

Draft Screening Assessment

***Lotus corniculatus*, extract**

Chemical Abstracts Service Registry Number

84696-24-2

**Environment and Climate Change Canada
Health Canada**

December 2019

Synopsis

Pursuant to section 74 of the *Canadian Environmental Protection Act, 1999* (CEPA), the Minister of the Environment and the Minister of Health have conducted a screening assessment of *Lotus corniculatus*, extract. The Chemical Abstracts Service Registry Number (CAS RN¹) for *Lotus corniculatus* extract is 84696-24-2. This substance was identified as a priority for assessment as it met categorization criteria under subsection 73(1) of CEPA.

Lotus corniculatus is a plant that is also known by the common name of bird's-foot trefoil. According to information reported in surveys under section 71 of CEPA, *Lotus corniculatus* extract was not manufactured or imported into Canada above the reporting threshold of 100 kg although *Lotus corniculatus* seed and flower extracts were notified as being present in cosmetic products in Canada.

The ecological risk of *Lotus corniculatus* extract was characterized using the ecological risk classification of organic substances (ERC), which is a risk-based approach that employs multiple metrics for both hazard and exposure, with weighted consideration of multiple lines of evidence for determining risk classification. Hazard profiles are based principally on metrics regarding mode of toxic action, chemical reactivity, food web-derived internal toxicity thresholds, bioavailability, and chemical and biological activity. Metrics considered in the exposure profiles include potential emission rate, overall persistence and long-range transport potential. A risk matrix is used to assign a low, moderate or high level of potential concern for substances on the basis of their hazard and exposure profiles. Based on the outcome of the ERC analysis, *Lotus corniculatus* extract is considered unlikely to be causing ecological harm.

Considering all available lines of evidence presented in this draft screening assessment, there is low risk of harm to the environment from *Lotus corniculatus* extract. It is proposed to conclude that *Lotus corniculatus* extract does not meet the criteria under paragraphs 64(a) or (b) of CEPA as it is not entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity or that constitute or may constitute a danger to the environment on which life depends.

The general population of Canada may be exposed to *Lotus corniculatus* extract through the use of cosmetics, including body lotion and lip balm. Some phenotypes of

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Lotus corniculatus are known to produce cyanogenic glycosides, and the possibility exists that products containing *Lotus corniculatus* extract could expose consumers to hydrogen cyanide. Although all plant extracts are multiconstituent mixtures of various phytochemicals, based on the known chemistry of *Lotus corniculatus*, hydrogen cyanide is considered the most toxicologically relevant substance.

Hydrogen cyanide is a systemic toxicant that interferes with the ability of cells to use oxygen by disrupting the electron transport chain, thereby inhibiting cellular respiration. In rodent studies, hydrogen cyanide has effects on the male reproductive system, whereas low levels of hydrogen cyanide exposure are associated with neuropathies and thyroid disturbances in humans. Margins between estimates of cyanide exposure from *Lotus corniculatus* extract used in cosmetics and critical effect levels are considered adequate to address uncertainties in the health effects and exposure databases for all endpoints.

On the basis of the information presented in this draft screening assessment, it is proposed to conclude that *Lotus corniculatus* extract does not meet the criteria under paragraph 64(c) of CEPA as it is not entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

It is therefore proposed to conclude that *Lotus corniculatus* extract does not meet any of the criteria set out in section 64 of CEPA.

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1. Introduction

Pursuant to section 74 of the *Canadian Environmental Protection Act, 1999* (CEPA) (Canada 1999), the Minister of the Environment and the Minister of Health have conducted a screening assessment of *Lotus corniculatus*, extract. This substance was identified as a priority for assessment as it met categorization criteria under subsection 73(1) of CEPA (ECCC and HC [modified 2007]).

The ecological risk of *Lotus corniculatus* extract was characterized using the ecological risk classification of organic substances (ERC) approach (ECCC 2016a). The ERC describes the hazard of a substance using key metrics including mode of action, chemical reactivity, food-web derived internal toxicity, bioavailability, and chemical and biological activity and considers the possible exposure of organisms in the aquatic and terrestrial environments on the basis of such factors as potential emission rates, overall persistence and long-range transport potential in air. The various lines of evidence are combined to identify substances as warranting further evaluation of their potential to cause harm to the environment or as having a low likelihood of causing harm to the environment.

This draft screening assessment includes consideration of information on chemical properties, environmental fate, hazards, uses and exposures, including additional information submitted by stakeholders. Relevant data were identified up to October 2018. Targeted literature searches were also conducted up to October 2018. Empirical data from key studies as well as results from models were used to reach proposed conclusions. When available and relevant, information presented in assessments from other jurisdictions was considered.

This draft screening assessment was prepared by staff in the Product Safety Program at Health Canada and the CEPA Risk Assessment Program at Environment and Climate Change Canada and incorporates input from other programs within these departments. The ecological portion of this assessment is based on the ERC document (published July 30, 2016), which was subject to an external review as well as a 60-day public comment period. The human health portion of this assessment has undergone external review and/or consultation. Comments on the technical portions relevant to human health were received from Theresa Lopez, M.S.; Jennifer Flippin, M.S.; and Joan Garey, Ph.D. While external comments were taken into consideration, the final content and outcome of this draft screening assessment remain the responsibility of Health Canada and Environment and Climate Change Canada.

