water

#### 4.2 Possibility of additional/combined human exposure

Since the main psychoactive constituent, salvinorin A, is only detected in *S. divinorum*, and is only used for recreational purposes in Europe, combined exposure from other sources than herbal preparations sold for recreational purposes is highly unlikely.

#### 4.3 Information on historical use of the ingredient

Fresh leaves of *S. divinorum* have been traditionally ingested by the Mazateca Indians living in forests in Oaxaca, Mexico. No information on the safe (historical) use of *S. divinorum* is available in the Commission E monographs, Martindale (38<sup>th</sup> edition), Hagers Enzyklopädie der Arzneistoffe und Drogen and the European Pharmacopoeia.

## 5 Toxicological data

This chapter describes the available toxicokinetic and toxicological studies of *S. divinorum* and its main constituent salvinorin A. Animal and human behavioral studies, in addition to toxicity studies are included. Studies describing the (possible) therapeutic effects are not included in this assessment.

### 5.1 Toxicokinetics

#### 5.1.1 Absorption

##### *In humans*

Both the lack of subjective effects and low concentrations of salvinorin A found in plasma and urine following exposure to 4000 µg sublingually administered salvinorin A suggest that the sublingual bioavailability of salvinorin A in humans is low (Mendelson et al., 2011).

After inhalation, salvinorin A is quickly absorbed in humans (Johnson et al., 2016; Maqueda et al., 2016; Ranganathan et al., 2012). In all three studies, individuals were exposed to a single dose through inhalation of vaporized salvinorin A.

Maqueda et al. (2016) conducted a placebo-controlled, randomized, double-blind study in which two groups of volunteers (n=11 and n=12) were exposed to 1 mg (14 µg/kg bw for a 70 kg person) through inhalation of vaporized salvinorin A. In both groups the effect of a different antagonist was tested to counter the effects of salvinorin A. For this assessment, the kinetics and effects are included only when volunteers were exposed to salvinorin A solely. The average time to reach the maximum concentration in plasma ( $T_{max}$ ) following sole salvinorin A exposure was 1.36 min and 1.5 min, showing rapid absorption, with in both cases a maximum plasma concentration of ( $C_{max}$ ) of 31.3 ng/mL. The area-under-the-curve values ( $AUC_{0-240 \text{ min}}$ ) for the two groups were 358 and 464 ng/mL\*min, respectively (Maqueda et al., 2016).

Johnson et al. (2016) exposed six volunteers to 18-21 µg/kg bw salvinorin A via inhalation. The dose chosen was the maximum tolerable dose determined in a previous test experiments. This was either the maximum dose tested or the dose just below the dose which they said they would refuse if repeated. While the average peak serum levels ( $T_{max}$ ) also occurred 1-2 min following exposure, they found variations between the six individuals (1-4 min post-inhalation). The average  $C_{max}$  was 19 ng/mL and ranged from 10-40 ng/mL.

Ranganathan et al. (2012) exposed 10 volunteers to 8 and 12 mg salvinorin A (114-171 µg/kg bw for a 70 kg person) via inhalation. At the first timepoint measured (15 min following exposure), a relatively low  $C_{max}$  of ~0.9-1.05 ng/mL was seen. The actual peak concentration was most likely within the time period not sampled. In all three studies, a rapid decline in plasma concentrations was seen in the first 30 min.

Notably, it has been suggested that other constituents in the herb may enhance the absorption of salvinorin A (Mendelson et al., 2011). In the discussion, Mendelson et al. (2011) suggested that crude extracts show higher potency compared to pure salvinorin A and that this was also seen for the isoflavone diadzin. However, Mendelson et al. (2011) do not cite the study in which higher potencies of the crude *S. divinorum* extracts were seen.

### 5.1.2 Distribution

#### *In vitro*

In an *in vitro* test with Madin-Darby canine kidney cells expressing MDR1 (MDCK-MDR1) monolayers the enhanced P-glycoprotein (P-gp) adenosine triphosphate (ATP)ase activity seen following exposure to 5 and 10  $\mu\text{M}$  suggests that salvinorin A is a substrate for the efflux transporter P-gp (Teksin et al., 2009). P-gp transporters are ATP-dependent efflux transporters important for both distribution and elimination since they are present in for example the blood-brain barrier (BBB), intestines, kidneys and liver.

#### *In animals*

Butelman et al. (2012) showed that behavioral effects of salvinorin A, and its levels in the CNS are enhanced when co-exposed to a competing P-gp substrate and P-gp blocker using adult captive-bred rhesus monkeys (*Macaca mulatta*). As a result, the authors suggest that effects of salvinorin A in recreational users can be affected by genotype or by the (chronic) use of another P-gp substrate (Butelman et al., 2012).

Using male Sprague-Dawley rats, the kinetics and distribution of a bolus intraperitoneal (IP) dose of 10.000  $\mu\text{g}/\text{kg}$  bw salvinorin A was evaluated over a 240 min period (Teksin et al., 2009). At 10 and 15 min respectively,  $C_{\text{max}}$  was reached in the brain and in the plasma. It was suggested that due to its molecular weight of 432 Da and high lipophilicity, salvinorin A will easily cross the blood-brain barrier (BBB) and end up in the brain. Teksin et al. (2009) also reported a large volume of distribution ( $V_d = 47.1 \text{ L}/\text{kg}$ ). The  $C_{\text{max}}$  in the brain was much lower (23.9 ng/mL) than that in plasma (345 ng/mL) leading to a brain-to-plasma ratio of 0.05. The area under the curve (AUC) was 410  $\mu\text{g h}/\text{L}$  in plasma and 20.6  $\mu\text{g h}/\text{L}$  in the brain. The low brain-to-plasma ratio is probably due to the fact that salvinorin A is a P-gp substrate. The half-lives ( $t_{1/2}$ ) in plasma and the brain were short, being 75.4 min and 36.1 min, respectively (Teksin et al., 2009).

Schmidt et al. (2005a) used four (two males, two females) adult captive-bred rhesus monkeys (*Macaca mulatta*) with complex drug histories (no history of chronic drug administration or salvinorin A exposure in the last 30 days). Immediately after intravenous (IV) injection of 32  $\mu\text{g}/\text{kg}$  bw Salvinorin A (in a vehicle) in the saphenous vein, plasma concentrations ranged between  $\sim 11$  and  $\sim 59$  ng/mL after which they rapidly decreased. Distribution differed between gender with  $t_{1/2}$  for distribution being lower for male subjects compared to female subjects. The same group published two more studies, Butelman et al. (2009) and Butelman et al. (2012), in which they exposed adult rhesus monkeys from both genders to either 10 or 32  $\mu\text{g}/\text{kg}$  bw salvinorin A IV, respectively. Cerebrospinal fluid (CSF) samples showed average peak

concentrations 2 min after the end of the 32 µg/kg bw injection (~20 sec injection time;  $C_{max}$  = 1.01 ng/mL) and a gradual decline up to 180 min (0.21 ng/mL still measured in CSF after 180 min). The concentrations reached are in the reported range of the brain-receptor affinity of salvinorin A. Consistent with this finding, behavioral effects, like facial relaxation and ptosis, were detected within 1-2 min following salvinorin A exposure (Butelman et al., 2009). Following IV exposure to 10 µg/kg bw salvinorin A, peak concentrations in the CSF were on average 1.3 ng/mL. At the last timepoint taken (30 min), concentrations had declined with 75% (Butelman et al., 2012).

Hooker et al. (2008) determined the distribution of salvinorin A by exposing adult female baboons (*Papio anubis*) to carbon-11 labeled salvinorin A (1.18-2.8 millicuries) IV in the radial arm vein. Salvinorin A is lipophilic and therefore binds to plasma proteins (~83%). Using positron emission tomography (PET) scans, it was determined that the maximum concentration in the brain was reached in ~40 sec yet cleared rapidly ( $t_{1/2}$  = 8 min, 75% decrease in less than 30 min). The  $C_{max}$  in the brain was 0.0175% of injected dose/cm<sup>3</sup>, which corresponds to 3.3% of the administered dose (1.18-2.8 millicuries). Salvinorin A was widely distributed throughout the brain, with the highest concentrations found in the cerebellum. A statistically significant amount of activity was also seen in the striate (visual) cortex. Twenty to forty min after exposure, a small amount of <sup>11</sup>C-salvinorin A was still concentrated in the cerebellum. The highest concentration short after exposure was found in the kidneys (0.11% of injected dose/cm<sup>3</sup>), and the greatest amount in the liver (due to its size; 0.04% of injected dose/cm<sup>3</sup>). After 10 min, accumulation took place in the gall bladder. At 60 min post-exposure, 0.12% of injected dose/cm<sup>3</sup> was accumulated there, which was the highest concentration in any organ (Fig. 1). Whilst not in the brain anymore, <sup>11</sup>C was visible in the spine two hours post-exposure.

