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Prof. Dr. G. Eisenbrand - President

SKLM

Toxicological Assessment of Furocoumarins in Foodstuffs

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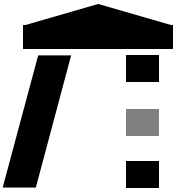

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Owing to the more frequent use of parsnips, which may contain phototoxic furocoumarins, in domestic and industrial food products, the DFG Senate Commission on Food Safety (SKLM) has studied toxicological evaluations of furocoumarins in foods and has assessed data relating to exposure, metabolism, kinetics, toxicity, carcinogenicity, reproductive and developmental toxicity, as well as the effects of these substances on xenobiotic metabolism. After reviewing the available data, the subject was discussed on 23-24 September 2004, and the following opinion was passed:

Toxicological Assessment of Furocoumarins in Foodstuffs

1 Introduction

Furocoumarins are compounds that contain a coumarin fused to a furan ring. Furocoumarins are divided into psoralens or angelicins, depending on the position of the furan ring (APPENDIX, Table 1). In combination with UVA radiation they exhibit phototoxic properties and may trigger cytotoxic and mutagenic effects (Ashwood-Smith et al., 1980; Berkley et al., 1986; Schlatter, 1988). Some compounds, such as 5- and 8-methoxypsoralen (5- and 8-MOP) are used in combination with UVA radiation in the so-called PUVA therapy (**P**soralen + **UVA)** to treat skin diseases such as vitiligo (pigment defect) and psoriasis (Schlatter, 1988).

Furocoumarins occur naturally in a number of fruits and vegetables and in cold-pressed oils from citrus fruits. Flavoured foodstuffs and cosmetic products may contain furocoumarins if these oils are used as ingredients.

Because of their aromatic, sweetish flavour, the roots of furocoumarin-containing parsnips have become more popular in domestic cooking and in convenience food products, particularly baby foods in the recent past. This has prompted the Senate Commission on Food Safety (SKLM) of the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) to assess food safety aspects of furocoumarins. This statement of the Senate Commission only addresses dietary intake of furocoumarins. On the basis of the available data furocoumarin concentrations in foods and the related exposure situation are reviewed. Metabolism, kinetics and toxicology of furocoumarins are primarily discussed based on data obtained for 5- and 8-MOP, which because of their therapeutic use are already well investigated. This is followed by a risk assessment based on the available data, by final conclusions and suggestions for further research.

2 Occurrence and concentrations

2.1 Fruits and vegetables

Furocoumarins are natural constituents of a number of plant species. They are particularly prevalent in the carrot family *(Apiaceae, Umbelliferae)*, e.g. *Ammi* (bullwort), *Pimpinella* (pimpernel), *Angelica* and *Heracleum* (hogweed), in the legume family *(Fabaceae)* and in the citrus family *(Rutaceae)* (Ramaswamy, 1975; Wagstaff, 1991). Furocoumarins are secondary plant metabolites (phytoalexins) that are produced by plants in response to attack by pests and to stressful challenge.

Edible fruits and vegetables may also contain furocoumarins, e.g. celery *(Apium graveolens L.)*, parsnips *(Pastinaca sativa)*, parsley *(Petroselinum crispum)*, carrots *(Daucus carota L.)*, oranges *(Citrus sinensis L.)*, lemons *(Citrus limon)* and limes *(Citrus aurantifolia)* (Wagstaff, 1991). Many citrus oils may contain considerable quantities of furocoumarins, such as those obtained by cold-pressing the peel of bergamot, orange, lime or grapefruit. Table 2 gives a summary of the natural furocoumarin concentrations in various foods (APPENDIX).

Furocoumarin concentrations in fruits and vegetables may vary considerably depending on the cultivation and storage conditions. The highest concentrations are found in stored samples of celery and parsnip infected by microorganisms. Furocoumarin concentrations of less than 2.5 mg/kg fresh weight (Ostertag et al., 2002) or approx. 3 mg/kg (Mongeau et al., 1994) have been found in freshly harvested, uninfected **parsnips**. Storage of such freshly harvested tubers for several weeks at -18°C did not affect the furocoumarin concentration. However, just one-week storage of the whole tubers at +4°C led to a ten-fold increase in the total furocoumarin concentration to approx. 30 mg/kg (Ostertag et al., 2002).

Microbially infected parsnips have been shown to contain concentrations of 570 mg/kg (Ostertag et al., 2002) up to 2500 mg/kg (Ceska et al., 1986b). The average concentration in retail parsnips lies between 20 and 124 mg/kg (Ivie et al., 1981; Ceska et al., 1986b; Baumann et al., 1988; MAFF UK, 1993; Ostertag et al., 2002); 8-MOP (Ivie et al., 1981) or 8-MOP and angelicin were identified as the main constituents (Baumann et al. 1988; Ostertag et al., 2002). Furocoumarin concentrations between 1.3 and 1.8 mg/kg were found in freshly harvested **celeriac tubers** (Beier et al., 1983; Chaudhary et al., 1985). Retail samples contained

furocoumarin concentrations of up to 25 mg/kg with approximately equal proportions of isopimpinellin, 5-MOP and 8-MOP (Baumann et al., 1988). Samples infected intentionally with microorganisms and subsequently stored for more than 29 days at 4°C and a relative humidity of about 75% showed a large increase in the furocoumarin concentrations from about 2 mg/kg to 44 mg/kg, about 50 % of the concentration being attributable to 8-MOP (Chaudhary et al., 1985).