This draft screening assessment focuses on information critical to determining whether the substance meets the criteria set out in section 64 of CEPA by examining scientific

information and incorporating a weight of evidence approach and precaution.² This draft screening assessment presents the critical information and considerations on which the proposed conclusions are based.

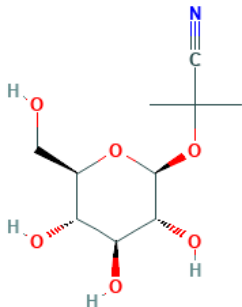
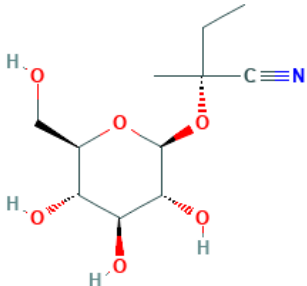
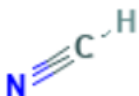
2. Identity of *Lotus corniculatus* extract

Lotus corniculatus is a near globally distributed flowering plant of the pea family that is known by the common name of bird's-foot trefoil. As a botanical derivative, *Lotus corniculatus* extract is a complex mixtures of various phytochemicals, or what is referred to by the acronym for Unknown or Variable composition Complex reaction products and Biological material (UVCB). These materials are derived from natural sources or complex reactions and cannot be characterized in terms of constituent chemical compounds because their composition is too complex or variable. A UVCB is not an intentional mixture of discrete substances and is considered a single substance.

To inform the ecological and human health assessments of *Lotus corniculatus* extract as a whole, the components of the extract with potential for toxicological relevance were identified on the basis of available empirical data. Specifically, *Lotus corniculatus* is notable for being polymorphic in the ability to produce cyanogenic glycosides, which are plant secondary compounds involved in metabolism and predator defense. Cyanogenic phenotypes produce the glycoside linamarin and its methylated form lotaustralin, both of which may liberate hydrogen cyanide (HCN) upon enzymatic hydrolysis. The highest glycoside concentrations are observed in the leaves of immature plants, while the seeds as well as leaves of mature plants generally contain low concentrations (Grant and Sidhu 1967). Flowers have been reported to contain glycoside levels similar to developing leaves (Gebrehiwot and Beuselinck 2001). The chemical structures of these compounds as well as their hydrolysis product appear in Table 2-1.

Table 2-1. Substance identity for *Lotus corniculatus* extract

²A determination of whether one or more of the criteria of section 64 of CEPA are met is based upon an assessment of potential risks to the environment and/or to human health associated with exposures in the general environment. For humans, this includes, but is not limited to, exposures from ambient and indoor air, drinking water, foodstuffs, and products available to consumers. A conclusion under CEPA is not relevant to, nor does it preclude, an assessment against the hazard criteria specified in the *Hazardous Products Regulations*, which are part of the regulatory framework for the Workplace Hazardous Materials Information System for products intended for workplace use. Similarly, a conclusion based on the criteria contained in section 64 of CEPA does not preclude actions being taken under other sections of CEPA or other acts.

CAS RN	Name	Representative chemical formula	Representative chemical structure	Molecular weight (g/mol)
554-35-8	Linamarin	$C_{10}H_{17}NO_6$		247.248
534-67-8	Lotaustralin	$C_{11}H_{19}NO_6$		261.27
74-90-8	Hydrogen cyanide; Prussic acid	HCN		27.025

If cyanogenic phenotypes of *Lotus corniculatus* were used to derive the botanical extracts, the possibility exists that these products might contain linamarin and lotaustralin. The potential toxicity of these substances depends principally on their capacity to generate HCN, which can also interconvert with the cyanide anion (CN⁻) depending on pH and temperature (EFSA 2004). Acyanogenic plants may lack the glycosides, the enzymes to hydrolyze them, or both (Compton and Jones 1985) and are therefore also potentially a source of cyanide.

3. Physical and chemical properties

A summary of physical and chemical property data of the key components in *Lotus corniculatus* extract are presented in Table 3-1. When experimental information was limited or not available for a property, (Q)SAR models were used to generate predicted values for the substance. Additional physical and chemical properties are reported in ECCC 2016b.

Table 3-1. Experimental physical and chemical property values (at standard temperature) for the representative structures of *Lotus corniculatus* extract.

Property	Linamarin	Lotaustralin	HCN	Key reference(s)
CAS #	554-35-8	534-67-8	74-90-8	N/A
Physical state	Solid	Solid	Liquid or gas	Kim et al. 2019; WHO 2004
Melting point (°C)	145	147	-13.24 to -13.4	PhysProp 2013; Gail et al. 2012
Boiling point (°C)	434	446	25.6 to 25.7	PhysProp 2013; Gail et al. 2012
Vapour pressure (Pa) (at 25°C)	8.17×10^{-12}	1.08×10^{-12}	98 900 to 100 000	PhysProp 2013; Chatwin et al., 1987; Daubert and Danner 1985
Henry's law constant (Pa·m ³ /mol)	7.6×10^{-9}	1.01×10^{-8}	13.5 to 5167.6	PhysProp 2013; Gaffney et al., 1987; Yoo et al., 1986
Water solubility (mg/L)	5.65×10^5	1.8×10^5	1×10^6 ; miscible	PhysProp 2013; Lide, 1990
Log K _{ow} (dimensionless)	-1.91	-1.41	-0.25 to 0.66	PhysProp 2013; US EPA 1984; Hansch et al. 1995

Abbreviations: N/A, Not Applicable; K_{ow}, octanol-water partition coefficient

4. Sources and uses

Lotus corniculatus extract was included in a survey issued pursuant to section 71 of CEPA. For the 2012 calendar year, there were no reports of manufacture or import into Canada above the reporting threshold of 100 kg.

