Retrospective cumulative dietary risk assessment of craniofacial alterations by residues of pesticides

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Abstract

In 2022, the European Food Safety Authority (EFSA) conducted a dietary cumulative risk assessment for active substances of plant protection products on two types of craniofacial alterations: 1) craniofacial alterations due to abnormal skeletal development and 2) head soft tissue alterations and brain neural tube defects. These effects were selected based on developmental biology knowledge and a hypothetical teratogenic process. Cumulative risk assessment was conducted for 14 European populations of women in childbearing age. The dietary cumulative exposure was determined using individual consumption data collected under national food consumption surveys, and the calculations were based on occurrence data collected by Member States under their official monitoring programmes. A rigorous uncertainty analysis was performed using expert knowledge elicitation. Considering all sources of uncertainty, their dependencies, and differences between populations, it was concluded that the total margin of exposure* (MOET) resulting from cumulative exposure to residues of

* MOET: total margin of exposure

pesticides is above 100 for both types of craniofacial alterations and therefore the threshold for regulatory consideration is not exceeded. For the head soft tissue alterations and brain neural tube defects the MOET was even above 500 while for the alterations due to abnormal skeletal development, it was found about as likely as not that the MOET is above 500 in most populations. These results need to be interpreted in the light of the conservatism of the hazard assessment methodology. This review is a summary of the EFSA report on a retrospective cumulative dietary risk assessment of craniofacial alterations by residues of pesticides published in 2022.

Keywords: craniofacial alterations, cumulative risk assessment, residues of pesticides, monitoring, European population

1. Introduction

Regulation (EC) No 1107/2009 concerning the placing of plant protection products (PPP) on the market is the key legislation governing the authorization of pesticides for agricultural use in the European Union. While it primarily focuses on the authorization and use of individual PPPs, it also addresses cumulative risk assessment (CRA) of pesticides, stipulating that known harmful effects of the PPP on human and animal health, including known effects on vulnerable groups, and any cumulative and synergistic effects should be considered. While Regulation (EC) No 1107/2009 does not provide detailed methodologies or specific requirements for conducting CRAs, it establishes the principle that such assessments should be considered when evaluating the safety of PPPs. As a European authority entrusted with the task of carrying out risk assessments, the European Food Safety Authority (EFSA) developed a methodology for CRA since 2008 (EFSA PPR Panel, 2008, EFSA PPR Panel, 2012, EFSA PPR Panel, 2013); this involves examining the combined effects of mixtures of different substances that may have similar toxicological properties or modes of action on consumers exposed through food.

Following the CRA done in 2020 for substances having effects on nervous system and thyroid (EFSA, 2020a, b) and in 2021 for substances inhibiting acetylcholinesterase (EFSA, 2021a), EFSA continued with a CRA for craniofacial alterations. This was motivated by the severity of such defects, the frequency of their occurrence in regulatory toxicological studies, the fact that these effects are among the most frequently recorded abnormalities in new-borns (Bartzela et al., 2017), the high plausibility for craniofacial alterations to result from a combined action of chemicals (Zoupa et al., 2020) by triggering common molecular initiating events (MIE), and by the fact that there is at least one adverse outcome pathway (AOP) described at the time the risk assessment was performed.

CRA: cumulative Risk Assessment
CAG: cumulative assessment group
AOP: adverse outcome pathway
NOAEL: no observed adverse effect level
LOAEL: lowest observed adverse effect level
EKE: expert knowledge elicitation

Following the identification and characterisation of hazard for CRA purposes, exposure calculations are done via probabilistic methodology, using monitoring data on pesticides residues collected by Member States under their national and the EU coordinated official monitoring programmes during a 3-year period (from beginning 2017 until end 2019) and using individual food consumption data from European national surveys.

Total Margin of Exposure (MOET) concept is applied in CRA as method for calculation and expression of cumulative risk. The MOET is one of the existing metrics to quantify the cumulative risk resulting from the exposure to a mixture of chemicals under the assumption of dose-addition. It expresses the ratio between reference points (NOAEL, BMDL) and levels of exposure. It is calculated as the reciprocal of the sum of the reciprocals of the individual Margins of Exposure (MOE) of chemicals in the mixture. Member States agreed on a MOET of 100 at 0.1st percentile for the whole population as a general threshold for regulatory consideration[†]. In other words, a MOET below 100 would be interpreted as a situation of unacceptable risk and would require risk mitigation measures. In case of very severe and irreversible effects, the probability of the MOET at the 0.1st percentile being above 500 may also be considered, by analogy to the use of additional safety factors for the setting of reference values. This is the case of craniofacial alterations, and, for this reason, this alternative probability is also considered in this review to provide Member States with complete information.

Estimates of dietary cumulative risk from combined exposure to multiple pesticides are necessarily subject to a degree of scientific uncertainty, due to limitations in the data and to assumptions used to address those limitations. The assessment related to craniofacial alterations therefore includes a rigorous analysis of the assumptions and uncertainties involved, leading to a semi-quantitative assessment of the degree of certainty that the MOET at the 0.1st percentile is either above 100 or 500.

To assess the cumulative risk of craniofacial alterations, the working hypothesis question was finally formulated as:

What was the cumulative risk of craniofacial alterations for European consumers resulting from dietary exposure to pesticide residues from 2017 to 2019[‡]?

2. Hazard identification and characterisation

The identification of specific effects of relevance in view of performing retrospective CRA of pesticide residues causing craniofacial alterations, and the establishment of respective cumulative assessment groups (CAG) was performed according to the following steps:

1) Identification and definition of specific toxicological effects considered relevant for performing CRA

[†] A technical report can be found on the website of the EU Commission here: https://food.ec.europa.eu/plants/pesticides/maximum-residue-levels/cumulative-risk-assessment/technical-annex_en

[‡] Retrospective dietary cumulative risk assessments are conducted using a 3-year cycle of official pesticide residues monitoring data. At the time of the present assessment, the most recent cycle comprised data collected in 2017, 2018 and 2019.

2) Definition of the indicators[§] describing the specific effects identified
3) Collection of data from regulatory assessment reports on indicators observed in toxicological studies conducted with active substances/metabolites
4) Establishment of CAGs for each specific effect identified
5) Hazard characterisation of the pesticides included into the CAGs

The identification of craniofacial alterations relevant for CRA (Step 1) was performed based on the criteria established in the Opinion of the EFSA Panel on plant protection products and their residues (PPR Panel) on the identification of pesticides to be included in cumulative assessment groups on the basis of their toxicological profile (EFSA PPR Panel, 2013) and considering the Guidance Document of the EFSA Scientific Committee on scientific criteria for grouping chemicals into assessment groups for human risk assessment of combined exposure to multiple chemicals (EFSA Scientific Committee, 2021).