In contrast, only low concentrations were found in industrially manufactured products, such as **celery salad and celery juice**, which contained up to approx. 2 mg/kg (Baumann et al., 1988), and in other processed foods, such as soups or purées, with concentrations of 0.04 to 8 mg/kg (MAFF UK, 1993). In contrast, industrially produced baby food in jars (Germany) with parsnips as the sole vegetable contained up to 12.6 mg/kg in some cases. Furocoumarins were not detected in products containing parsnips mixed with other types of vegetables (Chemisches und Veterinäruntersuchungsamt Karlsruhe, 2004 - Chemical and Veterinary Investigation Laboratory Karlsruhe, 2004).

In **citrus fruits**, most of the furocoumarins are contained in the peel. The concentrations in **lime peel** differed, depending on the variety between 334 (West Indian) and 502 mg/kg (Persian). The main constituents were 5-MOP and limettin in the latter variety and limettin in the former. The flesh of both varieties contained significantly lower furocoumarin concentrations (5-6 mg/kg), whereby isopimpinellin was the main compound (Nigg et al., 1993).

The flesh of **Seville oranges** contained approx. 13 mg/kg furocoumarins, marmalade made from Seville oranges contained approx. 2 mg/kg and lime jam contained approx. 5 mg/kg (MAFF UK, 1993). **Grapefruit juice** was found to contain approx. 2-10 mg/kg of 6',7'-dihydroxybergamottin (Tassaneeyakul et al., 2000).

2.2 Flavoured foods

Up-to-date analytical data on furocoumarin concentrations in flavoured foods are not available in the literature. The highest concentrations are expected to be found in products that contain added lime oil or bergamot oil. However, the furocoumarin concentrations in such oils differ, depending on whether they were obtained by distillation or cold-pressing.

According to the Deutscher Verband der Aromenindustrie (Association of the German Flavour Industry), approx. 1500 t/year of lime oil are produced world-wide. The majority is obtained by distillation, which, according to the producers, allows the furocoumarins to be separated. In a comparative study of cold-pressed versus distilled lime oils, furocoumarins were only detected in cold-pressed oil and not in the distilled oil. Distilled lime oil is used to flavour beverages exhibiting a taste of cola. The global production of cold-pressed lime oil is estimated to be 100 to 150 t per year. According to data from industry, lime oil has a furocoumarin concentration of 3-6 %, which is in accordance with literature values (Stanley and Vannier, 1967). The manufacturers have stated that the amount of oil used in such beverages is approx. 50 ppm, which corresponds to a furocoumarin concentration of up to 3 mg/l. More accurate information on its use in other products is currently not available.

According to relatively old information, Bergamot oil (containing up to 0.4 % 5-MOP; Opdyke, 1973) was used in the following concentrations to flavour certain foods (Furia and Bellanca, 1971): non-alcoholic beverages (9 µg/g), ice cream (8 µg/g), confectionary (27 µg/g), bakery products (29 µg/g), gelatine and dairy puddings (5 - 90 µg/g), chewing gum (43 µg/g) and icings (1 - 130 µg/g). The Commission does not have any more recent data.

3 Exposure

The furocoumarin intake may vary considerably, depending on the overall diet. The consumption of microbially infected celeriac or parsnip roots, in particular, may lead to a **maximum acute exposure**. Consumption of 200 g of such parsnips may result in intakes of up to 100 mg per person, based on a furocoumarin concentration of approx. 500 mg/kg. However, the estimated intake is approx. **4 to 10 mg** per person from 200 g of commercial parsnips or celery that have average furocoumarin concentrations of approx. 20-50 mg/kg (Table 2, Appendix).

Estimates of the **average daily furocoumarin intake** in the diet are about one order of magnitude below this value. In the USA, the average intake was estimated to be **1.3 mg per person** per day (Wagstaff, 1991). This estimate was based on the

assumption that citrus fruits, citrus juices and foods flavoured with citrus oils each contain 0.25 % citrus oils. According to this estimate, the main source of furocoumarins are limes, which make up approx. 97 % of the estimated daily intake, including soft drinks flavoured with lime oil.

In Great Britain, the daily furocoumarin intake has been estimated to be up to 0.02 mg/kg bw, corresponding to **1.2 mg** per person based on a body weight of 60 kg (COT, 1996).

In Germany, on the basis of the described database, the estimated average daily furocoumarin intake via fruit and vegetables is **0.04 mg** per person. The contribution from flavoured foods to the total intake still appears to be unclear. If it is assumed that flavoured foods only contain cold-pressed citrus oils, the estimated average furocoumarin intake via flavoured foods is about **1.41 mg** per person per day. The estimated total exposure is thus about **1.45 mg** per person per day (APPENDIX, Table 3).

4 Kinetics and metabolism

In mammals, most of the psoralens are metabolised predominantly in the liver via cytochrome P450 (CYP)-dependent monooxygenases (Bickers and Pathak, 1984). After oral intake they are almost completely absorbed in the gastrointestinal tract (Pathak et al., 1977). In mice and humans, more than 90 % of an orally administered dose of 8-MOP was found within 12 h as metabolites in the urine. The main biotransformation pathways are epoxidation, hydroxylation, glucuronide conjugation and hydrolytic opening of the lactone ring (Pathak et al., 1977; Schmid et al., 1980a; Bickers and Pathak, 1984). 5-MOP and 8-MOP bind to human serum proteins, particularly to albumin (Artuc et al., 1979). Furthermore, 5-MOP has been found to bind to low-density lipoproteins in serum (Melo et al., 1984). The main excretion route is via the kidneys, while 5-10 % are excreted via the faeces (Pathak et al., 1977).

8-MOP has been shown to undergo metabolic activation with subsequent covalent binding of the metabolites to microsomal protein (Sharp et al., 1984). The mechanism underlying this irreversible binding is thought to involve formation of an epoxyfuran or an unsaturated dicarbonyl compound that could react with the sulfhydryl or amino

groups in proteins (Mays et al., 1989). This reaction is catalysed by two or more isoforms of CYP (Mays et al., 1989) and may inhibit CYP enzymes by "suicide-inactivation" through covalent binding (Labbe et al., 1989). Further metabolites of 8-MOP are 5,8-dihydroxypsoralen and its conjugates (Mays et al., 1987).