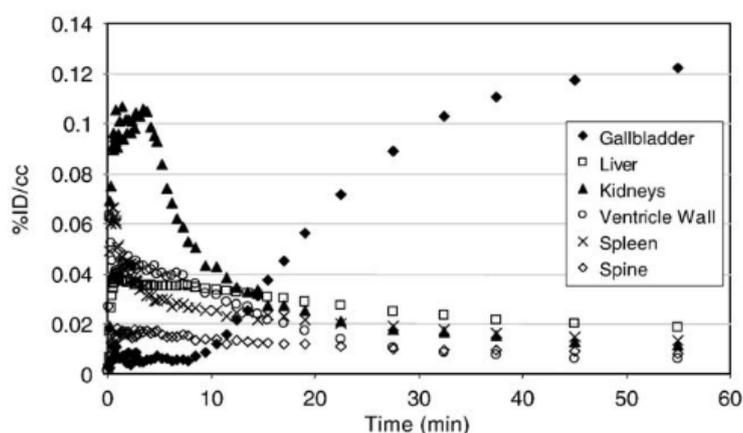


Figure 1 Concentration-time profile of peripheral organs in baboons after IV administration of salvinorin A (Hooker et al., 2008).

#### In humans

After a single inhalatory dose of 1 mg salvinorin A (14 µg/kg bw for a 70 kg person), an apparent volume of distribution ( $V_d/F$ ) of 166-237 L was found indicating a large distribution (Maqueda et al., 2016).

### 5.1.3 Metabolism

#### *In vitro*

Teksin et al. (2009) stated that based on the chemical structure, salvinorin A may be a substrate for oxidative metabolism via cytochrome P450 (CYP450) enzymes and hydrolysis via uridine glucuronyl transferase (UGT) or carboxylesterases (ChEs).

Schmidt et al. (2005b) studied the metabolism of spiked salvinorin A in whole blood taken from adult rhesus monkeys. Both salvinorin A and salvinorin B (inactive) concentrations were determined over 60 min. Salvinorin B was seen to increase steadily, while salvinorin A decreased. Around 30 min, similar concentrations of salvinorin A and B were measured in whole blood. Sixty min post-incubation, the concentration of salvinorin B was close to the initial concentration of salvinorin A, suggesting that salvinorin B is the main metabolite formed in blood (*ex vivo*, without the interference with other pathways of metabolism). The authors suggest salvinorin A is metabolized by the present blood esterases (Schmidt et al., 2005b).

Tsujikawa et al. (2009) studied the esterases responsible for the degradation of salvinorin A in addition to estimating the degradation products using *in vitro* methods. *Ex vivo*, in rat plasma, the degradation of salvinorin A followed a first-order reaction at 37°C ( $k_{\text{obs}} = 3.8 \pm 0.3 \times 10^{-1} \text{ h}^{-1}$ ). Twenty-four hours post-incubation, less than 2% of salvinorin A was still present whilst 47.7% of salvinorin B was present. As the sum of salvinorin A and B is less than 100% after 24 h, these results suggest that salvinorin B degradation took place as well. The estimated degradation constants of salvinorin A and B are  $4.3 \times 10^{-1}/\text{h}$  and  $2.7 \times 10^{-2}/\text{h}$ , respectively (Tsujikawa et al., 2009).

To determine the esterases responsible for salvinorin A degradation, rat plasma was incubated with salvinorin A in combination with various esterase inhibitors. Results suggest that salvinorin A is rapidly metabolized by blood esterases, mainly carboxylesterase (Fig. 2). Notably, blood esterase distribution differs between species, with carboxylesterase activity in rat plasma and CES activity in human in red blood cell membranes (Tsujikawa et al., 2009).

Tsujikawa et al. (2009) also determined degradation products of salvinorin A using liquid chromatography-mass spectrometry. They included the deacetylated form of salvinorin A, named salvinorin B, and the lactone-ring-open forms of salvinorin A and B (Fig. 2). It is suggested that these were produced via the activity of calcium-dependent lactonase. *Ex vivo*, in rat plasma, the metabolic pathway from salvinorin A to salvinorin B and from salvinorin B to its metabolite was advantageous compared to the metabolite of salvinorin A formed by lactonase. However, the latter metabolic pathway may be major in human serum since the divergent CES activity between rat and human and the similar activity of lactonase between rat and human (Tsujikawa et al., 2009).

Teksin et al. (2009) investigated the CYP isoforms involved in the metabolism of salvinorin A using single enzyme systems that express a specific human CYP isoforms. After 60 min of incubation with 50 µM salvinorin A, a statistically significant decrease (6-10%) in salvinorin A concentration was seen when incubated with CYP2D6, CYP1A1, CYP2C18

and CYP2E1, but not with CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP3A4 and CYP3A5 (Fig. 2). Results suggest that since a statistically significant decrease was seen following incubation of 5  $\mu$ M salvinorin A with CYP1A1, CYP2C18 and CYP2E1, these isoforms follow Michaelis-Menten kinetics (Teksin et al., 2009). They also investigated the involvement of the major enzyme involved in the glucuronidation of most drugs, UGT2B7. The decrease in degradation of salvinorin A at higher concentrations compared to low concentrations of salvinorin A suggests UGT2B7 is involved (Fig. 2), but that it is saturable (Teksin et al., 2009).

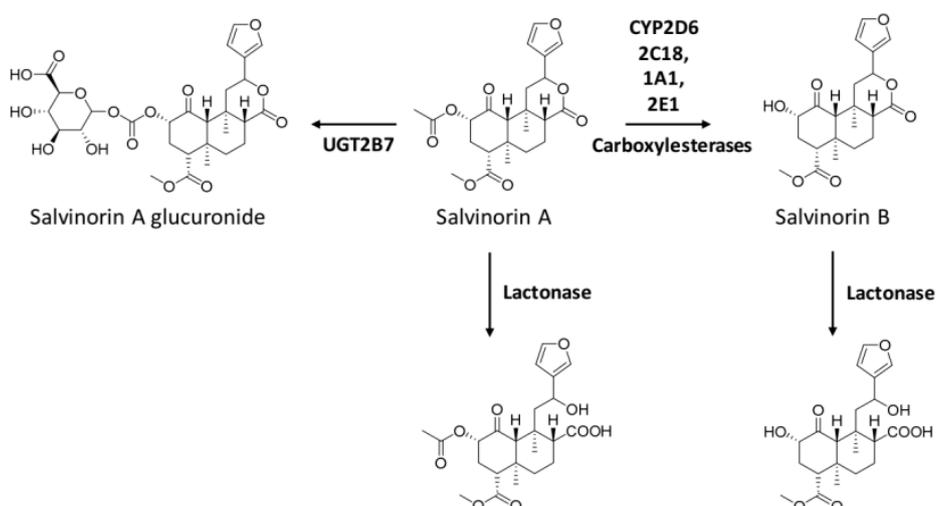


Figure 2 Proposed metabolic pathway of salvinorin A (Brito-da-Costa et al., 2021).

#### *In animals*

Whole-body images of female baboons taken 65-110 min post-exposure to  $^{11}\text{C}$ -labeled salvinorin A (IV) showed approximately equal amounts of  $^{11}\text{C}$  in the gallbladder and biliary tract, and the urinary bladder (Hooker et al., 2008). According to the authors this suggests that at least two modes of metabolism and excretion are present following salvinorin A exposure, namely renal filtration (hydrophilic metabolites) and collection in the gallbladder (lipophilic metabolites). They also measured the activity of cleaved  $^{11}\text{C}$ -labeled salvinorin A in plasma over time as a percentage of total  $^{11}\text{C}$ -labeled salvinorin A in plasma. While it did not show which metabolites were formed, it showed very fast metabolism of salvinorin A with 60% being metabolized within 5 min, and only 15% remaining unmetabolized after 60 min.

#### *In humans*

Salvinorin A displays a short duration of action after exposure, suggesting that it is rapidly cleared and that metabolism most likely plays a significant role in the dissipation of its effect. In addition, the rapidly declining plasma concentrations in volunteers following inhalatory exposure to salvinorin A as seen by Johnson et al. (2016); Maqueda et al. (2016); Ranganathan et al. (2012) in combination with clearance values of 2.33-3.32 L/min also support this (Maqueda et al., 2016).

#### 5.1.4 *Excretion In animals*

The elimination (rate) of salvinorin A was determined in rats and rhesus monkeys.

In male Sprague-Dawley rats relatively fast elimination was seen following a single IP dose of 10.000 µg/kg bw salvinorin A, with a half-life ( $t_{1/2}$ ) of 75 min and a clearance of (CL/F) of 26 L/h/kg (Teksin et al., 2009). The fast elimination is in accordance with its fast onset and short duration of action (see 5.2 Toxicological data), and the fact that salvinorin A is a substrate of the P-gp transporters.

In adult rhesus monkeys exposed to a single IV bolus of 32 µg/kg bw salvinorin A, elimination half-life was 57 min. In addition, gender differences were seen ( $t_{1/2}$  males= 38 min,  $t_{1/2}$  females= 80 min) (Schmidt et al., 2005a).