Lotus corniculatus extract is not listed in the Natural Health Products Ingredients Database (NHPID). However, *Lotus corniculatus* is listed in the NHPID as a homeopathic substance, with whole fresh flowering plant as the source material and a minimum homeopathic potency of 12C (i.e., a 10^{-24} dilution), although no homeopathic preparations containing *Lotus corniculatus* are currently licensed as natural health products in the Licensed Natural Health Products Database (LNHPD) (NHPID 2018; LNHPD 2018).

Notifications submitted under the *Cosmetic Regulations* to Health Canada identified *Lotus corniculatus* seed and flower extracts as being present in cosmetic products, including facial cleanser, skin moisturizer, face make-up, facial peel, shampoo and conditioner, and lip balm. *Lotus corniculatus* seed and flower extracts are listed in the Personal Care Products Council's International Nomenclature of Cosmetic Ingredients (INCI) dictionary with the stated function of skin-conditioning agents.

5. Potential to cause ecological harm

5.1 Characterization of ecological risk

The ecological risk of *Lotus corniculatus* extract was characterized using the ecological risk classification of organic substances (ERC) approach (ECCC 2016a). The ERC is a risk-based approach that employs multiple metrics for both hazard potency and exposure, on the basis of weighted consideration of multiple lines of evidence for determining risk classification. The various lines of evidence are combined to discriminate between substances of lower or higher potency and lower or higher potential for exposure in various media. This approach reduces the overall uncertainty with risk characterization compared to an approach that relies on a single metric in a single medium (e.g., median lethal concentration [LC₅₀]) for characterization. Since *Lotus corniculatus* extract is a UVCB substance and could not be suitably represented by a single chemical structure, a manual judgement-based approach to classification was used. The following summarizes the approach, which is described in detail in ECCC (2016a).

Hazard profiles were based principally on metrics regarding mode of toxic action, chemical reactivity, food web-derived internal toxicity thresholds, bioavailability, and chemical and biological activity. Exposure profiles were also based on multiple metrics, including potential emission rate, overall persistence, and long-range transport potential. Hazard and exposure profiles were compared to decision criteria in order to classify the hazard and exposure potentials for each organic substance as low, moderate or high. Additional rules were applied (e.g., classification consistency, margin of exposure) to refine the preliminary classifications of hazard or exposure. However, in the case of this UVCB, hazard and exposure could not be fully profiled because of the lack of a representative structure to estimate needed properties and the lack of empirical data for these properties. Therefore, manual classification of hazard and exposure was performed by examining the UVCB constituents and information obtained submitted under section 71 surveys under CEPA and making decisions on the basis of consideration of similar substances and application of expert judgement.

A risk matrix was used to assign a low, moderate or high classification of potential risk for each substance based on its hazard and exposure classifications. ERC classifications of potential risk were verified using a two-step approach. The first step adjusted the risk classification outcomes from moderate or high to low for substances

that had a low estimated rate of emission to water after wastewater treatment, representing a low potential for exposure. The second step reviewed low risk potential classification outcomes using relatively conservative, local-scale (i.e., in the area immediately surrounding a point-source of discharge) risk scenarios, designed to be protective of the environment, to determine whether the classification of potential risk should be increased.

ERC uses a weighted approach to minimize the potential for both over and under classification of hazard and exposure and subsequent risk. The balanced approaches for dealing with uncertainties are described in greater detail in ECCC (2016a). The following describes two of the more substantial areas of uncertainty. Error with empirical or modeled acute toxicity values could result in changes in classification of hazard, particularly metrics relying on tissue residue values (i.e., mode of toxic action), many of which are predicted values from (Q)SAR models (OECD QSAR Toolbox 2016). However, the impact of this error is mitigated by the fact that overestimation of median lethality will result in a conservative (protective) tissue residue used for critical body residue (CBR) analysis. Error with underestimation of acute toxicity will be mitigated through the use of other hazard metrics, such as structural profiling of mode of action, reactivity and/or estrogen-binding affinity. Changes or errors in chemical quantity could result in differences in classification of exposure as the exposure and risk classifications are highly sensitive to emission rate and use quantity. The ERC classifications thus reflect exposure and risk in Canada on the basis of what is calculated to be the current use quantity and may not reflect future trends.

Critical data and considerations used to develop the substance-specific profiles for *Lotus corniculatus* extract and the hazard, exposure and risk classification results are presented in ECCC (2016b).

On the basis of its low hazard and low exposure potential, according to information considered under ERC, *Lotus corniculatus* extract was classified as having a low potential for ecological risk. It is therefore unlikely that *Lotus corniculatus* extract is resulting in concerns for the environment in Canada.