Guinea pigs given **8-MOP** orally showed a linear relationship between the concentrations in the epidermis and in the serum, whereas those treated with **5-MOP** exhibited a non-linear relationship. Oral administration of equivalent doses of 5- and 8-MOP resulted in lower 5-MOP concentrations in the serum and epidermis as compared to 8-MOP, probably due to differences in absorption and metabolism. The observed phototoxicity correlated with the epidermal concentration (Kornhauser et al., 1984).

8-MOP given intravenously to dogs was rapidly distributed and excreted. The pharmacokinetic parameters varied considerably between individuals (Monbaliu et al., 1988).

Macaque monkeys given a dose of 0, 2, 6 or 18 mg **8-MOP**/kg bw (3 x week) exhibited non-linear kinetics as well as a saturable first-pass effect. The group with the lowest dose (3x2 mg/kg bw per week) exhibited a reduced plasma level after 26 test weeks. They were comparable to those exhibited by humans after administration of 0.4-0.6 mg/kg bw, corresponding to therapeutic doses of 8-MOP (Rozman et al., 1989).

Humans also exhibited a saturable first-pass effect (Schmid et al., 1980b; Brickl et al., 1984).

Healthy male volunteers given an oral dose of 40 mg **8-MOP** (Schmid et al., 1980 b) had a maximum plasma concentration (about 550 ng/ml) after approximately 1 h. The value had dropped to 50 ng/ml after 6 h. In another study, the furocoumarin plasma level 2 to 4 h after consumption of **300 g celery** (28.2 µg furocoumarins/g), corresponding to a dose of approx. 8.4 mg/person, was below the detection limit of 2 ng/ml. No phototoxic skin reactions occurred after UVA irradiation (Schlatter et al., 1991).

Data on oral absorption in humans indicate that bioavailability and kinetics vary greatly between individuals and cannot be predicted. After administration of therapeutic doses of 8-MOP, all investigated persons exhibited different kinetics (Herfst and De Wolff, 1982).

The pharmacokinetics of **8-MOP** administered intravenously is characterised by a rapid post infusion decrease of the plasma and blood levels, a large distribution volume and by rapid elimination (Billard et al., 1995).

5 Toxicity

5.1 Mechanisms

In combination with UVA irradiation (320 - 380 nm) furocoumarins exhibit phototoxic properties. The photochemical reactions taking place can be summarised as follows:
furocoumarins can intercalate between base pairs of DNA to form a non-covalent psoralen-DNA complex. UVA radiation both of angular furocoumarins, such as angelicin, as well as linear furocoumarins, such as psoralen or 8-MOP, leads to the formation of covalent photoadducts from these complexes. This may result in the formation of cyclobutane monoadducts with pyrimidine bases (e.g. 5,6-position of thymine) with cleavage of the 3,4 or 4',5' double bond of psoralen (Table 1, APPENDIX). Some of the 4',5'-monoadducts of linear psoralens can undergo another addition step and cross-link DNA when irradiated with UVA light. In contrast, 4',5'-monoadducts of angular compounds cannot undergo further photoreactions owing to their non-linear structure and thus are not expected to cause DNA cross-links (Musajo and Rodighiero, 1972; Dall'Acqua, 1977; Grossweiner, 1984; Dall'Acqua et al. 1984).
Furthermore, UVA irradiation may generate singlet oxygen from free or complexed furocoumarins or from the 4',5'-monoadducts (Joshi and Pathak, 1983; Grossweiner, 1984). Possible direct targets for singlet oxygen attack are membrane lipids and enzymes.
The reaction of singlet oxygen with the starting compounds, e.g. 8-MOP, leads to long-lived reactive products that bind covalently to proteins and DNA and which can initiate lipid peroxidation (Grossweiner, 1984; Midden, 1988). Furthermore, lysosome damage has also been observed (Fredericksen et al., 1989), as well as the formation of new antigens by covalent modification of proteins (Gasparro et al., 1990).

5.2 Animal toxicity

5.2.1. Acute toxicity

Data relating to acute toxicity of furocoumarins in the absence of UV light is conflicting. Doses of **8-MOP** given to rodents (mice, rats) led to LD_{50} values of 200 - 4000 mg/kg bw, depending on the formulation and route of administration (Apostolou et al., 1979; Herold et al., 1981). Values of 505 mg/kg bw were obtained for guinea pigs after oral administration (Herold et al., 1981). In the case of **5-MOP**, oral LD_{50} values of 8100 mg/kg bw were determined for mice, > 30 000 mg/kg bw for rats, and 9000 mg/kg bw for Hartley guinea pigs (Herold et al., 1981). The LD_{50} (i.p.) of **imperatorin** was 373 mg/kg bw for male mice (Booer et al., 1970). **Angelicin**, isolated from *Selinum vaginatum*, a plant from the Apiaceae family growing in the Himalayas, showed sedative, anti-convulsive and muscle-relaxing properties in rats, mice and rabbits after oral or i.p. administration. The LD_{50} for rats was 321 mg/kg (oral) or 165 mg/kg (i.p.) (Chandhoke and Ghatak, 1975).
Dogs given oral doses of 100 or 400 mg **5-MOP**/kg bw over a period of 8 days showed signs of behavioural disorders, bullous dermatitis, bilateral keratitis and reduced appetites (Herold et al., 1981[1]).