#### *In humans*

Two studies determined the elimination of salvinorin A using healthy volunteers (Maqueda et al., 2016; Pichini et al., 2005).

In Pichini et al. (2005), saliva, urine and sweat samples were collected, respectively, before and 1 hour after, <1.5 hour after, and 2 hour after ad lib smoking of 75 mg of dry *S. divinorum* leaves in a pipe for 3 min. Both volunteers experienced intense hallucinations, and no blood drawings could be performed. Salvinorin A was detected in saliva (11-25 ng/mL) and urine (2.4-11 ng/mL), yet not in sweat patches. In total, 0.4-1.2% of the theoretically administered dose of salvinorin A was detected unchanged in urine. In total, 77.1 to 92.7% was recovered (Pichini et al., 2005).

Maqueda et al. (2016) conducted a placebo-controlled, randomized, double-blind study in which two groups of volunteers (n=11 and n=12) were exposed to 1 mg (14 µg/kg bw for a 70 kg person) through inhalation of vaporized salvinorin A. Based on blood samples, the elimination rate ( $t_{1/2}$ = 49-50 min) and clearance (Cl/F= 2.3-3.3 L/min).

#### 5.1.5 *Summary on kinetic data*

In humans, the absorption of salvinorin A following inhalation is very rapid ( $t_{max}$  of 1-2 min); the bioavailability following sublingual exposure is low. Salvinorin A is widely distributed over the body as indicated by the large volume of distribution. Salvinorin A can pass the BBB, however since it is also a substrate of the efflux pump P-gp, it is rapidly transported back into the blood stream. This is also found in monkeys where salvinorin A was measured in CSF and a  $t_{1/2}$  in brain of ~8 min was observed. Moreover, effects subside quickly due to fast elimination ( $t_{1/2}$  of 49,8 min) and quick metabolism (0.4-1.2% is excreted via urine unchanged) seen in humans. Salvinorin A is metabolized by UGT2B7 and human CYP enzymes 2D6, 1A1, 2C18 and 2E1, lactonases and blood esterases. Salvinorin A is eliminated via urine.

## 5.2 Toxicological data

### 5.2.1 *Acute toxicity*

Mowry et al. (2003) exposed 17 male and female Long-Evan rats to vehicle or 1600 µg/kg bw salvinorin A via IP injection following baseline measurements. Electrophysiologic responses were measured every 10 min using electrodes placed for electrocardiography (ECG) recording, temperature was measured using an oral probe, and galvanic skin responses using an electrode placed on each forepaw. Compared to baseline measurements, no statistically significant effects were seen on heart rate, cardiac conduction, PR interval, QT interval, temperature and galvanic skin response at any timepoint. A non-statistically significant positive effect on pulse pressure was seen 20 to 40 min after exposure (Mowry et al., 2003). Notably, the first timepoint measured was 10 min, even though the onset of effects are often already seen at 1-2 min post-exposure.

González-Trujano et al. (2016) exposed 126 male Swiss albino mice IP to 0, 10000, 100000, 1000000 and 2000000 µg/kg bw non-polar, medium polar and polar crude extracts (extracted with hexane, ethyl acetate and methanol respectively) made from *S. divinorum*. The mice were observed and weighed for the following 14 days. No macroscopic injury or weight loss were noted in mice during the observation period. Diarrhea-type feces was observed in mice receiving the non-polar extract  $\geq 1000000$  µg/kg bw. The LD<sub>50</sub> value was determined to be 1000000 µg/kg bw IP for non-polar and medium polar extracts, whereas it was calculated to be 1414000 µg/kg bw IP for the polar extract.

### 5.2.2 *Short-term and sub-chronic toxicity*

The effects of sub-chronic exposure to salvinorin A through daily IP injection of 0, 400, 800, 1600, 3200 or 6400 µg/kg bw salvinorin A were investigated using 114 male and female Swiss-Webster mice (Mowry et al., 2003). After 14 days of exposure, a blood smear was collected in addition to tissue samples of the liver, spleen, kidney, bone marrow and brain. Using light microscopy, no histological differences were found between control and any of the exposure concentrations for either sex. Doses in this study are suggested to be in excess of those that could be expected to be used by humans (Mowry et al., 2003). It should be noted that the exposure route is not relevant for smoking, sublingual or oral use of salvinorin A.

### 5.2.3 *Genotoxicity*

No genotoxicity studies were identified.

### 5.2.4 *Chronic toxicity and carcinogenicity*

No chronic toxicity and carcinogenicity studies were identified.

### 5.2.5 *Reproduction and developmental toxicity*

No reproduction and developmental toxicity studies were identified.

### 5.2.6 *Other studies*

*Neurotoxicity (basal neurological effects and neuro-related behavioral effects)*

González-Trujano et al. (2016) investigated the sedative effects of *S. divinorum*. IP doses of 0, 10000, 30000 and 100000 µg/kg of crude non-polar (hexane extract), medium polar (ethyl acetate) and polar (methanol) extracts of *S. divinorum* were administered to male Swiss albino mice (without solvents). Effects on sedation were determined using the open-field (28 min post injection), hole-board (following open-field test) and exploration cylinder (following open-field test) ( $\leq 6$  mice per experimental group) tests. The number of squares explored in the open-field test (2 min) was registered as ambulatory activity which was not only taken as a measure of motor activity but also other factors such as exploratory drive (curiosity) and fear or anxiety-like behaviour. No effects were seen following exposure to the non-polar extracts. The medium polar and polar extracts reduced ambulatory activity at 30000 and 10000 µg/kg, respectively. During the 3 min hole-board test, the number of explored holes was measured by the number of head dips. All the extracts induced a statistically significant reduction in the number of head dips at all tested doses. In tests using the exploration cylinder, the number of spontaneous rearings on posterior limbs was recorded during the 5 min trail. A reduction in rearings, seen as a tranquilizing effect, was statistically significant following exposure to  $\geq 10000$  µg/kg,  $\geq 30000$  µg/kg or  $\geq 100000$  µg/kg for the polar, medium polar or non-polar extracts, respectively (González-Trujano et al., 2016).

An increase in latency was also seen by John et al. (2006) and McCurdy et al. (2006). John et al. (2006) studied the antinociceptive effect of salvinorin A via an intrathecal injection of 0, 11.6, 13.9, 18.5, 20.8 or 23.1 nM using 10 adult male CD-1 mice. Tail-flick latencies were used as a measure of antinociception. Salvinorin A affected tail-flick latencies in a dose- and time-dependent manner. At 5 min post-injection, only 23.1 nM statistically significantly affected latency, at 10 min post-injection all doses  $\geq 13.9$  nM did so (John et al., 2006). Highest dose at which no effects were seen by John et al. (2006) at any timepoint was 11.6 nM. Lower doses were not tested.

McCurdy et al. (2006) saw comparable results with male Swiss mice exposed to 0, 500, 1000, 2000 and 4000 µg/kg salvinorin A ( $n=8-10$  per group) in the tick-flick assay to determine the antinociceptive effects of salvinorin A. Following injection (type unknown), a dose- and time dependent effect on latency was seen. Doses of  $\geq 1000$  µg/kg salvinorin A induced statistically significantly different effects from control after 10 min, while after 20 min only doses of  $\geq 2000$  µg/kg salvinorin A statistically significantly affected latency. Moreover, 1000 µg/kg, yet not 500 µg/kg, statistically significantly increased latency when mice were put on a hotplate 10 min post-injection. The highest dose at which no effects on latency were seen by McCurdy et al. (2006) at any timepoint was 500 µg/kg salvinorin A. However, writing responses following acetic acid injections in an abdominal constriction assay decreased statistically significantly when co-exposed to doses of  $\geq 500$  µg/kg salvinorin A 10 min post-exposure (5 min post-exposure only statistically significant effects at doses  $\geq 1000$  µg/kg) (McCurdy et al., 2006).

Ansonoff et al. (2006) determined whether salvinorin A and B can act as kappa-opioid receptor agonists *in vivo* using a novel strain of kappa-opioid receptor-1 knockout mice (C57BL6/J x 129S6 or 129S6 inbred

background). Intracerebroventricular injection of 1.5-15 µg salvinorin A (in 50-100% DMSO; ED<sub>50</sub>: 1.4-1.5 µg) induced antinociception (tail-flick latency assay) in wild-type but not the knockout strain. Injection of DMSO alone also induced 10% antinociception, but was significantly lower compared to salvinorin A exposure. Also, a reduction in body temperature was noted at 50 µg salvinorin A, similar to conventional kappa-opioid receptor agonists. Also, salvinorin A showed high affinity for kappa 1-, yet not kappa 2-opioid receptors. No effects following exposure to salvinorin B were seen.

Zhang et al. (2005) determined the effects of salvinorin A on basal dopamine levels using male C57BL/6J mice exposed to 0, 320, 1000 and 3200 µg/kg salvinorin A IP (n= 5 per group). Using *in vivo* microdialysis, it was determined that exposure to 320 µg/kg salvinorin A IP did not affect dopamine levels. Exposure to 1000 and 3200 µg/kg IP salvinorin A statistically significantly decreased dopamine levels in the caudate putamen, yet not the nucleus accumbens. At the lowest dose tested in conditioned place preference or aversion and locomotor activity tests (1000 µg/kg IP) aversion was seen by the reduction in time spent on the conditioned site and the reduced number of crossovers (locomotor activity) (Zhang et al., 2005).