After a preliminary analysis of the most recurrent findings related to craniofacial alterations and reported in the toxicological studies available in regulatory assessment reports, the WHO global registry and database on craniofacial anomalies (WHO, 2001) was considered to define the most relevant effects induced by chemicals during craniofacial morphogenesis. A linear AOP for skeletal craniofacial defects, supported by experimental data, has been described (Menegola et al., 2021). This AOP, which appears to be of relevance in humans, relies on the inhibition of CYP26, a retinoic acid metabolising enzyme, as the molecular initiating event (MIE). Intermediate key events (KEs) are retinoic acid disbalance, aberrant Hox gene expression, disrupted specification, migration and differentiation of neural crest cells (NCCs). On the other hand, there are alterations of the head skeletal structures, which are secondary to the disruption of other head morphogenetic processes through other not yet documented AOPs. Based on this developmental biology knowledge and hypothetical teratogenic pathogenesis, two specific effects were identified, for which it is reasonable to assume that pesticides causing them contribute by dose-addition (i.e. they act as they were simple dilutions of one another), and which result from distinct mechanisms and pathways: 1) craniofacial alterations due to abnormal skeletal development (triggering the establishment of a CAG named CAG-DAC[**]) and 2) head soft tissue alterations and brain neural tube defects (triggering the establishment of a CAG named CAG-DAH[††]). These effects were both considered of acute nature (i.e. may be triggered by short-term exposure or even by a single exposure event).

The indicators of craniofacial alterations due to abnormal skeletal development (Step 2) include any abnormality directly correlated with abnormal head skeletogenesis (e.g. cleft palate, micrognathia, exencephaly) or considered as indicators of skeletal defects with an ectomesenchyme-derived structure (e.g. open eye). The indicators of head soft tissue alterations and brain neural tube defects include any abnormality not directly correlated by abnormal head skeletogenesis but due to any other head dysmorphogenic pathway (e.g. anencephaly, related to the abnormal head neural tube morphogenesis). An exhaustive list of indicators and their synonyms associated to the two specific effects and mainly based on

[§] An indicator describes a change in a toxicological endpoint considered relevant for the identification of a specific effect.
[**] CAG-DAC stands for 'Cumulative Assessment Group – Developmental toxicity/Acute/Craniofacial alterations
[††] CAG-DAH stands for 'Cumulative Assessment Group – Developmental toxicity/Acute/Head alterations

macroscopic and/or histopathological findings, potentially observable in toxicological studies, was then established.

For the collection of data on craniofacial alterations (step 3), a total of 85 active substances and 11 metabolites were selected on the basis of monitoring data and toxicological profiles. These substances were screened for indicators of craniofacial alterations using regulatory assessment reports such as draft assessment reports (DAR), draft renewal assessment reports (DRARs) related to EFSA conclusions on the pesticide risk assessment and submitted to EFSA under Regulation (EC) No 1107/2009. Similar reports produced under other jurisdictions were also considered, such as the Joint Meeting on Pesticide Residues (JMPR) evaluations, harmonised classification and labelling (CLH) reports submitted to the European Chemicals Agency and Committee for Risk Assessment (RAC) Opinions and other data in the context of the 'one-substance-one assessment' approach (e.g. Opinions by the Biocidal Products Committee (BPC) when the active substance has also been assessed as a biocide). All these were used as source documents using the established list of indicators and their synonyms identified under step 2. The studies scrutinised were mainly developmental toxicity studies in rats and rabbits, but information on other developmental studies in other species such as hamster and mice and reproductive toxicity studies were also considered. An excel spreadsheet served as database for the collection of the observations of interest. Entries in the spreadsheet were created when the indicators of the specific effects (defined by using a harmonised list of terminologies) were observed in studies reported in the scrutinised sources. One individual entry was created for each indicator of craniofacial alteration and per study. The data collection was performed by three independent experts and was followed by a quality check procedure. The criterion for the inclusion of a pesticide into a CAG (step 4) was the observation of one or more corresponding indicator/s in a toxicological study considered acceptable for the purpose of the assessment of craniofacial alterations. As result of this, each active substance or metabolite, for which at least one treatment-related indicator (i.e., observed with a dose-response relationship or observed at the highest tested dose only) was reported in the database, was included in the respective CAG, regardless of the presence of maternal toxicity. If indicators showed incidences within the historical control data and were concluded as not related to treatment during the most recent toxicological evaluation of the substance under EFSA peer review, they were disregarded. This resulted in 39 active substances and/or metabolites in CAG-DAC and 41 in CAG-DAH, with 29 active substances/metabolites belonging to both CAGs. One uncertainty in the selection of substances lays in the nature of the specific effects on craniofacial development which are of rare incidence and therefore could be overseen in regulatory studies conducted according to test guidelines. In addition, the uncertainties also lay in the fact that old study reports and evaluations of developmental studies may include lower level of detail or that studies were conducted according to different test guidelines versions in place over the decades reflecting also different practices in selection of appropriate staining for bone and cartilage.

Once allocated to the respective CAGs, the active substances/metabolites were characterized (step 5) by the assignment of a NOAEL (no observed adverse effect level) and LOAEL (lowest observed adverse effect level) for the most sensitive indicator/s of the specific effect of interest, by using all available information across studies and species from studies performed by oral route (gavage or dietary). All indicators were considered of equal relevance for the purpose of toxicological characterisation of substances included in the CAG. In the case a study

failed to identify a NOAEL and only provided a LOAEL for the indicators of interest, a default NOAEL was derived by applying an extra conservative uncertainty factor (UF) of 10 (EFSA Scientific Committee, 2012; WHO, 2011). In the case two or more studies performed in the same species, strain and by the same administration route and of equivalent quality were available and testing different doses, they were considered collectively to derive a combined NOAEL and LOAEL for the set of studies. The lowest of all observed LOAELs was adopted as the overall LOAEL of the substance in the respective CAG. The overall NOAEL of the substance was set at the highest tested dose in the same species and strain without any observable indicator.

