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Risk assessment of herbal preparations containing seed extracts of *Mucuna pruriens*

RIVM letter report 2024-0087
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Synopsis

Risk assessment of herbal preparations containing seed extracts of *Mucuna pruriens*

Herbal preparations (food supplements) that contain an extract of *Mucuna pruriens* seeds are sold in the Netherlands. These herbal preparations are mainly sold online. According to the manufacturers, *Mucuna pruriens* can support the immune system and increase energy levels.

RIVM has examined whether herbal preparations containing *Mucuna pruriens* seed extract are harmful to health. Very little scientific information about *Mucuna pruriens* is publicly available. Accordingly, it is not possible to determine a safe dose for this extract. However, there are indications that the extract has negative effects on the liver, kidneys, and the development of unborn children.

As a precaution, RIVM advises not to use these herbal preparations during pregnancy and breastfeeding, or in case of liver or kidney problems. In other cases, it is advised to be cautious. RIVM advises to be alert to side effects and to stop using the product in case side effects occur. If people choose to use herbal preparations containing *Mucuna pruriens,* they have to use it in accordance with the instructions on the packaging. And discuss the use with their doctor or pharmacist in case of medicine use.

In addition, it is known that one of the substances in *Mucuna pruriens* (levodopa) is the active substance in medicines used to treat Parkinson's disease. The quantity of levodopa that someone ingests from these herbal preparations is comparable to or higher than the quantity for people with Parkinson's who are starting to take these medicines. The side effects of these medicines, such as gastrointestinal symptoms, involuntary movement (dyskinesia) and psychological symptoms, can also occur in users of the herbal preparation.

Keywords: *mucuna pruriens,* levodopa*,* L-Dopa, food supplements, safety

Publiekssamenvatting

Risicobeoordeling van kruidenpreparaten met extract van de zaden van *Mucuna pruriens*

In Nederland worden kruidenpreparaten (voedingssupplementen) met extract van zaden van *Mucuna pruriens* verkocht. Deze kruidenpreparaten zijn vooral online verkrijgbaar. Volgens fabrikanten kan *Mucuna pruriens* het immuunsysteem ondersteunen en ervoor zorgen dat mensen meer energie hebben.

Het RIVM onderzocht of kruidenpreparaten met extract van zaden van *Mucuna pruriens* schadelijk zijn voor de gezondheid. Er is over *M. pruriens* heel weinig openbare wetenschappelijke informatie beschikbaar. Daarom is het niet mogelijk te bepalen wat een veilige dosis van dit extract is. Wel zijn er aanwijzingen dat het extract negatieve effecten heeft op de lever, nieren en de ontwikkeling van het ongeboren kind.

Daarom adviseert het RIVM uit voorzorg om deze kruidenpreparaten niet te gebruiken tijdens de zwangerschap en borstvoeding, of bij lever- of nierproblemen. In andere gevallen is het advies er voorzichtig mee te zijn. Het RIVM adviseert daarbij goed in de gaten te houden of er bijwerkingen optreden en met het product te stoppen als dat gebeurt. Áls mensen ervoor kiezen kruidenpreparaten met *Mucuna pruriens* te gebruiken, moeten ze de aanwijzingen op de verpakking volgen. En het gebruik met hun arts of apotheker bespreken wanneer ze medicijnen slikken.

Ook is bekend dat een van de stoffen in *Mucuna pruriens* (levodopa) de werkzame stof is in medicijnen voor de behandeling van de ziekte van Parkinson. De hoeveelheid levodopa die iemand via deze kruidenpreparaten binnenkrijgt is vergelijkbaar met of hoger dan de hoeveelheid voor mensen met Parkinson die het medicijn beginnen te slikken. De bijwerkingen bij dit medicijn, zoals maagdarmklachten, ongewilde bewegingen (dyskinesie) en psychische klachten, kunnen ook optreden bij gebruikers van het kruidenpreparaat.

Kernwoorden: *mucuna pruriens,* levodopa*,* L-Dopa, voedingssupplementen, veiligheid

Contents

Summary

Introduction

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 with substances/botanicals that are either forbidden or restricted (i.e., subject to a maximum level) 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 are sold on the Dutch market and (widely) used in the Netherlands and that there are 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 *Mucuna pruriens* seed extract.

Currently, there are no specific restrictions for the use of *M. pruriens* in herbal preparations included in the Herbal Preparations Decree of the Dutch Commodities Act[1]. In addition, there is no harmonized European legislation for *M. pruriens*.

Previous evaluations

M. pruriens seeds are listed in the EFSA Compendium of Botanicals with a reference to a paper describing outbreak of acute toxic psychosis attributed to the consumption of *M. pruriens seeds*[2].

The use of *M. pruriens* seeds in craft products has been evaluated by the Spanish Agency for Consumer Affairs, Food Safety and Nutrition (AECOSAN). The Spanish Agency concluded that the unintentional oral exposure via application of *M. pruriens* seeds in craft products did not pose a risk to adults or children (Navarro et al., 2016).

The toxicologically most relevant constituent of herbal preparations containing *M. pruriens* seed extracts is levodopa (L-Dopa). Levodopa is widely used as a medicine against the symptoms of Parkinson's disease. The toxicity of levodopa has been extensively investigated for its evaluation as medicine for Parkinson's disease. These studies are included in the confidential authorization dossiers of the medicines and are not publicly available.

Products on the Dutch market

An internet search identified several herbal preparations containing *M. pruriens* seed extracts available on the Dutch market. Many of these extracts are standardized to a high levodopa content. They are used,

[1] https://wetten.overheid.nl/BWBR0012174/2020-07-01. Accessed 7 July 2023.
[2] https://www.efsa.europa.eu/en/data-report/compendium-botanicals. Accessed 7 July 2023.

amongst others, in herbal preparations for more energy and in herbal preparations claiming to support the immune system.

Exposure

Based on the recommended use levels of the herbal preparations, the recommended daily exposure to *M. pruriens* seed extract and, in some cases, levodopa could be estimated. The exposure to *M. pruriens* seed extract varies between 200 and 2000 mg per day (i.e., 2.9 – 29 mg/kg bodyweight (bw) per day for a 70 kg person). The exposure to levodopa varies between 40 and 400 mg per day (i.e., 0.57 – 5.7 mg/kg bw per day for a 70 kg person). Three herbal preparations specifically provided a recommended use for children based on their body weight. For children weighing 10-20 kg, the estimated exposure was 500 mg *M. pruriens* seed extract (i.e., 25-50 mg/kg bw depending on the body weight) and 100 mg levodopa (i.e., 5-10 mg/kg bw depending on the body weight). For children weighing 20-30 kg, the estimated exposure amounts then to 1500 mg *M. pruriens* seed extract (i.e., 50-75 mg/kg bw depending on the body weight) and 300 mg levodopa (i.e., 10-15 mg/kg bw depending on the body weight).

Use of levodopa as a human medicine

Levodopa is also on the market as a human medicine (combined with inhibitors of its metabolism). The daily dose of levodopa is determined on an individual basis. The starting dose for patients that did not receive levodopa before is 200 mg per day (2.9 mg/kg bw per day for an individual weighing 70 kg). This dose may gradually be raised to a maximum dose of 2000 mg per day (29 mg/kg bw for a person weighing 70 kg). In this risk assessment, the possible combined exposure of herbal preparations containing *M. pruriens* and human medicinal products containing levodopa is not taken into account.

Biological data

- Levodopa is extensively metabolised by decarboxylation by dopa decarboxylase (DDC)[3] and by O-methylation by catechol-O-methyltransferase (COMT) as main pathways.
- Plasma concentrations were lower in Parkinson patients (n=3) after receiving *M. pruriens* powder containing 135 – 180 mg levodopa than after receiving 150 mg levodopa with a decarboxylase inhibitor (carbidopa or benserazide) (Cassani et al., 2016).
- There are only a few animal toxicity studies (publicly) available that studied the oral toxicity of *M. pruriens* seeds. Indications for adverse effects of *M. pruriens* seeds on the liver and/or kidney based on histopathological and biochemical changes were found (Swamy et al., 2019; Omeh et al., 2014; Gbotolorun et al., 2018; Iamsaard et al., 2020).
- For medicinal products containing levodopa, it is stated that the product should be administered with caution in people with hepatic or renal impairment. It is also recommended to evaluate hepatic and renal function periodically during long term use (EMA

[3] Also known as aromatic L-amino acid decarboxylase

2023a, 2023b). This is not conducted when people use herbal preparations.
- Some adverse effects on the testes and sperm cells in one study in rats were observed indicating an effect on male reproduction (Ashidi et al., 2019). In addition, effects on sex hormones were found in another study, also including female animals (Iamsaard et al., 2020). These effects are considered adverse in the current risk assessment.
- Studies on genotoxicity, developmental toxicity, chronic toxicity and carcinogenicity of *M. pruriens* seeds were not identified.
- According to the assessment report for Numient, levodopa has a weak mutagenic potential, which is reduced in the presence of a metabolic system (EMA, 2015).
- For levodopa, adverse effects on foetal development, such as an increase in the number of resorbed foetuses, a decreased litter weight and skeletal and visceral malformations in rabbits were mentioned in the reports for Stalevo and Numient, but the underlying data were not publicly available (EMA, 2004, 2015). According to the Summary of Product Characteristics (SmPC) of Stalevo and Corbilta, these medicines should not be used during pregnancy unless the benefits for the mother outweigh the possible risks to the foetus. In addition, since levodopa is excreted in milk and the safety of levodopa in the infant is not known, it is stated that women should not breast-feed during treatment with Stalevo or Corbilta (EMA 2023a, 2023b).
- Adverse effects including gastrointestinal complaints were observed in studies in Parkinson patients that assessed the safety and efficacy of *M. pruriens* seeds in comparison to synthetic levodopa treatment (with and without decarboxylase inhibitor) (Cilia et al., 2017; Cilia et al., 2018).
- In one case study a female experienced adverse effects including gastrointestinal complaints, amnesia, confusion and hallucinations after intake of five raw *M. pruriens* seeds (not further specified) (Maillot et al., 2022).
- Side effects of levodopa have been documented and include dyskinesia[4], gastrointestinal symptoms and psychological problems (EMA, 2023a, 2023b; Farmacotherapeutisch Kompas, 2024;).
- The website Natural Medicine gives indications of interactions of *M. pruriens* seed extracts with anaesthetics, drugs or supplements that decrease blood glucose levels, anti-psychotics, antihypertensive drugs and monoamine oxidase inhibitors, or methyldopa (Natural Medicines, 2021).