Using male albino Swiss mice, Socała et al. (2020) investigated the possible influence of salvinorin A (0, 100, 1000 and 10000 µg/kg IP, n= 9-20 per group) on seizure susceptibility. Three acute seizure tests were performed, namely the intravenous pentylenetetrazole (IV PTZ) seizure threshold test, the maximal electroshock seizure threshold test, and the 6-Hz psychomotor seizure threshold test. Moreover, effects on neuromuscular strength and motor coordination were determined. Results showed no statistically significant effects. It was therefore concluded that salvinorin A does not affect seizure thresholds or impact muscle strength and motor coordination (Socała et al., 2020).

Nemeth et al. (2010) exposed 10 male Sprague-Dawley rats to 0, 125, 250, 500, 1000, 2000, 4000 µg/kg salvinorin A IP to quantify attention using the food-motivated 5-Choice Serial Reaction Time Test (5CSRTT). Ten min following exposure, rats were placed in the 5CSRTT chamber. Following a stimulus (light) in one of the five response holes, correct identification (nose poke) of the right hole was rewarded with food. Exposure to 2000 µg/kg salvinorin A IP, yet not 1000 µg/kg, induced a statistically significant decrease in the percent of correct responses, increase in the latency to correct and an increase in the percentage omissions (Nemeth et al., 2010).

Hooker et al. (2009) investigated which brain regions were involved in salvinorin A-mediated effects. To do so, they examined regional changes (by studying metabolic activation) in 2-deoxy-2-[F-18]-fluoro-D-glucose (FDG) uptake in the male rat brain of 19 nonrestraint Sprague-Dawley rats following exposure to 0 or 2000 µg/kg salvinorin A IP. Results showed that several regions with a high kappa-opioid receptor (KOR) density showed an increased FDG uptake. However, uptake was not limited to regions with high KOR density. Increases in FDG uptake was also seen in brain regions without any anatomical connections, but who are functionally related due to the downstream effects of high KOR

density regions. The metabolic activation of brain regions due to exposure to salvinorin A was seen in the absence of any observable behavioral effects (Hooker et al., 2009).

Gehrke et al. (2008) determined whether dopamine levels decrease following (systemic and intrastriatal) exposure to salvinorin A is due to altered dopamine uptake or release in the dorsal striatum. Male Sprague-Dawley rats (n unknown) were exposed to 0, 1000 or 3200 µg/kg salvinorin A IP after which the dopamine levels in the dorsal striatum were determined using microdialysis. Both doses statistically significantly decreased dopamine levels compared to vehicle control following systemic exposure, however there was no difference in effect seen between both doses. To determine the effects of salvinorin A perfusion, rats were exposed to salvinorin A (20, 60 or 200 nM) in artificial CSF through a probe placed in the dorsal striatum. Time- and dose-dependent effects of salvinorin A were observed. At the first timepoint measured, doses  $\geq 20$  nM statistically significantly reduced basal dopamine levels. After 20 min,  $\geq 60$  nM statistically significantly affected basal dopamine levels. Using quantitative microdialysis under transient conditions, a statistically significant decrease in extracellular dopamine levels, yet not the extraction fraction (indirect measure of dopamine uptake), was seen. This indicates that dopamine release is decreased in response to intradorsal striatum perfusion of salvinorin A (Gehrke et al., 2008). It was also determined that no changes in basal dopamine levels and dopamine overflow were seen 48 hours after the cessation of a 5-day salvinorin A treatment regimen of 1000 µg/kg IP (Gehrke et al., 2008).

Carlezon et al. (2006) also investigated the effects of salvinorin A on extracellular dopamine concentrations, specifically in the nucleus accumbens, using *in vivo* microdialysis studies in which 129 male Sprague-Dawley rats were exposed to salvinorin A (0, 125 and 1000 µg/kg IP, n=7-11 per group). Effects were time- and dose-dependent. No statistically significant decrease in basal dopamine was seen following systemic exposure to 125 µg/kg salvinorin A, but statistically significant decreases in dopamine levels (between 40 and 15%) were seen after exposure to 1000 µg/kg salvinorin A. Exposure to salvinorin A did not affect serotonin basal levels. To determine whether systemic administration of salvinorin A (125-2000 µg/kg IP) would produce depressive-like effects in rats, the forced swim test (FST) and intracranial self-stimulation (ICSS) test were performed. In the FST, 125 µg/kg salvinorin A IP did not statistically significantly affect the behaviour of the exposed rats, while 250 µg/kg salvinorin A induced statistically significant effects on immobility and swimming, yet not on climbing. No effects were seen for any dose on the distance travelled (locomotor activity). At doses  $\geq 500$  µg/kg salvinorin A IP, a statistically significant increase in the intercranial stimulation thresholds was seen, which translates to a reduction in reward-facilitating effects following exposure over time. The decrease in maximal response rate was not statistically significant due to vehicle effects (Carlezon et al., 2006).

The effect of salvinorin A on attention and spatial, episodic and aversive memory was tested by Braida et al. (2011). Wistar rats were exposed to 0, 80, 160, 320 or 640 µg/kg bw subcutaneously (SC) administered

salvinorin A (n= 10 per group). All experiments were performed 20 min following exposure. At these concentrations no effects on locomotor activity were seen in the 15 min period 20 min post-exposure using a laser-equipped box. To determine effects on spatial memory, rats were placed in the 8-arm radial maze. It was determined that no effects on the total number of errors, the time to complete the task and the number of correct choices before the first error (re-entry of an arm already visited) were seen (working memory), even though an statistically significant increase in mean angle frequency (change in pattern) was seen at doses  $\geq 160 \mu\text{g}/\text{kg}$ . Long-term memory was studied by removing the rat from the maze after 4 choices and placing it back two hours later to identify the 4 unvisited arms. Statistically significant effects of this delay were seen in the total number of errors ( $\geq 80 \mu\text{g}/\text{kg}$ ), correct choices (not shown) and time needed to complete the task ( $\geq 160 \mu\text{g}/\text{kg}$ ). All groups of exposed rats performed worse than the vehicle exposed group. Again, effects were seen on the patterns of visiting the arms ( $\geq 80 \mu\text{g}/\text{kg}$ ). Novel object recognition was used to test for effects of salvinorin A on episodic memory. The amount of time spend exploring objects was determined in two situations; when all objects were new, followed by a situation in which one object was new compared to the first situation. Salvinorin A did not affect the time spend exploring in the first situation, but did statistically significantly decrease the time spend exploring the novel object compared to the familiar object in the second situation ( $640 \mu\text{g}/\text{kg}$ ) and the discrimination index ( $\geq 160 \mu\text{g}/\text{kg}$  bw (80 not tested); time spend novel object (N)- time spend familiar object (F) / N+F). Braida et al. (2011) studied effects on aversive memory using the passive avoidance test in which rats are given a shock when entering the dark room in a two room test situation. At  $\geq 160 \mu\text{g}/\text{kg}$  bw SC, a statistically significant decrease in retention latency was seen, whilst the number of amnesic animals was only statistically significant at the highest dose (animals that did not move within 300 sec into the dark compartment). The latent inhibition paradigm was carried out to determine attention deficits following salvinorin A exposure. Non pre-exposed (water) and pre-exposed (sucrose) groups were exposed to either vehicle or salvinorin A ( $160 \mu\text{g}/\text{kg}$  bw SC). The consumption of sucrose in the groups pre-exposed to sucrose was higher compared to the not pre-exposed groups in the vehicle exposed group, suggesting latent inhibition. No difference was seen between the not pre-exposed group and the pre-exposed group in the salvinorin exposed group, indicating the lack of latent inhibition. Moreover, salvinorin A exposure statistically significantly reduced the total sucrose consumed in the pre-exposed groups compared to the vehicle exposed groups. Since salvinorin A disrupted the latent inhibition, without reduction of fluid intake or sucrose intake, an effect on motivation can be excluded. In combination with the results from the object recognition test, results argue for an attention deficit induced by salvinorin A. In conclusion, the authors suggest salvinorin A impaired long-term spatial and nonspatial memory (Braida et al., 2011).

Schmidt et al. (2005a) investigated the pharmacokinetics of salvinorin A using two male and two female rhesus monkeys (see also 5.1. Toxicokinetics). In their research they state that immediately after IV injection of  $32 \mu\text{g}/\text{kg}$  (30 sec injection over the saphenous vein),

sedation-like behavioural effects (not further specified) were observed (Schmidt et al., 2005a).