Active substance	Type of indicator	NOAEL mg/kg bw per day*	LOAEL mg/kg bw per day*	Reference[(a)] and study type	Dose levels mg/kg bw per day and exposure window (days; p.c. = post coitum)	Source
Prothioconazole-sulfonic acid	Jaw/Nasopharynx: agnathia	**150**	**750**	[redacted] (2001), Developmental toxicity, Wistar rat, oral gavage	0, 30, 150, 750 days 6–20 p.c.	DAR 2004 (prothioconazole), EFSA Conclusion (2007) (prothioconazole)
Spirotetramat	Facial fissures: cleft palate	**40**	**160**	[redacted] (2004c), Developmental toxicity, Russian Himalayan (=Chbb:HM15) rabbit, oral gavage	0, 10, 40, 160 days 6–28 p.c.	DAR (2008), EFSA Conclusion (2013)
Spiroxamine	Facial fissures: cleft palate	**30**	**100**	[redacted] (1992), Developmental toxicity, Wistar rat, oral gavage	0, 10, 30, 100 days 6–15 p.c.	DAR (2009), EFSA Conclusion (2010)
Tebuconazole	Facial fissures: cleft palate Skull vault agenesis: exencephaly, partial acrania Eye: open eye	< 10 **(1)**	**10**	[redacted] (1995c), developmental toxicity Mouse NMRI, oral, gavage	0, 10, 30, 100 days 6–15 p.c.	DAR (2006) and Addendum (2012), EFSA Conclusion (2014)
	Skull vault agenesis: Rudimentary skull ossification centres	10	30	[redacted] (1988b), developmental toxicity oral, gavage Mouse NMRI	0, 10, 30, 100 days 6–15 p.c.	DAR (2006) and Addendum (2012), EFSA Conclusion (2014)
Tebufenpyrad	Facial fissures: cleft palate	50	**150**	[redacted] (1992a), Developmental toxicity, Sprague Dawley rat, oral gavage	0, 15, 50, 150 days 6–15 p.c.	DAR (2007), EFSA Conclusion (2008)
	No indicators	90 **(90)**	–	[redacted] (1992b, Developmental toxicity, Sprague Dawley rat, oral gavage	0, 15, 50, **90** days 6–15 p.c.	DAR (2007), EFSA Conclusion (2008)

Figure 1 shows an example of how the substances allocated to CAG-DAC were characterised based on the analysis of the data collected:

Active substance	Type of indicator	NOAEL mg/kg bw per day*	LOAEL mg/kg bw per day*	Reference[(a)] and study type	Dose levels mg/kg bw per day and exposure window (days; p.c. = post coitum)	Source
Prothioconazole-sulfonic acid	Jaw/Nasopharynx: agnathia	**150**	**750**	[redacted] (2001), Developmental toxicity, Wistar rat, oral gavage	0, 30, 150, 750 days 6–20 p.c.	DAR 2004 (prothioconazole), EFSA Conclusion (2007) (prothioconazole)
Spirotetramat	Facial fissures: cleft palate	**40**	**160**	[redacted] (2004c), Developmental toxicity, Russian Himalayan (=Chbb:HM15) rabbit, oral gavage	0, 10, 40, 160 days 6–28 p.c.	DAR (2008), EFSA Conclusion (2013)
Spiroxamine	Facial fissures: cleft palate	**30**	**100**	[redacted] (1992), Developmental toxicity, Wistar rat, oral gavage	0, 10, 30, 100 days 6–15 p.c.	DAR (2009), EFSA Conclusion (2010)
Tebuconazole	Facial fissures: cleft palate Skull vault agenesis: exencephaly, partial acrania Eye: open eye	< 10 **(1)**	**10**	[redacted] (1995c), developmental toxicity Mouse NMRI, oral, gavage	0, 10, 30, 100 days 6–15 p.c.	DAR (2006) and Addendum (2012), EFSA Conclusion (2014)
	Skull vault agenesis: Rudimentary skull ossification centres	10	30	[redacted] (1988b), developmental toxicity oral, gavage Mouse NMRI	0, 10, 30, 100 days 6–15 p.c.	DAR (2006) and Addendum (2012), EFSA Conclusion (2014)
Tebufenpyrad	Facial fissures: cleft palate	50	**150**	[redacted] (1992a), Developmental toxicity, Sprague Dawley rat, oral gavage	0, 15, 50, 150 days 6–15 p.c.	DAR (2007), EFSA Conclusion (2008)
	No indicators	90 **(90)**	–	[redacted] (1992b, Developmental toxicity, Sprague Dawley rat, oral gavage	0, 15, 50, **90** days 6–15 p.c.	DAR (2007), EFSA Conclusion (2008)

Figure 1: Example of allocation of substances into the CAG-DAC based on the indicators observed in the toxicological studies available in the regulatory assessment reports for active substances of PPPs together with their characterisation by assignment of a NOAEL and LOAEL for the effect of interest. The names of the authors of studies involving testing on vertebrates are blacked out in application of article 63 of Regulation (EC) No 1107/2009 which requires a confidential treatment of this information.*

The process of hazard identification for CAG establishment can be seen in Figure 2.

* : NOAEL and LOAEL of the specific indicator for craniofacial alteration. Values indicated in bold characters represent the overall NOAEL and LOAEL of the substance, after eventual collective evaluation of sets of studies of equivalent quality. Numbers in parenthesis represent the overall NOAEL when derived from the combination of different studies or when derived from a LOAEL divided by 10. (a): Reference as given in the respective DAR/DRAR and other source documents mentioned in the 'source and comment' column.