6. Potential to cause harm to human health

6.1 Exposure assessment

Between January 2015 and January 2018, *Lotus corniculatus* extract was notified as being present in cosmetics in Canada. The products notified included rinse-off and leave-on products for the face (cleanser, moisturizer, make-up, and facial peel), hair products (shampoo / conditioner), skin moisturizer, which could be used as a body lotion, as well as lip balm.

Lotus corniculatus extract is potentially a source of the cyanogenic glycosides linamarin and lotaustralin and/or their hydrolysis product HCN (see Appendix A for additional details). In a survey of various *Lotus corniculatus* cultivars, the highest HCN concentrations observed were between 70 and 139 µg/g fresh weight and between 296 and 784 µg/g dry weight (Borsos *et al.* 1976). These values are in general agreement with Gebrehiwot and Beuselinck (2001), who reported that, on a whole-plant basis, the HCN content of cyanogenic cultivars averaged 636 µg/g dry weight when field grown and 800 µg/g dry weight when greenhouse grown. Similarly, the average cyanide content in leaves of cyanogenic cultivars ($n=158$) surveyed by Briggs and Schultz (1990) was approximately 810 µg/g dry weight.

The highest cyanide concentration in *Lotus corniculatus* identified in the literature was 1000 µg/g wet weight, which was the maximum value observed among 204 strains harvested from 33 countries around the world (Ross and Jones 1983). Based on the ratios of Borsos *et al.* (1976) above, this value is estimated to correspond to approximately 5000 µg/g dry weight. Assuming *Lotus corniculatus* extract is not deliberately enriched for cyanide content and no measures are taken to prevent loss of HCN during the extraction process³, the HCN concentration in extracts would not be expected to exceed the maximum observed concentration on a dry weight basis. Therefore, an HCN content of 5000 µg/g (0.5%) for *Lotus corniculatus* extract was taken forward for exposure estimation.

Exposure estimates related to the use of cosmetics were derived on the basis of expected use patterns by the general population. The highest concentration in product notifications submitted to Health Canada (or the upper bound in the case of a range) was multiplied by the maximum total cyanide concentration (free and bound) in plant matter on a dry weight basis (5000 µg/g) in order to estimate the maximum cyanide content of products. In the case of products applied to the skin, body moisturizer was selected as a sentinel exposure scenario, with the maximum amount of HCN applied dermally estimated to be 250 µg/d, corresponding to a surface load of 0.014 µg/cm²/d for adults. Additional details of the HCN content of notified products as well as estimated exposure may be found in Appendix B.

Assuming an adult body weight of 70.9 kg and complete absorption, a dermal load of 250 µg/d corresponds to a systemic dose of 3.5 µg/kg bw/d. However, HCN is a highly volatile substance, with a boiling point of 25.7°C. Therefore, at a default skin surface

³ HCN is highly volatile and likely to be lost if the gas is not trapped and/or deliberate steps are not taken to prevent volatilization during the processing of the botanical extract as well as during formulation, packaging and storage of the cosmetic product. Therefore, it is likely that most finished cosmetic products containing *Lotus corniculatus* extract have little to no cyanogenic potential. However, for the purposes of this screening assessment, it was assumed that *Lotus corniculatus* extract is derived from cyanogenic phenotypes, and that the entire cyanide content of the glycosides is liberated, unbound, and present in the finished cosmetic product.

temperature of 32°C (Freitas 1999), the substance will rapidly volatilize. The Finite Dose Skin Permeation Calculator (NIOSH 2013), which implements solutions to Kasting and Miller's (2006) model of the kinetics of finite dose absorption through skin for volatile compounds, was used to predict the disposition of an applied surface load and estimate the fractional total mass that is potentially available for percutaneous absorption versus that lost to evaporation. The HCN quantities estimated to be distributed to the stratum corneum, viable epidermis, dermis and systemic compartment were summed to yield a percutaneous dose of $3.4 \times 10^{-5} \mu\text{g}/\text{cm}^2$, which corresponds to a systemic HCN dose of 0.009 $\mu\text{g}/\text{kg}$ bw/d (Appendix C).

HCN that evaporates from the skin surface rather than being absorbed may result in inhalation exposure. Therefore, HCN concentrations in air were derived using ConsExpo exposure modelling (RIVM 2016). The theoretical maximum HCN concentration in air was estimated to average 21 $\mu\text{g}/\text{m}^3$ during application of the body lotion, corresponding to a mean daily HCN exposure concentration of 0.15 $\mu\text{g}/\text{m}^3$ and a systemic dose of 0.03 $\mu\text{g}/\text{kg}$ bw/d, assuming 100% absorption via the inhalation route. Additional default parameters used in the exposure scenarios and further details of the calculations are provided in Appendix B.

The absorption of volatile substances through the skin directly from the gas phase can also be an important route of exposure (Weschler and Nazaroff 2014). However, Gaskin and colleagues (2013) have demonstrated that transdermal uptake of HCN gas is relatively poor. Therefore, dermal absorption from the vapour phase due to cosmetic use is considered to be negligible and no quantitative estimates were derived.