Later research performed by the same group also noted sedation-like effects in rhesus monkeys (4 males, 4 females) following exposure to salvinorin A. Butelman et al. (2009) showed time- and dose-dependent effects on sedation and postural changes after IV exposure to 0, 32 and 100 µg/kg salvinorin A (n= 6). At 5 (peak effect; first timepoint tested) and 15 min post-injection, the higher dose induced statistically significant increases in sedation scores compared to the vehicle, while statistically significant increases in postural relaxation scores were seen at 5, 15 and 30 min post-injection. The effects declined mostly within 30 min. Gender differences were seen, however not quantified. Butelman et al. (2009) also identified dose- and time-dependent increases in facial relaxation and ptosis with very rapid onset (1-2 min post end injection of 10 or 32 µg/kg IV). In the first time period measured (1-2 min), both doses showed statistically significant effects on both parameters.

In 2012, the same group showed comparable effects on ptosis (statistically significant increase in ptosis following exposure to 10 µg/kg in the 1-2 and 4-5 time window) in 4 male and 3 female rhesus monkeys. A lower dose of 3.2 µg/kg was also tested, but did not induce statistically significant effects on ptosis compared to control. At the same dose, no effects on facial relaxation were seen (Butelman et al., 2012).

#### 5.2.7 *Human data* *Case reports*

A 18-year old female was admitted to the psychiatric emergency service after she unknowingly smoked *S. divinorum* for the first time (amount unknown) (Paulzen and Gründer, 2008). She presented with acute onset of agitation, disorganization and hallucinating behaviour after smoking what she thought was cannabis. The patient had a long history of cannabis use but had never experienced a psychotic episode. The patient was first admitted to the general psychiatric ward with a presumptive diagnosis of substance-induced psychotic disorder and subsequent schizophrenia-spectrum disorder. Due to self-mutilating behaviour, massive disorganization, disorientation and intentions to quit treatment, she was admitted for involuntary treatment. No effect was seen following intramuscular olanzapine ( $\leq 30$  mg/day) or intravenous haloperidol ( $\leq 15$  mg/day). The patient was highly psychotic with disordered thinking, thought blocking, derealization or delusional perceptions and slow speech. She then developed symptomatic toxic psychosis with emerging and life-threatening medical consequences for which she needed treatment. Consequences were the long-lasting need for interdisciplinary medical treatment and physical impairment (tongue amputation, removal of part of gastrointestinal tract, temporary external cardiac pacemaker). Following treatment with clozapine ( $\leq 300$  mg) the psychopathology and psychotic symptoms slowly improved. Forensic-toxicological determination could identify cannabinoid usage in the past, yet not salvinorin A (also unknown if this would be possible due to short half-life) (Paulzen and Gründer, 2008).

Przekop and Lee (2009) described a case study regarding a 21-year old man who was admitted to the psychiatric unit for acute psychosis and paranoia following smoking *S. divinorum* (amount not mentioned). He was paranoid, demonstrated echolalia (unsolicited repetition of vocalizations made by another person), tried to escape and had psychomotor agitation. The patient remained agitated for two days, where after he was treated with risperidone (3 mg orally/ three times a day). Even though the treatment helped stabilize the patient, the treatment was slowly tapered since it induced parkinsonian features or rigidity, bradykinesia and masked facies. During tapering, the patient continued to improve. One day after the withdrawal, the patient's symptoms abruptly returned (agitation, paranoia, aggression, believing to be able to project and receive thoughts). Treatment with risperidone was reinstated to stabilize the patient and the patient was referred to a psychiatric facility for further treatment. No changes were seen at the 4-month follow up. The authors suggest that the patient was genetically predisposed to schizophrenia and that *S. divinorum* use precipitated the clinical manifestations.

Another case report describes the acute psychotropic effects of smoking *S. divinorum* (Winslow and Mahendran, 2014). A man in his thirties smoked a small amount of the 10X strength extract (internet bought) in a joint. He experienced vivid visual and auditory hallucinations for 12-14 min. Even though the effects were brief, the hallucinations were terrifying and unusual compared to those previously reported. He experienced extreme fear due to extracampine hallucinations of a female presence in the room, elementary- and second-person type auditory hallucinations, pareidolic experiences (walking and talking furniture), and hallucinations of talking ghostly figures. Half an hour later, the patient tried the '0' strength herb half hoping it would give a high without the vivid hallucinations. Nevertheless, he experienced similar symptoms not of lesser intensity or shorter duration. There were no after-effects reported and in the longer term, the patient did not experience any perceptual disturbances. The patient had a history of drug abuse, but did not experience similar psychopathological symptoms following *S. divinorum* use before with other drugs (Winslow and Mahendran, 2014).

Vohra et al. (2011) documented the reported exposures to *S. divinorum* to the California poison control system between January 1998 and May 2008. In total, 37 exposures to *S. divinorum* were identified. All were in the context of recreational use and classified as intentional exposure. No deaths were recorded. In 43% of the cases, patients were exposed to another drug in combination with *S. divinorum*. No doses were mentioned. For two patients solely exposed to *S. divinorum* vital signs were documented. One showed systolic hypertension and tachycardia, and the other only showed tachycardia. Other symptoms listed following *S. divinorum* use were "confusion or disorientation, hallucinations, giddiness or dizziness, flushed sensation, and tachycardia". Also mental or neurological effects were often seen. 73% of the patients solely exposed to *S. divinorum* showed psychotomimetic or neuromotor disturbances (Vohra et al., 2011).

Gonzalez et al. (2006) determined the pattern of use and subjective effects of *S. divinorum* using self-report questionnaires from 32 recreational salvia users from Spain. The Hallucinogenic Rating Scale (HRS), the addiction center research inventory (ARCI), State-Trait Anxiety Inventory-S (STAI-S), and the Altered States of Consciousness Questionnaire (Aussergewöhnliche Psychische Zustände, APZ) were used to retrospectively assess the subjective effects induced by salvia. 88% of the participants used salvia for the first time in the last year. All participants used extracts, and 9% also used dried leaves. The preferred route of administration was smoking (75%), and 22% combined smoking with sublingual uptake. Three out of four participants described the effects as "intense" to "very intense" and "extremely intense". The following effects were listed as positive effects: induction of the "trip", euphoria, dissociative effects. The short duration was seen as most negative, followed by lack of control and unpleasant after-effects. About 13% could not find any negative effect related to the experience. Fourteen participants (44%) reported having "some degree of malaise, hang-over or comedown" following the acute effects of exposure (mostly physical and mental tiredness). The scores of HRS, ARCI, APZ and STAI-S are listed, and compared to clinical trials performed with ayahuasca. These scores show that the scores are high compared to ayahuasca tested at fully psychotropic doses.

In personal communications with the Dutch Poison Information Center (NVIC), two situations in the last five years regarding possible *S. divinorum* exposure were mentioned. In 2018, the NVIC was called by a paramedic responding to a call regarding a 40-year old man who was having a 'bad trip' following *S. divinorum* use. The amount and route of exposure were unknown (presumed oral). In the second case, a male of unknown age was exposed to *S. divinorum* with one inhalation of a bong containing *S. divinorum*. He experienced tachycardia (146 beats/min). Hypertension could not be measured since the patient was restless. The NVIC recommended taking this patient to the hospital (personal communication with Chantal Roelen (NVIC)).

Also hardly any requests for information via the Drug Information line of Trimbos were recorded (personal communication with Steven Biemans (Trimbos)).

#### *Volunteer studies*

Siebert (1994) performed a volunteer study using an informal group (most likely friends) of twenty participants (non-randomized, not placebo controlled). In this study, six volunteers held 10 large fresh *S. divinorum* leaves (~30 gram) homogenized with 100 mL water using a blender in their mouth for 10 min without swallowing. Thereafter, the material was spit out. Effects (not specified) were seen within 5-10 min, with strength quickly building over a few minutes and maintaining at a plateau for about one hour. Within the next hour, effects gradually subsided.

The blended liquid was also swallowed by the same six volunteers to test the effects when bypassing the oral mucosa (mouth was rinsed with water after swallowing). No effects were reported. Furthermore, salvinin A was isolated and absorption through the oral mucosa was tested using 20 volunteers. Two mg salvinin A was

dissolved in 1 mL anhydrous ethyl ethanol before it was sprayed in the mouth using an aspirator. This method was found inefficient, and results were inconsistent.

In the next setup, salvinorin A ( $\leq 2.6$  mg) was inhaled using a butane micro torch held underneath the foil on which the extract was placed. Vapours were inhaled using a glass tube. Inhalation of 0.2-0.5 mg produced typical effects identical to the fresh herbs. Effects were observed starting from 0.2 mg salvinorin A onwards. Effects were seen in 30 sec and lasted 5-10 min before gradually subsiding over about 20-30 min. The duration of effects and out-of-body experiences increased when the dose was  $> 1$  mg. No other negative effects were reported at the highest dose tested (Siebert, 1994).

From this study, no NOEL or LOEL could be derived since the study setup was not clearly described and properly executed.

