NL questions and comments on the EFSA Scientific Opinion "Risk for animal and human health related to the presence of dioxins and dioxin-like PCBs in feed and food", version of 14 June 2018, doi: 10.2903/j.efsa.2018.5333

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INTRODUCTION

In preparing for the Information Session on the EFSA Opinion on Dioxins and DL-PCBs in food and feed in Parma, November 13th, we have formulated a series of questions and comments on the EFSA Scientific Opinion "Risk for animal and human health related to the presence of dioxins and dioxin-like PCBs in feed and food".

For now, we have only focussed on the human risk assessment elements and listed the questions and comments in a comprised way on:

- Selection of the Russian Children's Study
- Derivation of the TWI
- Interpretation of the TWI
- Exposure assessment
- Overall uncertainty.

QUESTIONS AND COMMENTS

Selection of the Russian Children's Study

General

Of the 516 boys originally selected at the onset of the Russian Children's Study, only 133 participated at follow-up 10 years later. It is not reported or known why these boys did not further participate.

Question 1. Can EFSA explain how the small number of boys followed up may have affected the results?

Presence of other organochlorine compounds

Section 3.1.4.1 cohorts

Based on the differences in half-life between animals and human (human is worst case), EFSA prefers an epidemiological study for the derivation of a HBGV. There are however some points of concern in using the Russian Children's Cohort Study: the presence of high concentrations of organochlorines. In 70% of the participants also other organochlorides were measured, HCB (hexachlorobenzene) was approximately 8-fold higher compared to average levels in the US, while DDE is in a similar range.

As the POD taken from the Russian Children's Study seems highly affected by NDL-PCBs and OCPs, alternatively the lowest value of the Seveso study of 22 WHO2005-TEQ/g fat could be selected (Table 14, section 3.1.8.1, page 149). This would result in a 3-fold higher POD and TWI.

Question 2: Could EFSA explain the choice of selecting the Russian Children's Study as key study, over the other epidemiological studies, as the Russian Children's Study contains important confounders?

HCB levels were also associated with delayed puberty and NDL-PCBs were associated with early puberty. If the onset of puberty is related to semen quality, the contribution of NDL-PBCs and HCB is unclear. Furthermore, as indicated by EFSA: the dose-response relations between the epidemiological studies are not consistent. The differences are attributed to the differences in exposure scenarios, congener composition and co-exposures, which highlights the uncertainty in both studies.

Question 3: How does this confounder and possible different congener pattern affect the observed association?

Section 3.1.4.3.1 (reproductive effects (human studies)) elaborates on this issue: adjustment for HCB did not affect TCDD associations with sperm parameters but association between PCDD-TEQ and several sperm parameters became stronger after adjusting for HCB, β-HCH and DDE. The association between TCDD and semen parameters became slightly stronger after adjustment for NDL-PBCs.

Question 4: While we recognise that EFSA takes into account the association of other chemicals, they could still contribute to the critical effect. Can EFSA explain how they investigated the contribution of several co-contaminants in relation to the critical effect?

Page 135: EFSA concluded that impaired semen quality is likely to be a causal effect of exposure to TCDD, other PCDDs and PCDFs (*3.1.7.1.1 developmental male reproductive endpoints)*. The relation between puberty onset and semen quality is, however, unclear. HCB levels as well as NDL-PCBs were –also- associated with delayed puberty. Furthermore, semen quality can also be affected by other factors, such as lifestyle.

Question 5: If the onset of puberty is related to semen quality (this cannot be excluded), can EFSA explain whether there is a potential contribution of NDL-PBCs and HCB to the critical effect, and if so, how does this contribute to the effect? Can EFSA further explain the contribution of other factors on semen quality? Furthermore, can EFSA show the relationship between dioxins and semen quality?