Use of *Lotus corniculatus* extract in lip balm was also notified, where potential exists for the product to be ingested. For the purposes of this screening assessment, it was assumed that lip balm contained *Lotus corniculatus* extract at up to 1%, which is the upper bound of the highest concentration range notified to Health Canada, and that all product applied is ultimately ingested. The maximum potential systemic HCN exposure via the oral route from use of lip balm containing *Lotus corniculatus* extract is estimated to be 0.03 $\mu\text{g}/\text{kg}$ bw/d for a 70.9 kg adult. Lip balm may also be used by younger children and toddlers, and the maximum systemic dose for a toddler was estimated to be 0.07 $\mu\text{g}/\text{kg}$ bw/d. Additional details of the assumptions and default parameters used to estimate exposure via the oral route may be found in Appendix B.

Empirical data on concentrations of *Lotus corniculatus* extract in environmental media in Canada were not identified, but are expected to be negligible. *Lotus corniculatus* extract is not expected to be found in food or beverages.

6.2 Health effects assessment

Plant-associated cyanogenic glycoside toxicity depends on enzymatic hydrolysis of the parent molecules to liberate HCN. Cyanide toxicity has been studied extensively both in

humans and laboratory animals and the biochemical mechanisms of cyanide action are generally consistent in most mammalian species (NRC 2002). Cyanide disrupts cellular respiration leading to a state of hypoxia by binding to cytochrome c oxidase, the final enzyme complex of the electron transport chain (located in the membrane of the mitochondria of eukaryotic cells). Essentially, cyanide toxicity results from the inability of cells to utilize oxygen as an electron receptor, thereby disrupting synthesis of adenosine triphosphate (ATP), the primary source of cellular energy (Nelson 2006). Cyanide is a systemic toxicant and the organ systems most sensitive to low oxygen levels are also the most susceptible to acute cyanide toxicity, in particular the nervous, cardiovascular and respiratory systems. The following description is not intended to be exhaustive, but rather provides an overview of existing health-based guidance values for HCN and the lowest published doses associated with adverse effects. The health effects of HCN were previously reviewed by Health Canada (Canada 2018), and free/simple cyanides are the subject of various international assessments, most recently by the US EPA (EPA 2010) and FAO/WHO (2012).

The U.S. National Research Council (NRC 2000) has established an Acute Exposure Guideline (or AEGL-1⁴) for HCN of 1 ppm (1 mg/m³), based on human monitoring studies in workers occupationally exposed to HCN. AEGL values are intended to represent exposure thresholds that are applicable to the general public, including susceptible subpopulations such as infants, children, the elderly, persons with asthma, and those with other illnesses (NRC 2002). The AEGL-1 threshold for HCN is based on a weight of evidence evaluation that concluded an 8-h exposure to HCN at 1 mg/m³ would be without adverse health effects for the general population. Because the value is based on exposure over extended work periods (generally 8 h/d), it is thought to represent a conservative approach to AEGL derivation (NRC 2002). The 8-h AEGL-1 value was derived from a consideration of the dose response data obtained from several monitoring studies cited and subsequently time-scaled to the shorter AEGL exposure durations (see NRC 2002). Regression analyses have determined that the exposure duration (t) - concentration (C) relationship for HCN toxicity in a nonhuman primate is described by the equation $C^2 \times t = k$ (NRC 2002), where k denotes a constant. Therefore, an 8-h AEGL-1 of 1 ppm can be extrapolated to a 10-min AEGL-1 of 6.9 mg/m³.

In rodent studies, subchronic cyanide exposure via drinking water did not cause clinical signs associated with neurotoxicity or histopathological evidence of brain or thyroid effects in rats and mice at doses up to 12.5 mg and 26 mg cyanide/kg bw/d, respectively (NTP 1993). However, subtle changes in the male reproductive tract were observed, including decreased cauda and whole epididymis weights, decreased testes weight, and altered sperm parameters, with rats appearing to be the more sensitive species. There were no changes in epididymal sperm concentrations relative to controls

and the authors did not consider the effect to be biologically relevant for the rodent species. Humans, however, have lower rates of sperm production compared to rats and the potential impact of sperm quality decrements is greater for humans than for rats (EPA 2010). Therefore, the lower 95% confidence limit on the dose corresponding to a change in cauda epididymis weight in male rats equal to one standard deviation from the control mean (BMDL_{1SD}) was selected by the U.S. EPA as the point of departure for determining a chronic oral reference dose (RfD). Uncertainty factors totalling 3000 were applied to the BMDL_{1SD} of 1.9 mg/kg bw/d to yield a final chronic oral RfD of 0.6 µg/kg bw/d (EPA 2010). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) also derived a provisional maximum tolerable daily intake (PMTDI) for HCN based on the same point of departure (JECFA 2011).