No safe use level

It was investigated whether the presumption of safety could be applied to *Mucuna pruriens*. Botanical preparations for which an adequate body of knowledge exists, can benefit from a presumption of safety without any need for further testing (EFSA, 2009; EFSA, 2014). The presumption of safety could not be applied to *M. pruriens* and more information is needed to assess its safety.

[4] Dyskinesia refers to abnormal involuntary erratic muscle movements, which a person cannot control.

It is not possible to establish a health-based guidance value (HBGV) for *M. pruriens* seeds or for levodopa due to limited toxicological data. Adverse effects on the kidney, liver and reproductive system were found in animal studies. However, these studies do not allow a no observed adverse effect level (NOAEL) or lowest observed adverse effect level (LOAEL) to be derived. There are no studies available for genotoxicity, developmental toxicity, chronic toxicity and carcinogenicity of *M. pruriens* seed extracts. As an effect of levodopa, an increase in the number of resorbed foetuses, a decreased litter weight and skeletal and visceral malformations in rabbits were mentioned, but no details were available and also the information on other toxicity in the assessment reports was not sufficient to derive a reference value. The available human studies cannot be used as basis for an HBGV because not all aspects of toxicity were studied. Therefore, since no HBGV could be established, no safe use level for herbal preparations containing *M. pruriens* seed extracts can be determined. As the derivation of a NOAEL or LOAEL was not possible, a margin of exposure approach could not be carried out.

Risk assessment, conclusions and recommendations

In the risk assessment of *M. pruriens* seeds, the following aspects are considered:

- There are indications for adverse effects of *M. pruriens* seed extracts on liver, kidney, reproductive function and foetal development.
- Important information, e.g., studies on the genotoxicity, carcinogenicity and chronic toxicity, is lacking.
- The recommended use of herbal preparations containing *M. pruriens* seed extracts leads to an estimated exposure to levodopa that is similar or higher than the therapeutic starting dose prescribed to Parkinson patients. Pharmacological effects can be expected at the recommended use and the side effects (e.g., dyskinesia, gastrointestinal symptoms and psychological problems) reported for levodopa could occur. It should be noted that in the medicinal products levodopa is administered in combination with inhibitors of the metabolism of levodopa (e.g., carbidopa and entacapone). *M. pruriens* does not contain these inhibitors. Information about the effects of other constituents in *M. pruriens* or other ingredients in herbal preparations is lacking.

Therefore, it is concluded that herbal preparations containing *M. pruriens* seed extracts may pose a health risk.

As a precaution, RIVM advises consumers not to use herbal preparations containing *M. pruriens* seed extract during pregnancy, when breastfeeding or when having liver or kidney problems. In addition, RIVM advises consumers in other situations to be cautious with these herbal preparations. Consumers are advised to be alert to side effects and to stop using the product in case side effects occur. If people choose to use herbal preparations containing *Mucuna pruriens,* they have to use it in accordance with the instructions on the packaging and discuss the use with their doctor or pharmacist in case of medicine use.

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[5] with substances/botanicals that are either forbidden or restricted (i.e., subject to a maximum level) 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 are sold on the Dutch market and (widely) used in the Netherlands and there are 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 *Mucuna pruriens* seed (extract).

Mucuna pruriens seed extract is available on the Dutch market as a herbal preparation for amongst others more energy and supposed beneficial effect on the immune system. Its toxicologically most relevant constituent is levodopa (L-Dopa).

1.2 Information on existing assessments

M. pruriens has not been evaluated by the Committee on Herbal Medicinal Products (HPMC) of the European Medicines Agency (EMA). Levodopa is widely used as a medicine against symptoms of Parkinson's disease and assessment reports as well as product information is available at EMA (EMA, 2004, 2015, 2023a, 2023b). Details on the underlying studies are not publicly available as these studies are part of the confidential authorization dossiers.

M. pruriens is included in EFSA's Compendium of Botanicals with a reference to a paper describing an outbreak of acute toxic psychosis attributed to the consumption of *M. pruriens* seeds[6]. This paper described that in 1989 203 cases of acute psychosis were reported in Mozambique within 6 weeks. In that year there were severe food shortages and people turned to wild plants as food. In this particular area *M. pruriens* was a common famine food. The cases of acute toxic psychosis were attributed to the consumption of *M. pruriens* seeds. People experienced adverse effects, such as headaches, hallucinations and delusions. Symptoms were resolved within 2 weeks. The authors wrote that *M. pruriens* seeds contain N, N-dimethyltryptamine, 5-methoxy-N, N-dimethyltryptamine and levodopa (Infante et al., 1990 as cited in EFSA's Compendium of Botanicals).

[5] https://wetten.overheid.nl/BWBR0012174/2020-07-01. Accessed 7 July 2023.
[6] https://www.efsa.europa.eu/en/data-report/compendium-botanicals. Accessed 7 July 2023.

The use of *M. pruriens* in craft products has been evaluated by the Spanish Agency for Consumer Affairs, Food Safety and Nutrition (AECOSAN). In this evaluation the risk of unintentional oral exposure via application of *M. pruriens* seeds in craft products was assessed. The Spanish Agency concluded that the risk for acute toxicity by accidental ingestion is low. In addition, it calculated a safety margin for the intake of the seeds, both accidentally and continuously. The safety margins were calculated based on the lowest published toxic dose (TD_{Lo}) of 4286 mg/kg body weight (bw) and estimated exposures of 21.4 mg levodopa/kg bw for a 70-kg adult (safety margin of 200) and 50 mg levodopa/kg bw for a 30-kg child (safety margin of 85). The calculated safety margins were higher than 30 (factor 10 for intraspecies variation and a factor 3 since an effect level was used), which was considered safe by the agency. Overall the agency concluded that *"the accidental intake of seeds of Mucuna pruriens does not pose a toxicological risk due to acute and chronic exposure for adults and children due to the use in craft products. Nevertheless, due to the presence of L-Dopa, and other biologically active substances which may in turn have a synergic action, the voluntary intake of seeds of Mucuna pruriens in uncontrolled and unassessed conditions is a "risk factor" to be considered for the health of consumers"* (Navarro et al., 2016).

1.3 Information on existing legislations

Currently, there are no specific restrictions for the use of *M. pruriens* in herbal preparations included in the Herbal Preparations Decree of the Dutch Commodities Act[7]. In addition, there is no harmonized European legislation for *M. pruriens*.

[7] https://wetten.overheid.nl/BWBR0012174/2020-07-01. Accessed 7 July 2023.

2 Literature search

The risk assessment for herbal preparations containing *Mucuna pruriens* seeds extract was conducted using the template for the safety assessment of plant food supplements as a basis (De Wit et al., 2019).

A search strategy was developed to find relevant literature for the risk assessment of herbal preparations containing *M. pruriens* seed extracts. To this end, search terms were formulated to describe the herb of interest and included levodopa which is the toxicologically most relevant constituent of *M. pruriens*. The search strategy aimed to identify references describing toxicity, adverse outcomes and kinetics and to include animal data as well as human data (see Annex 1). Four databases, including Embase, Pubmed, Scopus and Toxcenter, were searched up to (and including) September 2021. Since the inclusion of the search term 'L-Dopa' or 'L-dopa' led to a very high number of results, it was decided to perform two different searches: one focused on *M. pruriens* and one focused on levodopa. The search that focused on *M. pruriens*, for which reviews were excluded, gave a total of 297 results. The search that focused on levodopa gave a total of 4702 results. The results of the literature search for *M. pruriens* were screened on relevance based on title and abstract and the results for levodopa were screened based on title, after which they were selected for inclusion in this report when deemed relevant. For *M. pruriens*, 14 articles containing toxicity and kinetic studies were selected for the risk assessment. No relevant publicly available toxicity studies for levodopa were found. On July 7th 2023 the literature search was updated and relevant literature between September 2021 and July 2023 was collected. The databases Embase, PubMed and Scopus were searched using the same search strategy. In total an additional 91 unique articles were found of which two were selected for the risk assessment. In addition, the grey literature was searched for assessments of *M. pruriens* by other organizations, including European Food Safety Authority (EFSA), European Pharmacopoeia, European Scientific Cooperative on Phytotherapy (ESCOP), EMA, World Health Organisation (WHO), Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES), Food and Drug Administration (FDA), Bundesinstitut für Risikobewertung (BfR), Danmarks Tekniske Universitet (DTU) and AECOSAN.

3 Description of the product

3.1 Identity and nature of the source material

3.1.1 Botanical (preparation)

M. pruriens is an annual plant native to tropical regions, especially Africa, India and the West Indies (Sathiyanarayanan & Arulmozhi, 2007). It is a vigorous climber and has trifoliolate leaves and white or purple flowers. The seed pods are covered with brown or grey irritant bristles, and each pod can contain up to 6 or 7 ovoid-shaped seeds. The name *pruriens* refers to the itching sensation resulting from contact with the seedpod hairs (Ingle, 2003; Navarro et al., 2016; Sathiyanarayanan & Arulmozhi, 2007). The plant (root, leaves and seeds) has since long been used in traditional Ayurvedic Indian medicine to treat different conditions, such as parkinsonism, nervous disorders, arthritis and venom poisoning. The roots are used for amongst others constipation, elephantiasis, dropsy and neuropathy. Examples where the leaves are used include tonics, aphrodisiacs, anthelmintics and extracts for the treatment of inflammation. The seeds are amongst others used for worms, dysentery, diarrhoea, snakebites, sexual debility, coughing, tuberculosis, impotence, rheumatic disorders, muscular pain, gonorrhoea, sterility, gout, delirium, dysmenorrhea, diabetes and cancer (Sathiyanarayanan & Arulmozhi, 2007; Lampariello et al., 2012). In India the seeds are also used as an aphrodisiac, emmenagogue, uterine stimulant, nerve tonic, diuretic and blood purifier. Powder of the seeds is in the Ayurvedic system used for treating Parkinson's disease (Sathiyanarayanan & Arulmozhi, 2007). Table 3.1 lists the classification of *M. pruriens*.

