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Poppy seeds in food: The content of opium alkaloid thebaine should be reduced as much as possible

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Thebaine is a component of the latex of the opium poppy plant. Like other opium alkaloids morphine, narceine or codeine, it is produced in the capsule as well as in the stems and leaves. The mature seeds of opium poppy, which are used as food, do not contain any naturally occurring opium alkaloids. However, when harvested by machine, poppy seeds may come into contact with the alkaloid-containing latex. “Thereby, edible poppy seeds may become contaminated with larger amounts of thebaine and other opium alkaloids. This has been shown in studies conducted by a regional authority of a German federal state (“Land”) and in data from the European Food Safety Authority EFSA.

The German Federal Institute for Risk Assessment (BfR) has provisionally assessed the health risk of thebaine concentrations in edible poppy seed and in food containing poppy seed. Since currently, only very few toxicological data are available on thebaine and information on consumption of poppy seed in Germany is incomplete, no conclusive assessment could be made. Therefore, the BfR utilized the TTC concept for the preliminary assessment of thebaine. This involves assignment of substances by means of their chemical structure to a corresponding substance class. Maximum intake levels are determined for each of these substance classes (TTC values) on the basis of extensive toxicological data of structurally related compounds. Below these TTC values, the occurrence of adverse health effects is considered to be less probable according to the TTC concept. When applying the TTC concept, it should be noted that an exceedance of the TTC value does not allow any precise conclusions to be drawn about the actual risk. If the TTC value is exceeded, this means that toxicological data is required to assess the actual risk. This model calculation done by the BfR for thebaine exposure shows that the respective TTC value was exceeded in almost all scenarios for intake of the substance, considered by the BfR .

In view of the currently insufficient data and unanswered questions, the BfR recommends to reduce the content of all opium alkaloids in poppy seeds used in food production - including those of thebaine - as far as technically feasible. Since EFSA's exposure assessment has shown that the acute reference dose (group ARfD) for morphine and codeine may be exceeded in humans of all ages, especially in the case of poppy seeds containing high levels of morphine and codeine, the BfR recommends to avoid excessive consumption of foods with a high poppy seed contents, especially during pregnancy (BfR 2018). In light of the indications for a potential risk through thebaine intake via poppy seed consumption and the lack of data on the hazard potential of thebaine, which could be used to clarify these points, this recommendation appears meaningful, too. Moreover, the BfR points out that the existing gaps in our knowledge about the consumption of poppy seeds in Germany and the toxic properties of thebaine should be filled by conducting studies to perform aconclusive assessment of possible health risks.

1 Subject of the assessment

The German Federal Institute for Risk Assessment (BfR) was asked by the Federal Ministry of Food and Agriculture (BMEL) to assess the content of opiates and, in particular, that of thebaine in poppy seeds, determined by a regional authority of a German federal state ("Land") in the years 2017 and 2018.

2 Result

There are very few toxicological data on thebaine. Studies with oral administration are only available for acute toxicity. Moreover, there is a lack of reliable data on poppy seed consumption, especially with regard to high short-term consumption. Therefore, based on currently available data, a conclusive assessment of the health risks that may result from oral intake of thebaine via poppy seed consumption is not possible.

The robustness of some of the available studies dealing with thebaine's potential hazards is also limited.IHowever, in sum, the findings suggest that the pharmacological effects of thebaine may differ from those of other opiates, such as morphine. Morever, the effects mediated by thebaine may be based on other modes of action. This does not support the inclusion of thebaine in the existing group ARfD (Acute Reference Dose) for the opium alkaloids morphine and codeine recently derived by EFSA.

The available toxicological data suggest that, after acute exposure, thebaine poses a higher toxic potential than morphine or codeine. From the point of view of the BfR, the range between intake levels, which can potentially be reached short term through consumption of poppy seeds with high thebaine concentrations, and the dose lethal for 50% of the treated mice (LD_{50}) is not very large.

Due to the lack of appropriate data to a risk assessment using the TTC concept (*threshold of toxicological concern*) was considered. According to the exposure model calculation, both the TTC value derived for genotoxic carcinogens as well as the TTC value for substances in "Cramer Class III" are exceeded in almost all exposure scenarios. When applying the TTC concept, it should be noted that exceeding the TTC value does not allow any precise conclusions to be drawn about the actual risk. However, an exceedance, requires toxicological data on the basis of which the *margin of safety* can then be calculated. In addition, this would require reliable studies on poppy seed consumption.

