Food and Chemical Toxicology 47 (2009) 1246–1254

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Application of the threshold of toxicological concern approach for the safety evaluation of calendula flower (*Calendula officinalis*) petals and extracts used in cosmetic and personal care products

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ARTICLE INFO

Article history:
Received 7 November 2008
Accepted 14 February 2009

Keywords:
TTC
Safety evaluation
Calendula
Cosmetics

ABSTRACT

Calendula flower (*Calendula officinalis*) (CF) has been used in herbal medicine because of its anti-inflammatory activity. CF and *C. officinalis* extracts (CFE) are used as skin conditioning agents in cosmetics. Although data on dermal irritation and sensitization of CF and CFE's are available, the risk of subchronic systemic toxicity following dermal application has not been evaluated. The threshold of toxicological concern (TTC) is a pragmatic, risk assessment based approach that has gained regulatory acceptance for food and has been recently adapted to address cosmetic ingredient safety. The purpose of this paper is to determine if the safe use of CF and CFE can be established based upon the TTC class for each of its known constituents. For each constituent, the concentration in the plant, the molecular weight, and the estimated skin penetration potential were used to calculate a maximal daily systemic exposure which was then compared to its corresponding TTC class value. Since the composition of plant extracts are variable, back calculation was used to determine the maximum acceptable concentration of a given constituent in an extract of CF. This paper demonstrates the utility and practical application of the TTC concept when used as a tool in the safety evaluation of botanical extracts.

1. Introduction

Calendula officinalis (CF) also known as Garden Marigold, God-Bloom, Holligold, Marigold, Marybud, and Pot Marigold, is part of the botanical family of Asteraceae/Compositae (Jellin et al., 2003). The dried flower is used as a spice and is considered to be generally recognized to be safe (GRAS) by the food and drug administration (FDA) (Food and Drug Administration, 2007) and the Flavors and Extracts Manufacturers Association (FEMA) (FEMA number 2658) (Hall and Oser, 1965). It is used topically as a natural anti-inflammatory medicine and for poorly healing wounds and leg ulcers. The dosages cited are 2–4 mL of tincture diluted to 250–500 mL with water or 2–5 g of herb in 100 g of ointment (Jellin et al., 2003). Other topical uses include treatment for 1st degree burns and scalds, bruises, boils, and rashes (Lueng and Foster, 1996; Blumenthal et al., 2000). A tea made from 1 to 2 g of the flower in 150 mL of boiling water has also been used up to three times a day as an antispasmodic (Jellin et al., 2003). Other oral uses include alleviation of the discomfort associated with stomach ulcers and inflammation of the oral and pharyngeal mucosa (Lueng and Foster, 1996; Blumenthal et al., 2000). Calendula preparations are therefore regarded as traditionally used medicines by the European Medicines Agency (EMEA) (EMEA, 2008a,b). In cosmetic or personal care preparations calendula extracts are used as skin conditioning agents at concentrations ranging up to 1% but are generally below 0.1% (Anonymous, 2008). All together, these data allow one to conclude that calendula extracts should be considered as having a long history of safe use as defined by Constable et al. (2007).

UNITIS, the European Organization of Cosmetic Ingredients Industries and Services, has provided the cosmetic ingredient review (CIR) (report available through the Cosmetic Ingredient Review, Washington, DC) with an extensive list of chemicals that comprise the calendula plant along with concentration ranges (UNITIS, 2006). Table 1 presents a summary of this list of chemical constituents that was derived through literature search. The concentrations

Abbreviations: CF, calendula flower (*Calendula officinalis*); CFE, *Calendula officinalis* extracts; GRAS, generally recognized to be Safe; TTC, threshold of toxicological concern; FDA, food and drug administration; FEMA, flavors and extracts manufacturers association; EMEA, European Medicines Agency; UNITIS, The European Organization of Cosmetic Ingredients Industries and Services; CIR, cosmetic ingredient review; SCCP, Scientific Committee on Cosmetic Products; NOAEL, no observable adverse effect level; NOEL, no observable effect level; EFSA, European Food Safety Authority; ADI, acceptable daily intake; JECFA, The Joint FAO/WHO Committee on Food Additives; MW, molecular weight.