Table 3.1 Information related to the classification of M. pruriens.

Scientific (Latin) names	Family: Leguminosae / Fabaceae Genus: *Mucuna* Adans. Species: *Mucuna pruriens* (L.) DC.
Synonyms	*Mucuna pruriens* subsp. Pruriens *Dolichos pruriens* L.
Common name	Velvet bean, Cowage, Cowitch (English), Juckbohne (German), Fluweelboon (Dutch), Atmagupta (Indian)
Parts used	Seeds (beans), roots, leaves
Geographical origin	Tropical regions, especially India, the West Indies and Africa

Source: Mansfeld's World Database of Agricultural and Horticultural Crops, USDA Plants Database, Katzenschlager et al., 2004, Sathiyanarayanan & Arulmozhi, 2007.

M. pruriens has not been listed in the European Pharmacopoeia and there is no monograph on it by ESCOP, EMA or WHO.

3.2 Manufacturing process

M. pruriens seed extract is used as a herbal preparation (in capsule, tablet form or as a powder). Different extraction methods have been described, for example using alcohol or water (Misra & Wagner, 2007;

Vora et al., 2017). The main focus of the different extraction methods is to optimize the levodopa content in the extract.

Levodopa can also be synthesized by oxidation of L-tyrosine (Ingle, 2003). The synthetic form is used as human medicinal product to treat Parkinson disease (Medicines Information Bank, 2021a).

3.3 Chemical composition

Analysis of air-dried seeds of *M. pruriens* showed the presence of several constituents: phenolics, trypsin inhibitors, saponins, phytic acid, levodopa, raffinose, stachyose, and verbascose (Table 3.2). The highest concentrations were found for trypsin inhibitor, total phenolics and levodopa (Adebowale et al., 2005).

Table 3.2 Composition of M. pruriens seeds (Adebowale, Adeyemi & Oshodi, 2005)

Constituent	**Presence in g/100 g dry matter** [a]
Total phenolics	7.75 ± 0.02
Trypsin inhibitor	24.2 ± 0.08
Saponins	1.46 ± 0.01
Phytic acid	1.97 ± 0.01
Levodopa	4.99 ± 0.02
Raffinose	1.65 ± 0.01
Stachyose	1.23 ± 0.02
Verbascose	0.93 ± 0.01

[a] Means ± SD of triplicate analysis

The final concentration of the constituents in *M. pruriens* preparations is dependent on the method of extraction, e.g. with alcohol or water, and the applied conditions (Misra & Wagner, 2007; Mugendi et al., 2010). Variability in concentration could also be caused by geographical origin and genetic nature between *M. pruriens* varieties. It has for example been observed that *Mucuna* species growing near the equator have higher levels of levodopa present than plants far away from the equator (Vadivel & Biesalski, 2012).

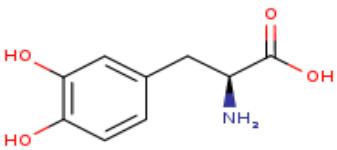
The concentration of levodopa also varies between the plant parts (Ingle, 2003). Table 3.3 shows the concentrations as found for the different plant parts. The seeds contain the highest concentrations of levodopa and extracts of the seeds are used in herbal preparations containing *M. pruriens*.

Table 3.3 Levodopa content in different M. pruriens plant parts (source: Ingle, 2003)

Plant part	**Levodopa (%)**
Seed	1.25-9.16
Pericarp	0.09-0.22
Leaf	0.35
Stem	0.31
Root	0.16
Whole bean	4.02
Endocarp	5.28

The chemical structure of the main constituent levodopa is presented in Table 3.4.

Table 3.4 Chemical structure of levodopa

Levodopa / L-Dopa		
Synonyms	a.o. (-)-3-(3,4-Dihydroxyphenyl)-L-alanine; 3,4-Dihydroxy-L-phenylalanine; L-beta-(3,4-Dihydroxyphenyl)-alpha-alanine	
Systematic name	L-Dopa; L-tyrosine, 3-hydroxy-; Alanine, 3-(3,4-dihydroxyphenyl)-, L-	
Molecular formula	C9-H11-N-O4	
Molecular weight	197.189 g/mol	
CAS no.	59-92-7	

Source: ChemIDplus, US National Library of Medicine.

3.4 Selected compound(s) for the risk assessment

This risk assessment reviewed *M. pruriens* seed extract and its toxicologically most relevant constituent levodopa. Levodopa is present in high concentrations in *M. pruriens* seeds and levodopa is used in human medicinal products.

The presence of levodopa in herbal preparations with *M. pruriens* is further confirmed by Hasegawa et al. (2011). They determined the levodopa content in 14 commercial dietary supplements (tablets and capsules) containing *M. pruriens* with a HPLC method. The supplements were bought on the internet in Japan. The levodopa content varied from 0.71 to 9.13 mg per capsule or tablet.

3.5 Use and use levels

Several herbal preparations containing seeds from *M. pruriens* can be bought on the Dutch market. According to their sellers, these products are recommended amongst others for a higher energy level and supposed beneficial effect on the immune system. Table 3.5 shows examples of these products that were found with an internet search on Dutch websites (August 2023). Most of them contain *M. pruriens* seed extract as single ingredient. For some products, the levodopa content is given but for the most of them this is not the case. Three herbal preparations also provide an adjusted use level for children, thereby suggesting that the product is suitable for children (product 4, 5 and 13).

Table 3.5 Examples of herbal preparations containing Mucuna pruriens extract available on the Dutch market with recommended daily use and recommended dose. body weight (bw)

Product	**Ingredients**	**Dose per unit**	**Recommended daily use**	**Total recommended daily dose *M. pruriens***	**Total recommended daily dose levodopa**	**Warnings[1]**
Product 1	*Mucuna pruriens* extract (min. 25% levodopa)	400 mg	one to two capsules	800 mg	200 mg	Yes[2]
Product 2	*Mucuna pruriens* extract (Sabina®, 25% levodopa)	250 mg	three to six capsules	1500 mg	375 mg	Yes[3]
Product 3	*Mucuna pruriens* extract (15% levodopa)	400 mg	1 to 2x one capsule	800 mg	120 mg	Yes[4]
Product 4	*Mucuna pruriens* extract (20% levodopa)	500 mg	bw>30 kg: 2x two tablets bw 20-30 kg: 3 tablets bw 10-20 kg: 1 tablet	2000 mg 1500 mg 500 mg	400 mg 300 mg 100 mg	Yes[5]
Product 5	*Mucuna pruriens* extract (20% levodopa)	500 mg	bw >30 kg: 2x two tablets bw 20-30 kg: 3 tablets bw 10-20 kg: 1 tablet	2000 mg 1500 mg 500 mg	400 mg 300 mg 100 mg	Yes[4]
Product 6	*Mucuna pruriens* extract (15% levodopa)	265 mg	one capsule	200 mg	40 mg	Yes[6]
Product 7	*Mucuna pruriens* extract (ratio 10:1)	375 mg	one to two capsules	750 mg	-	Yes[6]
Product 8	*Mucuna pruriens* extract (standardized)	400 mg	1x one to two capsules	800 mg	-	Yes[7]
Product 9	*Mucuna pruriens* extract	480 mg	two to four capsules	1920 mg	-	Yes[6]
Product 10	*Mucuna pruriens* extract	450 mg	2 to 3x one capsule	1350 mg	-	Yes[6]
Product 11	*Mucuna pruriens* extract	500 mg	2x one to two capsules	2000 mg	-	Yes[6]
Product 12	*Mucuna pruriens* extract (25% levodopa)	500 mg	2 capsules	1000 mg	250 mg	Yes[8]
Product 13	*Mucuna pruriens* extract (20% levodopa)	500 mg	bw >30 kg: 2x two tablets bw 20-30 kg: 3 tablets bw 10-20 kg: 1 tablet	2000 mg 1500 mg 500 mg	400 mg 300 mg 100 mg	Yes[6]

Product	**Ingredients**	**Dose per unit**	**Recommended daily use**	**Total recommended daily dose *M. pruriens***	**Total recommended daily dose levodopa**	**Warnings[1]**
Product 14	*Mucuna pruriens* (seed) extract (standardized at 60 mg levodopa)	400 mg	one to two capsules	800 mg	120 mg	Yes[8]
Product 15	*Mucuna pruriens* (seed) extract (15% levodopa)	350 mg	2x one capsule	700 mg	105 mg	Yes[9]
Product 16	*Rhodiola Rosea* extract, L-theanine, *Mucuna pruriens* extract, L-tyrosine, L-carnitine, caffeine, taurine, vitamin B6, zinc	100 mg	1x two capsules	200 mg	-	Yes[4]
Product 17	*Mucuna pruriens* extract, *Panax ginseng*, *Punica granatum*, *Muira puama*	100 mg	1x two capsules	200 mg	-	Yes[10]
Product 18	*Mucuna pruriens* extract (40% levodopa)	unknown (capsule) 400 mg (powder)	Capsules unknown One to two scoops	800 mg (powder)	320 mg (powder)	Yes[11]
Product 19	Carnosyn beta-alanine, creatine nitrate citrulline malate, caffeine anhydrous, *velvet bean seed* extract (levodopa), theacrine, vitamins (niacin, vitamine B6, B12) calcium silicate, citric acid, silicon dioxide, calcium silicate,	-	1 scoop	-	-	Yes[12]

Product	Ingredients	Dose per unit	Recommended daily use	Total recommended daily dose *M. pruriens*	Total recommended daily dose levodopa	Warnings[1]
	flavors, sucralose, acesulfame potassium					
Product 20	*Mucuna pruriens* extract (seed) (standardized for 20% levodopa) + 29 other ingredients	250 mg	1 scoop	250 mg	50 mg	Yes[13]