Chronic cyanide exposure via the inhalation route has also been documented in occupationally-exposed workers. The U.S. EPA (2010) has derived a chronic inhalation RfC on the basis of thyroid effects and neurological symptoms in workers in three electroplating factories (El Ghawabi *et al.* 1975). Male workers ($n = 36$) chronically exposed to HCN for 5-15 years showed significantly altered rates of iodine incorporation by the thyroid, enlargement of the thyroid, as well as CNS symptoms such as headache, weakness, and sensory changes for taste and smell (El Ghawabi *et al.* 1975). The results of individual breathing zone measurements indicated the mean HCN concentration across factories ranged from 7.07 to 11.5 mg/m³, and urinary excretion of thiocyanates was strongly correlated with individual exposure measurements. Twenty of the exposed workers (56%) were observed to have mild to moderate thyroid enlargement and altered iodide uptake. Neither exposure duration nor air concentrations were correlated with the incidence nor magnitude of effect, although given the small sample size and relatively narrow range of exposure concentrations this is not entirely unexpected. The EPA (2010) selected the lowest mean air concentration of 7.07 mg/m³ as the LOAEL. This number was adjusted to a continuous exposure of 2.5 mg/m³ for use as the point of departure for RfC derivation⁵. Uncertainty factors totalling 3000 were applied to the adjusted LOAEL to yield a final chronic inhalation RfC of 0.8 µg/m³ (EPA 2010).

The available data are not sufficient to assess the carcinogenic potential of cyanide in humans. A two-year feeding study in which groups of 10 male and 10 female rats were administered food fumigated with HCN at estimated doses of 4.3 and 10.8 mg cyanide/kg bw/d yielded no evidence of histopathologic lesions attributable to cyanide (Howard and Hanzal 1955), although the study has important methodological

⁵ The LOAEL of 7.07 mg HCN/m³ was adjusted for continuous daily exposure duration using a default occupational inhalation rate of 10 m³/8-hour day and a default inhalation rate for continuous ambient exposure of 20 m³/24-hour day. The LOAEL was also adjusted for continuous exposure from 5 days/week to 7 days/week (EPA, 2010). $LOAEL_{(ADJ)} = 7.07 \text{ mg/m}^3 \text{ HCN} \times 10/20 \times 5 \text{ days/7 days} = 2.5 \text{ mg/m}^3 \text{ HCN}$.

limitations. No studies evaluating the carcinogenicity of HCN via the inhalation route were identified.

6.3 Characterization of risk to human health

Consumer exposure to *Lotus corniculatus* extract is expected to be limited to the use of cosmetic formulations. *Lotus corniculatus* extract may contain cyanogenic glycosides and/or the hydrolysis product HCN. Although *Lotus corniculatus* extract may be present in skin products intended for topical application, given the very high volatility of HCN the principle route of exposure is expected to be inhalation. Ingestion may also occur through the use of lip balm containing *Lotus corniculatus* extract.

Chronic exposure to relatively low levels of cyanide has been associated with neuropathy and thyroid effects in occupationally-exposed workers as well as depressed growth and male reproductive dysfunction in rodent studies. Acute exposure to minimally toxic cyanide levels is known to cause mild CNS effects, especially headache (NRC 2000; Canada 2018). Critical effect levels associated with acute (inhalation) and chronic (oral, inhalation) cyanide toxicity were selected as points of departure for comparison with upper bounding estimates of cyanide exposure from the use of products containing *Lotus corniculatus* extract.

Table 6-1 provides relevant exposure and hazard values for potential HCN exposure from the use of sentinel cosmetic products containing *Lotus corniculatus* extract, as well as the resultant margins of exposure (MOEs).

Table 6-1. Relevant exposure and hazard values, and resulting MOEs

Exposure scenario	Estimated exposure	Critical effect level	Critical health effect	MOE
Body lotion - adult (daily inhalation)	0.15 µg/m ³	2.5 mg/m ³ (LOAEL) ^a	Thyroid enlargement and altered iodide uptake	>16 000
Body lotion - adult (daily dermal)	0.009 µg/kg bw/d	1.9 mg/kg bw/d (BMDL) ^b	Decreased cauda epididymis weight in male rats	>200 000
Body lotion - adult (per event inhalation)	21 µg/m ³	6.9 mg/m ³ (adj AEGL-1) ^c	The AEGL-1 is considered a “safe value” based on occupational studies	329
Lip Balm - adult (daily oral)	0.03 µg/kg bw/d	1.9 mg/kg bw/d (BMDL) ^b	Decreased epididymis weight in male rats	>60 000
Lip Balm - toddler (daily oral)	0.07 µg/kg bw/d	1.9 mg/kg bw/d (BMDL) ^b	Decreased epididymis weight in male rats	>25 000

Abbreviations: N/A, not applicable

^a El Ghawabi 1975; US EPA 2010

^b NTP 1993; US EPA 2010.

^c NRC 2002. The 10-min AEGL-1 was derived using the 8-hr AEGL-1 of 1 ppm and extrapolating for 10 min of exposure based on the equation of $C^2 = t \times k$, where C is concentration, t is time and K is a constant.

Based on the use of conservative parameters in estimating exposure from products, the margins between estimates of exposure and critical effect levels observed in both human epidemiological as well as animal studies are considered adequate to account for any uncertainties in the toxicological and exposure databases.

6.4 Uncertainties in evaluation of risk to human health

Although Canadian data were available concerning the levels of *Lotus corniculatus* extract used in cosmetics some significant limitations in the exposure database remain. Similarly, the toxicity of cyanide and its mode of action are well documented, yet some uncertainties in the health effects were identified. The key sources of uncertainty are presented in **Table 6-2** below.