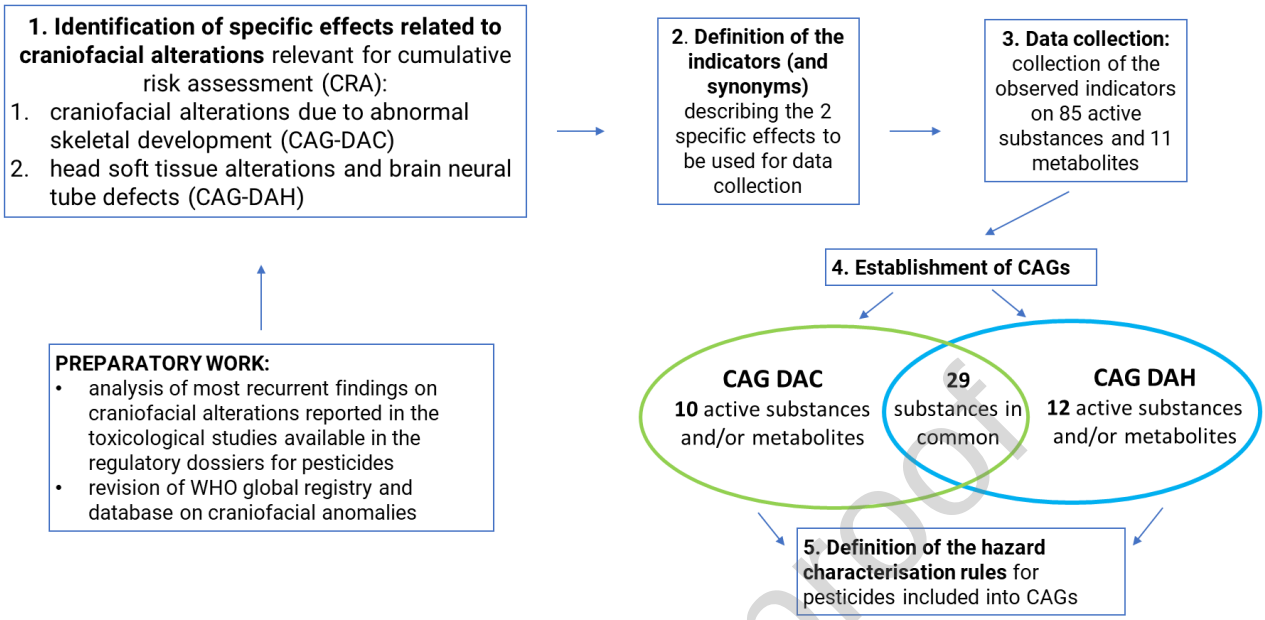


Figure 2: Overview of the steps required for the establishment of the CAGs for craniofacial alterations.

3. Exposure assessment

The cumulative exposure was determined in 14 populations of women in childbearing age (i.e. adult women aged from 18 to 45 years old) from different European countries (Belgium, Czechia, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Romania, Spain and Sweden) using individual consumption data collected under national food consumption surveys and stored in the EFSA Comprehensive European Food Consumption Database (EFSA, 2011). The population sizes varied from about 300 to about 3000 subjects per survey. The calculations were based on official monitoring data reported by all EU Member States, Iceland and Norway to EFSA over a cycle of 3 years for 36 raw primary commodities widely consumed within Europe, and 2 processed commodities (olive oil and wine).

The exposure of subjects was modelled probabilistically considering their diet within a time window of 24 hours by means of an empirical Monte Carlo simulation. The Monte Carlo simulation was performed using the SAS® software with 100,000 iterations.

The basic iteration consisted in drawing at random one individual consumption day from the consumption dataset. For each food commodity consumed as raw primary commodity (RPC) or raw primary commodity derivative (RPCD) within this individual consumption day, one sample of the monitoring dataset was drawn at random to assign an occurrence level for each of the pesticides included in the CAG. For this individual consumption day, a reference point index (RPI) was then calculated, according to the equation in Figure 3, considering the NOAELs of the substances, the body weight of the subject, the variability factor (VF) representative of the variability of residues in individual commodity units and processing factors (PF) representing the effect of household or industrial treatment before the consumption of commodities. This inner loop execution resulted in an empirical distribution of RPIs, later converted into a distribution of MOETs, representing the variability of 24-hour exposures within the different populations.

$$RPI_{id} = \sum_{c}^{Commodities} \sum_{p}^{Processes} \sum_{s}^{Substances} \begin{cases} \dfrac{RPCD_{idcp} \cdot X_{idcps}}{BW_i \cdot NOAEL_s \cdot 10^3} & if\ X_{idcps}\ refers\ to\ the\ RPCD \\ \dfrac{RPC_{idcp} \cdot X_{idcps} \cdot WVF_{idcps}}{BW_i \cdot NOAEL_s \cdot 10^3} & if\ X_{idcps}\ refers\ to\ the\ RPC\ and\ PF_{cps}\ is\ not\ available \\ \dfrac{RPCD_{idcp} \cdot X_{idcps} \cdot WVF_{idcps} \cdot PF_{cps}}{BW_i \cdot NOAEL_s \cdot 10^3} & if\ X_{idcps}\ refers\ to\ the\ RPC\ and\ PF_{cps}\ is\ available \end{cases}$$

where

RPI_{id}	is the RPI of individual i on day d;
RPC_{idcp}	is the amount of commodity c with processing type p consumed by individual i on day d, expressed in g of RPC;
$RPCD_{idcp}$	is the amount of commodity c with processing type p consumed by individual i on day d, expressed in g of RPC derivative;
BW_i	is the body weight of individual i, expressed in kg;
X_{idcps}	is the average concentration of substance s in the sample that was randomly assigned to individual i on day d for commodity c with processing type p, expressed in mg/kg;
WVF_{idcps}	is the weighted VF that was randomly assigned to individual i on day d for substance s in commodity c with processing type p;
PF_{cps}	is the PF for substance s in commodity c with processing type p;
$NOAEL_s$	is the NOAEL level for substance s, expressed in mg/kg body weight.

Figure 3: Basic equation of the Monte Carlo simulation

After this, an outer loop execution repeated the inner loop execution 100 times, each time replacing the consumption and occurrence data sets with bootstrap data sets obtained by resampling the original datasets with replacement. This reflected the sampling uncertainty of occurrence and consumption data by producing 95% confidence intervals around any point of the MOET distribution.

The focus of the assessment was on the 0.1st percentile of the MOET distribution (corresponding to percentile 99.9 of the cumulative exposure), as this percentile was chosen by the Risk Managers of EU Member States as the reference point to trigger eventual regulatory measures.

At this percentile, median estimates of the MOET ranged from 73.5 to 298 for CAG-DAC in Ireland and Latvia, respectively, and from 534 to 1010 for CAG-DAH in Finland and Romania, respectively (Table 1).

Table 1: Estimates of the MOET and their corresponding 95% confidence intervals in women of childbearing age at the 0.1st percentile of distribution

Country	CAG-DAC	CAG-DAH
BE - Belgium	179 [133-240]	597 [488-716]
CZ - Czechia	119 [90-180]	573 [446-723]
DE - Germany	107 [84.5-151]	553 [474-653]
DK – Denmark	146 [98.4-194]	751 [622-898]

ES – Spain	194 [144-255]	674 [584-820]
FI – Finland	294 [242-392]	534 [386-754]
FR – France	148 [117-197]	659 [544-789]
HU – Hungary	267 [187-335]	775 [615-950]
IE -Ireland	73.5 [50.9-106]	562 [399-717]
IT -Italy	203 [162-266]	714 [579-930]
LV – Latvia	298 [237-359]	812 [606-1020]
NL - Netherlands	173 [130-236]	601 [499-737]
RO – Romania	288 [242-343]	1010 [739-1300]
SE - Sweden	134 [98.7-186]	684 [577-839]

The exposure estimates were driven by a few substance-commodity combinations, identified as risk drivers (i.e., contributing to at least 5% of the cumulative exposure below the 1st percentile of the MOET distribution in at least one population). Their relative contributions differed according to the population.

