European Industrial Hemp Association (EIHA) paper on:

Comparison of EFSA's rationale behind using uncertainty factors for plant ingredients in food

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Hürth (Germany), November 2016

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Take home messages

- Especially compared to caffeine (5.7 mg/kg bw is the EFSA-limit, even though anxiety can already occur at 3 mg/kg bw), nicotine (no LOAEL-NOAEL correction there) and alcohol (no risk assessment was performed for alcohol in food, even though research shows that it is warranted), THC is treated unfairly.

- WHO/EFSA advise an UF of 10 for interindividual differences, this was not done for amygdalin (histopathological changes) and tocopherol (deceleration of heart rate) which use UFs of 4.74 and 2 respectively.

- There seems to be a trend to have lower UFs for other substances compared to THC even though their endpoints are more severe. The mild and transient effects of THC should not be held to the same UF-guidelines as the severely adverse and possibly terminal effects opium alkaloids can have.

- **Actual adherence to EFSA's own guidelines and advice in practice would mean a total UF of 10 for THC.** This UF takes interindividual differences into account and does not use a LOAEL-NOAEL-UF (similar to nicotine). Based on the better data quality of THC compared to e.g. thujone and THC's mild, transient effect, the UF could be further reduced (compare with tocopherol and amygdalin using 2 and 4.74 respectively).

Purpose of this paper and approach

EIHA assigned nova-Institute the task of analyzing how uncertainty factors are applied to various botanicals by EFSA, to possibly detect a political bias towards THC-levels in food. To do this, nova contacted several experts at EFSA and BfR, and analyzed various scientific and EFSA/BfR papers with regards to which endpoints were used for deriving the NO(A)EL (no observed adverse effect level) and LO(A)EL (lowest observed adverse effect level).

A second priority was to see which uncertainty factors were applied and what the justification of using these were. Moreover, nova analyzed the guidelines published by BfR, EFSA and WHO for the risk assessment of botanicals and whether those were actually applied in their analyses of botanicals. In the next step, we analyzed the scientific literature regarding uncertainty factors and general toxicological measurements to detect possible pitfalls and variability in their application in a regulatory context. Additionally, we suggest several approaches how the risk assessment for potentially harmful substances could be improved by e.g. using a more refined methodology of NOAEL-measurements and better standardization of risk assessments comparable to the ones performed for chemicals.

Summary of the extensive table shown below

The substances in the table below were randomly selected, apart from the fact that we wanted to use EFSA journal entries starting from 2009 and we also included caffeine, nicotine and alcohol because they have a comparably long tradition in western cultures as cannabis.

The table clearly shows, that although advise from EFSA and WHO exists regarding the use of UFs, a lot of arbitrariness exists and there seems to be a trend to have lower UFs for other substances compared to THC.

In the case of tropanalkaloids a UF of 10 was selected "for the derivation also takes adequate account of possible increased sensitivity that could exist in infants, pregnant and breastfeeding women, unborn babies, sick people and the elderly, for example." This is in contrast to cyanide where sub factors where used and tocopherol where an UF of 2 for interindividual differences was applied.

Sometimes (e.g. for thujone) an extra UF is used for the fact that the NOAEL studies are based on few animals (statistical significance questionable), old studies, non-

Table 1: Summary comparison of health based guidance values (HBGV) and uncertainty factors (UF) for various inherent plant toxins and food contaminants (and vitamins; references can be found in the extensive table 2).

Substance	Uncertainty factor (UF)	Comment
THC	30	Factor of 10 was used for interindividual differences and 3 for the extrapolation of NOAEL from LOAEL measuring mood alteration
Opium alkaloids	3	Extrapolation of LOEL to NOEL was considered in the UF but neither the interindividual differences nor interactions between the alkaloids or that codeine gets metabolized to morphine
Tropanalkaloid	10	UF accounts for interindividual differences in the NOAEL measuring e.g. deceleration of heart rate
Vitamin E (alpha tocopherol)	2	UF is for interindividual differences for the NOAEL-end-point of blood clotting
Caffeine	0	EFSA does use any UFs for its guidance value of 5.7 mg/kg bw even though anxiety and behavioural changes already occur at 3 mg/kg
Alcohol	n/a	No EFSA risk assessment. Interestingly alcohol can cause dizziness in children starting from 1.5 g alcohol and apple juice can contain 0.77 g/l and a roll 1.2 g/100 g
Coumarin	100	The NOAEL for hepatotoxicity was measured in dogs so according to WHO/EFSA guidelines an UF of 10 was used for interspecies differences and another 10 for interindividual differences
Cyanide/ amygdalin	4.74	The UF is comprised of a toxicodynamic subfactor and 1.5 for women and children
Thujone	500	The NOAEL for convulsions and seizures was measured in mice so according to WHO/EFSA guidelines an UF of 10 was used for interspecies differences and another 10 for interindividual differences. An extra 5 was used for poor data quality (which was not done for e.g. vanillin)
Menthol	50	UFs and rationale behind them were not directly mentioned, also different NOAELs (for changes in body weight) were cited, ranging from 200-600 mg/kg bw. The UF of 50 is based on 200 mg/kg bw
nicotine	4.4	NO correction for LOAEL to NOAEL extrapolation. Correction factor of 0.44 because they used a study where they injected nicotine even though a 2006 study of smoked nicotine also exists