5.2.2 Sub-chronic toxicity

Daily doses of 0, 25, 50, 100, 200 or 400 mg **8-MOP**/kg bw were administered in the absence of UV light to 10 male and 10 female Fischer 344 rats (oral administration, gavage, over 90 days, 5 x per week). In all dosage groups, a dose-dependent and significant increase in the liver weight/body weight ratio was observed. Doses of 200 and 400 mg/kg bw led to increased lethality, a reduction in the body weight, lipid enrichment in the liver and adrenal glands, as well as to atrophy of the prostate, seminal vesicles and the tubuli semeniferi in the testicles (Dunnik et al., 1984; NTP, 1989).

[1] IARC, 1986, noted inadequate data report

In addition to this data, there are further studies on sub-chronic toxicity by Herold et al. (1981) that are, however, regarded as inadequate.

Oral administration of 60 mg **5-MOP**/kg bw for 28 days to dogs (beagles) resulted in anorexia and weight reduction as well as polycythemia and increased bilirubin levels in the blood 24 h after the final dose (Herold et al., 1981[1]).

One group of dogs was given oral doses of 3, 12 or 48 mg **5-MOP**/kg bw (7 x per week) for 13 weeks and another group was given oral doses of 12 or 48 mg 5-MOP/kg bw (4 x per week) for 26 weeks. Both groups showed increased liver weights, disturbance in biliary function, as well as liver necroses and inflammations (Herold et al., 1981[1]).

Wistar AF rats were given oral doses of 70, 280 or 560 mg/kg bw **5-MOP** over a year. The group given the highest dose exhibited slight changes, such as increased oedema, reduced weight gains, reduced blood urea levels and increased liver weights (Herold et al., 1981[1]). Thyroid hypofunction occurred at any early stage and persisted. Almost a third of the male animals of all dosage groups exhibited epidermoid cysts of the thyroid gland (number of control animals not stated). The females exhibited dosage-dependent proliferation of connective tissue in the region of the adrenal glands.

Male and female macaque monkeys given oral doses of 0, 2, 6 or 18 mg/kg bw 8-MOP (3 x week for 26 weeks) exhibited gastrointestinal toxicity characterised by dosage-dependent vomiting above a dosage of 3 x 6 mg/kg bw/week (Rozman et al., 1989).

5.2.3 Carcinogenicity

The carcinogenic effect on various strains of mice was studied by topical application of **5-MOP, 8-MOP and psoralen** in combination with exposure to UVA light or simulated sunlight. This led to papillomas and carcinomas of the squamous epithelium (Zajdela & Bisagni, 1981; Cartwright and Walter, 1983; Young et al., 1983).

To study the carcinogenicity of furocoumarins in rats, oral doses of 0, 37.5 or 75 mg/kg **8-MOP** were given 5 days a week for 103 weeks and in the absence of UVA

light. A carcinogenic effect was observed for male animals, with a dose-dependent increase in the incidence of tubular cell hyperplasia, adenomas and adenocarcinomas of the kidney, and of Zymbal gland carcinomas. In contrast, the females did not show any evidence of carcinogenic activity, even at the highest dosage (NTP, 1989).

5.2.4 Reproductive and developmental toxicity

A study of groups of 26 pregnant Sprague-Dawley rats given oral doses of 0, 70 or 560 mg/kg bw on days 6-15 of the gestation period revealed that, although the dose of 560 mg/kg bw led to maternal toxicity (reduced body weight), it did not lead to a significant increase in anomalies in the surviving offspring. Numbers of implantations in the uterus as well as the fetal and placenta weights were reduced (Herold et al., 1981).

Groups of 15 pregnant rabbits were given oral doses of 0, 70 or 560 mg/kg bw of 5-MOP on days 7-18 of the gestation period. The group given 560 mg/kg bw exhibited maternal toxicity (reduced body weight) (Herold et al., 1981). The finding of a dose-dependent increase in anomalies was classified by the IARC (1986) as inadequate because essential details on the type and extent of the anomalies were missing.

In another study carried out in the presence of UVA light, rats were given doses of 0, 250, 1250 or 2500 ppm 5-MOP or 8-MOP in their feed from day 21 to the birth (females) or days 21 to 61 (males). A dose-dependent reduction in the birth rate was found for female rats as well as a reduced weight gain for female and male rats. The birth weight of the offspring and the gestation period were not affected (Diawara et al., 1997 a and b). Female rats given 1250 or 2500 ppm 5-MOP or 8-MOP (corresponding to a dose of 100 or 200 mg/kg bw) in their feed from day 21 for approx. 39-49 days up to the expected birth date showed a decrease in the number of progeny. Furthermore, there were decreases in the weight of the uterus and the estradiol serum levels. During treatment, the animals were irradiated with UVA light for 45 min each day (Diawara et al., 1999).

Male rats that had received oral doses of 5-MOP or 8-MOP (0, 75 or 150 mg/kg bw) for 79 days without UVA irradiation exhibited a decrease in the weight of the pituitary

gland and in the number of sperm, as well as an increase in the relative testicle weights and the serum testosterone levels. With respect to mating, the number of pregnancies was reduced (Diawara et al., 2001).

5.3 Genotoxicity/mutagenicity

In the absence of UV light, furocoumarins are only weakly mutagenic; however, in combination with UVA radiation, 5- and 8-MOP exhibited genotoxic and mutagenic properties in various test systems (summary by IARC, 1980 and IARC, 1986).

In *in vitro* assays ***with isolated DNA***, **5-MOP** and **8-MOP** form non-covalent complexes in the dark (Dall'Acqua et al., 1978, 1979; Isaacs et al., 1984) and bind covalently if exposed to light (Musajo et al., 1966; Musajo & Rodighiero, 1972; Rodighiero et al., 1970). Photoinduction leads to "interstrand cross-links" (Dall'Acqua et al., 1979).