Nevertheless, in CAG-DAC, folpet in wine was by far the largest risk driver in all population groups. Other risk drivers, of minor importance and present in varying amount according to the population, included folpet in apples, mancozeb in head cabbage, lettuce and oranges, tebuconazole in apples and peaches, 2,4-D in oranges, chlorpyrifos in potatoes and thiabendazole in oranges.

In CAG-DAH, several major risk drivers were observed. 2,4-D in oranges, essentially through the consumption of orange juice was the largest risk driver in 12 populations. In Ireland the largest risk driver was folpet in wine grapes. In Romania, the main risk drivers were deltamethrin in wheat and chlorpyrifos on potatoes. Thiabendazole in oranges was an additional significant risk drivers in the majority of populations. Minor risk drivers included 2,4-D in mandarins, chlorpyrifos in tomatoes and thiabendazole in mandarins.

4. Uncertainty

To assess the impact of toxicological uncertainties and limitations and assumptions affecting the exposure assessment, an uncertainty analysis was performed following the guidance of the EFSA Scientific Committee (2018) for each CAG.

Forty sources of uncertainty affecting the input data, model assumptions and the assessment methodology were identified. Their impact was assessed using a combination of expert knowledge elicitation (EKE) techniques (EFSA, 2014) and Monte Carlo simulations, in 4 successive steps, as depicted on the right-hand side of Figure 4 and described in the following paragraphs:

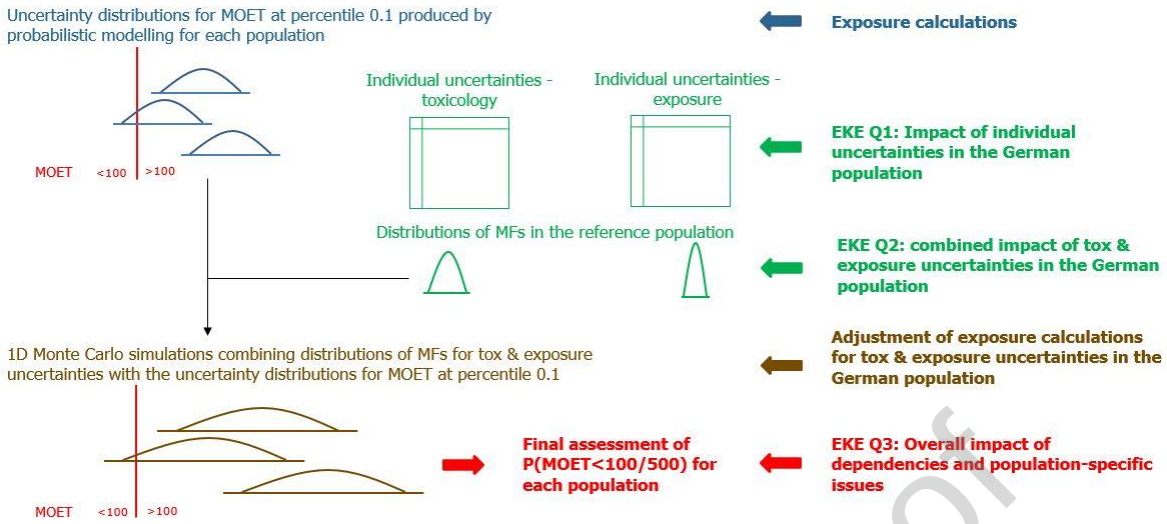


Figure 4: Overview of the approach to characterising overall uncertainty in the CRA.

EKE Question 1: This was the first of three sessions of EKE. Toxicology and exposure experts were required to consider separately each source of uncertainty related to their respective area of expertise and quantify its impact on the assessment in terms of how much the median estimate of the MOET at 0.1st percentile calculated by the probabilistic model for the German population would change if that source of uncertainty was resolved (e.g., by obtaining perfect information on the input or assumption affected by the uncertainty). Focussing the assessment primarily on the single German population, as a reference population, selected for the high number of subjects, avoided repeating this process 13 more times for each population, which would have been vulnerable to biases in judgement due to progressive expert fatigue. The experts expressed their judgements as ranges of multiplicative factors (MF) using the agreed conventional scale shown in Figure 5.

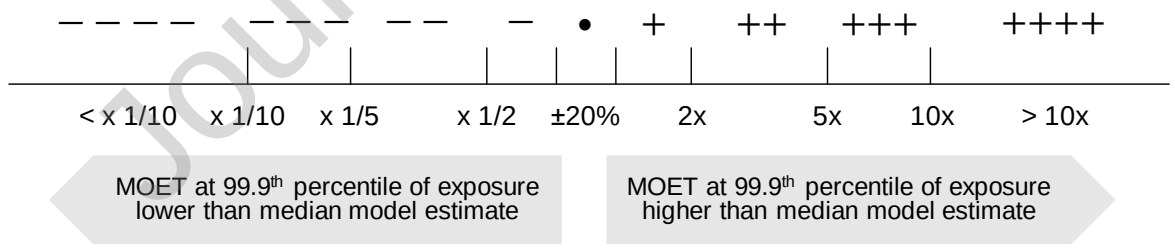


Figure 5: Scale used by the experts when assessing EKE Q1. They were asked to express their judgement as a range that they estimated has at least a 90% probability of containing the true MF. For example: '- - -/ •' means at least a 90% chance the true MF is between x1/10 and +20%; '++/++' means ≥ 90% chance between 2x and 5x etc.

EKE Question 2: This was the second of three sessions of EKE. Considering the outcome of EKE Question 1, the toxicology and exposure experts were asked to quantify their combined impact on the assessment in terms of how much the median estimate of the MOET at 0.1st percentile calculated by the probabilistic model for the German population would change if all those sources of uncertainty were resolved. The combined impact was elicited in the form of 2 distributions of MFs. This elicitation was conducted following the guidance for facilitation of consensus judgements in the Sheffield protocol provided by EFSA (2014) and in the SHELF framework. For both distributions, the experts first determined the plausible range for the

combined MF. Then, three further consensus judgements were elicited using the probability method (Oakley and O'Hagan, 2016) (described in EFSA (2014) as the fixed interval method). This consisted in eliciting the probability that the MF lies above (or below) three values in different parts of the plausible range. The experts' consensus judgements for these three values, together with their consensus for the plausible range, were entered into the SHELF Shiny app[‡‡] to display the best-fitting distribution. This distribution was adopted as consensus distribution, after eventual minor adjustment to accommodate the collective experts' view. Elicited distributions for CAG-DAC are presented in Figures 6 and 7**Error! Reference source not found.**. As can be seen, most of the distributions lies beyond a MF of 1, indicating that the real MOETs are more likely to be higher than the estimates from the cumulative exposure calculations, rather than lower, i.e. the calculated estimates are more likely to overestimate the real cumulative exposure than to underestimate it. For example, in the case of uncertainties affecting toxicology, the NOAEL of folpet (the main risk driver) was set by applying an UF of 10 to the LOAEL, as angulated hyoid bone was observed in 1 foetus in 1 litter at the lowest tested dose in the critical study. The toxicity of folpet was therefore considered as overestimated, and if perfect information would be available, for instance, studies with additional dose levels, it is highly plausible that a robust NOAEL would be set at a higher level. Similarly, in the case of uncertainties affecting exposure, the contribution of folpet through the wine consumption was overestimated because the consumption data were referring to populations of non-pregnant women, and therefore not representative of the wine consumption during pregnancy, expected to be significantly reduced.

