Lawsonia inermis, extract: Human health tier II assessment

08 March 2019

CAS Number: 84988-66-9

Preface

This assessment was carried out by staff of the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) using the Inventory Multi-tiered Assessment and Prioritisation (IMAP) framework.

The IMAP framework addresses the human health and environmental impacts of previously unassessed industrial chemicals listed on the Australian Inventory of Chemical Substances (the Inventory).

The framework was developed with significant input from stakeholders and provides a more rapid, flexible and transparent approach for the assessment of chemicals listed on the Inventory.

Stage One of the implementation of this framework, which lasted four years from 1 July 2012, examined 3000 chemicals meeting characteristics identified by stakeholders as needing priority assessment. This included chemicals for which NICNAS already held exposure information, chemicals identified as a concern or for which regulatory action had been taken overseas, and chemicals detected in international studies analysing chemicals present in babies' umbilical cord blood.

Stage Two of IMAP began in July 2016. We are continuing to assess chemicals on the Inventory, including chemicals identified as a concern for which action has been taken overseas and chemicals that can be rapidly identified and assessed by using Stage One information. We are also continuing to publish information for chemicals on the Inventory that pose a low risk to human health or the environment or both. This work provides efficiencies and enables us to identify higher risk chemicals requiring assessment.

The IMAP framework is a science and risk-based model designed to align the assessment effort with the human health and environmental impacts of chemicals. It has three tiers of assessment, with the assessment effort increasing with each tier. The Tier I assessment is a high throughput approach using tabulated electronic data. The Tier II assessment is an evaluation of risk on a substance-by-substance or chemical category-by-category basis. Tier III assessments are conducted to address specific concerns that could not be resolved during the Tier II assessment.

These assessments are carried out by staff employed by the Australian Government Department of Health and the Australian Government Department of the Environment and Energy. The human health and environment risk assessments are conducted and published separately, using information available at the time, and may be undertaken at different tiers.

This chemical or group of chemicals are being assessed at Tier II because the Tier I assessment indicated that it needed further investigation.

For more detail on this program please visit:www.nicnas.gov.au

Disclaimer

Acronyms & Abbreviations

Chemical Identity

Synonyms	henna, extract lawsonia inermis, ext
Structural Formula	**No Structural Diagram Available**
Molecular Formula	Unspecified
Molecular Weight (g/mol)	Unspecified
Appearance and Odour (where available)	greenish-grey powder

Import, Manufacture and Use

Australian

No specific Australian use, import, or manufacturing information has been identified.

International

The following international uses have been identified through Galleria Chemica; the Substances and Preparations in Nordic countries (SPIN) database; the European Commission Cosmetic Ingredients and Substances (CosIng) database; the United States (US) Personal Care Products Council International Nomenclature of Cosmetic Ingredients (INCI) Dictionary; the US Environmental Protection Agency's Aggregated Computer Toxicology Resource (ACToR); the US National Library of Medicine's Hazardous Substances Data Bank (HSDB); and the Scientific Committee Opinions on Cosmetic products (SCCNFP, 2002; SCCP, 2005; SCCS, 2013).

The chemical has reported cosmetic uses in hair dyes, masks, skin conditioners and as an antimicrobial and antioxidant agent. Aqueous pastes of the chemical are used for skin decoration.

Restrictions

Australian

No known restrictions have been identified.

International

No known international restrictions have been identified.

Existing Work Health and Safety Controls

Hazard Classification

The chemical is not listed on the Hazardous Chemical Information System (HCIS) (Safe Work Australia).

Exposure Standards

Australian

No specific exposure standards are available.

International

No specific international exposure standards are available.

Health Hazard Information

Lawsonia inermis, extract (CAS No. 84988-66-9) commonly known as 'henna' has been traditionally used for centuries by women in India, Egypt and other parts of Africa to dye hair, and stain skin and nails. Henna comes from the dried and powdered leaves of a plant, *Lawsonia inermis*, which has widespread use as medicinal plant. The main active ingredient of the *Lawsonia inermis* plant responsible for its dyeing properties is the chemical, 2-hydroxy-1,4-naphthoquinone (lawsone; CAS No. 83-72-7—not listed on the Inventory). The concentration of lawsone in henna varies from 0.5 to 1.5 % (Danish EPA, 2005; NSC, 2005; Hekmatpou, 2018).

Toxicokinetics

In an in vitro percutaneous absorption study, isolated pig skin pieces were exposed to 25 % chemical powder (containing 1 % lawsone) for 30 minutes. Following a 72-hour incubation period, about 0.28 % of the applied dose was found in the receptor fluid and 0.06 % remained on the skin. Absolute skin penetration rate of 703 µg lawsone/cm^2 was reported (SCCNFP, 2002; SCCP, 2005; SCCS, 2013).