In microorganisms, **8-MOP** is a weak frameshift mutagen in the absence of UV light and S9 mix (Clarke and Wade, 1975; Bridges and Mottershead, 1977; Ashwood-Smith, 1978) and exhibits mutagenic properties in the presence of S9 mix (NTP, 1989). **5-MOP** is also mutagenic in the dark (Ashwood-Smith et al., 1980). **Heraclenin** and **imperatorin** have been reported to show mutagenic effects in the dark in some test systems (Ivie et al., 1980); however, no mutageniticity was observed in other studies (Schimmer and Abel, 1986). On exposure to light, 5-MOP and 8-MOP bind covalently to DNA in bacteria and yeasts (Averbeck, 1985) and have a genotoxic/mutagenic effect (Ashwood-Smith et al., 1980; Pool and Deutsch-Wenzel, 1979; Pool et al., 1982). Heraclenin and imperatorin are also genotoxic/mutagenic (Schimmer and Abel, 1986).

In mammalian cells, **8-MOP** induces mutations in the absence of UV light (Bridges et al., 1978) as well as sister chromatid exchange (SCE) and chromosome aberrations (NTP, 1989); **Heraclenin** damages the chromosomes (Abel and Schimmer, 1986). Heraclenin and imperatorin are potentially clastogenic in the dark (Abel and Schimmer, 1986). When exposed to light, **5-MOP** and **8-MOP** bind covalently to DNA (Papadopoulo and Averbeck, 1985) and cause mutations (Loveday and Donahue, 1984), "interstrand cross-links" (Papadopoulo and Averbeck, 1985) and SCE

(Loveday and Donahue, 1984; Natarajan et al., 1981; Abel and Mannschedel, 1985; Abel et al., 1985).

In vitro experiments on human lymphocytes with **isopimpinellin** in combination with UVA radiation did not result in SCE, but it did show weak clastogenic potential, similar to 8-MOP. In contrast to 8-MOP/5-MOP, however, incubation with isopimpinellin resulted in atypical chromosomes (Abel et al., 1985).

In **in vivo** experiments, oral doses of 8-MOP (300 and 600 mg/kg) induced micronuclei in peripheral erythrocytes in mice (Stivala et al., 1995).

5.4 Human toxicity

There have been several reports of humans showing acute phototoxic effects after oral intake of furocoumarins in combination with sunlight or UVA light.

In a study on volunteers, for example, oral doses of 50 mg 8-MOP caused erythemas and oedemas after exposure to sunlight (Fitzpatrick and Pathak, 1984).

Severe skin burns (erythema, oedemas and blisters) were observed after consumption of approx. 450 g celery followed by approx. 30 min exposure to UVA radiation on a sunbed. The estimated intake of psoralen was 45 mg (Ljunggren, 1990).

The threshold dose (oral) for the occurrence of erythemas in humans (in combination with sunlight) was reported to be 14 mg 8-MOP (approx. 0.23 mg/kg bw for a body weight of 60 kg; Brickl et al., 1984). On the basis of exposure tests, a threshold value for the phototoxic effect in adults (in combination with UVA) was estimated to be 10 mg 8-MOP + 10 mg 5-MOP or 15 mg 8-MOP equivalents (0.25 mg/kg bw for 60 kg bw) (Schlatter et al.,1991).

Most of the available information on the toxicity of psoralens in humans was obtained from studies of psoriasis patients or patients with other skin diseases, such as vitiligo. The therapeutic oral dose used to treat psoriasis is **500** - **600 µg 8-MOP/kg bw** or **1200 µg 5-MOP/kg bw** in combination with UVA (0.5 - 7 J/cm^2, wavelength range 315-400 nm, maximum at 355 nm). Outdoor exposure of naked skin for 5-30 minutes between 10 a.m. and 14 p.m. is probably sufficient, even in winter, to reach the UVA dose generally used in PUVA therapy (Schlatter, 1988).

In the prospective PUVA follow-up study on 1380 orally treated psoriasis patients, oral therapeutic doses of **psoralen + UVA** (PUVA) were associated with a dose-dependent increase in the incidence of squamous epithelium carcinomas (Stern et al. 1998), basal cell carcinomas (Stern et al.,1998; Katz et al., 2002) and melanomas (Stern et al., 2001). The group of 892 men in the study also exhibited a dose-dependent increase in genital tumours (Stern et al., 2002).

5.5 Impact on xenobiotic metabolism

6',7'-Dihydroxybergamottin and its dimeric furocoumarin derivatives, which are found e.g. in grapefruit juice, are highly potent inhibitors of cytochrome P450 (CYP) 3A and other CYP isoenzymes that play a central role in the metabolism of many drugs (Tassaneeyakul et al., 2000; review by Evans, 2000). Imperatorin and isopimpinellin proved to be e.g. inhibitors of CYP 2B, whereas bergamottin and coriandrin inhibited the activity of CYP 1A1 and 1A2 in the liver. Furthermore, bergamottin inhibited the enzyme activity of CYP 3A (Wen et al., 2002). Therefore, the consumption of typical amounts of grapefruit juice may increase the bioavailability or the maximum plasma concentrations or the elimination half-life of certain drugs (review by Bailey et al., 1998). Furthermore, this may inhibit CYP-dependent activation of drugs from the prodrug into its effective form.
In mice, pretreatment with juice pressed from celery or parsley lengthened the pentobarbital sleep time (Jakovljevic et al., 2002).