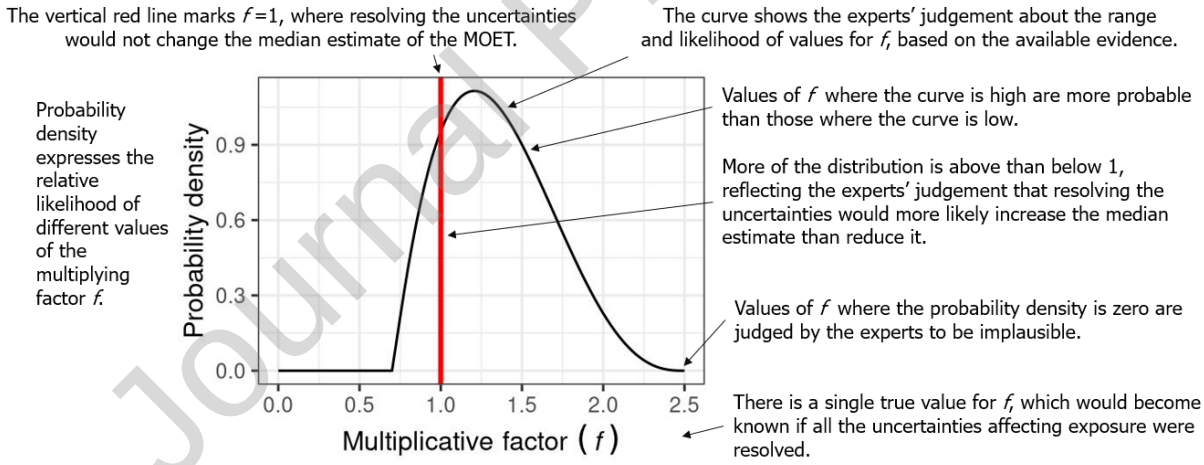
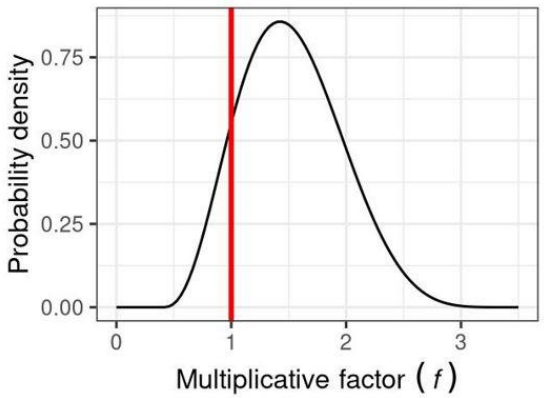


Figure 6: CAG-DAC: Consensus distribution of the experts for the combined impact of the quantified uncertainties affecting ***toxicology*** *(if resolved) on the MOET at 0.1st percentile for the German population.*

[‡‡] https://jeremy-oakley.shinyapps.io/SHELF-single/



- This is the **consensus distribution** provided by the exposure experts for the combined impact of the uncertainties they assessed
- The red line shows $f = 1$, i.e. no change in MOET

*Figure 7: CAG-DAC: Consensus distribution of the experts for the combined impact of the quantified uncertainties affecting **exposure** (if resolved) on the MOET at 0.1st percentile for the German population.*

Combination of distributions using Monte Carlo simulations: In this step, the distributions for the MFs quantifying the exposure and toxicology uncertainties, elicited in EKE Q2, were combined by multiplication with the uncertainty distribution for the MOET at 0.1st percentile produced by the probabilistic model. This results in a new distribution for the MOET at 0.1st percentile which incorporates the experts' assessment of the impact of the exposure and toxicology uncertainties. This was done for each of the 14 modelled populations. The results are shown in Figure 6 (CAG-DAC). In this figure, the 'Model' boxplots show the MOET estimates and their confidence intervals at 0.1st percentile in each consumer population, as estimated by the SAS® software. The 'Model+experts' boxplots show the result of the combination of these estimates with the elicited distributions of MFs quantifying the impact of uncertainties related to toxicology and exposure, assuming perfect independence between them.

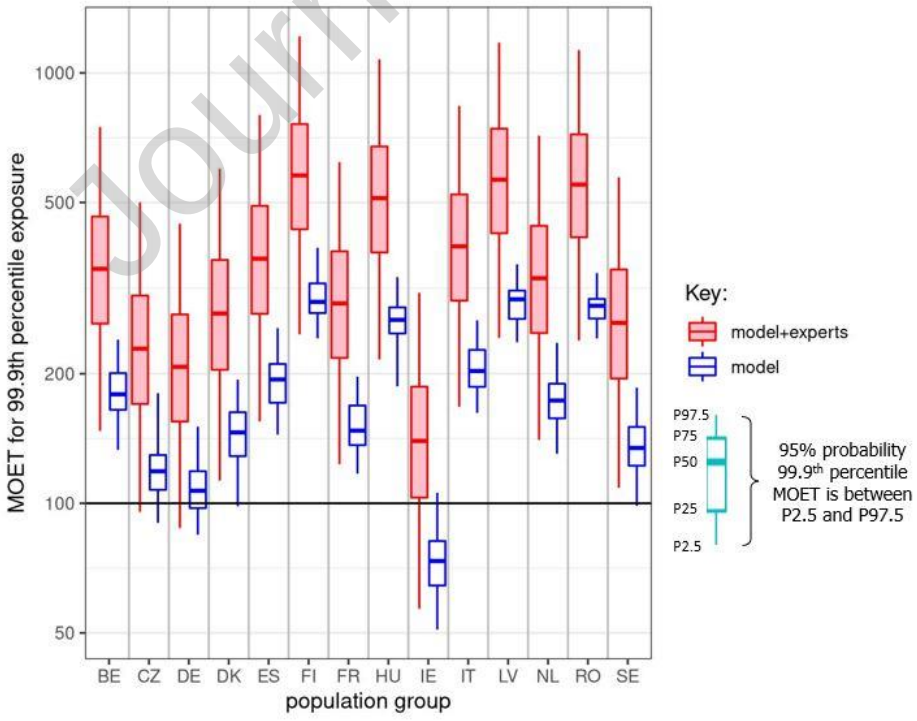


Figure 8: CAG-DAC: Combination of MOET estimates and confidence intervals at 0.1st percentile (equivalent to MOET for 99.9th percentile of exposure) in each consumer population, with the elicited distributions of MFs quantifying the impact of uncertainties related to toxicology and exposure.