[1] Translated from warning phrases in Dutch
[2] Keep out of the reach and sight of young children. Consult an expert in case of pregnancy (wish) or breastfeeding.
[3] Not suitable during pregnancy and lactation.
[4] Do not use during pregnancy and lactation. Keep out of reach of children.
[5] Better not to use during pregnancy and lactation.
[6] Keep out of reach of young children. Consult an expert before using supplements in case of pregnancy, lactation, medication and illness.
[7] Do not use during pregnancy or breastfeeding. Keep out of reach of young children. Consult an expert before using supplements in case of pregnancy, lactation, medication and illness.
[8] Always keep it out of reach of young children. Consult an expert if you are pregnant, breast-feeding, or if you are taking medication and/or are ill.
[9] Do not use this product if you are using an MAO inhibitor. Consult your physician prior to use if you suffer from high blood pressure, heart or lung disease, or if you are using products containing levodopa. Keep out of reach of children.
[10] Only suitable for men aged 16 and over. May affect the effect of anticoagulants. Keep out of reach of children.
[11] If you are going to use supplements, consult your doctor first in case of medication use, health problems or pregnancy/ breastfeeding.
[12] WARNING: THIS PRODUCT IS ONLY INTENDED TO BE CONSUMED BY HEALTHY ADULTS, 18 YEARS OF AGE OR OLDER. Do not use this product if you are pregnant, nursing, or are currently taking nitrates for chest pain or if you are taking medication used to treat erectile dysfunction such as PDE-5 inhibitors. Before using this product, consult a licensed, qualified, healthcare professional, including but not limited to, if: you are taking antidepressants such as MAOI (Monoamine Oxidase Inhibitor) or SSRI, blood thinners, nonsteroidal anti-inflammatory drugs, pseudoephedrine, or you are taking any other dietary supplement, prescription drug or over-the-counter medication; or if, you suspect you have or have been treated for, diagnosed with or have a family history of, any medical condition, including but not limited to: high or low blood pressure, diabetes, glaucoma, anxiety, cardiovascular, psychiatric or seizure disorders, cardiac arrhythmia, stroke, heart, liver, kidney or thyroid disease, or difficulty urinating due to prostate enlargement. This product contains caffeine and should not be used by individuals wishing to eliminate caffeine from their diet or in combination with caffeine or stimulants from other sources including but not limited to, coffee, tea, soda, or other dietary supplements and medications. Discontinue 2 weeks prior to surgery. Immediately discontinue use and contact a medical doctor if you experience any adverse reaction to this product. Do not exceed recommendations for Suggested Use. Use only as directed. Do not use if safety seal is broken or missing. Store in a cool dry place. KEEP OUT OF REACH OF CHILDREN."
[13] Not recommended for children, pregnant or breastfeeding women

4 Exposure: extent and duration

4.1 Exposure from food supplement use

Based on the recommended use levels of the herbal preparations containing *M. pruriens* seed extract that are available on the Dutch market and described in Table 3.5, recommended daily exposure to *M. pruriens* seed extract and, in some cases, levodopa could be estimated. The exposure to *M. pruriens* seed extract varies between 200 and 2000 mg per day (i.e., 2.9 – 29 mg/kg bw per day for a 70 kg person). The exposure to levodopa varies between 40 and 400 mg per day (i.e., 0.57 – 5.7 mg/kg bw per day for a 70 kg person).

Three products specifically provide a recommended use for children based on their body weight. For children weighing 10-20 kg, the estimated exposure amounts was 500 mg *M. pruriens* seed extract (i.e., 25-50 mg/kg bw depending on the body weight) and 100 mg levodopa (i.e., 5-10 mg/kg bw depending on the body weight). For children weighing 20-30 kg, the estimated exposure amounts was 1500 mg *M. pruriens* seed extract (i.e., 50-75 mg/kg bw depending on the body weight) and 300 mg levodopa (i.e., 10-15 mg/kg bw depending on the body weight).

4.2 Possibility of additional/combined human exposure

Levodopa is also on the market as a human medicine. Several medicinal products containing levodopa are registered at the Dutch Medicines Evaluation Board (MEB; Medicines Information Bank, 2021a). Besides levodopa these medicinal products also contain a decarboxylase inhibitor (e.g., carbidopa, benserazide) and/or a COMT (catechol-O-methyltransferase) inhibitor (e.g., entacapone) to prevent the breakdown of levodopa before it has passed the blood brain barrier. The daily dose of levodopa is determined on an individual level. In patients receiving levodopa for the first time, a starting dose of 200 mg per day, equivalent to 2.9 mg/kg bw per day for an individual weighing 70 kg, is recommended (Farmacotherapeutisch Kompas, 2024). This dose may gradually be raised to a maximum dose of 2000 mg per day equivalent to 29 mg/kg bw per day for an individual weighing 70 kg, depending on an individual's needs. It is recommended to build up the dose with 100 mg levodopa each week, which means that the starting dose would be built up to the maximum dose in a period of 18 weeks. In this risk assessment, the possible combined exposure of herbal preparations containing *M. pruriens* and human medicinal products containing levodopa is not taken into account.

The use of (seed extracts of) *M. pruriens* as an alternative treatment for Parkinson's disease to registered medicines containing levodopa is not recommended. The reason is that the evidence for beneficial effects of *M. pruriens* on Parkinson specific symptoms and motoric and non-motoric side-effects is scarce. It is recommended to physicians to ask patients for their use of food supplements and herbal preparations, including *M. pruriens* (Richtlijnendatabase, 2021).

5 Biological data

5.1 Toxicokinetics

5.1.1 Absorption, distribution, metabolism, excretion

No studies on the toxicokinetics of *M. pruriens* seed extracts have been identified. Only one study that reported on the pharmacokinetics of levodopa when administered via *M. pruriens* seed powder and when administered as a single compound in combination with decarboxylase inhibitor in Parkinson patients was identified (Cassani et al., 2016). The plasma concentrations of levodopa were measured at fixed time points over a three hour period after the intake of levodopa with a decarboxylase inhibitor (carbidopa or benserazide) or *M. pruriens* powder in the morning on two consecutive days, after overnight withdrawal of usual anti-Parkinson's disease therapy. Three patients receiving 150 mg of levodopa with a decarboxylase inhibitor showed a C_{max} of 2.4 to 5.7 µg/ml. The time to peak concentration was 15 to 45 minutes and the area under the plasma concentration time curve (AUC) varied from 212 to 241.1 µg/min/mL. When these patients received 135 to 180 mg of levodopa via *M. pruriens* powder, the C_{max} varied from 1.2 to 2.05 µg/mL. The time to peak concentration was 15 to 45 minutes and AUC was 65.2 to 98.4 µg/min/mL. The authors calculated a median ratio between the AUCs obtained after 150 mg of levodopa and a decarboxylase inhibitor and after the *M. pruriens* powder of 3.47 (Cassani et al., 2016).

Information about the toxicokinetics of levodopa have been described in the Summary of Product Characteristics (SmPC) of Stalevo and Corbilta, the assessment report for the no longer authorised medicinal product Numient and on the website Farmacotherapeutisch Kompas and is summarized below (EMA, 2015[8], 2023a, 2023b; Farmacotherapeutisch Kompas, 2024).
In humans, levodopa is rapidly absorbed and has a bioavailability of 15 to 33% after an oral dose of 200 mg, when given without inhibitors of its metabolism. Bioavailability of levodopa is significantly higher in women than in men. In addition, the absorption of levodopa is greater and elimination is slower in elderly than in young people. Levodopa has a short half-life of 0.6 to 1.3 h as it is extensively metabolised. The main metabolization pathways are decarboxylation of levodopa to dopamine by dopa decarboxylase[9] (DDC) and O-methylation of levodopa to 3-O-methyldopa by catechol-O-methyltransferase (COMT) (EMA 2023a, 2023b). Levodopa is fully excreted as metabolites in urine via the kidneys (Farmacotherapeutisch Kompas, 2024)

The enzymatic metabolism in mammalian species does not involve the CYP450 family of enzymes and therefore CYP450-derived drug-drug interactions are unlikely to manifest. Interactions with common CYP450 inhibitors or substrates have not been investigated (EMA, 2015).

[8] The authorization for Numient has been withdrawn at the request of the marketing-authorization holder. However, its content was concluded to be still usable for this report.
[9] Also known as aromatic L-amino acid decarboxylase

In studies with rats and mice, levodopa is rapidly taken up from the blood by the pancreas and other glands involved in rapid protein synthesis. Also, high initial concentrations of levodopa were observed in the kidney, liver and small intestine. Levodopa passes freely into the brain (EMA, 2015). In a scientific discussion document about the medicinal product Stalevo, it is also mentioned that levodopa can cross the placenta (EMA, 2004).

Animal studies have shown levodopa and its metabolites to be predominantly excreted via the urine with between 60-90% of the administered dose recovered in urine. Excretion is rapid, with rat studies showing 90% recovery within 24h after oral administration. In fasted patients, approximately 85% of the total administered dose was recovered after excretion in 24h. Levodopa is almost completely cleared by metabolism through the action of DDC and COMT (EMA, 2015).

5.2 Toxicological data

There are limited toxicological data available for *M. pruriens* seed extracts. Below, the relevant toxicity studies that have been found for *M. pruriens* have been summarized. In addition, a short overview of the toxicologically most relevant issues with respect to levodopa has been given. Despite the fact that this substance is available as a medicine and has been evaluated extensively for its toxicological effects, pre-clinical toxicity data could not be found as these are owned by the companies applying for the authorization of the levodopa containing medicine. Although human data is available in the assessment reports from EMA and the evaluation reports by MEB, details about the conducted studies were not publicly available.

5.2.1 Acute toxicity

Mice

In 2015, Hadimani et al. investigated the acute oral toxicity of methanolic extracts of seeds of *M. pruriens*. Female albino Swiss mice were monitored for mortality in a study that was reportedly performed according to OECD guideline no. 420. No mortality was seen up till 2000 mg/kg bodyweight *M. pruriens* seed extract. Details of the study were not given and no other toxicological endpoints were described.

