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ORIGINAL ARTICLE

Parthenolide in Danish biodynamic and organic milk: A new source of exposure to an allergenic sesquiterpene lactone

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Summary

Background: The sunflower family of plants (Compositae = Asteraceae) is currently the most allergenic plant family worldwide, according to the number of sensitizing species. Secondary plant metabolites, including the allergenic sesquiterpene lactones present in Compositae plants, may occur in food items either through their presence in, or through contamination of, plant-based raw materials, or through their occurrence in products of non-plant origin.

Objective: To analyse biodynamic, organic and conventional milk for the presence of the sesquiterpene lactone parthenolide.

Methods: The content of parthenolide in the milk samples was investigated in dichloromethane extracts obtained by liquid-liquid extraction, followed by gas chromatography-mass spectrometry analyses.

Results: The concentration of parthenolide was 0.07 ±0.004 ppm in biodynamic milk, 0.05 ±0.002 ppm in organic milk, and not detectable (<0.002 ppm) in conventional milk.

Conclusions: This is the first report of a potent contact allergen in milk. There seems to be an association between the time that the dairy cattle spend grazing and the amount of parthenolide detected. Although the concentration is low, it is estimated to be high enough to elicit dermatitis in the most sensitive persons by direct contact with the milk.

KEYWORDS

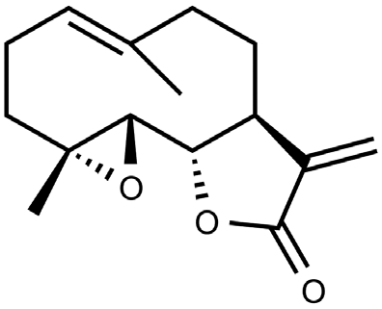
allergic contact dermatitis, biodynamic milk, organic milk, parthenolide, sesquiterpene lactones, *Tanacetum vulgare*

1 | INTRODUCTION

On the basis of the number of sensitizing species, the daisy or sunflower family of plants (Compositae = Asteraceae) is currently the most allergenic plant family worldwide. It is also one of the largest flowering plant families, comprising >27 000 species, the majority of which occur in temperate regions. The Compositae plants are ubiquitous in nature, ornamental gardens, and vegetable gardens; furthermore, some important medicinal plants, such as German chamomile (*Matricaria chamomilla* L.), dandelion (*Taraxacum officinale* F. H. Wigg), and arnica (*Arnica montana* L.), belong to this family, which makes it difficult for sensitized persons to avoid direct or indirect contact with the plants or plant extracts.[1,2] The major contact allergens are sesquiterpene lactones (SLs), which may occur in glandular hairs (trichomes) on the plant surface or in the milky latex.[3,4] Sensitized persons may experience flares of eczema after ingestion of edible Compositae plants or teas made from Compositae plants.[5,6] Furthermore, Amorim et al, in their review of the adverse health effects and toxicity mechanisms of SLs, mention experimental evidence of alternative routes of exposure, including excretion of the SL tenulin in milk from a cow fed with this compound.[7,8]

Parthenolide (PHL) (Figure 1) is a strong SL contact allergen that occurs in several Compositae species, such as tansy (*Tanacetum vulgare* L.) and feverfew (*Tanacetum parthenium* [L.] Sch.Bip.).[9–15] Tansy is a common weed in temperate Europe, including Denmark, and the roadsides are brimming with their yellow flowers in late summer (Figure 2). In addition to PHL, the essential oil of tansy may contain the poisonous monoterpene thujone. An experimental study has

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Parthenolide (PHL)

FIGURE 1 The sesquiterpene lactone parthenolide

shown that PHL may be disseminated to the surroundings of PHL-containing plants through airborne plant particles.[16] Thus, theoretically, dairy cows may graze on PHL-"polluted" grass, even though they allegedly avoid most of the yellow-flowered Compositae plants and probably also the slightly poisonous tansy.

The aim of the present study was to analyse the milk from biodynamically, organically and conventionally raised cows for the presence of PHL.