In a commentary on the current EFSA-Opinion on Opium Alkaloids in Poppy Seeds (EFSA 2018), the BfR has already recommended to investigate the hazard potential of further opiates, such as thebaine, which occur in poppy seeds beside morphine and codeine, as well as to lower the levels of pharmacologically active opium alkaloids in poppy seeds to the technologically achievable minimum. Since EFSA's exposure assessment has shown that the group ARfD (Acute Reference Dose) for morphine and codeine may be exceeded in humans of all ages, especially in the case of poppy seeds containing high levels of morphine and codeine, the BfR discourages excessive consumption of foods with a high poppy seed contents, especially during pregnancy (BfR 2018). Also in the context of the potential risk through thebaine intake via poppy seed consumption and the lack of data on thebaine's hazard characterization, which could be used to clarify these points, this recommendation appears meaningful

3 Rationale

3.1 Risk assessment

3.1.1 Hazard identification (agent)

Thebaine (paramorphine, IUPAC name: (5*R*,9*R*,13*S*)-3,6-dimethoxy-*N*-methyl-4,5-epoxymorphine-6,8-dien, empirical formula: $C_{19}H_{21}NO_3$; CAS number: 115-37-7) is a pharmacologically active alkaloid formed in the opium poppy (*Papaver somniferum* L., family: *Papaveraceae*) (EFSA 2011b, 2018). In addition to morphine and other alkaloids such as codeine or narceine, it is a constituent of opium, the dried latex of immature poppy capsules (BfR 2006; Hager ROM 2006). High contents of latex and the alkaloids contained therein can be found i.a. in the roots, stems, leaves and especially in the poppy capsules (BfR 2006; Hager ROM 2006; EFSA 2018). The total alkaloid concentration in the opium poppy depends on different factors, such as the variety or cultivar, the location and the harvest time (BfR 2006; Hager ROM 2006). For pharmaceutical use, opium and opium alkaloids are mainly obtained from the capsules and the poppy straw of opium poppy (mostly cultivars with high morphine or alkaloid concentrations). However, the mature poppy seeds are usually obtained from alkaloid-poor varieties and are used i.a. for the production of various foods, such as oils, baked goods (e.g. poppy seed rolls, poppy seed cake) and desserts (e.g. poppy seed pies) (BfR 2006; EFSA 2018). Although the seeds of opium poppy are the only part of the plant not containing latex (Frohne & Pfänder 2004), opium alkaloids, such as morphine, codeine and thebaine, are also found in mature poppy seeds, albeit in relatively small quantities (Hager ROM 2006; EFSA 2011b, 2018).

A possible explanation for the occurrence of opium alkaloids in poppy seeds could be that the capsules are squeezed during mechanical harvesting, leading to contamination of the seeds with latex (BfR 2006). In this context, It should also be borne in mind that different cultivars of opium poppy are grown around the world for different purposes, e.g. for pharmaceutical use or food production (BfR 2006; EFSA 2018). In Europe, especially in Germany and Austria, particularly cultivars low in alkaloids are grown exclusively for the extraction of poppy seeds, which are then used i.a. for the production of baked goods and oil. Likewise, in some countries, such as Spain, Hungary, Bulgaria and Australia, alkaloid-rich varieties are grown for pharmaceutical use (BfR 2006; EFSA 2018). Some of these varieties are used simultaneously for the production of opium alkaloids and for the production of poppy seeds (BfR 2006; Hager ROM 2006), which could lead to poppy seeds with comparatively high alkaloid concentrations entering the food chain.

In addition to poppy seeds with high concentrations of morphine (EFSA 2011b, 2018), samples with particularly high concentrations of other alkaloids, such as thebaine or codeine have recently been identified (Sproll *et al.* 2016; EFSA 2018). In this context, it has been stated in several publications that it should be examined whether the intake of these alkaloids may pose a potential health risk to consumers (Sproll *et al.* 2016; BfR 2018; EFSA 2018).

3.1.1.1 Key results from previous opinions

Possible health risks from the oral intake of thebaine have already been addressed in previous opinions on health risks of opium alkaloids in poppy seeds, which were published by the European Food Safety Authority (EFSA) and the German Federal Institute for Risk Assessment (BfR) (BfR 2006; EFSA 2011b; BfR 2018; EFSA 2018).

In its 2006 Opinion, the BfR concluded that a final assessment of health risks, which may be associateded to the intake of thebaine through poppy seed consumption, is not possible due to insufficient data (BfR 2006).

In its recent opinion, EFSA stated that the results of animal studies indicate a higher acute toxicity of thebaine compared to morphine (EFSA 2018). However, it was emphasised that no adequate scientific studies were available to comprehensively assess the sensitive endpoints for the toxicity of thebaine after oral intake. Regarding thebaine's mode of action, EFSA reported that activation of μ-opioid receptors (ORs) would occur only at very high concentrations, but further noted that to some extent thebaine could be metabolised to other opiates such as morphine or oripavine. In turn. these metabolites can lead to morphine-like effects via the activation of μ-OR ("*morphine-like toxicity*"). This contribution, however, is considered to be low (EFSA 2018). Due to insufficient data, EFSA concluded that it was not possible to fully assess the health risks that could be associated with the oral intake of thebaine. In this context, EFSA has identified needs for, in particular, toxicological and toxicokinetic studies as well as for concentration data on thebaine and other opium alkaloids in foods containing poppy seeds (EFSA 2018).