Keys: BE (Belgium), CZ (Czechia), DE (Germany), DK (Denmark), ES (Spain), FI (Finland), FR (France), HU (Hungary), IE (Ireland), IT (Italy), LV (Latvia), NL (Netherlands), RO (Romania), SE (Sweden). Note that the vertical axis is plotted on a logarithmic scale. The lower and upper edges of each boxplot represent the quartiles (P25 and P75) of the uncertainty distribution for each estimate, the horizontal line in the middle of the box represents the median (P50) and the 'whiskers' above and below the box show the 95% probability interval (P2.5 and P97.5).

As shown in Figure 8, the median estimates for 'model+experts' are about twice higher than those for 'model'. They range from 140 to 565 in Ireland and Latvia, respectively. This indicates that the exposure calculations overestimated the cumulative risk, due to conservative assumptions. In addition, the boxplots for 'model+experts' and 'whiskers' are much wider than those for 'model', indicating that the contribution of sampling uncertainty for consumption and occurrence data, which is quantified in the calculation model, represents a fraction only of the overall uncertainty.

EKE Question 3: This was the third and last session of EKE. For reasons of practicality, the preceding steps involved two important simplifications. First, the uncertainties were assessed with reference to only one reference population (German population), and following this, the distributions elicited for the reference population were applied to all other populations. In addition, it was assumed that the model distributions and the distributions for exposure and toxicology uncertainties are independent of one another. Therefore, the experts were asked to judge how the distribution for the MOET at 0.1st percentile calculated would change if it was adjusted for any dependencies between the exposure and toxicology uncertainties and for differences in uncertainty between the reference population and each of the other populations. In addition, the experts were also asked to consider the impact of the conservatism of the criteria used to decide on the inclusion of pesticides in the CAGs. As a result of this conservatism, some active substances/metabolites included in the CAGs might, in reality, not cause the respective craniofacial alterations as a primary effect (e.g. as a result of maternal toxicity, and not as a result of a biochemical event altering directly the craniofacial morphogenesis). To assess the soundness of the inclusion of substances in the CAGs, a series of lines of evidence was therefore defined (chemical structure, strength of the dose-response relationship, absence of maternal toxicity, multiplicity of observations…), and an indicative weight (low, medium or high) was assigned to each line of evidence. Subsequently, for each risk driver, a probability that it actually causes the effect (CAG-membership probability) was elicited based on the line of evidence specifically available. This was done using the approximate probability scale from the EFSA Guidance on uncertainty analysis (EFSA, 2018).

For example, for CAG-DAC the CAG-membership probabilities of risk drivers were elicited as follows:

- Folpet: 40-70%
- Mancozeb: 75-90%
- Tebuconazole: 90-99%
- 2,4-D: 33-90%
- Chlorpyrifos: 10-50%
- Thiabendazole: 33-90%

These probabilities were used to repeat the exposure calculations with the 100 executions of the inner loop performed with or without the most important risk drivers (folpet in the case of CAG-DAC) in proportion of either the lower or the upper bound of their CAG-membership probabilities. These sensitivity analyses showed how the median estimate of the MOET at 0.1st percentile decreased and the confidence interval extended, as a result of the impact of the uncertainty on the CAG-membership probability of folpet. This helped the experts to quantify the impact generated by the conservatism of the criterion used to populate CAG-DAC.

5. Risk characterisation

The risk of the 2 types of craniofacial alterations for each population was ultimately expressed in terms of the probability ranges that the MOET at 0.1st percentile in 2017–2019 is equal or greater than the thresholds of 100 and 500. These probability ranges were associated with verbal probability terms, based on the approximate probability scale recommended for harmonised use in EFSA assessments (EFSA Scientific Committee, 2018).

Considering all sources of uncertainty, it was concluded that cumulative exposure results in a MOET at 0.1st percentile above 100 for all population groups considered, with varying degrees of certainty. In the case of CAG-DAC, this certainty exceeded 90% for the Irish population, 93% for the German population, 97% for the Czech population and 99% for all other populations. In the case of CAG-DAH, this certainty is 100% for all populations.

Because craniofacial alterations are severe and irreversible effects, and by analogy with the risk assessment principles applied for this type of effects under the approval process of active substances under Regulation (EC) No 1107/2009 (e.g., use of an additional safety factor of 5), the probability of the MOET at 0.1st percentile being above 500 was also assessed. In the case for CAG-DAH, this probability exceeded 66% for the German population, 90% for the Czech, Danish and Romanian populations, and 95% for all other populations. In contrast, in the case of CAG-DAC, this probability was only between 33 to 66% in most countries. The probability was even lower in Germany (5 to 33%) and in Romania (10 to 50%). In Sweden, the probability was higher (50 to 80%).

The results of the assessment for all populations are summarised in Table 2.

Table 2: CAG-DAC and CAG-DAH: Outcome of the CRA for craniofacial alterations resulting from dietary exposure to pesticides residues in 2017 to 2019

Country	Probability for the MOET at 0.1st percentile to be above 100	Probability for the MOET at 0.1st percentile to be above 500
CAG-DAC		
BE - Belgium	99-100% (almost certain)	33-66% (about as likely as not)
CZ - Czechia	97-100% (extremely likely to almost certain)	33-66% (about as likely as not)
DE - Germany	93-100% (very likely to almost certain)	5-33% (very unlikely to unlikely)
DK – Denmark	99-100% (almost certain)	33-66% (about as likely as not)
ES – Spain	99-100% (almost certain)	33-66% (about as likely as not)
FI – Finland	99-100% (almost certain)	33-66% (about as likely as not)
FR – France	99-100% (almost certain)	33-66% (about as likely as not)
HU – Hungary	99-100% (almost certain)	33-66% (about as likely as not)
IE -Ireland	90-100% (very likely to almost certain)	33-66% (about as likely as not)
IT -Italy	99-100% (almost certain)	33-80% (about as likely as not to likely)
LV – Latvia	99-100% (almost certain)	33-90% (about as likely as not to likely)