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doi:10.1016/j.fct.2009.02.016

Table 1
Analysis of *Calendula officinalis*.

Component class	Main fraction and % concentration	Sub-fraction	CAS number
Mineral matter	Major elements as salts	Potassium (≈6.0%)	7440-09-7
		Sodium (≈1.7%	7440-23-5
		Magnesium (≈0.9%)	7439-95-4
		Calcium (≈0.5%)	7440-70-2
		Iron (0.15%)	7439-89-6
Carbohydrates	12–25%, dry matter	Arabinogalactan PSII 25 kDa (arabinose, galactose)	Not found
		Arabinogalactan PSIII 35 kDa (arabinose, galactose)	Not found
		Mucilage (1.5%): rhamnoarabingalactan PSI 15 kDa (arabinose, galactose, rhamnose)	Not found
Lipids	Fatty acids mainly as esters (5%, dry matter)	9-Hydroxy-*trans*-10,*cis*-12-octadecadienic-acid	15514-85-9
		Capric acid	334-48-5
		Caprylic acid	124-07-2
		Dimorphecolic acid	Not found
		Lauric acid	143-07-7
		Linoleic acid	60-33-3
		Linolenic acid	463-40-1
		Myristic acid	544-63-8
		Palmitic acid	57-10-3
		Palmitoleic acid	2091-29-4
		Pentadecanoic acid	1002-84-2
		Stearic acid	57-11-4
		Calendic acid	5204-87-5
	Hydrocarbon/paraffin/waxes (0.015%, fresh petals)	C32H62	not found
		Dotriacontane	544-85-4
		Hentriacontane	630-04-6
		Heptacosane	593-49-7
		Hexacosane	630-01-3
		Nonacosane	630-03-5
		Octacosane	630-02-4
		Tetratriacontane	14167-59-0
		Tritriacontane	630-05-7
Phenolic compounds	Phenolic acids in free and esterified forms (0.1%, dry matter)	Lignin	9005-53-2
		Caffeic acid	331-39-5
		Chlorogenic acid	327-97-9
		Coumaric acid	Not found
		Ferulic acid	1135-24-6
		Gentisic acid	490-79-9
		trans-*O*-Coumaric acid	614-60-8
		O-Hydroxyphenylacetic acid	614-75-5
		4-Coumaric acid	7400-08-0
		4-Hydroxy benzoic acid	99-96-7
		Protocatechuic acid	156-38-7
		Quinic acid	77-96-2
		Salicylic acid (traces)	99-50-3
		Sinapinic acid	69-72-7
		Syringic acid	530-59-6
		Vanillic acid	530-57-4
		Veratric acid	121-34-6
	Flavanoids (<1.5%)	Astragalin	93-07-2
		Hyperoside	482-36-0
		Calendoflaside	Not found
		Calendo flavo side	Not found
		Calendo flavobio side	Not found
		Isoquercitrin	21637-25-2
		Isorhamnetin	480-19-3
		Isorhamnetin-3-neohesperidoside	Not found
		Isorhamnetin-3-*O*-(2″,6″-dirhamnosyl)-glucoside	Not found
		Isorhamnetin-3-*O*-(2″-rhamnosyl)-glucoside	Not found
		Isorhamnetin-3-*O*-glucoside	Not found
		Isorhamnetin-3-rhamnosyl-(1,2)-rhamnoside	Not found
		Isorhamnetin-3-β-D-glucopyranosyl-6-1-β-1-rhamnofuranoside	Not found
		Kaempferol	520-18-3
		Manghaslin	Not found
		Narcissin	604-80-8
		Neohesperidin (Closest Match to Neoliesperoside)	13241-33-3
		Quercetin	117-39-5
		Quercetin-3-neohesperidoside	Not found
		Quercetin-3-*O*-(2″,6″-dirhamnosyl)-glucoside	Not found
		Quercetin-3-*O*-(2″-rhamnosyl)-glucoside	Not found
		Quercetin-3-rutinoside	Not found
		Rutin	153-18-4
		Syringin (Closest Match to Syringentin)	118-34-3

(*continued on next page*)