Recently, the BfR commented on EFSA's Opinion (EFSA 2018), noting that the hazard characterisation of other opiates, such as thebaine, which occur in poppy seeds in addition to morphine and codeine, should be considered. The BfR recommended in this communication to make every effort to reduce the contents of pharmacologically active opium alkaloids in poppy seeds to the technologically achievable minimum. Since EFSA's current exposure assessment has shown that the group ARfD (Acute Reference Dose) for morphine and codeine may be exceeded in humans of all ages, especially in the case of poppy seeds containing high levels of morphine and codeine, the BfR discourages excessive consumption of foods with a high poppy seed contents, especially during pregnancy (BfR 2018).

3.1.2 Hazard characterisation

The typical effects of opiates, such as analgesia, sedation and respiratory inhibition, are mediated via agonistic effects on μ-, δ- and κ-OR found in both the central and peripheral nervous system. Since the three receptor subtypes in part mediate different effects[1], the specific activity profile of a substance is determined i.a. by which receptor a particularly high activity exists (Aktories *et al.* 2009). An *in vitro study* by Zhang *et al.* (2012) showed that morphine is a potent full agonist of the μ-OR and an equally potent partial agonist of the κ-OR. However, activation of δ-OR was induced by morphin only at higher concentrations. Compared to morphine, the activation of μ- or κ-OR by codeine was less pronounced and occurred only at higher concentrations. It did not cause activation of δ-OR.

Data from different studies indicate that unlike morphine, thebaine has no or only limited analgesic effect, does not act in a morphine-like manner or even counteracts the effect of morphine, respectively (Gilbert & Martin 1978; UNODC 1980; Kettenes-van den Bosch *et al.* 1981). In contrast to codeine, and morphine in particular, thebaine did not show any activation of μ-OR in the study by Zhang *et al.* (2012) and exhibited only very low effects on the activation of other ORs. The results of a mouse study performed by Aceto *et al.* (1999), showing that naturally occurring thebaine exhibits a very low affinity for μ- and δ-OR, substantiated the *in vitro* findings (Aceto *et al.* 1999). An earlier receptor binding study from Nikolaev *et al.* (2007) demonstrated that the affinity of thebaine to μ-OR is about 1000 times

[1] Action profile of OR according to Aktories *et al.* (2009):
μ-OR: Analgesia (+++), euphoria (+++), sedation (++), respiratory inhibition (+++), inhibition of gastrointestinal motility (++)
δ-OR: Analgesia (+), respiratory inhibition (++), inhibition of gastrointestinal motility (++)
κ-OR: Analgesia (++), dysphoria (+++), sedation (++), inhibition of gastrointestinal motility (+)

lower than that of morphine. A study by Goldinger *et al.* from 1981 gives an indication of how thebaine might mediate its pharmacological effects (Goldinger *et al.* 1981). In the described studies on rat nerve or brain tissue, an antagonistic effect of thebaine on glycine or γ-aminobutyric acid (GABA) receptors was identified, which was significantly stronger than that of morphine or codeine. The inhibitory effect was reported as IC_{50}, (inhibitor concentration at which fifty percent inhibition occurs). For thebaine, the IC_{50} (in relation to the effect of strychnine) was 1.0 µM for glycine receptors and 300 µM (in relation to the effect of GABA) for GABA receptors. The levels for morphine were significantly higher (IC_{50} = 40 µM for glycine receptors and 1300 µM for GABA receptors, respectively). The authors concluded from these data that the effect of thebaine in the nervous system could be mediated in particular by the inhibition of glycine receptors and is comparable to the effect of strychnine (Goldinger *et al.* 1981). In a publication by Sproll *et al.* (2016) this view is shared and linked to the convulsive effect of thebaine. However, the studies mentioned above are subject to some uncertainties, such as the limited size of the experimental groups, the lack of appropriate controls, or insufficient information on the purity of the substances used.

In sum, the available experimental data indicate that thebaine's mode of action may differ from that of other opiates, such as morphine and codeine. However, more robust studies are needed to allow a conclusive assessment of the mode of action.

3.1.2.1 Toxicokinetics

The availability of information regarding the toxicokinetic properties of thebaine is limited. With regard to bioavailability after oral intake, the BfR has no relevant data available.

However, it is known that thebaine is extensively metabolised in the gastrointestinal tract (GIT), in the liver and in other tissues, e.g. demethylated and glucuronidated (EFSA 2011b, 2018). For example, studies on rats or rhesus monkeys revealed that thebaine administered subcutaneously (s.c.) is metabolised to various metabolites, including oripavine as the major product, as well as norcodeine, codeine and morphine in lower levels (Misra *et al.* 1973, 1974; Yamazoe *et al.* 1981). These results were confirmed in various *in vitro* and *ex vivo* studies (Yamazoe *et al.* 1981; Kodaira & Spector 1988; Mikus *et al.* 1991). *In vitro* experiments on rat cells (from the liver and brain) showed that O-demethylation mediated by enzymes of the cytochrome P450 (CYP) superfamily (CYP2D1, 2D6, 3A4 and 3A5) play an important role in the metabolism of thebaine i.a. to oripavine (Mikus *et al.* 1991; Guengerich *et al.* 2010; Kramlinger *et al.* 2015).