NL - Netherlands	99-100% (almost certain)	33-66% (about as likely as not)
RO – Romania	99-100% (almost certain)	10-50% (unlikely to about as likely as not)
SE - Sweden	99-100% (almost certain)	50 to 80 (about as likely as not to likely)
CAG-DAH		
BE - Belgium	100%	95-100% (extremely likely to almost certain)
CZ - Czechia	100%	90-99% (very likely to extremely likely)
DE - Germany	100%	66-95% (likely to very likely)
DK – Denmark	100%	90-100% (very likely to almost certain)
ES – Spain	100%	95-100% (extremely likely to almost certain)
FI – Finland	100%	95-100% (extremely likely to almost certain)
FR – France	100%	95-100% (extremely likely to almost certain)
HU – Hungary	100%	95-100% (extremely likely to almost certain)
IE -Ireland	100%	95-100% (extremely likely to almost certain)
IT -Italy	100%	95-100% (extremely likely to almost certain)
LV – Latvia	100%	95-100% (extremely likely to almost certain)
NL - Netherlands	100%	95-100% (extremely likely to almost certain)
RO – Romania	100%	90-100% (very likely to almost certain)
SE - Sweden	100%	95-100% (extremely likely to almost certain)

The probabilities reported in Table 2 need to be interpreted in the light of the estimated extra risk (i.e., the incidence of foetuses affected minus the incidence in the control group divided by the non-affected fraction of the population) at the NOAELs set for craniofacial alterations in the context of this report. This extra risk was estimated to range between 0 and 1%, with a median value of 0.5%, i.e., lower than the average size of the estimated effect at the NOAEL (5 and 10% for continuous and quantal effects, respectively) of toxicological effects (EFSA Scientific Committee, 2022). The low level of extra risk handled in this CRA was explained by the high number of pups that can be examined in developmental toxicity studies and the fact that the statistical significance of the observations was not taken into account in the setting of the NOAEL, considering the high toxicological specificity and biological relevance of these observations. This indicates that the present assessment was conducted with a high conservatism.

6. Conclusions and recommendations

EFSA established CAGs and conducted CRAs for two types of craniofacial alterations (alterations due to abnormal skeletal development and head soft tissue alterations and brain neural tube defects). Cumulative exposure calculations were performed by probabilistic modelling using monitoring data collected by Member States for 14 European populations of women of childbearing age. Considering all sources of uncertainty, their dependencies, and

differences between populations, it was concluded that the MOET resulting from dietary cumulative exposure to pesticides is above 100 for both types of craniofacial alterations and therefore the threshold for regulatory consideration is not reached. For the head soft tissue alterations and brain neural tube defects even the MOET of 500 was not exceeded while for the alterations due to abnormal skeletal development, it was found about as likely as not that the MOET is above 500 in most populations. These results are reassuring based on the relative conservatism of the hazard assessment methodology resulting from the low level of extra risk that was taken into consideration. As residues of pesticides in wine were found as risk drivers, EFSA recommended to further investigate the possible impact of co-exposure to pesticide residues and alcohol, as alcohol consumption is associated to the foetal alcohol syndrome, a pattern of multiple anomalies in offspring of women, including the impairment of craniofacial development.

7. Disclaimer

The authors Maria Anastassiadou, Anna Federica Castoldi, Federica Crivellente, Bruno Dujardin, Samira Jarrah and Luc Mohimont are employed with the European Food Safety Authority (EFSA) in the Pesticides Peer Review Unit, the Methodology and Scientific Support Unit or the Feed and Contaminants Unit that provide scientific and administrative support to the assessment of chemical risks in the food chain from farm to fork, in general, and in the area of combined effects of pesticides in particular. However, the present article is published under the sole responsibility of these authors and may not be considered as an EFSA scientific output. The positions and opinions presented in this article are those of the authors alone and are not intended to represent any official position of EFSA. To know about the views or scientific outputs of EFSA, please consult its website under http://www.efsa.europa.eu

8. Glossary

AOP (adverse outcome pathway): Grouping chemicals together that are shown to activate the same AOP based on results of assays or predictions of the Molecular initiating events (MIEs) or Key events (KEs).

BMDL (lower confidence limit of the benchmark dose): Lower confidence limit of the dose that causes a low but measurable target effect

CAG (cumulative assessment group): Pesticides being sorted into groups based on their toxicological profile.

CRA (cumulative Risk Assessment): Analysis, characterisation and possible quantification of the combined risks to health or the environment from multiple agents or stressors.

EKE (expert knowledge elicitation): Methods for gathering and validating judgements, insights and information from experts.

LOAEL (lowest observed adverse effect level): The lowest tested dose at which adverse effects were observed.

MOET (total margin of exposure): A safety margin between the real exposure to humans and the exposure levels that would lead to a certain adverse health effect.

NOAEL (no observed adverse effect level): The highest tested dose that is without adverse effect.

PF (processing factor): ratio between the concentration of a pesticide residue in a processed commodity and its concentration in the raw commodity.

RPC (raw primary commodity): unprocessed food commodity (e.g., orange)

RPCD (raw primary commodity derivative): processed food commodity (e.g., orange juice)

VF (variability factor): factor representative of the unit-to-unit variability of pesticide residues within a lot of commodities.

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10. Appendices

a. List of figures

population, with the elicited distributions of MFs quantifying the impact of uncertainties related to toxicology and exposure.
Keys: BE (Belgium), CZ (Czechia), DE (Germany), DK (Denmark), ES (Spain), FI (Finland), FR (France), HU (Hungary), IE (Ireland), IT (Italy), LV (Latvia), NL (Netherlands), RO (Romania), SE (Sweden). Note that the vertical axis is plotted on a logarithmic scale. The lower and upper edges of each boxplot represent the quartiles (P25 and P75) of the uncertainty distribution for each estimate, the horizontal line in the middle of the box represents the median (P50) and the 'whiskers' above and below the box show the 95% probability interval (P2.5 and P97.5).

b. List of tables

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Declaration of interest

Authors do not have any interest to declare

Highlights

- European Food Safety Authority (EFSA) conducted a dietary cumulative risk assessment for two types of craniofacial alterations: 1) craniofacial alterations due to abnormal skeletal development and 2) head soft tissue alterations and brain neural tube defects.
- Cumulative risk assessment was conducted for 14 European populations of women in childbearing age.
- A rigorous uncertainty analysis was performed using expert knowledge elicitation.
- Considering all sources of uncertainty, it was concluded that the threshold for regulatory consideration is not exceeded.
- This review is a summary of the EFSA report on a retrospective cumulative dietary risk assessment of craniofacial alterations by residues of pesticides published in 2022.