In 2016, Saikarthik et al. performed an acute toxicity study with methanol extracts of *M. pruriens* seeds in albino mice (n=3), administering 2000 mg/kg bw orally and observing the animals for signs of mortality and toxicity for 14 days. Symptoms like changes in colour of fur and eyes, mucous membrane and parameters like tremors, convulsions, salivation, diarrhoea and lethargy were monitored for a 14-day period after administration. After that, blood was collected for haematological and biochemical research and the animals were killed and investigated for histopathological effects. Results showed that during the observation period no signs of toxicity were present. Also, haematological parameters like red blood cell (RBC) count, white blood cell (WBC) count, haemoglobin estimation, packed cell volume were within normal range. No effects were seen either in biochemical parameters such as blood glucose level, serum alanine aminotransferase (ALT), serum aspartate transaminase (AST) and alkaline phosphatase

(ALP) levels. The histopathological studies of the liver, kidney, spleen, lungs and pancreas did not reveal signs of toxicity either. The authors stated that the study was performed according to the OECD guideline no. 423. It is noted that the haematological and biochemical measurements were performed two weeks after a single exposure, which is too late for measuring possible effects.

The acute toxicity was assessed of an extract of *M. pruriens* seeds containing only protein hydrolysates and an ultrafiltered fraction (<1 kDa) in male ICR mice by Nuñez-Aragón et al. (2019). Mice were given a single oral dose of 10, 100, 1000, 1600, 2900 or 5000 mg/kg bw of extract and compared to a control group for mortality. Each treatment group consisted of 3 mice and was observed for a 14-day period during which mortality and other signs of abnormal behaviour and toxicity were monitored. The results showed no behavioural alterations, weight loss or other signs of toxicity (the exact signs of toxicity were not described).

Rats
Suresh et al. (2009) studied the acute oral toxicity of 0, 250 and 2500 mg/kg bw ethanol *M. pruriens* seed extract in female Albino Wistar (3 per dose). The animals were observed for the occurrence of mortality and signs of toxicity such as convulsion, hyperactivity, grooming, sedation, increased respiration and loss of righting reflex, continuously for four hours, followed by intermittent check-ups until ten hours past dosing and again after 24 and 48 hours past dosing. No adverse effects were observed. According to the authors the study was conducted following the up and down method in accordance with OECD guidelines from 2001 (not further specified).

The acute oral toxicity of a *M. pruriens* seed extract was assessed in female albino rats (Swamy et al., 2019). Rats received a single dose of 175, 550 or 2000 mg/kg bodyweight (bw) via oral gavage. Each treatment group consisted of six rats and no control group was included. Shortly before administration and on the 7^{th} and 14^{th} day after administration, rats were monitored for changes in body weight. The rats were checked for mortality and clinical signs during the first 30 minutes after administration, followed by four more times in the first 4 hours and twice daily until the end of the study thereafter. At the end of the study, rats were killed and examined for histological changes in the brain, heart, liver and kidney tissues. The seed extract of *M. pruriens* did not appear to cause any mortality or behavioural changes. However, between 1h and 4h after administration clinical symptoms such as piloerection and dullness were observed in all dose groups. No significant changes in the histological structure of the investigated organs were shown, except for mild congestion and hydropic changes in liver tissue that was seen in rats given 2000 mg/kg bw of *M. pruriens* extract. The authors stated that the study was performed according to the OECD guideline no. 425.

Sardjono et al. (2017) studied the acute toxicity of ethanol extracts of *M. pruriens* seeds in Wistar rats. Rats were orally given a single dose of 0, 5, 50, 300, 2000 or 5000 mg/kg bodyweight and were observed for 14 days. However, the bodyweight and the bodyweight gain of animals was not consistent with that of Wistar rats. It is more likely that mice

were used in the study. In addition, there was a large difference in bodyweight gain and bodyweight on day one between the groups, which suggests that different age groups were used. Therefore, the results of this study were not considered to be reliable. The same authors also published a subchronic toxicity study in which the same discrepancies were noted (Sardjono et al., 2018). Therefore, this study is not included in the risk assessment.

5.2.2 Short-term to sub-chronic toxicity

Mice

Manalisha et al. tested the oral toxicity of methanolic extracts of *M. pruriens* seeds in albino mice (male and female) in 2012. Animals were given a daily oral dose of 150, 300, 500, 1000, 2000, 3000 or 4000 mg/kg bw (plus a control group receiving saline) for four consecutive days and their mortality, loss of body weight and general behaviour was recorded from the first dose up to 72 hours after the last administration. Each treatment group consisted of five animals. No changes in body weight or general behaviour were seen and no mortality occurred either.

Rats

Suresh et al. (2009) orally exposed albino Wistar rats to 0, 150, 200 and 250 mg/kg bw *M. pruriens* seed extract for 45 days. Body weight, signs of toxicity and feed and water intake were monitored daily. On day 15, 30 and 45, blood samples were collected and tests were conducted for mating behaviour, libido and potency. At the end of the study, the rats were killed. Feed and water intake did not differ between the groups. For mating behaviour, libido and potency no dose-dependent changes were observed. The authors state that the levels of testosterone and oestradiol increased, but it was not mentioned what the extent of the increases were and if these differences were statistically significant. The number of spermatozoa increased from 185 million/ml in the control group to 200, 250 and 210 million/ml in respectively the 150, 200 and 250 mg extract/kg bw groups. No statistical analysis was performed. Sperm motility was graded on a scale from 0 (no movement) to 5 (maximum movement) as 5, 4, 5 and 3 for respectively the 0, 150, 200 and 250 mg extract/kg bw groups. No histological changes were observed in the liver and kidney and no dose-dependent changes were seen in organ weights of reproduction organs. However, the animals in the highest dose group showed slight erosion in the mucosal layer of the stomach.

Iamsaard et al. (2020) studied the subacute toxicity of a 14-day oral administration of (Thai) *M. pruriens* seed extract in male and female Wistar rats. Groups of rats (8 of each sex) were given daily doses of 0, 150 or 300 mg/kg bw by oral gavage. During the administration period the body weight of the rats was checked. After the administration period the animals were killed and blood samples were collected. Also, the weights, morphology and histopathology of the liver, kidney and reproductive organs were assessed. In female rats, serum oestradiol was statistically significantly increased and serum progesterone was statistically significantly decreased at both dose levels. Also, ovarian follicles were well-developed. The absolute weights of the ovaries in female rats in both treatment groups were shown to be statistically significantly lower than in the controls but this was not seen when

relative weights were compared. In male rats, the density of sperm masses stored in the epididymal lumen treated with *M. pruriens* extract was higher than in the controls. Both doses of *M. pruriens* statistically significantly decreased serum and intratesticular testosterone levels in male rats. This was, however, concluded not to have an effect on the spermatogenesis. The authors concluded *M. pruriens* not to have any toxic effect on the reproductive system. Furthermore, it was concluded that *M. pruriens* seed extract had a phytoestrogen effect on female rats. In addition, levels of creatinine, urea nitrogen, AST, ALT and ALP were measured. No statistically significant differences were seen in the levels of these biochemical parameters in treated male rats compared to controls. In female rats, AST was statistically significantly higher (approximately a factor of 4) in the highest dose group compared to the control group, which could suggest liver damage. ALT levels were also statistically significantly higher in female rats (a factor of 1.2 at 300 mg/kg bw) and serum ALP levels were statistically significantly lower (approximately a factor 1.1 at 300 mg/kg bw). These small effects on ALT and ALP levels were not considered toxicologically relevant. No statistically significant changes were observed for creatine and urea nitrogen in female rats.

The short-term toxicological effects of a diet based on *M. pruriens* seed oil as compared to a palm oil diet was assessed by Omeh et al. (2014). Male Wistar albino rats, 5 per group, received a diet with 10% *M. pruriens* oil or a casein based diet with 10% palm oil for 28 days (exact doses unknown). After 28 days the rats were killed and blood samples were collected. Biochemical analyses showed statistically significant increases ($p<0.05$) of AST, ALT and ALP activities in rats fed with *M. pruriens* seed oil compared to the palm oil group. Also, the serum total and conjugated bilirubin, total proteins, albumin and urea concentrations were statistically significantly increased ($p<0.05$) in the rats receiving *M. pruriens* seed oil compared to the rats receiving palm oil. The statistically significant changes were small and therefore not toxicological relevant. The authors state in the discussion that histological examination showed lesions in the liver and kidney, tubular atrophy in the kidney and mild oedema indicating liver and kidney damage, but the type of lesions was not specified. The results of the histological examination were not provided in the article.

Rajesh et al. (2016) studied the effects of *M. pruriens* seed extract in diabetic Wistar rats. Diabetes was induced using an intraperitoneal injection of streptozotocin. After 12 weeks, the diabetic rats (6 per group) were orally exposed to 0 or 200 mg/kg bw *M. pruriens* seed extract for 28 days. Body weight and fasting blood glucose levels were measured every week. At the end of the study, rats were killed and blood samples, pancreas and liver were collected. Blood glucose, total protein and LDL levels were statistically significantly reduced and serum insulin and HDL levels were statistically significantly increased in the experimental group compared to the control group. The histopathological examination showed that *M. pruriens* reduced damage to pancreas and liver seen in the control group. The focus of this article was on the positive effects of *M. pruriens* in diabetic rats.

The toxicity of a *M. pruriens* seed methanol extract on the kidney function of Sprague-Dawley rats (sex not described) was investigated by Gbotolorun et al. (2018). Rats (5 per group) received oral doses of 0, 50, 100 or 200 mg/kg bw daily for two weeks. During the dosing period the rats were monitored for signs of toxicity and mortality, and body weight was measured. After the administration period the rats were killed and the kidneys were examined for haematological, biochemical and histopathological parameters. No mortality and no signs of toxicity were observed during the dosing period. No effect on body weight was observed. Biochemical analysis showed that lipid peroxidation in the kidneys was higher in the treated groups compared to the control animals. Superoxide dismutase activities and glutathione hydroxylase were statistically significantly lower in the treated animals. Furthermore, a dose-dependent decrease that reached statistically significance at the two highest doses, was shown for creatinine in serum. The concentration of urea in serum was statistically significantly higher in animals given 50 and 100 mg/kg bw compared to control animals while it was lower in animals given 200 mg/kg bw compared to control animals. Rats given 100 or 200 mg extract/kg bw showed an increase in epithelial degeneration with mild to severe haemorrhage in the interstitial spaces (tubular necrosis) when compared to the control. It was concluded by the authors that at high doses, *M. pruriens* seed extract reduces renal clearance probably by an oxidative stress mechanism.