A broad tissue distribution was described for thebaine after parenteral administration. It was detected in various organs, such as the stomach, large intestine and brain. Moreover, it is excreted mainly via urine or to a lesser extent via faeces (Misra *et al.* 1973, 1974; Yamazoe *et al.* 1981; Donnerer *et al.* 1986; Enginar *et al.* 2013).

3.1.2.2. Toxicity

At present, only few information is available about thebaine's hazard potential. Most data came from older studies (around 1960 to 1985), mostly investigating effects after s.c. administration. Effects of thebaine after oral administration were investigated only in few studies. In particular, excitatory neurological effects have been observed in most animal studies (EFSA 2011b).

3.1.2.2.1 Experimental data on toxicity

Acute toxicity

Available data indicate that thebaine has a higher acute toxicity than morphine (UNODC 1980; EFSA 2018). The dose at which 50% of testanimals die within a certain time after a single administration (lethal dose 50%, LD_{50}) was significantly lower for thebaine (oral: LD_{50} = 54 mg/kg BWT in mice; LD_{50} = 114 mg/kg body weight (BWT) in rats) than for morphine (oral: LD_{50} = 524 mg/kg BWT in mice; LD_{50} = 335 mg/kg BWT in rats) or codeine (oral: LD_{50} = 250 mg/kg BWT in mice; LD_{50} = 427 mg/kg BWT in rats) (summarised in (EFSA 2018). Determination of the LD_{50} values for other application pathways (s.c. or intraperitoneal (i.p.)) led to comparable results (UNODC 1980; Kettenes-van den Bosch *et al.* 1981; EFSA 2018). High acute toxicity was also observed in other studies. In this context, intravenous (i.v.) administration of 2.5 mg/kg BWT thebaine in rabbits led to seizures and rapid death of the animals (Navarro & Elliott 1971; Navarro *et al.* 1976).

Chronic toxicity (including carcinogenicity), genotoxicity and immunotoxicity

No reliable data are available for these endpoints.

Developmental / reproductive toxicity

In 1975, Geber and Schramm performed a study on hamsters to investigate possible teratogenic effects of thebaine (Geber & Schramm 1975). On day 8 of pregnancy, dams were treated with different doses of thebaine (0, 110, 140 and 193 mg/kg BWT, respectively) administered s.c. as single dose . In contrast to controls, this let to increased mortality of dams in the two highest dose groups (0% at 0 and 110 mg/kg BWT, 10% at 140 mg/kg BWT, 75% at 193 mg/kg BWT). Viability of the offspring (foetuses) was not impaired. However, there was a dose-dependent increase in the occurrence of malformations in the offspring (0% at 0 and 110 mg/kg BWT, 2% at 140 mg/kg BWT, 4.2% at 193 mg/kg BWT). In the highest dose group, only cranial obstruction (cranioschisis) was observed (Geber & Schramm 1975). However, due to the strong maternal toxicity, the validity of these findings was classified as very low.

Neurotoxicity

Thebaine causes stimulating effects, especially in the central nervous system, such as an increased irritability and reflex excitability as well as an increased motor activity in mice, rabbits and dogs (UNODC 1980). In this regard, Navarro & Elliott (1971) demonstrated that the administration of relatively low doses of thebaine to cats (2 - 2.5 mg/kg BWT; i.p.) or to rabbits (0.4 - 1.5 mg/kg BWT; i.v.) led to an increase in the respiratory rate. In cats, higher doses of thebaine (12.5 mg/kg BWT, i.p.) led to a significant increase in brain activity, increased voiding and defecation as well as increased salivation. In rabbits, thebaine doses of 2.5 mg/kg BWT (i.v.) caused severe convulsions, consequently leading to death of the animals (Navarro & Elliott 1971). In particular, the convulsive effect of thebaine after parenteral administration was also observed in further experimental studies in other species, such as mice, rats, frogs, rhesus monkeys or dogs (Pinto Corrado & Longo 1960; McClane & Martin 1967; Gilbert & Martin 1975; UNODC 1980; Tortella *et al.* 1984).

Observations in humans

At present, little information is available about the effects of thebaine on humans.

Thebaine itself is not used therapeutically, but serves as a precursor in the production of other drugs such as codeine (EFSA 2011b, 2018). So far, there is no information regarding toxic

effects of thebaine in humans in legal applications. Furthermore, no cases of thebaine abuse were known until 1980 (UNODC 1980). There is only one publication from 2016 relating to a case of poisoning (Martinez *et al.* 2016). It describes the death of a man (32 years, also suffering from epilepsy) associated with the consumption of poppy capsules and the included opiate-containing latex (in an unknown amount) for the purpose of euphoria. This resulted in seizures and respiratory arrest, which consequently led to the man's death. At the autopsy, thebaine (2.81 mg) and morphine (1.25 mg) were found in the man's gastrointestinal tract, leading the authors to the conclusion that the cause of death was *"poly-drug toxicity"* with a predominant role of thebaine and morphine. They also discussed that the victim's pre-existing medical condition of epilepsy might also have contributed (Martinez *et al.* 2016). Further data on possible effects of thebaine in humans are not available.