6 Assessment

It is not possible to specify a no observed effect level for the repeated intake of furocoumarins. In sub-chronic studies of dogs, 48 mg 5-MOP/kg bw/day was still hepatotoxic. In monkeys, a dose of 6 mg 8-MOP/kg bw/day still led to gastrointestinal toxicity (vomiting). Both, 5-MOP and 8-MOP are genotoxic. In a 2-year study of 8-MOP in rats, even the lowest tested dose of 37.5 mg/kg bw/day was nephrotoxic and carcinogenic. According to Brickl et al. (1984), the lowest furocoumarin dose in

combination with UVA that led to detectable phototoxic effects in adult humans is approx.14 mg 8-MOP, corresponding ton about 0.23 mg/kg bw for 60 kg bw or, according to Schlatter et al. (1991) 10 mg 8-MOP + 10 mg 5-MOP corresponding to 15 mg 8-MOP equivalents (0.25 mg/kg bw for 60 kg bw).

The average daily furocoumarin intake via foods was estimated to be 1.3 mg (USA) or maximum 1.2 mg (Great Britain) per person, which corresponds to 0.020 - 0.023 mg/kg bw (Wagstaff, 1991; COT, 1996). An initial estimate for Germany, based on the assumption that only distilled citrus oils are used to flavour foods, arrives at a significantly lower average daily intake of approx. 0.04 mg per person. However, if this estimate is based on the exclusive use of cold-pressed citrus oils in flavoured food, the resulting average daily intake is approx. 1.45 mg per person, which is similar to the value estimated for the USA.

These intakes calculated from the average consumption of furocoumarin-containing foods lie approximately two to three orders of magnitude below the lowest doses reported as being toxic in animal studies of sub-chronic and chronic toxicity. They are closer to the values of therapeutic doses of 0.5 - 0.6 mg 8-MOP/kg bw (factor of 30) and to the lowest phototoxic dose of 0.23 mg/kg bw (factor of 10).

A similar result is obtained from an estimation not based on average consumed quantities in all foods contained in the diet, but based on the consumption of 200 g celery or parsnips containing the highest furocoumarin concentrations found in retail goods, namely 25 mg/kg (celery) or 50 mg/kg (parsnips). In this case as well, the consumed quantities of furocoumarins lay between 5 and 10 mg, which are still, although to a lesser extent, below the lowest known phototoxic dose for adults of 14 mg (8-MOP) or 20 mg (8-MOP + 5-MOP). Owing to matrix effects, it can be assumed that the bioavailability of furocoumarins from foods is lower than if pure furocoumarins are administered in an isolated form, as was the case in studies to derive the phototoxic dose. The phototoxic dose for children is not known.

The situation for infants requires separate consideration. The tubers of parsnips and celeriac as well as parsley roots are being used as vegetables more frequently in baby foods, both in domestic cooking and in industrially manufactured convenience products. Exploratory studies were carried out on baby foods in glass jars available on the German market containing parsnips as the sole vegetable. In some cases, furocoumarin concentrations of up to approx. 13 mg/kg were found (Chemisches und Veterinäruntersuchungsamt Karlsruhe, 2004). The consumption of 200 g of such a

product with the highest analysed concentration thus would correspond to a furocoumarin intake of approx. 2.5 mg. For a body weight of 7 kg, this would correspond to a dose of approx. 0.36 mg/kg bw, higher than the lowest phototoxic dose known for adults.

For baby food cooked at home, it is not expected that the intake reaches phototoxic doses of furocoumarins provided that freshly harvested or frozen parsnips are used. However, toxic effects cannot be ruled out if incorrectly stored parsnips are used. A meal including around 100 g of parsnips with a high furocoumarin concentration (50 mg/kg) would result, for example, in the intake of 5 mg furocoumarins, which corresponds to a dose of approx. 0.71 mg/kg bw for a child weighing 7 kg.

Particular care should be taken concerning storage and preparation of home-cooked foods and avoiding consumption of celeriac and parsnips stored for long periods or even mouldy vegetables. For example, storage of parsnips for 53 days at room temperature with initial mould infection increased the furocoumarin concentration to approx. 500 mg/kg (Ostertag et al., 2002).

There is insufficient data available to estimate the risk of a carcinogenic effect of furocoumarins contained in the diet. However, studies on the increase of certain types of skin cancer after PUVA therapy suggest that excessive quantities of furocoumarins in the diet in combination with UVA radiation could increase the risk of skin cancer. Evidence for this was obtained from epidemiology of PUVA treatment. A dose-dependent increase in skin tumours was observed after long-term oral doses at phototoxic levels in combination with therapeutic UVA treatment. In contrast, the additional risk of skin cancer is regarded as insignificant for the consumption of furocoumarin-containing foods that remain significantly below the range of phototoxic doses.

Furocoumarins can also influence xenobiotic-metabolizing enzymes that play a role in drug metabolism with corresponding consequences for the effectiveness of the drug. Thus the intake of furocoumarins via e.g. grapefruit juice can lead to an increase in the bioavailability of the drug. Corresponding warnings should thus be included in patient information sheets.

In summary, the SKLM concludes that the consumption of typical quantities of correctly stored, processed foods that may contain furocoumarins does not represent a significant risk for the occurrence of phototoxic effects. However, for celery and parsnips, in particular, there is a risk for significant increases in

furocoumarin concentrations, depending on the storage, processing and production conditions. In such cases, the consumption of phototoxic quantities cannot be ruled out for these foods. There is still not enough data available to estimate the risk presented by foods flavoured with citrus oils. A final estimation of the carcinogenic risk is currently not possible due to the complexity of the influencing factors, particularly the levels of exposure, the metabolism and how it is affected, as well as the influence of light. The additional risk of skin cancer arising from the consumption of typical quantities of furocoumarin-containing foods, which remain significantly below the range of phototoxic doses, is regarded as insignificant. The consumption of large quantities of incorrectly stored tubers as well as the consumption of extreme quantities should be avoided, particularly by children.