A double-blind, placebo-controlled, randomized study to determine the immediate subjective experiences following acute exposure and the consequences thereof eight weeks later was performed using 30 middle-aged, well-educated, experienced hallucinogen users (Addy, 2012). Participants used a metal pipe to smoke either 1017 or 100  $\mu\text{g}$  (non-dose, used as control, no further justification) two weeks apart, in a counterbalanced order. At the high dose, salvinorin A statistically significantly affected hallucinogenic experiences, as rated with the HRS one hour after exposure (affect, cognition, intensity, perception, somaesthesia and volition). Throughout the session, participants showed increased movement, laughing, physical contact, paranoia and talking. No effects of salvinorin A on systolic and diastolic pressure, pulse, temperature and respiration were observed. The authors state that since the effects on these physiological measures were determined 60 min post-exposure and the subjective effects lasted  $\sim 20$  min, the physiological reactions may have resolved before the measurement. Even though based on self-report, 70% ( $n=16$ ) of all participants reported effects lasting more than 24h following exposure (not noted how long). Three of them reported negative aftereffects like persisting headaches, feeling unsure of things, impatient, and having a labile effect (Addy, 2012).

From this study, a LOEL of 1017  $\mu\text{g}$  salvinorin A for inhalation could be derived based on psychoactive effects observed at this dose. This was the only dose tested.

Ranganathan et al. (2012) investigated the behavioral, subjective, cognitive, psychophysiological and endocrine effects of 0, 8 and 12 mg inhaled salvinorin A in 10 healthy salvinorin A-experienced individuals during a 3-day, double-blind, randomized, crossover, counterbalanced study. Doses were inhaled using a vaporizer. Subjective, psychotomimetic and perceptual effects were determined using self-reporting and various scales (visual analog scale (VAS), positive and negative syndrome scale (PANSS), psychotomimetic state inventory (PSI), clinician administered dissociative symptoms scale (CADSS) and the HRS). Except on the CADSS, all scales were affected by exposure to the low dose of salvinorin A, while more limited effects were seen when exposed to the high dose (assessments were performed 30 min post-exposure). Effects on cognition (tested 7 min following exposure) were not found using a cognitive battery of digit forward, digit backward or

letter number sequencing tasks. Neuroendocrine effects, determined by statistically significant increases in hormone levels at 10, 15, 30 and 90 min following exposure, were observed following the low dose (increase in cortisol and prolactin) and the high dose (increase in prolactin). No effects on heart rate, systolic or diastolic blood pressure were observed in any participant (measured every 5 min until 30 min post-exposure, 60 min and 90 min post-exposure). Psychophysiological effects were determined using EEG in the first 3 min after exposure. Only the reductions in the  $\beta$ -band were statistically significant. No acute or delayed adverse effects were seen during this study (Ranganathan et al., 2012).

From this study, a LOEL of 8 mg salvinorin A for inhalation could be derived based on psychoactive effects observed at this dose. This was the lowest dose tested.

Johnson et al. (2011) published a double-blind, placebo-controlled study of the effects of salvinorin A in four healthy subjects with experience in using hallucinogens (two males, two females). Sixteen ascending doses (0.375, 0.75, 1.5, 3.0, 4.5, 6.0, 7.5, 9.0, 10.5, 12, 13.5, 15, 16.5, 18, 19.5, 21  $\mu\text{g}/\text{kg}$  bw) and four intermixed placebo doses were inhaled over 40 sec using a round-bottom flask heated with a butane micro torch. The total study took place over several weeks in which each dose was tested once. The two males were reported unresponsive (no reaction to stimuli) on at least one timepoint for at least one dose. A dose-dependent but rapid increase in drug strength was seen following exposure. These effects subsided quickly and within 20 min effects were seen as 'possibly mild'. After the session, the drug strength was rated and all but one dose  $\geq 4.5$   $\mu\text{g}/\text{kg}$  bw were rated statistically significantly different from placebo. Statistically significant effects were observed on all subscales of HRS and the mysticism (M) scale performed 60 min post-exposure. The lowest dose affecting the HRS subscale 'intensity' was  $\geq 4.5$   $\mu\text{g}/\text{kg}$  bw, which is consistent with the pattern seen in the recognition of drugs strength. No statistically significant effects on heart rate, blood pressure and no resting or kinetic tremors were observed (Johnson et al., 2011).

From this study, a NOEL of 3  $\mu\text{g}/\text{kg}$  bw salvinorin A for inhalation could be derived based on psychoactive effects observed at a dose of 4.5  $\mu\text{g}/\text{kg}$  bw (LOEL).

The same group published another double-blind, placebo-controlled study in which the data of the four volunteers discussed in Johnson et al. (2011) were incorporated with four other volunteers (three males + one female) (MacLean et al., 2013). The setup of the study was similar, with exposure to 0.375-21  $\mu\text{g}/\text{kg}$  bw salvinorin A. Five participants (four males) were unresponsive on at least one time point for at least one dose. Effects on drug strength and dissociative effects were statistically significant at  $\geq 4.5$   $\mu\text{g}/\text{kg}$  bw and  $\geq 6.0$   $\mu\text{g}/\text{kg}$  bw salvinorin A, respectively. In addition to the HRS and M scales, the APZ, quantitative pharmacological class questionnaire (QPCQ), perception scale, ARCI were assessed. On the HRS scale, the lowest concentration statistically significantly different from placebo (1.5  $\mu\text{g}/\text{kg}$  bw) affected the intensity. On the APZ scale, the OSE score was statistically significantly affected at 12  $\mu\text{g}/\text{kg}$  bw. On the M scale, statistically significant effects were seen at the two highest concentrations. In the SOCQ, the lowest concentration

statistically significantly affected the ineffable score at 4.5 µg/kg bw. The total score and the detachment score on the perception scale were also statistically significantly affected at 4.5 µg/kg bw. No statistically significant effects were seen at the ARCI and STAI assessments. The dose was strongly negatively correlated with the number of words recalled and the accuracy of recognition while weakly positively correlated with recognition response bias. No statistically significant effects on blood pressure and heart rate was seen. One patient showed resting (doses 6.0 and 10.5 µg/kg bw) and kinetic (dose 10.5 µg/kg bw) tremors at 15 min, yet not 30 min post-exposure. Within 3 days after each session, participants wrote an open-ended narrative about the subjective effects, which included “disruptions in vestibular and interoceptive signals, contact with entities, revisiting childhood memories, cartoon-like imagery, and recurring content across sessions” One month after the last session, a follow-up assessment was performed. No statistically significant changes between screening and the follow-up assessment were seen in depressive symptoms, anxiety, affective mood state, vigor, fatigue, confusion, psychiatric symptoms or visual disturbances (tested using the following tests: Beck depression inventory (BDI), STAI, profile of mood states (POMS) and brief symptom inventory (BSI)) (MacLean et al., 2013).

From this study, a NOEL of 0.75 µg/kg bw salvinorin A for inhalation could be derived based on psychoactive effects observed at a dose of 1.5 µg/kg bw (LOEL).

Another study from Johnson and MacLean, focused on the hormonal effects of salvinorin A (Johnson et al., 2016). Six healthy volunteers inhaled a single high dose of vaporized salvinorin A. The dose was based on the maximum tolerated dose in a dose run-up experiment (4x 21 µg/kg bw, 2x 18 µg/kg bw). Subjective drug strength and monitor-rated effects (drug strength, distance from usual daily reality, unresponsiveness, psychological distress, paranoia, anxiety/fear, motor activity, joy/peace and physical distress) and physiology measures (systolic and diastolic blood pressure, heart rate) were assessed every 2 min for 60 min after inhalation. Prolactin increased to on average ~21 ng/mL 16 min post-exposure ( $C_{max}$  range: 11-36 ng/mL) and reached a plateau in individual participants before gradually decreasing. Effects on cortisol were difficult to distinguish due to large interindividual variability. A statistically non-significant increase in cortisol levels was observed for most participants 10 min post-exposure. The effects on hormones could not be correlated to salvinorin A exposure. The effects of salvinorin A were statistically significant on the participant- and monitor ratings of drug strength, monitor ratings of distance from usual daily reality, unresponsiveness, psychological distress and paranoia. No statistically significant effects were seen on any other monitor ratings (anxiety/fear, motor activity, joy/peace, physical distress). Also no statistically significant effects were seen on physiological measures including systolic and diastolic blood pressure and heart rate (Johnson et al., 2016).

From this study, a LOEL of 18 µg/kg bw salvinorin A for inhalation could be derived based on psychoactive effects observed at this dose. This was the lowest dose tested.