3.1.3 Exposure

The currently available toxicological data are insufficient to conclusively characterize the hazard potential of thebaine. Furthermore, the currently available data on poppy seed consumption are insufficient to adequately estimate the intake levels. For these reasons, no exposure assessment representative for Germany was included in this opinion. Instead, based on model calculations only potential intake levels of thebaine via poppy seed consumption were pointed out.

Data from in the current EFSA opinion on opium alkaloids in poppy seeds (EFSA 2018) on intake levels in the German population were utilized as **consumption data** of poppy seeds for model calculations. These data refer to the current German consumption studies in children and adults (VELS, ESKIMO, NVS II). However, it should be noted that poppy seed is a foodstuff whose assessment in consumption studies is accompanied by methodological difficulties. This is due to the fact that poppy seeds are no frequently / regularly consumed foods. This results in a lower probability that poppy seed consumption events were within the survey period, compared to regularly consumed foods. For example, a one-off high intake of poppy seed cake is a consumption event relevant to the assessment of the risk of acute exposure, but under-represented in short-term surveys.

The use of poppy seeds as a food ingredient also contributes to uncertainty. The poppy content in food is usually unknown and can only be estimated with corresponding uncertainties. Greater uncertainty further results from inadequate specification of foods in consumption studies. For example, some consumption studies only record if people have eaten a bread roll. Specification of the bread roll is not requested, systematically or otherwise, so it is left to chance, whether the respondent mention this additional information (poppy seed role) unsolicited or not. This leads to a systemic underestimation of the proportion of poppy seed-containing foods.It should also be taken into account that poppy seed consumption is subjected to strong seasonal fluctuations. For example, in Germany, poppy seeds are traditionally consumed at Christmas time. An non consideration of seasonal variations and the resulting potentially higher levels of consumption leads to a possible underestimation of the acute exposure percentile.

Taken together, these uncertainties may potentially represent an underestimation of high intake levels on single days, which are relevant for the acute risk assessment. Therefore, model calculations show only potential intake levels of thebaine via poppy seed consumption.

Poppy seed consumption is present in all three studies in Germany (VELS, ESKIMO, NVS II). However, the recorded consumption days per age group with N=2 (0.02%/ VELS, infants) to N=151 (0.7%/ NVSII, adults) are very low. Accordingly, it was only possible to calculate a

95th percentile for two age groups (adults aged ≥18 to <65 years and children aged 36 months to <10 years). Therefore, only the consumption data of the ESKIMO study (group: "*other children*" aged 36 months to <10 years) and NVS II (group: "*adults*") were used for the model calculation, because sufficient data were reported in these studies for both the medium and the high consumption. The data can be found in Annex B of the above-mentioned EFSA opinion. For adults, an average long-term consumption of about 23 g of poppy seeds and a high short-term consumption of about 55 g of poppy seeds per person per day are assumed for the model calculation. For children, however, an average long-term consumption of about 5 g of poppy seeds and a high short-term consumption of about 19 g of poppy seeds per person are assumed for the model calculation.

A plausibility check of the high short-term consumption levels can be made using conventional recipes. Using 250 g of poppy seeds for a cake and assuming that 12 pieces are made, an adult consuming 2 to 3 pieces of poppy seed cake would be equivalent to eating about 42 to 63 g of poppy seeds, while a child consuming one piece corresponds to almost 21 g of poppy seeds. These consumption levels largely correspond to the high short-term consumption levels of about 55 g in adults and about 19 g in children.

In the BfR opinion from 2006, on the other hand, a *worst-case* scenario was assumed where in the short-term adults consume up to 150 g of poppy seeds and children up to 75 g of poppy seeds by eating poppy seed cake (BfR 2006). This consumption would also lead to about 3 to 4 times higher intake levels of thebaine.

For calculation of the intake per kg of bodyweight, a standard bodyweight of 70 kg is assumed for adults and a standard bodyweight of 23 kg for children (EFSA 2012a). Again, it should be noted that the use of standard values is associated with additional uncertainties. The intake levels of thebaine via poppy seed consumption determined within the model calculation can be seen in Tables 1 and 2, with the acute high intake highlighted in grey. The intake estimates must be interpreted against the background of the aforementioned uncertainties.

The **concentration data** for thebaine in poppy seeds that form the basis of the model calculation are also taken from the recently published EFSA opinion. In its opinion, EFSA separates concentration data by country of origin with UN authorisation for poppy cultivation for pharmaceutical applications (*high-morphine group*) and a group of countries of origin that are authorised exclusively for food consumption (*low-morphine group*). The data used here do not take account of this distinction, but rather reflect the average of all concentration data, since it is not known which varieties are available on the German market. In addition, thebaine concentrations (68 samples from the years 2017 and 2018) provided by CVUA Sigmaringen are also taken into account.