7 Research needs

There is a need for further research concerning factors responsible for increased furocoumarin formation in raw vegetables. This applies, in particular, to studies on the influence of storage and manufacturing conditions on furocoumarin concentrations. Priority should be given to checking and promoting preventive measures based on food technology, crop farming and plant breeding that are aimed at reducing furocoumarin concentrations, particularly in baby foods.

With respect to the toxicological significance of furocoumarins in the diet, urgent issues include clarification of absorption, metabolic pathways and excretion. The mechanisms of action and dose-response relationships of toxic/genotoxic effects must be elucidated with respect to individual influencing factors. Finally, studies should be carried out on potential combination effects of different furocoumarins that may occur together in foods. The influence of dietary furocoumarins on the metabolism of drugs and other xenobiotics should also be investigated.

There still is a lack of up-to-date analytical data on the occurrence and concentrations of furocoumarins in citrus oils, particularly in lime oil, and on the foods to which they have been added.

8 Literature

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APPENDIX

Table 1: Examples of psoralen- and angelicin-type furocoumarins

(Schlatter, 1988; Römpp, 1995)

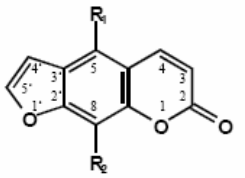
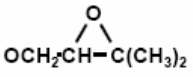
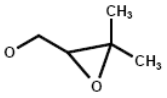
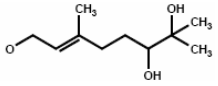
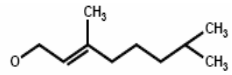
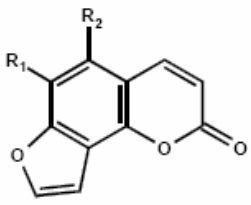
		R_1	R_2
Type of psoralen 	Psoralen (P)	H	H
	5-Methoxypsoralen; Bergapten (5-MOP)	OCH_3	H
	8-Methoxypsoralen; Xanthotoxin (8-MOP)	H	OCH_3
	Isopimpinellin (IP)	OCH_3	OCH_3
	Imperatorin (I)	OCH_2-CH=$C(CH_3)_2$	H
	Heraclenin (H)		H
	Oxypeucedanin (O)		H
	Phellopterin (Ph)	OCH_3	O-CH_2-CH=$C(CH_3)_2$
	6,7'-Dihydroxy-bergamottin (DHP)		H
	Bergamottin (B)		H
Type of angelicin 	Angelicin (A)	H	H
	Pimpinellin (Pi)	OCH_3	OCH_3
	Isobergapten (IB)	H	OCH_3
	Sphondin (S)	OCH_3	H

Table 2: Examples of furocoumarin concentrations in foods

Type of food	Furocoumarin concentration [mg/kg]	Analyzed furocoumarins	References
Celery, freshly harvested	up to 1.3	P, IP, 5-MOP, 8-MOP	Beier et al., 1983
	1.8	P, 5-MOP, 8-MOP	Chaudhary et al., 1985
Celery, retail	up to 25.2	P, 5-MOP, 8-MOP	Baumann et al., 1988
	0.08 - 0.24	5-MOP	Avalos et al., 1995
	0.9 - 8	P, 5-MOP, 8-MOP	MAFF UK, 1993
Celery, intentionally infected with microorganisms	43.8	P, 5-MOP, 8-MOP	Chaudhary et al., 1985
Celery salad	0.3 - 0.7	P, 5-MOP, 8-MOP	Baumann et al., 1988
Celery juice	0.9 - 2.2	P, 5-MOP, 8-MOP	Baumann et al., 1988
Parsnips, freshly harvested	1 - 2.5	P, A, IP, 5-MOP, 8-MOP	Ostertag et al., 2002
	3.3	P, A, IP, 5-MOP, 8-MOP	Mongeau et al., 1994;
Parsnips, retail	up to 49	P, A, IP, 5-MOP, 8-MOP	Ostertag et al., 2002
	43 (edible parts)	P, A, 5-MOP, 8-MOP	Baumann et al., 1988
	20 - 48	P, 5-MOP, 8-MOP	MAFF UK, 1993
Parsnips, infected with microorganisms	up to 570	P, A, IP, 5-MOP, 8-MOP	Ostertag et al., 2002
	up to 400	P, A, IP, 5-MOP, 8-MOP	Mongeau et al., 1994
Baby food, glass jars containing parsnips	0.06 - 0.41	P, A, IP, 5-MOP, 8-MOP	Ostertag et al., 2002
	0 - 12.6	P, A, IP, 5-MOP, 8-MOP	Chemisches & Veterinäruntersuchungsamt Karlsruhe, 2004
Industrial products e.g. soups, purées	0.04 - 8	P, 5-MOP, 8-MOP	MAFF UK, 1993
Parsley (flat and curly leafed)	11.4 - 14.6	P, 5-MOP, 8-MOP (A: nn)	Baumann et al, 1988
	38	P, 5-MOP, 8-MOP	MAFF UK, 1993

Parsley roots	1.3	5-MOP, 8-MOP (P, A: nn)	Baumann et al, 1988
Carrots	0.02	5-MOP, 8-MOP	Ceska et al., 1986a
Seville oranges (flesh)	13	P, 5-MOP, 8-MOP	MAFF UK, 1993
Limes (Persian variety)			Nigg et al., 1993
Peel	502	P, 5-MOP, 8-MOP, IP, L	
Flesh	6	P,5-MOP, 8-MOP, IP, L	
Limes (West-Indian variety)			Nigg et al., 1993
Peel	334	5-MOP, IP, L (P, 8-MOP: nn)	
Flesh	5	5-MOP, IP, L (P, 8-MOP: nn)	
Orange marmalade (Seville oranges)	2	P, 5-MOP, 8-MOP	MAFF UK, 1993
Lime marmalade	5	P, 5-MOP, 8-MOP	MAFF UK, 1993
Grapefruit juice	2-10	DHP	Tassaneeyakul et al., 2000
Lemon oil	33	5-MOP	Shu et al., 1975
Lime oil	46 700	B, I, II, IP, Ph, O, CAS 69239-53-8, CAS 71612-25-4	Stanley and Vannier, 1967
	1 700 -3 300	5-MOP	Cieri, 1969
Grapefruit oil	120	5-MOP	Shu et al., 1975
Bergamot oil	up to 3900 3 000-3 600	5-MOP 5-MOP	Opdyke, 1973 Cieri, 1969