Table 6-2. Sources of uncertainty in the risk characterization

Key source of uncertainty	Impact
The maximum potential HCN level in <i>Lotus corniculatus</i> extract is unknown.	+/-
There is no information available concerning whether <i>Lotus corniculatus</i> extract are prepared from cyanogenic phenotypes. For the purposes of this assessment, all extracts were considered to be cyanogenic.	+
Due to the volatility of HCN, it is unknown whether the final product would contain any HCN even if the extract was from a cyanogenic phenotype. For the purposes of this assessment, it was considered that no HCN was lost between processing of the extract and consumer use of the product and there was full conversion of parent glycosides to HCN.	+
No information on cyanide carcinogenicity via the inhalation route was identified and the lone oral study has methodological limitations, precluding assessment of the carcinogenic potential of HCN.	+/-
There is uncertainty concerning the extent to which cyanide-induced reproductive toxicity in male rats is relevant to humans as the cyanide database lacks human male reproductive studies.	+/-
There are no chronic animal studies for oral exposure, nor subchronic or chronic animal studies for inhalation exposure.	+/-
The 90-day National Toxicology Program (NTP, 1993) study for oral exposure in rat did not assay for thyroid endpoints.	+/-
The chronic human study used for inhalation exposure (El Ghawabi et al., 1975) did not assay for male reproductive endpoints (focus was on thyroid gland and iodine uptake). Further, there was no temporal	+/-

Key source of uncertainty	Impact
correlation between the time of exposure and the degree of thyroid enlargement in this study.	
There are numerous other sources of HCN other than <i>Lotus corniculatus</i> extract as well as the potential for interaction with other chemical exposures such as carbon monoxide.	-

+ = uncertainty with potential to cause over-estimation of risk; - = uncertainty with potential to cause under-estimation of exposure risk; +/- = unknown potential to cause over or under estimation of risk.

Although no data were identified concerning the levels of cyanogenic glycosides or HCN in either *Lotus corniculatus* extract or finished products, the assumptions used are believed to be sufficiently conservative.

7. Conclusion

Considering all available lines of evidence presented in this draft screening assessment, there is low risk of harm to the environment from *Lotus corniculatus* extract. It is proposed to conclude that *Lotus corniculatus* extract does not meet the criteria under paragraphs 64(a) or (b) of CEPA as it is not entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity or that constitute or may constitute a danger to the environment on which life depends.

On the basis of the information presented in this draft screening assessment, it is proposed to conclude that *Lotus corniculatus* extract does not meet the criteria under paragraph 64(c) of CEPA as it is not entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

It is therefore proposed to conclude that *Lotus corniculatus* extract does not meet any of the criteria set out in section 64 of CEPA.

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Appendix A – Cyanogenic glycosides and the potential for HCN exposure from cosmetics containing *Lotus corniculatus* extract

Cyanogenic glycosides are relatively small molecules, which are typically found in low concentrations in plants (Briggs and Schultz 1990), although concentrations vary seasonally with spring and summer having consistently higher HCN levels than fall and winter (Gebrehiwot and Beuselinck 2001). The highest cyanide levels are found in developing leaves, and decrease as leaves mature and toughen, while the seeds contain little to no cyanogenic glycosides (Jones and Turkington 1986; Vetter 2000). There is less information on cyanide content of *Lotus* flowers, although in one greenhouse-grown cultivar cyanide concentration in flowers was similar to that of leaves (Gebrehiwot and Beuselinck 2001).

In intact plant material, the cyanogenic glycosides are stored in separate cellular compartments from hydrolytic enzymes such as β -glucosidases. Damage to the plant through chewing by herbivores or processing to produce extracts may disrupt this compartmentalization and initiate the formation of HCN. On hydrolysis, one gram of linamarin can liberate 109.3 mg HCN (equivalent to 105.2 mg CN⁻) and one gram of lotaustralin can liberate 103.4 mg HCN (equivalent to 99.6 mg CN⁻). Humans and other mammals were long thought to lack the endogenous hydrolytic enzymes relevant to the metabolism of dietary glucosides (Dorr and Paxinos 1978; Frakes *et al.* 1986; Hernandez *et al.* 1995), although partial hydrolysis of glycosides and the release of HCN may be accomplished by the action of microbial enzymes in the gastrointestinal tract (EFSA 2007). Evidence of endogenous human β -glucosidases has since emerged (Berrin *et al.* 2002; Németh *et al.* 2003), although these enzymes do not appear to have any significant capacity to hydrolyze linamarin, the dominant glycoside produced by *Lotus corniculatus* as well as several other plant species that are important human foodstuffs. Therefore, if not metabolized by gut microbiota, the parent glycosides are absorbed and excreted in urine without causing HCN exposure (EFSA 2007).

It is reasonable to assume that HCN may be liberated from cyanogenic glycosides during the production and processing of *Lotus corniculatus* extract if plant material is homogenized or otherwise subject to technical processes that compromise the integrity of the material. Extracts derived from acyanogenic phenotypes that produce the glycoside but lack the enzyme may also contain intact glycosides. Although no data were identified concerning the capacity of skin microflora to hydrolyze cyanogenic glycosides, the possibility exists that unhydrolyzed glycosides that remain in plant extracts may also liberate HCN via this mechanism. As per the oral route, any cyanogenic glycosides that are percutaneously absorbed without being metabolized by skin biota are not expected to result in HCN exposure due to the lack of endogenous hydrolytic enzymes in mammals.