For both adults and children, the possible long-term thebaine intake is calculated using average thebaine levels and mean poppy seed intake.

Furthermore, an estimatimation was done for the acute exposure to thebaine via poppy seed consumption. In principle, a high intake (95th percentile) is assumed and for calculation the thebaine levels in the 95th percentile and maximum concentrations are used.

Table 1. *Model calculation for intake of thebaine via poppy seeds for adults (age ≥18 to <65 years; only consumers; N=151 consumption days).*

Data basis for thebaine concentration in poppy seeds		Thebaine concentration in poppy seeds* [mg/kg]	Consumption# Poppy seeds [g]	Thebaine intake [µg/kg BWT]
EFSA 2018 (a) (N=356)	Average	15.8	23.1	5.2
	P95	101	54.9	79
	Max.	783	54.9	614
EFSA 2018 (b) (N=869)	Average	12.6	23.1	4.2
	P95	64.0	54.9	50
	Max.	455	54.9	357
CVUA Sigmaringen (N=68)	Average	6.13	23.1	2.0
	P95	27.3	54.9	21
	Max.	54.7	54.9	43

EFSA 2018 (a): EFSA concentration data, collected up to the year 2011
EFSA 2018 (b): EFSA concentration data, collected from the year 2011
* Concentration values in *upper bound*
# Average consumption levels (white) or consumption in 95th percentile (grey), based on data from NVS II for adults
N=Number of samples

Table 2. *Model calculation for intake of thebaine via poppy seeds for children (age 36 months to <10 years; only consumers; N=61 consumption days).*

Data basis for thebaine concentration in poppy seeds		Thebaine concentration in poppy seeds* [mg/kg]	Consumption# Poppy seeds [g]	Thebaine intake [µg/kg BWT]
EFSA 2018 (a) (N=356)	Average	15.8	5.4	3.7
	P95	101	18.8	83
	Max.	783	18.8	640
EFSA 2018 (b) (N=869)	Average	12.6	5.4	2.9
	P95	64.0	18.8	52
	Max.	455	18.8	372
CVUA Sigmaringen (N=68)	Average	6.13	5.4	1.4
	P95	27.3	18.8	22
	Max.	54.7	18.8	45

EFSA 2018 (a): EFSA concentration data, collected up to the year 2011
EFSA 2018 (b): EFSA concentration data, collected after the year 2011
* Concentration values in *upper bound*
# Average consumption levels (white) or consumption in 95th percentile (grey), based on data from the ESKIMO study
N=Number of samples

Conclusion from exposure analysis

The EFSA data can be used to first consider possible scenarios for the intake of thebaine, but they are associated with considerable uncertainties and may represent an underestimation of the high single-dose intake levels that are relevant for acute risks. To reduce the uncertainties, a specific survey on the consumption of poppy seed-containing foods would be required. However, this is not possible in the short-term. Therefore, the shown intake levels have to be considered as potential values and do not provide a basis for a conclusive assessment.

3.1.4 Risk characterisation

There are very few toxicological data on thebaine. Studies with oral administration are only available for acute toxicity. There is also a lack of reliable data on poppy seed consumption, especially with regard to high short-term consumption. Therefore, based on currently available data, a conclusive assessment of the health risks that may result from oral intake of thebaine via poppy seed consumption is not possible.

The robustness of some the available studies on thebaine's hazard potential is also limited. However, in sum, the data suggest that the pharmacological effects of thebaine may differ from those of other opiates, such as morphine. The effects mediated by thebaine may also underlie other modes of action. Older studies, which robustness have to be considered as limited, provide evidence that the effects of thebaine may be mediated via inhibition of glycine and GABA receptors. Inhibition of these receptors would also match the observed convulsive effects. However, due to limited robustness of these older studies, this mode of action cannot be considered as certain.

Animal studies indicate that thebaine can be converted to a small extent i.a. into codeine and morphine. Overall, the inclusion of thebaine in the group ARfD (Acute Reference Dose), which were recently derived by EFSA for the opium alkaloids morphine and codeine, appears to be of little value with regard to the available data and evidence of divergent, potentially counteractive modes of action.

Available toxicological data suggest that thebaine has a greater acute toxicity than morphine and codeine. However, the data do neither allow the identification of the most sensitive endpoint for the toxic effects of thebaine nor is it possible to establish reference points such as NOAEL (*no observed adverse effect level*), LOAEL (*lowest observed adverse effect level*) or BMDL (*benchmark dose lower confidence*), which are usually used for deriving health-based guidance values. Accordingly, no *margin of safety* can be calculated. Therefore, hereafter, only the potential intake levels resulting from different scenarios for long-term or short-term exposure are compared with the oral LD_{50} from a mouse study (Tables 3 and 4). Comparison of the LD_{50} (acute toxicity) with intake levels of thebaine, which can be achieved after short-term high intake (P95) of poppy seeds with high thebaine levels (P95 and maximum values) is of particular relevance, whereby a high intake (P95) of poppy seeds with a maximum of measured thebaine concentration would be regarded as a *worst-case* scenario.