In an in vivo percutaneous absorption study, aqueous solution containing 23.5 % of the chemical mixed with 75 % deionised water and spiked with 1.5 % of [^{14}C]-lawsone was applied onto the skin of Sprague-Dawley (SD) rats (n=4/sex/group) for 40 mins. Mean percutaneous absorption of 0.20 % of administered radioactivity was achieved, indicating absolute absorption of 1.70 µg/cm^2 after 72 hours of exposure (SCCNFP, 2002; SCCP, 2005; SCCS, 2013).

Acute Toxicity

Oral

The chemical has low acute toxicity based on results from one animal test conducted according to OECD Test Guideline (TG) 401 following oral exposure. The median lethal dose (LD50) in SD rats is >2000 mg/kg bw (SCCNFP, 2002; SCCP, 2005; SCCS, 2013).

Dermal

The chemical has low acute toxicity based on results from one animal test conducted according to OECD TG 402. The median lethal dose (LD50) in Wistar rats (n = 5/sex) is >2000 mg/kg bw (SCCNFP, 2002; SCCP, 2005; SCCS, 2013).

Inhalation

No data are available.

Corrosion / Irritation

Skin Irritation

No irritation studies are available. Results from the acute dermal toxicity study, indicate that the chemical is not irritating to skin.

No skin irritation was reported after a single occlusive application of 2 g/kg bw chemical for 24 hours in an acute dermal toxicity study in Wistar rats (n=5/sex) performed in accordance with OECD TG 402 (SCCNFP, 2002; SCCP, 2005; SCCS, 2013).

Eye Irritation

The chemical was reported to slightly irritate the eyes. The effects were not sufficient to warrant hazard classification.

In an eye irritation study conducted according to OECD TG 405, 0.1 mL of the chemical (approximately 58 mg) was instilled into the right eye of three New Zealand White (NZW) rabbits which remained unwashed. The chemical caused transient inflammation of the iris and moderate conjunctival irritation. Mean total scores of 17.0, 15.7, 10.0 and 2.7 were recorded after 1, 24, 48 and 72 hours, respectively. The effects were reversible within 7 days after application. No corneal effects were observed. (SCCNFP, 2002; SCCP, 2005; SCCS, 2013).

Sensitisation

Skin Sensitisation

Based on the available data, the chemical is not a skin sensitiser.

In a Buehler study (non-adjuvant test; OECD TG 406), 0.5 mL of the chemical (50 % w/w in petrolatum) was applied to the left flank of female albino Dunkin Hartley guinea pigs (n=20) under occlusive dressing for 6 hours. The topical application was repeated using the same concentration of the chemical (50 % w/w) during induction and at challenge. No signs of adverse reactions were observed. The chemical is not considered to be a skin sensitiser (SCCNFP, 2002; SCCP, 2005; SCCS, 2013).

Observation in humans

It was reported that the chemical was not sensitising in human studies.

In a human repeat insult patch test (HRIPT), 10 volunteers were exposed to 10 % of the chemical in petrolatum for three weeks in the induction phase, followed by a week of no treatment, then a challenge phase. No skin reaction was observed during induction and challenge (SCCNFP, 2002; SCCP, 2005; SCCS, 2013).

Some rare cases of contact allergy to the chemical have been reported (SCCNFP, 2002; SCCP, 2005; SCCS, 2013).

Repeated Dose Toxicity

Oral

Based on the available data, the chemical is not expected to be harmful to health following repeated oral exposure.

In a 90-day study (OECD TG 408), the chemical was administered (as a suspension in 0.5 % aqueous methylcellulose) to groups of SD rats (n=10/sex/group) by gavage at 0, 40, 200 or 1000 mg/kg bw/day for 13 weeks. No mortality was reported. Loud breathing (2/40 animals), and brown urine was reported in all treated animals. No changes in mean food consumption and mean body weight gain in treated males was reported. Mean body weight gain in females at 40 and 1000 mg/kg bw/day was significantly lower as compared to controls. Haematological effects observed at 1000 mg/kg bw/day included: slight but statistically significant reductions in the erythrocyte count (-7 % for males and -8 % for females) and haemoglobin (-6 % for males and -5 % for females). At this dose, statistically significant increases in mean relative kidney weights (+18 % for males and +13 % for females), relative spleen weights (+29 % for males and +33 % for females), relative liver weights (+10 % for males) and relative testes weights (+15 % for males) were seen. Orange stained hair and extremities (5/10 females), forestomach (all animals), mucosa of the urinary bladder (1/10 males and 1/10 females) were also seen at the highest dose. Microscopic findings observed included minimal to moderate accumulation of acidophilic globules in cortical tubular epithelium of the kidneys at 1000 mg/kg bw/day; haemosiderosis in the spleen at 200 and 1000 mg/kg bw/day; and extramedullary haemopoiesis at 1000 mg/kg bw/day. All treatment-related effects, except haemosiderosis of the spleen and orange staining observed at 1000 mg/kg bw/day, were reversible in the recovery period. A no observed adverse effect level (NOAEL) of 40 mg/kg bw/day was established (SCCNFP, 2002; SCCP, 2005; SCCS, 2013).