Appendix B - Default parameters and assumptions in estimating hydrogen cyanide exposure from cosmetics via the inhalation and oral routes.

Sentinel exposure scenarios were used to estimate the potential exposure to cyanide from use of cosmetic products containing *Lotus corniculatus* extract; scenario assumptions are summarized in Tables B-1. Exposures were estimated on the basis of the assumed weight of 70.9 kg for adults and 15.5 kg for toddlers (Health Canada 1998) as well as an adult inhalation rate of 16.2 m³/d. Air concentrations were estimated using ConsExpo Web version or algorithms from the model (ConsExpo Web 2016).

Table B-1. Sentinel exposure scenario assumptions

Exposure scenario	Assumptions
Body lotion	<p>Max HCN concentration in product: 25 mg/kg (max cyanide concentration in <i>Lotus corniculatus</i> extract (see section 5.1) multiplied by max concentration of extract in product per cosmetic notifications).</p> <p>Product amount: 10 g (Ficheux <i>et al.</i> 2016)</p> <p>Frequency: 1/day (Ficheux <i>et al.</i> 2015)</p> <p>Retention factor: 1 (RIVM 2006)</p> <p>Room volume: 10 m³ (RIVM 2014)</p> <p>Ventilation rate: 2/hr (RIVM 2014)</p> <p>Application duration: 1 minute (expert judgement)</p> <p>Exposure duration: 10 minutes (EPA, 2011)</p> <p>Release area: 17,530 cm² (Ficheux <i>et al.</i> 2016)</p> <p>Temperature: 32°C (default skin temperature NIOSH 2013).</p> <p>Vapour pressure: 959 torr at 32°C (calculated using Antoine equation – see Appendix C)</p> <p>Mass transfer rate: 0.167 m/min (Meesters <i>et al.</i> 2018)</p>
Lip Balm	<p>Max HCN concentration in product: 50 mg/kg (max cyanide concentration in <i>Lotus corniculatus</i> extract (see section 5.1) multiplied by max concentration of extract in product per cosmetic notifications).</p> <p>Product amount: 0.022 g (Ficheux <i>et al.</i> 2016)</p> <p>Max HCN conc. (mg/kg): 50</p> <p>Frequency: 2/d adults; 1/d toddlers (Ficheux <i>et al.</i> 2015)</p> <p>Amount ingested: 100% (theoretical worst case)</p>

	Retention factor: 1 (RIVM 2006)
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Appendix C – Finite dose skin permeation of volatile substances.

The Antoine equation may be used to estimate vapour pressure within a specific range of temperatures. The equation is

$$\log_{10} p = A - \frac{B}{C + T}$$

where p is the vapour pressure (mmHG), T is temperature (°C) and A , B and C are component-specific constants. The Antoine constants for hydrogen cyanide were obtained from EPA (2012).

A	B	C	T_{\min} °C	T_{\max} °C
7.52823	1329.49	260.418	-16.4	46.2

Accordingly, the vapour pressure of hydrogen cyanide at 32°C (default skin surface temperature) may be calculated as follows:

$$\log_{10} p = 7.52823 - \frac{1329.49}{260.418 + 32}$$

$$p = 959 \text{ mmHG}$$

The finite dose skin permeation calculator (NIOSH 2013) solves for the disposition of an applied surface load and can be used to estimate fluxes, skin concentrations and the amount of a chemical that is absorbed. In the case of highly volatile substances such as cyanide, exposure via the dermal route will depend not only on the surface load but also the rate of evaporation relative to the rate of absorption. Indeed, the finite dose skin permeation calculator predicts that at a skin temperature of 32°C the maximum evaporative flux will vastly exceed the maximum absorptive flux (Table).

Table C-1. Results of the Finite Dose Skin Permeation Calculator for topically applied products containing HCN.

Maximum Fluxes and Concentrations	
Maximum Absorptive Flux (max(J_{abs}))	1.645E-03 $\mu\text{g}/\text{cm}^2/\text{h}^1$
Time to Max Abs Flux	0.471 hours
Maximum Evaporative Flux	2789.242 $\mu\text{g}/\text{cm}^2/\text{h}^1$
Time to Max Evap Flux	2.778E-10 hours

The finite skin permeation calculator was used to estimate permeant distribution following dermal application (Table C-2). The sum of the total absorbed solution in all skin layers plus that systemically absorbed was estimated to be $6.9 \times 10^{-3} \mu\text{g}/\text{cm}^2$. Assuming an HCN concentration of 0.5%, this corresponds to a dose per unit area of $3.4 \times 10^{-5} \mu\text{g}/\text{cm}^2$. This value may be multiplied by a body surface area of $17,530 \text{ cm}^2$ and divided by an adult body weight of 70.9 kg to yield a systemic HCN dose of 0.009 $\mu\text{g}/\text{kg bw}/\text{d}$.

Table C-2. Estimated permeant distribution following dermal application based on Kasting and Miller (2006).

HCN Disposition	Mass per unit area ($\mu\text{g}/\text{cm}^2$)
Evaporated	0.564
Skin surface	0.0
Stratum corneum	1.57×10^{-06}
Viable epidermis	4.18×10^{-06}
Dermis	5.71×10^{-05}
Systemically absorbed	6.84×10^{-03}