Table 3. *Deviation between LD_{50} (54 mg/kg BWT) and the thebaine intake in adults (aged ≥18 to <65 years; only consumption; N=151 consumption days)*

Data basis for thebaine concentration in poppy seeds	Thebaine concentration in poppy seeds* [mg/kg]	Consumption# Poppy seeds [g]	Thebaine intake [µg/kg BWT]	Deviation from LD_{50} [Factor]

EFSA 2018 (a) (N=356)	Average	15.8	23.1	5.2	10,385
	P95	101	54.9	79	684
	Max.	783	54.9	614	88
EFSA 2018 (b) (N=869)	Average	12.6	23.1	4.2	12,857
	P95	64.0	54.9	50	1,080
	Max.	455	54.9	357	151
CVUA Sigmaringen (N=68)	Average	6.13	23.1	2.0	27,000
	P95	27.3	54.9	21	2,571
	Max.	54.7	54.9	43	1,256

EFSA 2018 (a): EFSA concentration data, collected up to the year 2011
EFSA 2018 (b): EFSA concentration data, collected after the year 2011
* Concentration values in *upper bound*
# Average consumption levels (white) or consumption in 95th percentile (grey), based on data from NVS II for adults
N=Number of samples

Table 4. *Deviation between LD_{50} (54 mg/kg BWT) and the thebaine intake in children (aged 36 months to <10 years; only consumption; N=61 consumption days).*

Data basis for thebaine concentration in poppy seeds		Thebaine concentration in poppy seeds* [mg/kg]	Consumption# Poppy seeds [g]	Thebaine intake [µg/kg BWT]	Deviation from LD_{50} [Factor]
EFSA 2018 (a) (N=356)	Average	15.8	5.4	3.7	14,595
	P95	101	18.8	83	651
	Max.	783	18.8	640	84
EFSA 2018 (b) (N=869)	Average	12.6	5.4	2.9	18,621
	P95	64.0	18.8	52	1,038
	Max.	455	18.8	372	145
CVUA Sigmaringen (N=68)	Average	6.13	5.4	1.4	38,571
	P95	27.3	18.8	22	2,455
	Max.	54.7	18.8	45	1,200

EFSA 2018 (a): EFSA concentration data, collected up to the year 2011
EFSA 2018 (b): EFSA concentration data, collected from the year 2011
* Concentration values in *upper bound*
# Average consumption levels (white) or consumption in 95th percentile (grey), based on data from the ESKIMO study
N=Number of samples

For example, based on the EFSA concentration data collected after 2011, taking into account high concentrations (P95) and high short-term intake levels (P95), the model calculations for exposure yield intake levels for thebaine of approximately 50 µg/kg BWT for both adults and children. This corresponds to a deviation of about a factor of 1,000 from the oral LD_{50} for mice. Using the CVUA Sigmaringen concentration data instead of the EFSA concentration data with otherwise the same approach, result in a thebaine intake of about 22 µg/kg BWT in both adults and children. The resulting deviations from LD_{50} are around a factor of 2,500. Using the maximum measured thebaine concentration (described by EFSA) to map a *worst-case* scenario, deviations from LD_{50} may fall below a factor of 100 (Tables 3 and 4). Furthermore, if the short-term consumption levels of 150 g in adults and 75 g in children postulated in the BfR opinion (BfR 2006) as *worst-case* were taken as the basis, the deviations from LD_{50} after short-term exposure to poppy seeds with high thebaine concentrations would be about 3 to 4 times lower than indicated in the table.

It is to be expected that LD_{50} is clearly in the effect range, since adverse effects in subchronic and chronic toxicity studies are usually observed even at significantly lower doses compared to LD_{50}. In addition, data on the dose-response course of lethality or other adverse effects are not available. An assessment of safe intake levels based solely on the LD_{50} would not be toxicologically robust. The range between intake levels, which can potentially be reached in the short-term through the consumption of poppy seeds with high thebaine concentrations, and the dose that is lethal for 50% of the treated mice is, in the perpective of the BfR, not very large.

Due to the lack of appropriate data to characterise the hazard potential of thebaine, a risk assessment using the TTC concept (*threshold of toxicological concern*) was considered. In the TTC concept, substances for which no or only insufficient toxicological data are available are assigned to a corresponding substance class on the basis of their chemical structure. On the basis of extensive toxicological data on structurally related compounds, maximum intake level (TTC values) were derived for each of these substance classes, before which the occurrence of adverse health effects is considered to be less probable (EFSA 2012b).