2 | MATERIALS AND METHODS

2.1 | Analysis of PHL in milk samples

The content of PHL in biodynamic, organic and conventional milk samples, bought in August 2016 and 2017, was investigated in dichloromethane extracts obtained by liquid-liquid extraction followed by gas chromatography-mass spectrometry (GC-MS) analysis, as described in the following. One litre of dichloromethane was added to 1 L of milk sample in a separating funnel, and the mixture was shaken vigorously. The organic layer was collected, and the residue was extracted with a further 3 L of dichloromethane by use of the same procedure as described above. The collected organic layers were dried with anhydrous sodium sulfate, concentrated in vacuo by rotary evaporation at 40°C, dissolved in 0.5 mL of dichloromethane, and analysed by GC-MS for PHL. The extraction process was repeated on a second batch of milk samples; hence, the extracts were considered to be representative samples. The extracts were analysed directly by GC-MS, and each extract was analysed in triplicate. GC-MS data were obtained with a DSQ II Single Quadrupole mass spectrometer (Thermo Fisher Scientific, San Jose, California) operated at an ionization potential of 70 eV and coupled to a Trace GC Ultra gas chromatograph equipped with a ZB-5 column (30.0 m × 0.25 mm, 0.25 μm; Phenomenex, Torrance, California). The temperature programme was as follows: 32°C for 1 minute, and then an increase to 260°C at a rate of 10°C/min, with an isothermal step at 260°C for 20 minutes. The injection volume was 1 μL with a split ratio of 1:50, and the temperature of the injector and the transfer line was 200°C. Helium was applied as a carrier gas at a flow rate of 1.4 mL/min. The mass spectrometer was operated in scan mode (1 scan/s) over a mass range from 39 to 650 u (Da).

Compounds suggested by the National Institute of Standards and Technology MS Database (NIST 2008) were verified by comparison of gas chromatography retention times and mass spectra of an available authentic reference compound of PHL (Sigma-Aldrich; Merck, Darmstadt, Germany). The GC-MS analysis of the extracts from the organic milk resulted in a peak in the total ion chromatogram at a retention time 39.56 minutes with a molecular ion peak at *m/z* 248 $[M]^+$ and a fragmentation pattern corresponding to PHL.[16]

3 | RESULTS

PHL in the milk extracts was quantified from a calibration curve (R^2 > 0.998) of the PHL standard in the range 0.02 to 1000 ppm (data not shown). The precision of the GC-MS method used for



FIGURE 2 Flowering tansies (*Tanacetum vulgare* L.) along the roadside. Photograph: Professor Niels K. Veien, Denmark

quantification of PHL was determined by 3 injections of the milk extract within 1 day (intraday variation) and on 3 different days (interday variation). The overall intraday and interday variations were <6% for PHL. The concentration of PHL in the investigated biodynamic milk samples was determined to be 0.07 ±0.004 ppm, and that in the organic milk samples was 0.05 ±0.002 ppm. PHL could not be detected in the conventional milk samples, and was either not present or was below the detection limit (<0.002 ppm).

4 | DISCUSSION

Secondary plant metabolites may occur in food items either through their presence in, or through contamination of, plant-based raw materials, or, indirectly, through their occurrence in products of non-plant origin. Toxic secondary plant metabolites, such as pyrrolizidine alkaloids, which also occur in the Compositae family of plants and are suspected of being genotoxic carcinogens, have attracted attention because of the risk of inadvertent ingestion by both livestock and humans.[17,18] In a European survey on the occurrence of pyrrolizidine alkaloids in animal-derived and plant-derived food, the metabolites were rarely detected in animal products and only in trace amounts, whereas 60% of herbal food supplements and 92% of herbal teas contained pyrrolizidine alkaloids, with a mean concentration of 460 μg/kg dry tea.[18]