5.2.3 Genotoxicity

No genotoxicity studies have been identified for *M. pruriens* seed extract.
However, the assessment report about Numient states that levodopa has been shown to have weak mutagenic potential. This has been shown in both non-mammalian and mammalian *in vitro* studies. The mechanism for genotoxicity could be via oxidative intermediates formed. The presence of a metabolic system generally reduced the mutagenic potential (EMA, 2015).

5.2.4 Chronic toxicity and carcinogenicity

No chronic toxicity or carcinogenicity studies have been identified for *M. pruriens* seed extracts. The assessment report about Numient states that increasing doses of levodopa in combination with a fixed dose of carbidopa (a decarboxylase inhibitor) at ratios up to 1:10 were shown not to be carcinogenic in rats when administered orally for up to 106 weeks. The estimated systemic exposure to levodopa and carbidopa was lower than exposure in Parkinson patients. Therefore, the lack of carcinogenic effects in rats was concluded to provide little evidence for humans (EMA, 2015). It is however unknown how the exposure to levodopa resulting from the intake of herbal preparations containing *M. pruriens* seed extract (that are in the lower end of the therapeutic window and without the presence of carboxylase inhibitors) relates to the estimated systemic exposure, due to the fact that further details of this carcinogenicity study were not available.

5.2.5 Reproduction and developmental toxicity

Ashidi et al. (2019) assessed the effect of *M. pruriens* seed powder on the reproductive function in 32 adult male albino rats for eight weeks. Rats in different groups (n=8/group) received feed (12% of their

bodyweight) with 0, 0.75, 1.5 or 2.25 g of *M. pruriens* seed powder daily (total dosage unknown). At the end of the study, the rats were killed and blood samples were collected. At the lowest dose level improvements of the reproductive function were seen by monitoring follicle stimulating hormone, testosterone, luteinizing hormone, oxidative stress markers in the testis, epididymal sperm quality and cytoarchitectural structure of the testis. Necrotic tissue was observed in testicular tissue in mid-dose rats. Toxic effects as oxidative stress, severe degenerative architectural lesions in the testis as well as statistically significant reductions in epididymal sperm count, percentage motility and a statistically significant increase in abnormal sperm cells were observed at the highest dose. At the highest dose also statistically significantly reduced levels of testosterone, luteinizing hormone and follicle stimulation hormone were observed.

Adjei et al. (2023) exposed male Sprague Dawley rats (7 per group) daily to 0, 50, 1000, 2000 mg/kg bw *M. pruriens* seed powder by oral gavage for 90 days to study the effect on fertility. During the study the weight of the rats was measured weekly. At the end of the study, the rats were killed and blood samples, semen samples and organs were collected. A histological examination was performed. The relative weights of the spleen and liver were statistically significantly decreased in the dose groups compared to the control, but the decrease was not dose-dependent. Testosterone levels increased and oestrogen levels decreased in the dose groups compared to the control group, but the differences were not statistically significant. FSH levels did not differ. No statistically significant differences were observed for biochemical and haematological parameters, except for a decrease in the percentages of basophils (not further specified). However, basophils count did not statistically significantly differ. Sperm motility was statistically significantly increased and sperm immotility was statistically significantly decreased in the mid and high dose groups compared to the control group. Sperm count was statistically significantly increased in the mid dose group and was decreased in the low and high dose groups compared to the control group. No abnormalities were observed in the prostate, testes, seminal vesicle, liver, kidney and heart of the test or control animals.

The assessment report about Numient states that no detailed publications of nonclinical female reproduction studies with levodopa are available. Yet it states that chronic toxicity studies in rats or monkeys did not report effects on the gonads of either sex. In addition, in mice, mating performance and fertility were unaffected in animals when levodopa was administered via the diet in doses of 10 of 20 mg/g diet. However, the number of pregnancies and offspring born to females receiving 40 mg levodopa/g diet were reduced. For foetal development, the assessment report mentioned that adverse effects, such as an increase in the number of resorbed foetuses and a decreased litter weight, were observed in rabbits. Details of these studies, such as the exact dose per kg bw and duration of treatments, were not described (EMA, 2015). Furthermore, the scientific discussion document about Stalevo stated that in rabbits levodopa has caused skeletal and visceral malformations. It is also mentioned that levodopa can cross the placenta and as a result the foetus will be exposed to levodopa and its

metabolites (EMA, 2004).

5.2.6 *Other studies*

No studies were identified reporting other endpoints of toxicity for *M. pruriens* seed extracts. However, in preclinical outcomes of *in vitro* models of neurodegeneration levodopa has been shown to have neurotoxic effects. The neurotoxic effect is mostly thought to be due to its damaging effect on dopaminergic cells of the substantia nigra as a consequence of reactive oxygen species generated by the oxidative metabolism of levodopa. However, in animal studies with rodents or nonhuman primates no behavioural or pathological changes were observed (Olanow et al. 2015).

5.2.7 *Human data*

M. pruriens

The efficacy and safety outcomes on motor response of roasted *M. pruriens* seed powder was investigated by Cilia et al. in 2017. Tolerability of Parkinson patients receiving the treatment was assessed by looking at any adverse event occurring, changes in blood pressure and heart rate, and the severity of dyskinesias[10]. In this study, a total of 18 Parkinson patients received six different randomized treatments during six consecutive days. They received each of the following treatments: 1) levodopa (3.5 mg/kg bw) and benserazide (decarboxylase inhibitor), 2) high-dose *M. pruriens* powder containing 17.5 mg/kg bw levodopa, 3) low-dose *M. pruriens* powder containing 12.5 mg/kg bw levodopa, 4) levodopa at 17.5 mg/kg bw without benserazide, 5) *M. pruriens* powder containing 3.5 mg/kg bw levodopa with benserazide and 6) placebo. The ratio between levodopa and benserazide inhibitor was 4:1. The motor response and dyskinesias were assessed 90 and 180 minutes after treatment. The patients were monitored for adverse events such as nausea, somnolence, dizziness, psychiatric complaints and changes in blood pressure, heart rate and the severity of dyskinesias during 180 minutes after treatment. The low and high dose *M. pruriens* treatments showed respectively similar and qualitative better effects on motor response compared to the levodopa treatment with a decarboxylase inhibitor and were superior to the placebo group. The treatment with *M. pruriens* and a decarboxylase inhibitor showed a similar response as the low dose *M. pruriens* treatment at 90 minutes after treatment, but at 180 minutes the effects were statistically significantly less compared to the low dose treatment.

There were no major adverse events and no patients dropped out of the study. At 90 minutes after treatment, there were less dyskinesias with the high dose *M. pruriens* and treatment with levodopa without a decarboxylase inhibitor than for the treatment with levodopa with a decarboxylase inhibitor. The number of adverse events that did occur, was statistically significantly lower after treatment with the low dose *M. pruriens* than after levodopa treatment with or without decarboxylase inhibitor. The treatment with *M. pruriens* and a decarboxylase inhibitor showed a similar amount of adverse effects as the low dose *M. pruriens* treatment, however statistical significance was not measured. The number of adverse events was also lower in the high dose *M. pruriens*

[10] Dyskinesia refers to abnormal involuntary erratic muscle movements, which a person cannot control.

treatment compared to the levodopa treatment with decarboxylase inhibitor, but this change was not significant. Despite a similar dose of levodopa, the treatment of levodopa without decarboxylase inhibitor was associated with more adverse events than the treatment with the high dose *M. pruriens*. There was no difference among the different treatments with regard to changes in blood pressure and heart rate.

In a second part of the study described above, Cilia et al. investigated the efficacy and safety of long-term intake of *M. pruriens* powder, prepared from roasted seeds, among fourteen patients with advanced Parkinson's disease in comparison with a marketed levodopa/carbidopa preparation (LD/CD; 250/25 mg) in an open-label, noninferiority, randomized, crossover, phase 2b pilot trial (Cilia et al., 2018). Treatment consisted of two phases of 8 weeks each (maintenance/treatment phase) and two dose-adjustment periods (≤3 weeks) before each treatment phase. It is not known if a washout period between the first treatment phase and the second dose-adjustment period was included. The daily dose was adjusted per person in the dose-adjustment period and ranged from 36.6 to 87.2 mg levodopa/kg bw in the *M. pruriens* treatment and from 7.1 to 25.5 mg LD/CD kg bw in the marketed treatment. There were seven patients on *M. pruriens* powder that reported one or more adverse events and three patients that were on LD/CD. Adverse events reported by patients on *M. pruriens* included revulsion/nausea (n=4), worsening of Parkinson's disease symptoms (n=5) and excessive daytime somnolence (n=2). The first two adverse events were reasons to discontinue with the trial for seven patients. No patients receiving LD/CD discontinued prematurely. Compared to baseline levels, treatment with *M. pruriens* powder was also associated with increased blood urea nitrogen, ferritin, vitamin B12, folates levels and lowered levels of total proteins. These differences were in most cases nonsignificant and within normal range. In patients who tolerated the *M. pruriens* powder, the efficacy on motor outcomes was similar to that in patients on LD/CD. After this part of the study, the 7 patients, who did not tolerate *M. pruriens* powder and discontinued the study, tested the tolerability of *M. pruriens* powder supernatant (duration 2-48 weeks, median duration 16 weeks). Daily, the patients added *M. pruriens* powder to a glass of water. After 10 minutes, the patients only drank the supernatant, leaving the powder in the glass. The authors assumed that the same dose of levodopa was given as in the first experiment with *M. pruriens* powder. The patients did not experience the side effects they experienced in the first experiment. This may suggest that *M. pruriens* seed powder may contain other toxicologically active substances.

Maillot et al. (2022) described the case of a 58-year-old woman who was brought to the hospital with severe digestive symptoms, confusion, hallucinations and amnesia after ingestion of five raw *M. pruriens* seeds. The digestive symptoms started around 40 minutes after ingestion of the seeds, followed by the neurological symptoms. The woman arrived at the hospital two hours after ingestion of the seeds. After arrival blood pressure (160/80 mm Hg), heart rate (63 beats/minute), percutaneous oxygen saturation (99%) and blood sugar levels (7.9 mmol/L) were measured. The neurological examination did not show reduced consciousness. No blood tests or electrocardiogram were performed as

the condition of the woman improved rapidly. The symptoms disappeared five hours after ingestion. The woman had no medical history (not further specified).