Maqueda et al. (2015) performed a placebo-controlled, double-blind, randomized study in which eight healthy participants with an interest in the altered state of consciousness induced by psychoactive substances were exposed via inhalation to 0, 0.25, 0.5 and 1 mg salvinorin A. Placebo and salvinorin A were placed in a round-bottom flask to heat and vaporize the residue. Psychological effects were determined using HRS, ARCI, STAI, self-administered and experimenter-administered visual analog scales (VAS), and multidimensional assessment of interoceptive awareness (MAIA) test. Dose-response effects were observed on all subscales of the HRS. Statistically significant effects on 4 out of 6 subscales of the HRS were seen at 0.25 mg, while effects on the other subscales were statistically significant when exposed to 0.5 mg salvinorin A. Only two of the five subscales of the ARCI were statistically significantly affected by salvinorin A, at either the lowest or the middle dose. The low and middle dose statistically significantly affected the STAI, yet the highest dose did not. All three subscales of the APZ were statistically significantly increased when participants were exposed to the 0.5 mg dose. All 10 self-administered VAS subscales were affected by 1 mg exposure, 8 by the 0.5 mg dose and 4 by the 0.25 mg dose. The experimenter-administered VAS showed that all doses induced maximal intensity at 2 min post-exposure and gradually decrease to baseline after 20 min. Maximum intensity was dose-dependent. In three subscales of MAIA statistically significant effects were seen. The 0.25 mg dose induced effects on the subscale trusting, the 0.5 mg dose on the subscale attention regulation and the 1.0 mg dose on the subscale emotional awareness. Narratives from users following exposure stated "the sudden onset of effects, changes in bodily sensations, changes in the perception of time, depersonalization, and derealization, modifications of visual and auditory perception, the intensity and brevity of effects, pleasurable effects, and impairment of the capacity to interact with surroundings". Moreover, participants described interoceptive effects as "being pulled, pressured, or divided, changes in body temperature, tingling, sweating, relaxation, vibrations, loss of contact with the body, and out-of-body experience" (Maqueda et al., 2015). From this study, a LOEL of 250 µg salvinorin A for inhalation could be derived based on psychoactive effects observed at this dose. This was the lowest dose tested.

Based on this research, the same group published a highly comparable study to determine the effects of a fully psychoactive dose of 1 mg salvinorin A in a double-blind, randomized, cross over study with 24 volunteers (divided in 2 groups) with previous experience in the use of psychedelics (Maqueda et al., 2016). This study investigated the antagonistic effects of naltrexone and ketanserin on the effects induced by salvinorin A (KOR and serotonin-2A receptor (5-HT<sub>2A</sub>)). For this risk assessment, only the results in groups exposed solely to salvinorin A were stated. Statistically significant effects of 1 mg salvinorin A were detected for both groups of volunteers at the 6 subscales of the HRS, the 3 subscales of the APZ and 8 out of 10 subscales of the VAS. In 2 of the 10 subscales of the VAS and the STAI, only one group of volunteers showed statistically significant effects whilst the other group showed a close to statistically significant effect. The effects on all tests showed that salvinorin A reduced external sensory perception and induced intense visual and auditory modifications. Cardiovascular and

neuroendocrine effects were also determined following inhalation of 1 mg salvinorin A. Increases in blood pressure, cortisol and prolactin were seen, whilst 1 mg salvinorin A did not affect heart rate and growth hormones (Maqueda et al., 2016).

From this study, a LOEL of 1000 µg salvinorin A for inhalation could be derived based on psychoactive effects observed at this dose. This was the only dose tested.

Doss et al. (2020) investigated the effects of inhaled salvinorin A on the static, dynamic and enteropic functional connectivity using volunteers in a single-blind, placebo-controlled, within-subject study. Static connectivity (the strength of association between brain regions over time (sFC)), dynamic connectivity (the variance in connectivity (dFC)) and entropy of connectivity (lack of order of strength over time (eFC)) was measured using functional magnetic resonance imaging (fMRI) since it was hypothesized that salvinorin A, like other hallucinogens, would strongly modulate default mode network (DMN) connectivity. Twelve healthy male participants with previous experience with hallucinogen use were exposed to 15 µg/kg bw salvinorin A and placebo 45 sec after the start of the fMRI. Drug strength ratings showed a rapid increase of strength with a peak effect 2 min post-exposure. fMRI results showed that salvinorin A decreased within-network sFC but increased between-network sFC. Peak effects were seen within the first 20 min following exposure. dFC was reduced brain wide (statistically non-significant) following salvinorin A exposure while eFC was increased brain wide (statistically significant). Both the decrease in sFC within the DMN and the increase in eFC brain wide was predictive of the effects of salvinorin A on brain function compared to other network interactions (Doss et al., 2020).

From this study, a LOEL of 15 µg salvinorin A/kg bw for inhalation could be derived based on affected neuronal connectivity at this dose. This was the only dose tested.

Mendelson et al. (2011) performed a placebo-controlled ascending dose study using eight *S. divinorum*-experienced volunteers to characterize the physiological and subjective effects of sublingual doses up to 4000 µg salvinorin A. The first two doses (placebo and 100 µg) were given double-blind, the remaining doses were single-blind (250, 500, 1000, 2000 and 4000 µg). Salvinorin A solutions were administered sublingually using a needle-less syringe and were kept in the mouth without swallowing for five min. The mouth was rinsed following disposal of the solution. There were no significant physiological or subjective effects at any of the doses.

From this study, a NOEL of 4000 µg salvinorin A for oral (sublingual) exposure could be derived based on the absence of any effects at this dose.

An overview of the lowest-observed-effect-levels (LOELs) and no-observed-effect-levels (NOELs) in the human volunteer studies are given in Table 7.

Table 7 Overview of the no observed effect levels (NOEL) and lowest observed effect levels (LOEL) obtained from the volunteer studies describing the toxicological effects of salvinorin A. N: number of volunteers in the study.

Dose salvinorin A	Value	N	Effect seen?	Reference
<b>Inhalation</b>				
1017 µg (14.5 µg/kg bw <sup>1</sup> ) <sup>2</sup>	LOEL	30	Yes, psychoactive effects	(Addy, 2012)
8000 µg (114 µg/kg bw <sup>1</sup> ) <sup>3</sup>	LOEL	10	Yes, psychoactive effects, ↑hormone levels	(Ranganathan et al., 2012)
3 µg/kg bw	NOEL	4	No, no psychoactive effects	(Johnson et al., 2011)
4.5 µg/kg bw	LOEL	4	Yes, psychoactive effects	(Johnson et al., 2011)
0.75 µg/kg bw	NOEL	8 <sup>5</sup>	No, no psychoactive effects	(MacLean et al., 2013)
1.5 µg/kg bw	LOEL	8 <sup>5</sup>	Yes, psychoactive effects	(MacLean et al., 2013)
18 µg/kg bw <sup>3</sup>	LOEL	6	Yes, psychoactive effects	(Johnson et al., 2016)
250 µg (3.6 µg/kg bw <sup>1</sup> ) <sup>3</sup>	LOEL	8	Yes, psychoactive effects	(Maqueda et al., 2015)
1000 µg (14 µg/kg bw <sup>1</sup> ) <sup>2</sup>	LOEL	24	Yes, psychoactive effects, ↑blood pressure, ↑hormone levels	(Maqueda et al., 2016)
15 µg/kg bw <sup>3</sup>	LOEL	12	Yes, affected functional connectivity	(Doss et al., 2020)
<b>Dose salvinorin A</b>				
	<b>Value</b>	<b>N</b>	<b>Effect seen?</b>	<b>Reference</b>
<b>Oral (sublingual)</b>				
4000 µg (47 µg/kg bw <sup>1</sup> ) <sup>4</sup>	NOEL	8	No, no psychoactive or physiological effects	(Mendelson et al., 2011)
<b>Dose salvinorin A</b>				
	<b>Value</b>	<b>N</b>	<b>Effect seen?</b>	<b>Reference</b>
<b>Oral (ingestion)</b>				
No studies available				

<sup>1</sup>: calculated for a 70 kg person

<sup>2</sup>: only dose tested

<sup>3</sup>: lowest dose tested

<sup>4</sup>: highest dose tested

<sup>5</sup>: data for 4 novel volunteers was added to the data the 4 volunteers of Johnson et al. (2011)

### 5.2.8 *Interactions*

No studies were identified studying the interaction between *S. divinorum* and other products. Since the enzymes involved in the metabolism of salvinorin A are common to other drugs of abuse and pharmaceuticals, it is important to highlight the increased probability of pharmacokinetic drug–drug interactions. Pharmaceuticals or drugs of abuse metabolized by UGT2B7, CYP2D6, CYP2C18, CYP1A1, CYP2E1, carboxylesterases or lactonases can reduce metabolism and, subsequently, prolong exposure to (higher concentrations of) salvinorin A. *S. divinorum* users are reported to be more often under treatment for depressions and other mental health problems and have a high prevalence of substance use disorders. In addition, *S. divinorum* is often used in combination with other hallucinogens and alcohol. Since *S. divinorum* is often used in combination with other drugs of abuse, co-exposure and interactions with medications treating these mental health problems could pose a health concern (Vohra et al., 2011; Wu et al., 2011).

## 5.3 **Derivation of toxicological reference value**

It is not possible to establish a health-based guidance value (HBGV) for *S. divinorum* or for salvinorin A due to limited toxicological information. There are some studies available that look at the behavioural or psychoactive effects of salvinorin A. No information on genotoxicity, chronic toxicity, carcinogenicity or reproduction and developmental toxicity for either *S. divinorum* or salvinorin A is available at all.