adherence to GLP/GMP guidelines, studies which were not originally designed to assess toxicity or studies where only two measurements were taken (e.g. effects tested at 50 mg and 500 mg of a substance). The difference between acute and chronic studies is also not always taken into account in the EFSA opinions. The studies used for THC are more recent compared to the ones mentioned for thujone.

From a methodological perspective, one could also question whether the relatively simple and transient adverse effects of THC should be held to the same UF-guidelines as the severely adverse and possible terminal effects opium alkaloids can have. Severity and transient vs. permanent effects should be accounted for to avoid comparing apples with oranges.

Polymorphisms of hepatic enzymes were only specifically mentioned in the THC case. Generally speaking, the studies, the EFSA-THC-opinion is based on, are relatively new and based on humans. Moreover, studies exist which show that children have less CB-Rs so they wouldn't be more vulnerable. This provides and additional argument to Renwick et al. (1998) showing that children are not necessarily more susceptible, which in turn raises the question whether an extra UF for children should be employed. Moreover, statistical analyses of UFs exist which show that they can be too conservative (Pieters et al., 1998; Gaylor and Godell, 2000).

It is interesting that EFSA has not performed a risk assessment at all for alcohol in food. Nonetheless, dizziness in children can occur with 100 mg/1 or 1.5 g alcohol. But one burger roll can contain 1.2 g/100 g and apple juice 0.77 g/1 (see table for reference).

For caffeine no UFs were proposed even though the adverse endpoints are the most comparable to THC. EFSA advised a daily intake of 400 mg even though children and adolescent have 1-13 mg/kg bw for the endpoints "sleep deprivation, withdrawal symptoms and tolerance". In the general population anxiety symptoms can occur at a caffeine amount of 3 mg/kg bw, but EFSA suggests 5.7 mg/kg bw (=400 mg caffeine daily). Therefore, its limits are **above** the LOAEL. They only suggest 200 mg/day for pregnant women which translates to 2.85 mg/kg bw, which is still close to caffeine levels, which can elicit anxiety.

In only 2 cases the UFs were higher than for THC (not taking into account the additional factor when limits were set based on studies in animals). In case of thujone, which causes seizures and convulsions, the higher UF seems to be justified based on the severity of the effects.

It might be worthwhile to consider the whole risk assessment formula, seen as we only analyzed NOAEL variability and UF arbitrariness here. It remains to be seen if similar randomness on EFSA's side can be detected when exposure scenarios are thoroughly analyzed.

Even though it might be unrealistic, Europe should have its own food laboratory (using strict GLP/GMP procedures) which can perform up-to-date NOAEL tests itself and according to an agreed-upon/ standardized set of endpoints and methods (including a factor which takes into account differences in endpoint severity), perhaps comparable to doping test procedures. This should lead to better comparability of the limits set by EFSA and could render the UF more or less redundant. As a short term solution a study should be issued, ideally by EFSA to determine the NOAEL in humans.

Taking all of these inconsistencies into account (e.g. mild transient effects of cannabis compared to the handling of caffeine, nicotine and alcohol with similar short-term mild transient effects, relatively good data quality for cannabis) and by trying to streamline (no LOAEL-NOAEL-extrapolation-UF for nicotine) the different EFSA opinions with EFSA's own guidelines, **a total UF for THC of 10 would be justified.**

Literature

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WHO (2008): Harmonization Project Document No. 6.