Levodopa
As levodopa is available on the market as a medicine for patients with Parkinson's disease, the possible side effects have been documented (EMA, 2004, 2015, 2023a, 2023b; Medicines Information Bank, 2021b; Farmacotherapeutisch Kompas, 2024). During levodopa therapy, dyskinesia, akinesia, tremor and stiffness are common (reported in more than 1% of patients). Other often seen side effects are anorexia, headache, paraesthesia, muscle cramps and psychic effects such as hallucinations, sleepiness, confusion, dizziness, insomnia, nightmares and depression, pain in the chest, weakness, palpitations, orthostatic hypotension and dyspnoea. Also, effects on the gastrointestinal tract have been observed (Medicines Information Bank, 2021b; EMA 2023a, 2023b; Farmacotherapeutisch Kompas, 2024). In the product information, it is stated that products should be administered with caution in people with hepatic or renal impairment. It is also recommended to evaluate hepatic and renal function periodically during long term use (EMA 2015, 2023a, 2023b).

5.2.8 Interactions

Interactions in general are possible with substances that have an effect on the enzymes responsible for the transformation of levodopa into dopamine (dopa decarboxylase; DDC) or the O-methylation of levodopa by catechol-o-methyltransferase (COMT). It is important to note that the metabolism in mammalian species does not involve cytochrome P450 (CYP450) enzymes. Therefore, it is unlikely that induction or inhibition of these enzymes will affect the metabolism of levodopa. Interactions with common CYP450 inhibitors or substrates have not been investigated (see also paragraph 5.1.1).

In addition, the website Natural Medicine mentioned seven interactions with drugs or supplements, which will be discussed one by one below. Concomitant use of *M. pruriens* and antidiabetic drugs or herbs or supplements with hypoglycaemic potential possibly decreases blood glucose levels and thereby increases the risk of hypoglycaemia. This is based on the study of Akhtar et al. (1990), which showed that *M. pruriens* might have hypoglycaemic effects in rabbits. The concomitant use of anaesthetics based on cyclopropane and halogenated hydrocarbons and *M. pruriens* possibly results in cardiac arrhythmia. Therefore other anaesthetics are advised for patients taking *M. pruriens* (only anecdotal evidence). The concomitant use of *M. pruriens* and antipsychotics possibly decrease the effectiveness, if the antipsychotic drugs are based on antidopaminergic effects (only theoretical evidence). The concomitant use of *M. pruriens* and guanethidine, a antihypertensive drug, might cause additive hypotension (only theoretical evidence) and the use of methyldopa and *M. pruriens* might increase the risk of hypotension (only theoretical evidence). The concomitant use of levodopa and *M. pruriens* will likely increase the risk of adverse effects, related to levodopa (only theoretical evidence). Lastly, the concomitant use of non-selective monoamine oxidase

inhibitors and *M. pruriens* might increase the risk of hypertensive crisis (only theoretical evidence) (Natural Medicines, 2021).

Furthermore, the website mentioned three interactions with drugs or supplements. Tricyclic antidepressants, kava and vitamin B might decrease the effects of *M. pruriens* (Natural Medicines, 2021).

5.3 Derivation of toxicological reference value

The toxicological data available for *M. pruriens* seed extracts are limited. The available data do not allow the derivation of a health-based guidance value (HBGV).

Most studies focused on the acute toxicity of *M. pruriens* seed extracts, of which one study indicated adverse effects on the liver. Adverse effects on liver and kidney were also observed in three repeated-dose toxicity studies. Two studies showed adverse effects on the reproductive system. These studies however do not allow a no observed adverse effect level (NOAEL) of lowest observed adverse effect level (LOAEL) to be derived. There are no studies available for genotoxicity, developmental toxicity, chronic toxicity and carcinogenicity of *M. pruriens* seed extracts. As an effect of levodopa, an increase in the number of resorbed foetuses, a decreased litter weight and skeletal and visceral malformations in rabbits were mentioned, but no details were available and also the information on other toxicity in the assessment reports was not sufficient to derive a reference value. In humans, two studies are available, but in these studies not all relevant toxicological endpoints were studied.

6 Risk assessment

6.1 Risk assessment

As a first step in the risk assessment, it was investigated whether the presumption of safety could be applied to *Mucuna pruriens*. Botanical preparations for which an adequate body of knowledge exists, can benefit from a presumption of safety without any need for further testing (EFSA, 2009; EFSA, 2014). This generally means that when there is a history of safe use and the intended use of the botanical preparation in herbal preparations does not exceed the historical levels of intake, the intended use in herbal preparations is assumed to be safe. *M. pruriens* has a history of use as Ayurvedic medicine, however, safety is not adequately documented and the level of exposure to *M. pruriens* in Ayurvedic medicine is unknown. The presumption of safety could therefore not be applied to *M. pruriens* and more information is needed to assess its safety.

Furthermore, no HBGV could be derived for *M. pruriens* seeds or levodopa and therefore, no safe use level can be determined.

Based on the few toxicity studies publicly available, there are indications for adverse effects of *M. pruriens* seed extract on the kidney, liver and reproductive system in animal studies. In addition, adverse effects such as gastrointestinal complaints have been reported in humans during trials with *M. pruriens* seed extract. However, the available studies did not allow for the derivation of a NOAEL or LOAEL. Therefore the calculation of a margin of exposure was not possible.

In the risk assessment of *M. pruriens* seeds, the following aspects are considered.

- In animal studies mild congestion and hydropic changes in liver tissue were observed after a single dose of *M. pruriens* seed extract in rats. After repeated doses of *M. pruriens* seed extract, AST levels increased (only in female) and epithelial degeneration with haemorrhage in interstitial spaces (tubular necrosis) was observed in rats. Furthermore, lesions and other histopathological changes in liver and kidney (not further specified) were observed in rats exposed to *M. pruriens* seed oil in feed. Reproductive effects on sex hormones and spermatogenesis were observed in animal studies. One study concluded that *M. pruriens* seed extract had a phytoestrogenic effect in female rats. Although most of the effects were perceived as positive by the study authors, the effects are considered as adverse in this risk assessment. Also necrotic tissue in testicular tissue, a decrease of hormone levels, sperm count and sperm motility and an increase of abnormal sperm cells were observed.
- No studies are available on genotoxicity, developmental toxicity, chronic toxicity and carcinogenicity.
- In the assessment report for Numient it is stated that levodopa has a weak mutagenic potential, however no other information is

available on genotoxicity. No data are available on the mutagenic potency of other substances in *M. pruriens* preparations.
- Developmental effects on foetal development and the number of offspring induced by levodopa were stated in EMA reports about Numient and Stalevo, but underlying data were not publicly available.
- For adults and children, the estimated exposure to levodopa via *M. pruriens* seed extracts in herbal preparations can be around or higher (up to a factor 5 for children weighing 20-30 kg) than the therapeutic starting dose of levodopa in humans of 2.9 mg/kg bw per day, indicating that a pharmacological effect and possible side effects (e.g., dyskinesia, gastrointestinal symptoms and psychological problems) can be expected for these herbal preparations, specifically in children. It should be noted that in the medicinal products levodopa is administered in combination with inhibitors of the metabolism of levodopa (e.g. carbidopa and entacapone). *M. pruriens* does not contain carbidopa or entacapone. Information about the effects of other constituents in *M. pruriens* or other ingredients in herbal preparations is lacking.
- For medicinal products containing levodopa, it is stated that the product should be administered with caution in people with hepatic or renal impairment. It is also recommended to evaluate hepatic and renal function periodically during long term use. This is not conducted when people use herbal preparations.

Taken into account that adverse effects on liver, kidney, reproductive function and foetal development were observed, important information, such as genotoxic potential and effects after long-term exposure is lacking, and the levodopa dose in herbal preparations is similar to the therapeutic starting dose, it is concluded that herbal preparations containing *M. pruriens* seed extracts may pose a health risk.

6.2 Interactions

The website Natural Medicine gives indications of interactions of *M. pruriens* seed extracts with anaesthetics, drugs or supplements that decrease blood glucose levels, anti-psychotics, antihypertensive drugs and monoamine oxidase inhibitors or methyldopa (Natural Medicines, 2021).

Also, interactions are possible with substances that have an effect on the enzymes responsible for the transformation of levodopa into dopamine (DDC) or the O-methylation of levodopa by COMT (section 5.2.8).

6.3 Sensitive/vulnerable groups

In the SmPC of Stalevo and Corbilta, it is stated that these medicines should not be used during pregnancy unless the benefits for the mother outweigh the possible risks to the foetus. This is based on the reproductive toxicity observed in studies in animals. In addition, since levodopa is excreted in milk and the safety of levodopa in the infant is not known, it is stated that women should not breast-feed during treatment with Stalevo or Corbilta. Further it is stated that the product

should be administered with caution in people with hepatic or renal impairment. It is also recommended to evaluate hepatic and renal function periodically during long-term use. (EMA, 2023a, 2023b). When people use herbal preparations this periodic review is not conducted.

6.4 Uncertainties

Exposure
The levodopa content of the herbal preparations containing *M. pruriens* seed extracts was not available for all products and this may differ between different brands and batches. This makes the estimation of the exposure more uncertain. Also, the exposure to levodopa was estimated based on the recommended daily use. It is possible that this is not in line with the actual exposure, due to the intake of more than recommended use or the fact that the actual content can be different from what is described on the label. In order to improve the exposure assessment, more information on the intake of herbal preparations and the actual content of levodopa in herbal preparations containing *M. pruriens* seed extracts is needed.

Toxicology
There is limited toxicological data available for *M. pruriens* seed extracts. The available data focused mainly on the acute and short-term toxicity and some data was available for reproductive toxicity. For genotoxicity, carcinogenicity, developmental toxicity and chronic toxicity no studies were found with *M. pruriens* seed extracts. The publicly available data on levodopa was too limited to use for a quantitative assessment.
In addition, it is not known which effects can occur after use of herbal preparations containing a combination of different herbs and other substances.
There are indications that, apart from levodopa, other constituents in *M. pruriens* preparations may also cause adverse effects (Cilia et al., 2018).