Likewise, some SLs may cause livestock poisoning, and tansy is one of the plants that has been reported to be poisonous.[7] PHL is an alkylating SL, owing to an α-methylene-γ-butyrolactone moiety and an electrophilic epoxide group in its structure (Figure 1); hence, PHL is reactive towards, for example, thiols in proteins, which may explain its anti-inflammatory and cytotoxic properties in addition to its allergenic potential.[19,20] Both PHL and its synthetic analogue dimethylamino-parthenolide are promising anticancer agents.[7,21,22] In a phase I dose escalation trial in patients with cancer, PHL at a daily dose of up to 4 mg orally was well tolerated. There was no detectable concentration in the plasma, with a detection limit of 0.5 ng/mL.[23]

In the experimental study with tenulin, a bitter SL without the α-methylene-γ-butyrolactone moiety, occurring in bitter sneezeweed (*Helenium amarum* [Rafin.] H.Rock), a total of 15.5 g of the compound was given orally to a lactating cow over a period of 3 days.[8] Less than 0.1% of the tenulin appeared in the milk, as estimated by taste comparison with fresh whole milk fortified with tenulin and with a minimal detectable concentration of 0.5 ppm. The maximum concentration in the milk was 1-1.5 ppm.[8] The total dose of 15.5 g was estimated to be equivalent to the tenulin content of 2 kg of *H. amarum* plants.[8] Even though PHL, with its reactive functional groups, may be metabolized differently from tenulin, the results suggest that ingestion of relatively large quantities of SL-containing plants may be necessary to make SLs detectable in the milk.

Although only traces of PHL were detected in the biodynamic and organic milk samples, our results show an association between the time that the dairy cattle spend grazing and the amount of PHL detected. In Denmark, dairy cattle in organic farms are let out to graze from 15 April to 1 November, whereas dairy cattle from biodynamic farms are outdoors all year round, weather permitting. In general, biodynamic farmers have to follow the regulations for organic farming in addition to several more: for instance, the fodder has to be biodynamic or organic, and steaming of the soil prior to sowing, to kill weeds and their seeds, is not allowed. Thus, the biodiversity of weeds in biodynamic and organic pastures is probably greater than in conventional pastures. Furthermore, some Danish organic farmers sow herbs, including chicory (*Cichorium intybus* L., Compositae), to offer their cows a more varied feed and to obtain possible benefits of mineral supplementation and medical effects from the herbs. Some farmers have reported that the cows, especially in spring, seem to prefer herbs and leaves from bushes and trees rather than grass.[24] As cows do not have incisors in the upper part of the mouth, they use their tongues to tear off grass and herbs. Although cows allegedly prefer taste-neutral herbs, their indiscriminate way of eating means that they may inadvertently eat less tasty, bitter or withered plants, including tansy. As mentioned in the Introduction, grass and herbs growing near tansy or other PHL-containing plants may be contaminated with PHL carried on airborne plant parts. Even though the concentration of PHL in European tansy varies according to chemotype, the ubiquity of the weed suggests a role in this connection. In a study of 14 Dutch tansy samples, 6 contained no or only trace amounts of PHL, whereas the rest contained between 0.06% and 1.33% dry weight of PHL in the flower heads and 0.05% to 0.46% in the leaves.[12] Feverfew, which may have an even higher concentration of PHL, may contribute, as it is naturalized around cities, villages, and old farms.[15] Furthermore, it has been shown that plant material from tansy and other Compositae, collected from the ground and dried under glass, still contains SLs, and dried plant material such as hay used for fodder could thus be a source of SLs from weeds.[25] In addition, silage from herb fields in organic dairy farms is also used as fodder, and may contain weeds.[24]