Overview of NOAEL and uncertainty factor combinations for selected botanicals and food contaminants by EFSA

Table 2: Comparison of health based guidance values (HBGV) and uncertainty factors (UF) for various inherent plant toxins and food contaminants (and vitamins).

Substance	HBGV	HBGV based on...	UF	UF based on...	Nature of adverse effect	Reference
WHO guideline on botanical substances	ARfD (acute effects) or ADI/TDI (chronic effects)	NOAEL	100	10 = interspecies differences 10 = interindividual differences	varies	WHO 2008 Harmonization Project Document No. 6
EFSA guideline on botanical substances	Depending on nature of the effect	NOAEL (or BMD, LO(A)EL)	100	10=interspecies differences 10=interindividual differences (including genetic variability, correction for more susceptible groups e.g. children) or inter-species variability in toxicokinetics: 4.0 · for inter-species variability in toxicodynamics: 2.5 · for intra-human variability in toxicokinetics: 3.16 · for intra-human variability in toxicodynamics: 3.16	varies	EFSA Scientific Committee. (2012). Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA journal, 10(3), 2579. Draft of updated versions indicates same values: https://www.efsa.europa.eu/en/consultations/call/150618
THC	ARfD = 1 ug/kg bw	LOAEL = 36 ug/kg bw in humans	30	3 = extrapolation of NOAEL from LOAEL ("The CONTAM Panel concluded that an UF of 3 is sufficient to allow for extrapolation from the LOAEL to a NOAEL considering that the LOAEL is based on effects of low or moderate severity.) 10 = interindividual differences ("interindividual differences because although the data on adverse effects are partly derived from studies in patients with severe diseases, data on adverse effects in infants and children are not available and there are interindividual differences in metabolism (CYP2C polymorphism).")	mood alteration and sedation ("are the most sensitive endpoint and thus, these dose-response relationships are most suitable for the derivation of the ARfD for Δ9-THC.")	EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2015. Scientific Opinion on the risks for human health related to the presence of tetrahydrocannabinol (THC) in milk and other food of animal origin. EFSA Journal 2015;13(6):4141, 125 pp. doi:10.2903/j.efsa.2015.4141
Opium alkaloids	ARfD = 10 ug/kg bw	LOEL = 30 ug/kg bw	3	"an uncertainty factor of 3 was sufficient to allow for extrapolation from the LOEL to a no observed effect level (NOEL), considering that the LOEL was derived from patients and not from the general population." BUT no alkaloid interactions taken into account and the fact that codeine gets metabolized to morphine	no dose-response relationship for poppy containing foods known⇒ lowest known single therapeutic dose for treatment of pain or dyspnoea	Panel, E. C. (2011). Scientific Opinion on the risks for public health related to the presence of zearalenone in food. EFSA J, 9(2)
Tropanalkaloid (hyoscyamine, scopolamine)	ARfD = 0.016 ug/kg bw	NOAEL= 0.16 ug/kg bw in humans	10	Interindividual differences (includes susceptible groups e.g. unborn children, breastfeeding, elderly); "The Panel decided to apply an uncertainty factor of 10 for interindividual differences to allow for the fact that this was a small study in young healthy male volunteers."	deceleration in the heart rate and CNS effects, such as drowsiness,headaches and nausea	Beuerle, T., Benford, D., Brimer, L., Cottrill, B., Doerge, D., Dusemund, B., & Mulder, P. P. J. (2013). Scientific Opinion on Tropane alkaloids in food and feed. EFSA Journal, 11(10), 1-113
Vitamine E (alpha-tocopherol)	UL = 300 mg/day (rounded fr. 270 mg/day)	NOAEL= 540 mg/day in humans	2	Interindividual differences ("A larger uncertainty factor was not considered necessary because data from a number of other older but less well controlled studies showed no adverse effects at considerably higher intakes.)	blood clotting	E. Schmidt, A. Weißenborn, B. Wörner, R. Ziegenhagen. Verwendung von Vitaminen in Lebensmitteln Toxikologische und ernährungsphysiologische Aspekte Teil I 2004, Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Vitamin E (2003)