Following to the Benigni/Bossa (Benigni *et al.* 2008) rules contained in the Toxtree software (v2.6.13), thebaine would be considered a potentially genotoxic substance due to the included alkenylbenzene structure and have to be grouped into the substance class for which there are structural indications of a possible genotoxic potential according to the decision criteria of Kroes *et al*. (2004). For such substances, a TTC value of 0.0025 µg/kg BWT was derived (EFSA 2012b). However, the alkenylbenzene structure present in thebaine differs sterically from the structure of other alkenylbenzenes, such as safrole or estragole, which are genotoxic and carcinogenic in animal studies. Therefore, the reliability of predicting the genotoxic potential of thebaine solely on the basis of this structural feature is questionable.

Assuming that thebaine has no genotoxic activity, the alkaloid would be classified, with regard to its potential toxicity, within “Cramer Class III” according to the Cramer, Ford and Hall decision criteria set forth in the Toxtree software (v2.6.13) (Cramer *et al.* 1978). These substances have a TTC value of 1.5 µg/kg BWT (EFSA 2012b).

According to the exposure model calculation, both the TTC value derived for genotoxic carcinogens and the TTC value for substances in “Cramer Class III” are exceeded in almost all exposure scenarios. Only, when based on the average thebaine concentration of 6.13 mg/kg of poppy seeds measured by CVUA Sigmaringen and assuming average intake levels of 5.4 g of poppy seeds in the model calculation yields for children weighing 23 kg led to a thebaine intake of 1.4 µg/kg BWT, which is just below the TTC value of 1.5 µg/kg BWT. When applying the TTC concept, it should be noted that exceeding the TTC value does not allow any precise conclusions to be drawn about the actual risk. However, an exceedance requires toxicological data on the basis of which the *margin of safety* can then be calculated.

In order to avoid exceeding the TTC value of 1.5 µg/kg BWT for “Cramer Class III” substances, it was calculated that the thebaine concentrations have to be less than 1.9 mg/kg poppy seed (for adults) or 1.8 mg/kg poppy seed (for children), assuming a high short-term intake of 54.9 g poppy seeds in adults (70 kg BWT) or 18.8 g poppy seeds in children (23 kg BWT). Taking into account higher levels of poppy seed consumption, as assumed in the BfR opinion of 2006 as a *worst-case*, the concentrations would have to be correspondingly lower.

Comparing the possible exposure determined via model calculation with the relevant TTC value indicates a potential health risk through the intake of thebaine via consumption of poppy seeds. However, regarding its nature and scope, the currently available data are not suitable to fully characterise the potential risk.

3.2 Other aspects

3.2.1 Quality of available data

Overall, only few data on thebaine's hazard potential are available. In this context, the available information comes mainly from older studies offering only limited robustness. Currently, it must be assumed thatthebaine poses a greater toxic potential after acute exposure than morphine or codeine. However, basing of the available data, a precise characterisation of the course of the dose-response is not possible. In addition, no information is available about other potential adverse effects. Therefore, no reference points can be determined for the derivation of health-based guidance values.

Further uncertainties arise regarding to exposure to thebaine, since in particular the amounts of poppy seed intake found in consumption studies are subject to various uncertainties.

4 Risk management options

At a minimum, the following studies would be required to ensure more robust risk characterisation:

- analyses of genotoxicity of thebaine according to the EFSA guideline "*Scientific opinion on genotoxicity testing strategies for food and feed safety assessment*" (EFSA 2011a).
- Investigations allowing the identification of relevant toxicological endpoints and the derivation of reference points for the establishment of health-based guidance values. Basing on preliminary considerations, a study on subchronic toxicity in rodents appears to be suitable. In this case, additional parameters, in particular for neurotoxicity, should be taken into account for examining the effects of such opium alkaloids. Depending on the results, further studies may be required.
- Toxicokinetic studies
- Robust studies to adequately estimate the short-term and long-term consumption of poppy seeds.

In a commentary on the current EFSA-Opinion on Opium Alkaloids in Poppy Seeds (EFSA 2018), the BfR has already recommended to investigate the hazard potential of further opiates, such as thebaine, which occur in poppy seeds beside morphine and codeine, as well as to lower the levels of pharmacologically active opium alkaloids in poppy seeds to the technologically achievable minimum. Since EFSA's exposure assessment has shown that the acute reference dose (group ARfD) for morphine and codeine may be exceeded in humans of all ages, especially in the case of poppy seeds containing high levels of morphine and codeine, the BfR recommends to avoid excessive consumption of foods with a high poppy seed contents, especially during pregnancy (BfR 2018). In light of the indications for a potential risk through thebaine intake via poppy seed consumption and the lack of data on thebaine's hazard potential, which could be used to clarify these points, this recommendation appears meaningful, too

Further information on food supplements is available from the BfR website

https://www.bfr.bund.de/cm/343/bfr_empfiehlt_vorlaeufige_maximale_taegliche_aufnahmemenge_und_einen_richtwert_fuer_morphin_in_mohnsamen.pdf

https://www.bfr.bund.de/cm/343/gehalte-an-pharmakologisch-aktiven-opiumalkaloiden-in-mohnsamen-sollten-auf-das-technologisch-erreichbare-mindestmass-gesenkt-werden.pdf

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