A = angelicin, B = bergamottin, DHP= 6',7'-dihydroxybergamottin, I = imperatorin, II = isoimperatorin, IP = isopimpinellin, L = limettin, 5-MOP = bergapten, 8-MOP = xanthotoxin, P = psoralen, Ph = phellopterin, O = oxypeucedanin hydrate

APPENDIX

Table 3
Proposed exposure estimate
The exposure was estimated according to the following suppositions:

- the average furocoumarin concentrations in foods [µg/g] given in Table 2
- the average consumed quantities for all age groups, consisting of men and women, as well as the maximum quantity consumed by one age group (95th percentile)
  is given by [1]
- citrus fruits, citrus juices and food flavoured with citrus oils contain 0.25 % citrus oils (based on Wagstaff, 1991).

	Foodstuff[1]	**Average consumption of all age groups**[1] **[g/day]**	**Average maximum consumption [g/day]**[2]	**Average furocoumarin concentration (from Tab. 2) [µg/kg]**	**Average/maximum furocoumarin intake**[3] **[µg/person/day]**
Non-flavoured foods	**Carrots**	7.9	40.8	0.02	**0.2/** 0.8
	Misc. fresh vegetables	9.95	57.75		
	of which acc. to [4]				
	parsley	1.31		13	**17**
	celery	0.41		17	**7**
	parsnips	0.1		58	**6**
	Frozen vegetables	3.8	33.9		
	of which 20% estimated to be carrots	0.76	6.78	0.02	**0.02**/ 0.1
	Vegetable conserves	14.6	63,5		
	of which 20% estimated to be carrots	2.92	12.7	0.02	**0.06**/ 0.3

	Oranges of which[6] 0.25% orange oil	13.25 0.03	96.0 0.24	 **0.5** [5]	 **0.02**/ 0.1
	Misc. citrus fruits estimated to contain: 1% limes with 0.25% lime oil 30% lemons with 0.25% lemon oil 20% grapefruit with 0.25% grapefruit oil	7.5 0.07 0.000175 2.24 0.0056 1.49 0.003725	50.4 0.5 0.00125 15.1 0.03775 10.1 0.025	 46 700 33 120	 **8**/58 **0.2**/1.3 **0.5**/3
	Fruit/vegetable juices estimated to contain: 10% grapefruit juice with 0.25 % grapefruit oil 60 % orange juice with 0.25 % orange oil	80.5 8.05 0.02 48.6 0.12	521.5 52.2 0.13 312 0.78	 120 0.5	 **2**/ 16 **0.06**/ 0.4
	sum of dietary furocoumarin intake via non-flavoured foods [µg/person/day]				**41/**110
	Miscellaneous confectionary (minus ice-cream, honey, chocolate) estimated to contain: 0.25 % citrus oils with 1% lime oil 1 % bergamot oil 98 % lemon oil	2.5 0.006 0.0006 0.0006 0.0058	29.05 0.07 0.007 0.007 0.0686	 46 700 3 900 33	 **28**/330 **2**/27 **0.2**/ 2 Σ **30**/ 360
	Caffeine-containing soft drinks estimated to contain:	48.9	490.85		

	0.25 % citrus oils with 10% lime oil 87 % lemon oil 3 % bergamot oil	0.12 0.012 0.104 0.004	1.23 0.123 1.07 0.04	 46 700 33 3 900	 **560** / 5740 **3**/35 **16**/156
					Σ **579**/ 5930
	Misc. **soft drinks** estimated to contain: 0.25 % citrus oils with 10% lime oil 87 % lemon oil 3 % bergamot oil	60.45 0.15 0.015 0.13 0.005	569.9 1.42 0.142 1.24 0.04	 46 700 33 3 900	 **700**/6631 **4**/ 41 **20**/156 Σ **724**/6830
	Bakery products/pastries: estimated to contain: 0.25 % citrus oils 1% lime oil 1 % bergamot oil 98 % lemon oil	55.8 0.14 0.0014 0.0014 0.137	183.3 0.46 0.0046 0.0046 0.45	 46 700 3 900 33	 **65/**215 **5**/ 15 **6**/ 18 Σ **76**/248
	Sum of dietary furocoumarin intake via flavoured foods [µg/person/day]				**1409**/13 370
	Overall sum of dietary furocoumarin intake via flavoured and non-flavoured foods [µg/person/day]				**1 450** / 13 500

[1] Classification of the foodstuffs according to the literature: Standards zur Expositionsabschätzung, Arbeitsgemeinschaft der leitenden Medizinalbeamtinnen und -beamten der Länder, published by: Behörde für Arbeit, Gesundheit und Soziales, Hamburg (1995), Pages 94-95, 98-99. The data was acquired for the study without differentiation between vegetarians and non-vegetarians

[2] 95th percentile

[3] Average or maximum consumed amounts for both sexes, mean
[4] Baumann et al., 1988
[5] Supposition based on calculations by Wagstaff, 1991
[6] Supposition that all citrus fruits contain 0.25 % citrus oil; see also Wagstaff, 1991