Another possible source of exposure to PHL is watering. Cows may drink from surface water such as puddles or small lakes, as well as from ground water, including tap water. Surface water is likely to contain plant material in varying amounts, and hence also SLs. In an experimental study, Picman added the SL isoalantolactone to samples of organic and mineral soil, and, after 90 days, isoalantolactone was undetectable in organic soil and was present in trace amounts only in the mineral soil.[26] The disappearance was attributed to the trapping of the SLs by the humic material in organic soil and to microbial degradation. The author stated that large quantities of SLs could enter the soil via decomposing plant material.[26] Likewise, another experimental study on the degradation rate of the SL artemisinin from *Artemisia annua* L. (sweet wormwood) showed a 2-phase decay process, and artemisinin was detectable for 60 and 35 days in sandy and loamy soils, respectively. The soil concentration of artemisinin in a Danish field of sweet wormwood was up to 11.7 mg/kg, and, based on the water solubility of artemisinin, the SL was considered to be potentially leachable and thus capable of polluting surface water.[27] Another SL, parthenin from *Parthenium hysterophorus* L., had a half-life of 59 hours under conditions similar to those reported by Jessing et al.[28] Thus, SLs are degraded differently according to their molecular structure, and no data are available for PHL.

In Denmark, mainly synthetic pesticides are measured in groundwater.[29] It has been shown, however, that secondary plant metabolites, which, in fact, are naturally occurring pesticides used to defend the plants against herbivores and microorganisms, may occur in both surface water and groundwater.[30] This is of major concern in the case

of carcinogenic substances such as the norsesquiterpene glucoside ptaquiloside from the ubiquitous bracken ferns (*Pteridium* spp.), but the SL artemisinin is also considered to be a hazard because of its adverse effects on plants combined with its leaching potential.[27]

The most important aspect, however, is the clinical relevance of these findings. Denmark is an agricultural country, and, at the beginning of 2016, there were 3200 dairy farms, 38 of which were biodynamic. Denmark has the highest market share of organic food products in the world, and 10% of Danish milk production is organic, meaning that 1 in 3 L of milk bought is organic.[31] In general, both organic and biodynamic food are becoming more popular. The Danish Veterinary and Food Administration recommends 500 mL of milk per day for children, and between 250 and 500 mL for adults. Thus, a child or an adult drinking 500 mL of organic milk per day would be exposed to 25 μg of PHL. This may seem very modest as compared with the above-mentioned clinical study with PHL at a daily dose of up to 4 mg orally. Although not specified, these patients were probably not sensitized to PHL: none of them had cutaneous side-effects.[23] In contrast, PHL-sensitive patients may react to a patch concentration of PHL as low as 8 ng per patch.[16] Therefore, it cannot be ruled out that ingestion of organic or biodynamic milk may contribute to the cheilitis that is so often seen in Compositae-allergic individuals. Likewise, direct contact with milk, spilled or otherwise, will probably elicit flares of dermatitis in the most sensitive persons. Traditionally, milk has been used in homemade beauty products because of its alleged cleansing, moisturizing, calming and skin-lightening properties. It may be used "as is" for facial washes or as an ingredient of facial masks—and, of course, also in baths in the style of Queen Cleopatra. These practices will be risky for even moderately PHL-sensitive persons if they use organic or biodynamic milk in larger quantities.

A systemic reaction to ingested PHL in organic or biodynamic milk is less likely in view of the fact that no PHL was detectable in plasma, with a detection limit of 0.5 ng/mL, after ingestion of 4 mg of PHL. However, PHL was given as a component of commercial feverfew (*T. parthenium* [L.] Sch.Bip.) capsules, and the authors suggested poor bioavailability as the most likely explanation.[23] Other potent allergens, such as gold, may elicit systemic symptoms with oral exposure to 449.3 μg per month.[32] A daily intake of 500 mL of organic or biodynamic milk would result in a monthly dose of 750 to 1050 μg of PHL—at least in the summer. Thus, a systemic reaction cannot be completely ruled out, although metal and plant haptens probably differ significantly in their pharmacokinetics: these have not been investigated for PHL in humans.

In conclusion, this is the first report on a potent plant contact allergen detected in organic and biodynamic milk, but not in conventional milk. Although the concentration of PHL is low, it is estimated to be high enough to elicit dermatitis in the most sensitive persons by direct contact with the milk. Further studies are warranted to investigate the clinical implications, including possible systemic effects.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interests

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