Several studies used species with nervous systems not especially prone to the effects of hallucinogens (rat, mice) (Mowry et al., 2003). Also, differences in effect concentrations between species indicate species differences. Moreover, most animal studies were performed using male subjects even though gender differences were observed. Additionally, the route of exposure in animal studies makes comparison to the human exposure situation less relevant. As a consequence, only human data was used to derive a toxicological reference value.

### *Inhalation*

Several case reports and human volunteer studies are available for the exposure following the inhalation of *S. divinorum* or salvinorin A showing adverse effects, such as visual and auditory hallucinations. This information can however not be used as a basis for an HBGV or as a point of departure due to limited information on the dosage and/or the fact that they cover only a limited set of toxicological endpoints.

In humans, an overall lowest LOEL of 1.5 µg/kg bw salvinorin A could be derived for psychoactive effects after a single inhalatory dose. In the same study, a NOEL of 0.75 µg/kg bw could be derived. However, since solely psychoactive effects were tested in this study, it cannot be selected as an overall NOEL following inhalation. These values can be used for assessing psychoactive effects, but cannot be used as a point of departure for establishing an HBGV due to missing data on other toxicological endpoints. Other studies exposing volunteers via inhalation also showed psychoactive effects around or above this LOEL.

*Sublingual*

For sublingual exposure, a NOEL of 47 µg/kg bw salvinorin A (the highest dose tested) could be derived for the absence of psychoactive or physiological effects in a study in humans. Due to the lack of other well-performed sublingual studies, this NOEL is not supported by other studies and, as a result, less confident.

*Ingestion*

No human data were available from which a LOEL or NOEL could be derived for adverse effects after oral ingestion (consumption of tea) of salvinorin A.

## 6 Risk assessment

### 6.1 Risk assessment

Safety of a herbal preparation can be presumed when “available data would allow concluding that exposure to known levels of the botanical ingredient has occurred in large population groups for many years without reported adverse effects” (EFSA, 2009). For botanical preparations with *S. divinorum* and/or salvinorin A, safety cannot be presumed since *S. divinorum* is listed in the EFSA Compendium of Botanicals, in which botanicals and botanical preparations are listed with a potential to contain toxic, addictive, psychotropic or other substances that may be of concern (EFSA, 2009).

No HBGV could be established for *S. divinorum* or its main active ingredient salvinorin A for exposure through inhalation, ingestion or sublingual exposure.

#### *Inhalation*

From human volunteer studies, a LOEL for psychoactive effects after a single inhalation exposure of 1.5 µg/kg bw salvinorin A was derived.

Exposure to salvinorin A through smoking or vaping of (products containing) *S. divinorum* was, for a 70 kg individual, estimated to be 1.46-111 µg/kg bw when smoking dried leaves and 19-77 µg/kg bw when smoking the 5X, 10X and 20X strength extracts. These exposure ranges exceed the LOEL for psychoactive effects after single inhalation exposure of 1.5 µg/kg bw salvinorin A and therefore acute psychoactive effects can be expected. Therefore, the use of (herbal preparations containing) *S. divinorum* on the Dutch market poses a health concern. Indeed, the case reports obtained from literature described adverse health effects, like agitation, confusion, dizziness, flushed sensation, hypertension, tachycardia, symptomatic toxic psychosis and acute psychotropic effects (including psychosis, paranoia, hallucination, disorientation).

In addition, negative effects from inhaling over the lungs can also be expected when *S. divinorum* products are smoked regularly.

#### *Sublingual*

A NOEL of 47 µg/kg bw was obtained from a study describing the lack of psychoactive effects following sublingual exposure of volunteers to salvinorin A.

Exposure to salvinorin A through chewing *S. divinorum* (extract) leaves or using a quid was calculated to be 12-891 µg/kg bw for dried leaves and 19-26 µg/kg bw for the 5X strength extract (for an individual of 70 kg). Using the NOEL for psychoactive effects after single sublingual exposure of 47 µg/kg bw, a Margin of Exposure (MOE) of 0.053-3.92 and 1.81-2.47 was calculated for dried leaves and 5X strength extract, respectively. In this case, a MOE of at least 10 is deemed sufficient,

based on a factor 10 for interindividual differences. Given this small margin, psychoactive effects cannot be excluded.

#### *Ingestion*

No data were available to derive a NOEL or LOEL after oral exposure to (ingestion of) *S. divinorum* or salvinorin A. However, since tea is made and ingested for its effects, any adverse effects following ingestion of *S. divinorum* can be expected although a quantitative assessment is not possible.

## **6.2 Interactions**

Salvinorin A can be added to other herbs for mixtures used for recreational purposes. These herb mixtures can contain other psychoactive substances next to salvinorin A. In addition, *S. divinorum*/salvinorin A extracts are reported to be used in combination with cannabis, alcohol, mushrooms and synthetic hallucinogenic drugs in one third of the time. Co-exposure may affect the severity and/or duration of the physical and hallucinogenic effects.

Interactions are of concern especially when co-exposed to substances or medication affecting the mechanism of action of salvinorin A or its kinetics. Co-exposure with other substances or medication may also affect dopamine concentrations in the brain and can also affect the kappa-opioid receptor. Moreover, distribution and elimination of salvinorin A in the body can be affected by substances and medications affecting the P-gp transporter involved in removing salvinorin A from the brain and out of the body, and the enzymes involved in the metabolism of salvinorin A. Further, online smartshops advise people using medications or monoamine oxidase (MAO) inhibitors to consult the general practitioner or doctor before use.

## **6.3 Sensitive/vulnerable groups**

Gender differences are seen in the pharmacokinetics and -dynamics in rhesus monkeys, but have not yet been investigated in other species or humans (Butelman et al., 2009; Schmidt et al., 2005a). The mechanism behind the gender differences has not been investigated yet. Also, it is speculated that the genotype of P-gp transporter could affect salvinorin A induced effects (Butelman et al., 2012; Doran et al., 2005; Levran et al., 2008). Similarly, the genotypes of enzymes involved in the metabolism of salvinorin A could affect the (time of) effects induced following salvinorin A exposure. As such, individuals with liver disorders or slow metabolizers might be more prone to adverse effects.

Wu et al. (2011) and Vohra et al. (2011) stated that *S. divinorum* users have a higher prevalence of substance use disorders, mental health problems and depressions. El-Khoury and Sahakian (2015) hypothesized as well that the consumption of *S. divinorum* could be associated with the development of psychotic disorders, however, more research was needed. As a result, users might be more vulnerable to develop a psychotic event and have co-exposure with medications affecting the dynamic of salvinorin A.

In addition, online smartshops warn against the use when pregnant, lactating, suffering from depressions, heart and vascular diseases and liver disorders.

#### **6.4 Uncertainties**

The concentrations of salvinorin A in the products sold for recreational use vary greatly and depend on geographic origin, cultivation, extraction method and brand. For this assessment, the concentrations of salvinorin A in the extracts were based on the claimed concentration. Since most measured concentrations in products sold outside of the Netherlands are lower than the claimed concentrations, this may lead to an overestimation of exposure. The concentration of salvinorin A in dried leaves is based on the concentrations measured in *S. divinorum* leaves obtained elsewhere (not from the Dutch market) since the concentrations of salvinorin A in leaves sold in Dutch web shops were not listed. As a result, this may lead to an under- or overestimation of an individual's exposure.

The number of times an individual uses (herbal preparations containing) *S. divinorum* varies roughly between once a year to multiple times a day. In this risk assessment, only the risk of acute adverse effects following a single exposure (inhalation/sublingual) were assessed. In addition, toxicological data regarding genotoxicity, chronic exposure, reproduction or developmental toxicity are lacking.

The NOEL and LOEL for psychoactive effects after single inhalation exposure were based on a human clinical study including both male and female volunteers. The volunteers included all had a history of recreational hallucinogen and/or salvinorin A use. This history may limit the generalizability of these findings to users naïve to *S. divinorum*/salvinorin A and/or hallucinogenic drugs, since users with negative experiences following the use of *S. divinorum*, salvinorin A, or hallucinogenic drugs most probably did not volunteer (selection bias). Also, it is mentioned in online user platforms that users become more sensitive to the effects of salvinorin A following prolonged use.



## 7 Conclusions and recommendations

Acute adverse health effects may occur following inhalation or sublingual use of (herbal preparations containing) *S. divinorum* currently available on the Dutch market. The adverse effects are caused by the psychoactive compound salvinorin A and can already occur at the recommended quantity. Acute effects that may occur include affected attention, latency, sedation-like effects and visual and auditory hallucinogenic effects. Effects following oral exposure (ingestion) can be expected, although no quantitative risk assessment was possible for this route of exposure.

Toxicological data regarding long-term use, genotoxic or carcinogenic effects or effects on reproduction and development are lacking and no firm conclusions can be drawn on these aspects.

Based on the acute adverse health effects, RIVM advises consumers to not use (herbal preparations containing) *S. divinorum*. RIVM advises VWS to consider regulation of (herbal preparations containing) *S. divinorum*.



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