Substance	HBGV	HBGV based on...	UF	UF based on...	Nature of adverse effect	Reference
Caffeine	400 mg/day (ca. 5.7 mg/kg bw)	NOAEL = 3 mg/kg bw in humans	NO	BUT anxiety is already observed at 3 mg/kg bw. Therefore, the EFSA advise of 400 mg/day (or 5.7 mg/kg bw) is ABOVE the NOAEL	anxiety, behavioural changes	EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2015. Scientific Opinion on the safety of caffeine. EFSA Journal 2015;13(5):4102, 120 pp. doi:10.2903/j.efsa.2015.4102
Alcohol (NO risk assessment of alcohol in food by EFSA)	No TDI defined (20 g abs. alcohol for men, 10 g for women)	n/a	n/a	BUT Dizziness in children with 100 mg/l or 1.5 g alcohol but burger roll can contain 1.2 g /100 g and apple juice 0.77 g/l	Endpoint could be e.g. dizziness	http://www.eurocare.org/media_centre/eurocare_newsletter/2013/issue_7_2013_10_june/in_focus/alcohol_and_cancer, Gorgus, E., Hittinger, M., & Schrenk, D. (2016). Estimates of Ethanol Exposure in Children from Food not Labeled as Alcohol-Containing. Journal of Analytical Toxicology, 40(7), 537-542
Coumarin	TDI = 0.1 mg/kg bw	NOAEL= 10 mg/kg bw in dogs	100	10 for interspecies differences + 10 for interindividual differences	hepatotoxicity	European Food Safety Association. (2008). Coumarin in flavourings and other food ingredients with flavouring properties. EFSA J, 793, 1-15
Thujone	TMDI = 0.11 mg/kg bw	NOEL = 5mg/kg bw in mice	500	10 for interspecies differences + 10 for interindividual differences 5 poor data quality, interestingly not applied to vanillin	convulsions and seizures	Lachenmeier, D. W., & Uebelacker, M. (2010). Risk assessment of thujone in foods and medicines containing sage and wormwood–evidence for a need of regulatory changes? Regulatory Toxicology and Pharmacology, 58(3), 437-443
Cyanide/ amygdalin (in e.g. apricot kernels)	ARfD = 0.02 mg/kg bw	NOAEL = 0.36 mg/kg bw in rats BUT 0.105 mg/kg = 20 uM in human blood used as "toxicity threshold" for ARfD calculation	4.74	3.16= toxicodynamic subfactor; in the absence of cyanide-specic data on individual sensitivity 1.5= Women have a smaller distribution volume of blood than men and children have a larger blood volume (per kg/bw) than adults. "Therefore, the CONTAM Panel concluded that a default factor of 3.16 was not required and that a factor of 1.5 was sufcient to cover any additional variability in toxicokinetics. The CONTAM Panel noted the lack of information on whether potentially sensitive individuals (e.g. children) were included in the database underpinning the assumption that a blood cyanide level of 20 uM is a toxicity threshold, and that the bioavailability study was conducted in a small number of healthy volunteers."	histopathological changes and changes in organ weight of rats	EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2016. Scientific opinion on the acute health risks related to the presence of cyanogenic glycosides in raw apricot kernels and products derived from raw apricot kernels. EFSA Journal 2016;14(4):4424, 47 pp. doi:10.2903/j.efsa.2016.4424
Menthol	ADI = 4 mg/kg/gw	NOAEL = 200 mg/kg bw in rats	50	UF and rationale behind it was not directly mentioned, also different NOAELs were cited, ranging from 200-600 mg/kg bw. The UF of 50 is based on 200 mg/kg bw	changes in body weight	Larsen, J. C., Nørby, K. K., Beltoft, V. M., Lund, P., & Binderup, M. L. (2010). Scientific Opinion on Flavouring Group Evaluation 9, Revision 2 (FGE. 09Rev2): Secondary alicyclic saturated and unsaturated alcohols, ketones and esters containing secondary alicyclic alcohols from chemical group 8 and 30, and an ester of a phenol derivative from chemical group 25
Nicotine	ARfD = 0.0008 mg/kg	LOAEL = 0.0035 mg/kg bw in humans	4.4	"using an overall uncertainty factor of 10 and a correction factor of 0.44 for oral bioavailability of nicotine (extrapolation from the intravenous route to the oral route). The LOAEL is considered to be close to the no observed adverse effect level (NOAEL) and the overall uncertainty factor of 10 would be sufficient to cover the intra-species variability and the extrapolation from the LOAEL to NOAEL for the pharmacological effects."	increase of the heart rate	European Food Safety Authority, 2009. Potential risks for public health due to the presence of nicotine in wild mushrooms. EFSA Journal 2009;7(5):RN-286, 47 pp. doi:10.2903/j.efsa.2009.286r