Dermal

No data are available.

Inhalation

No data are available.

Genotoxicity

Based on weight of evidence from available in vitro and in vivo genotoxicity studies, the chemical is not considered to be genotoxic.

In vitro studies

In vitro genotoxicity assays conducted using the chemical showed either positive or negative results. These results included the following:

- In a bacterial reverse mutation assay conducted according to OECD TG 471, the chemical in DMSO gave negative responses in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 at concentrations of 50, 100, 500, 1000, 2500 or 5000 µg/plate with and without metabolic activation (SCCNFP, 2002; SCCP, 2005; SCCS, 2013).
- In a bacterial reverse mutation assay (non-guideline study), the chemical in DMSO and water gave negative results in *S. typhimurium* strains TA98, TA100, TA1535, TA1537, TA 1538 and streptomycin-resistant strains TA98strp, TA100strp, TA1535strp, TA1537strp and TA1538strp at concentrations up to 5000 µg/plate, with and without metabolic activation (SCCNFP, 2001; SCCP, 2005; SCCS, 2013).
- In an in vitro gene mutation test (OECD TG 476), the chemical gave negative mutagenic results in hprt locus of Chinese hamster lung (V79) cells, in the presence and absence of metabolic activation, at concentrations up to 3000 µg/mL (SCCNFP, 2002; SCCP, 2005; SCCS, 2013).
- In a thymidine kinase (tk) gene mutation test (OECD TG 476), concentration dependent and statistically significant increases in the mutant frequency were observed when the chemical in Fischer's medium was tested in L5178Y mouse lymphoma cells, in the presence and absence of metabolic activation, at concentrations up to 1250 µg/mL (SCCNFP, 2002; SCCP, 2005; SCCS, 2013).
- In another tk gene mutation test (OECD TG 476), negative results were observed when the chemical in deionised water was tested in L5178Y mouse lymphoma cells, in the presence and absence of metabolic activation, at concentrations up to 1200 µg/mL (SCCP, 2005; SCCS, 2013).
- In a mammalian chromosome aberration assay (OECD TG 473), the chemical induced an increase in cells with structural chromosomal aberrations in human lymphocytes at concentrations up to 1250 µg/mL, with and without metabolic activation (SCCNFP, 2001; SCCP, 2005; SCCS, 2013).
- In an in vitro micronucleus assay (OECD TG 487) using cultured human peripheral blood lymphocyte, the chemical (10 % in purified water) at concentrations up to 70 µg/mL induced an increase in the number of lymphocytes with micronuclei, with metabolic activation (SCCS, 2013).
- Negative results were seen in a sister chromatid exchange (SCE) assay in V79 Chinese hamster cells at doses up to 800 µg/mL, in the presence and absence of metabolic activation (SCCNFP, 2001; SCCP, 2005; SCCS, 2013).

In vivo studies

Negative in vivo genotoxicity assay results were reported using the chemical. These included:

- In an in vivo micronucleus assay (OECD TG 474), the chemical was administered in CD-1 mice (n=5/sex) at 300 mg/kg bw/day, via an intraperitoneal injection. No increase in the number of micronucleated polychromatic erythrocytes was observed. A significant change in the normochromatic to polychromatic erythrocytes ratio was observed, indicating that the chemical reached the bone marrow cells (SCCNFP, 2001; SCCP, 2005; SCCS, 2013).
- In an unscheduled DNA synthesis (UDS) study according to OECD TG 486, the chemical did not induce UDS in the hepatocytes of treated Wistar rats (n=3 males/group) at doses of 1000 or 2000 mg/kg bw/day in corn oil (SCCP, 2005; SCCS, 2013).

Carcinogenicity

No data are available.

Reproductive and Developmental Toxicity

In a single teratogenicity study, results observed in foetuses appeared to be evidence of developmental delay consistent with non-specific maternal toxicity at the highest dose.