7 Conclusions and recommendations

In the risk assessment of *M. pruriens* seeds, the following aspects are considered:

- There are indications for adverse effects of *M. pruriens* seed extracts on liver, kidney, reproductive function and foetal development.
- Important information, e.g., studies on the genotoxicity, carcinogenicity and chronic toxicity, is lacking.
- The recommended use of herbal preparations containing *M. pruriens* seed extracts leads to an estimated exposure to levodopa that is similar or higher than the therapeutic starting dose prescribed to Parkinson patients. Pharmacological effects can be expected at the recommended use and the side effects (e.g., dyskinesia, gastrointestinal issues and psychological problems) reported for levodopa could occur.

Therefore, it is concluded that herbal preparations containing *M. pruriens* seed extracts may pose a health risk.

As a precaution, RIVM advises consumers not to use herbal preparations containing *M. pruriens* seed extract during pregnancy, when breastfeeding or when having liver or kidney problems.
Consumers are advised to be alert to side effects and to stop using the product in case side effects occur. If people choose to use herbal preparations containing *Mucuna pruriens,* they have to use it in accordance with the instructions on the packaging and discuss the use with their doctor or pharmacist in case of medicine use.

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Annex 1 Search strategies

Mucuna pruriens

Embase

#13	#9 OR #10 OR #11 OR #12	75
#12	(#1 OR #2 OR #3) AND #8	5
#11	#2 AND #3 AND (#4 OR #5 OR #6)	13
#10	#3 AND #7	25
#9	(#1 OR #2 OR #3) AND (#4 OR #5 OR #6) AND #7	61
#8	'physical disease'/exp/mj/dm_co,dm_si OR 'mental disease'/exp/mj/dm_co,dm_si	1,380,126
#7	toxic*:ti OR intoxic*:ti OR toxin*:ti OR poison*:ti OR genotox*:ti OR neurotox*:ti OR hepatotox*:ti OR cytotox*:ti OR immunotox*:ti OR mutagen*:ti OR carcinogen*:ti OR phototox*:ti OR embryotox*:ti OR risk*:ti OR safe*:ti OR photocytotox*:ti	1,527,844
#6	'risk'/exp	2,660,415
#5	'toxicokinetics'/exp/mj OR 'pharmacokinetics'/exp/mj OR 'metabolism'/exp/mj	1,642,054
#4	'toxic substance'/exp OR 'toxicity and intoxication'/exp OR 'exposure'/exp	2,576,509
#3	'velvet bean'/exp/dd_ae,dd_to OR 'mucuna pruriens extract'/exp/dd_ae,dd_to OR 'n,n dimethyltryptamine'/exp/dd_ae,dd_to OR 'bufotenine'/exp/dd_ae,dd_to OR '5 methoxy n,n dimethyltryptamine'/exp/dd_ae,dd_to	102
#2	'mucuna pruriens*':ti OR 'cowage*':ti OR 'velvet bean*':ti OR 'n,n-dimethyltryptamin*':ti OR 'bufotenin*':ti OR '5-methoxy-n,n-dimethyltryptamin*':ti	713
#1	'velvet bean'/exp/mj OR 'velvet bean extract'/exp/mj OR 'mucuna pruriens extract'/exp/mj OR 'n,n dimethyltryptamine'/exp/mj OR 'bufotenine'/exp/mj OR '5 methoxy n,n dimethyltryptamine'/exp/mj	1,566

Scopus
(TITLE(mucuna-pruriens* OR cowage* OR velvet-bean* OR n-n-dimethyltryptamin* OR bufotenin* OR 5-methoxy-n-n-dimethyltryptamin*)) AND (TITLE(*toxic* OR *toxin* OR poison* OR mutagen* OR carcinogen* OR risk* OR safe* OR acute))
n=35

Pubmed
((("mucuna-pruriens*"[Title] OR "cowage*"[Title] OR "velvet-bean*"[Title] OR "n-n-dimethyltryptamin*"[Title] OR "bufotenin*"[Title] OR "5-methoxy-n-n-dimethyltryptamin*"[Title])) AND ((toxic*[Title]) OR intoxic*[Title] OR toxin*[Title] OR poison*[Title] OR genotox*[Title] OR neurotox*[Title] OR hepatotox*[Title] OR cytotox*[Title] OR immunotox*[Title] OR mutagen*[Title] OR carcinogen*[Title] OR phototox*[Title] OR embryotox*[Title] OR risk*[Title] OR safe*[Title] OR photocytotox*[Title] OR acute[Title]))
n=18

Total articles: n=53 ; unique articles: n=42

Toxcenter
L1 (mucuna-pruriens? OR cowage? OR velvet-bean? OR n-n-dimethyltryptamin? OR bufotenine? OR 5-methoxy-n-n-dimethyltryptamin?)/TI

=> **s (?toxic? OR ?toxin? OR poison? OR mutagen? OR carcinogen? OR risk? OR safe? OR acute)**
5046965 ?TOXIC?
1270841 ?TOXIN?
369200 POISON?
455848 MUTAGEN?
503315 CARCINOGEN?
1307582 RISK?
837808 SAFE?
784810 ACUTE
L2 7775176 (?TOXIC? OR ?TOXIN? OR POISON? OR MUTAGEN? OR CARCINOGEN? OR RISK? OR SAFE? OR ACUTE)

=> **s (person# OR human? OR volunteer# OR man OR men OR woman OR women OR boy# OR girl# OR child? OR infant# OR worker# OR employee# OR case OR cases)**
108400 PERSON#
5112913 HUMAN?
95932 VOLUNTEER#
112750 MAN
153759 MEN
57380 WOMAN
296684 WOMEN
24594 BOY#
23665 GIRL#
460565 CHILD?
195982 INFANT#
173320 WORKER#
21139 EMPLOYEE#

680755 CASE
630095 CASES
L3 6038731 (PERSON# OR HUMAN? OR VOLUNTEER# OR MAN OR MEN OR WOMAN OR WOMEN OR BOY# OR GIRL# OR CHILD? OR INFANT# OR WORKER# OR EMPLOYEE# OR CASE OR CASES)

=> **s (rat OR rats OR mouse OR mice OR dog# OR hamster# OR pig# OR monkey# OR rabbit# or mammal#)**
911718 RAT
1139273 RATS
693568 MOUSE
1114554 MICE
139192 DOG#
83038 HAMSTER#
176942 PIG#
51583 MONKEY#
253821 RABBIT#
1793655 MAMMAL#
L4 4283213 (RAT OR RATS OR MOUSE OR MICE OR DOG# OR HAMSTER# OR PIG# OR MONKEY# OR RABBIT# OR MAMMAL#)
75% OF LIMIT FOR TOTAL ANSWERS REACHED

=> **s L1 AND L2 AND (L3 OR L4)**

Unique articles: n=180

Levodopa

Embase

#16	#14 AND #15	1,392
#15	'l-dopa*':ti,ab OR 'levodopa*':ti,ab	34,821
#14	#13 AND ('Article'/it OR 'Article in Press'/it)	1,809
#13	#9 OR #10 OR #11 OR #12	3,398
#12	(#1 OR #2 OR #3) AND #8	2,665
#11	#2 AND #3 AND (#4 OR #5 OR #6)	478
#10	#3 AND #7	294
#9	(#1 OR #2 OR #3) AND (#4 OR #5 OR #6) AND #7	493
#8	'physical disease'/exp/mj/dm_co,dm_si OR 'mental disease'/exp/mj/dm_co,dm_si	1,388,468
#7	toxic*:ti OR intoxic*:ti OR toxin*:ti OR poison*:ti OR genotox*:ti OR neurotox*:ti OR hepatotox*:ti OR cytotox*:ti OR immunotox*:ti OR mutagen*:ti OR carcinogen*:ti OR phototox*:ti OR embryotox*:ti OR risk*:ti OR safe*:ti OR photocytotox*:ti	1,547,855
#6	'risk'/exp	2,696,611
#5	'toxicokinetics'/exp/mj OR 'pharmacokinetics'/exp/mj OR 'metabolism'/exp/mj	1,657,493
#4	'toxic substance'/exp OR 'toxicity and intoxication'/exp OR 'exposure'/exp	2,600,048
#3	'levodopa'/exp/dd_ae,dd_to	6,331
#2	'l-dopa*':ti OR 'levodopa*':ti	12,925
#1	'levodopa'/exp/mj	20,18

Scopus

(TITLE ("l-dopa*" OR "levodopa*")) AND (TITLE (*toxic* OR *toxin* OR poison* OR mutagen* OR carcinogen* OR risk* OR safe* OR acute))

N=498

Pubmed

(("l dopa*"[TITLE] OR "levodopa*"[TITLE]) AND (toxic*[Title] OR intoxic*[Title] OR toxin*[Title] OR poison*[Title] OR genotox*[Title] OR neurotox*[Title] OR hepatotox*[Title] OR cytotox*[Title] OR immunotox*[Title] OR mutagen*[Title] OR carcinogen*[Title] OR phototox*[Title] OR embryotox*[Title] OR risk*[Title] OR safe*[Title] OR photocytotox*[Title] OR acute[Title]))

N=417

Total articles 1392 + 498 + 417 = 2307 ; unique articles: n=1822

Toxcenter
L1 8649 (L-DOPA? OR LEVODOPA?)/TI

L2 7789865 (?TOXIC? OR ?TOXIN? OR POISON? OR MUTAGEN? OR CARCINOGEN? OR RISK? OR SAFE? OR ACUTE)

L3 6050096 (PERSON# OR HUMAN? OR VOLUNTEER# OR MAN OR MEN OR WOMAN OR WOMEN OR BOY# OR GIRL# OR CHILD? OR INFANT# OR WORKER# OR EMPLOYEE# OR CASE OR CASES)

L4 4288737 (RAT OR RATS OR MOUSE OR MICE OR DOG# OR HAMSTER# OR PIG# OR MONKEY# OR RABBIT# OR MAMMAL#)

L5 3587 L1 AND L2 AND (L3 OR L4)

L6 2880 DUP REM L5 (707 DUPLICATES REMOVED)

L7 2880 S L6

Literature update July 7th 2023

	Mucuna pruriens	**Levodopa**
Databases	Embase; PubMed; Scopus	
Period	September 2021-July 7th 2023	
Search strategy	Copied from original search strategy	
Total number of articles	28	109
Number of unique articles	17	74
Selected for risk assessment	2	0

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Committed to
health and sustainability