In a teratogenicity study (OECD TG 414), groups of 25 pregnant female SD rats received the chemical in 0.5 % aqueous methyl cellulose solution at 0, 40, 200 or 1000 mg/kg bw/day by gavage during gestation days (GD) 6–15. No deaths were observed. Slight, but statistically significant decrease in the body weight gain and food consumption were observed in the dams at 1000 mg/kg bw/day. No treatment-related effects on pre and post-implantation loss, foetal body weight and sex-ratios were reported at any dose. At 1000 mg/kg bw/day, dilation of cerebral ventricles in two foetuses; cleft palate in one foetus; significant reduction in ossification of caudal vertebra; unossification of the 5^{th} sternebrae and caudal vertebra; reduction in ossification of the 1^{st} to 4^{th} metatarsals and of the pubic bone were observed. Foetal findings at 200 mg/kg bw/day included reduced ossification of the pubic bone and cleft palate in one foetus. A NOAEL for maternal animals was 200 mg/kg bw/day and 40 mg/kg bw/day for the foetuses (SCCNFP, 2002; SCCP, 2005; SCCS, 2013).

Risk Characterisation

Critical Health Effects

The chemical is not considered to have high toxicity. There is a potential for contact allergy when exposed repeatedly to high concentrations of the chemical.

Public Risk Characterisation

Information on Australian use of the chemical has not been made available. International information is considered representative for Australian use and indicates that it has cosmetic uses including use as hair dye and in preparations for skin decoration (see **Import, manufacture and use** section).

Considering the range of cosmetic products that may contain the chemical, the main route of public exposure is expected to be through the direct application of the products onto the skin and hair from hair dye and skin products.

The chemical is expected to be used as a cosmetic product at low concentrations due to its dyeing properties. Given the low hazards identified for the chemical and its low concentrations in cosmetic products, doses associated with systemic toxicity are not expected. The chemical is not considered to pose an unreasonable risk and further risk management is not considered necessary for public safety.

Occupational Risk Characterisation

During product formulation, dermal exposure might occur, particularly where manual or open processes are used. These could include transfer and blending activities, quality control analysis, and cleaning and maintaining equipment. Worker exposure to the chemical at lower concentrations could also occur while using formulated products containing the chemical. The level and route of exposure will vary depending on the method of application and work practices employed.

Whilst the chemical is not recommended for classification as a hazardous chemical, the chemical could pose an unreasonable risk to workers unless adequate control measures to minimise repeated exposure to high concentrations are implemented.

NICNAS Recommendation

The risk to workers and public from this chemical is not considered to be unreasonable. The chemical is not recommended for classification and labelling under the adopted GHS. This report does not consider classification of physical hazards and

environmental hazards. No recommendations or further assessment is required.

Regulatory Control

Advice for industry

Control measures

Control measures to minimise the risk from dermal exposure to the chemical should be implemented in accordance with the hierarchy of controls. Approaches to minimise risk include substitution, isolation and engineering controls. Measures required to eliminate, or minimise risk arising from storing, handling and using a hazardous chemical depend on the physical form and the manner in which the chemical is used. Examples of control measures that could minimise the risk include, but are not limited to:

- minimising manual processes and work tasks through automating processes;
- work procedures that minimise splashes and spills;
- regularly cleaning equipment and work areas; and
- using protective equipment that is designed, constructed, and operated to ensure that the worker does not come into contact with the chemical.

Guidance on managing risks from hazardous chemicals are provided in the *Managing risks of hazardous chemicals in the workplace—Code of practice* available on the Safe Work Australia website.

Personal protective equipment should not solely be relied upon to control risk and should only be used when all other reasonably practicable control measures do not eliminate or sufficiently minimise risk. Guidance in selecting personal protective equipment can be obtained from Australian, Australian/New Zealand or other approved standards.

Obligations under workplace health and safety legislation

Information in this report should be taken into account to help meet obligations under workplace health and safety legislation as adopted by the relevant state or territory. This includes, but is not limited to:

- ensuring that hazardous chemicals are correctly classified and labelled;
- ensuring that (material) safety data sheets ((M)SDS) containing accurate information about the hazards (relating to both health hazards and physicochemical (physical) hazards) of the chemical are prepared; and
- managing risks arising from storing, handling and using a hazardous chemical.

Your work health and safety regulator should be contacted for information on the work health and safety laws in your jurisdiction.

Information on how to prepare an (M)SDS and how to label containers of hazardous chemicals are provided in relevant codes of practice such as the *Preparation of safety data sheets for hazardous chemicals—Code of practice* and *Labelling of workplace hazardous chemicals—Code of practice*, respectively. These codes of practice are available from the Safe Work Australia website.

A review of the physical hazards of the chemical has not been undertaken as part of this assessment.

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