Associations

3.1.7.2 page 143 (decreased sperm concentrations Russian children's study)

Total TEQ levels in serum varied between 1.9 and 107 pg TEQ/g fat. Levels of PCBs (sum of more than 30 PCBs are relatively high with a median of 235 ng/g fat (58-1500). PCB-126 shows a relatively high contribution. There was an association with TCDD, PCDD-TEQ and PCDD/F-TEQ but not with total TEQ (thus including DL-PCBs), PCDF-TEQ and DL-PCB-TEQ: EFSA explains this is due to uncertainty of the relative potency of PCB-126. The POD was therefore based on the inverse association between PCDD/F-TEQ and sperm concentrations.

Question 6: The dependency of the association to the TEF factor of PCB-126 only is valid if the relative contribution of PCB-126 to the total TEQ is expected to vary largely between individuals, as the DL-PCB-TEQs would otherwise have the same relative decline due to the decline in TEF. Can EFSA explain, if such a change in congener patterns is expected?

Question 7: As exclusion of PCB-126 is essential for the association, can EFSA further elaborate on the uncertainty of the existing TEF scheme? In particular on the impact of the uncertainty in the potency of 2,3,4,7,8-PeCDF and 1,2,3,4,7,8-HxCDF (Section 3.1.7.2.1, page 138) on the observed associations, in comparison to the uncertainty around the potency of PCB-126?

Absence of dose-response

The three human studies suggest associations with sperm concentration. The LOAEL in the Russian Children's Study is lower compared to the Seveso NOAEL with no further decrease at high dose levels, i.e. *no dose-response relationship*. The differences are attributed to the differences in exposure scenarios, congener composition and co-exposures, which contributes to uncertainty in both studies. The TEQ levels in LOAEL quartile Russian study are lower compared to the (estimated) control group in the Seveso cohort.

Question 8: Can EFSA explain the inconsistency in the epidemiological data, and how uncertainties are dealt with. Has EFSA considered the option that since there is lack of a dose-relationship in this study for the 2nd-4th quartiles of exposure, there may be no effect at all, and that the somewhat lower sperm quality in these higher quartiles of exposure may just be chance finding? EFSA concludes that this Russian study provides a NOAEL for sperm quality effects caused by TEQ and goes into a lengthy discussion why this NOAEL conflicts with the NOAEL from the Seveso cohorts, but the option that in absence of a good exposure-response relationship, the Russian Cohort in fact does not show any effect seems easily dismissed.

Significant effect was observed in the second quartile, but no further decrease at higher levels. It was decided by EFSA (Section 3.1.8.3) to base the HBGV on the NOAEL of 7.0 pg PCDD/F-WHO2005-TEQ/g serum fat, being the median serum level in the lowest quartile, for effects on sperm concentration in the Russian Children's Study. In the absence of individual data and a clear dose response, it was decided not to perform BMD modelling (section 3.1.7.2.3).

Question 9: The sentence "In the absence of individual data and a clear dose response,….." (page 143) is unclear. Does EFSA mean "In the absence of individual data and ***the absence of*** *a clear dose response,…." or "In the absence of individual data and* ***the presence of*** *a clear dose response,…."? If the first interpretation is meant, why was a NOAEL derived from a study lacking*

any dose-response? How does this the lack of a dose-response relationship affect the reliability of the critical effect? If the latter interpretation is meant, why did EFSA not perform a dose response analysis (and derive a BMD(L)) to verify the assumption that the dose response is obvious, and to obtain an objective point of departure, which does not depend on the number of quantiles? Note that individual data is not required to perform a dose response analysis. Summary data (like in Table 10 of the opinion) would suffice provided that it is interpreted correctly.

At the first quantile the PCDD/F-TEQ is between 1.95 and 9.13 pg/g fat and the sperm concentration between 52 and 77.8 million/mL.

Question 10: How did EFSA determine that the effect at the first quantile can be regarded as a non-adverse or negligible effect? How does EFSA rule out that the sperm concentration could be higher than 77.8 million at lower PCDD/F-TEQ concentrations (than 1.95 pg/g fat), possibly warranting a lower NOAEL? Does EFSA consider the sperm concentration at the first quantile as a proxy for the background response, i.e. the response when exposure to dioxins would not occur? If yes, the NOAEL would heavily depend on the choice of the number of quantiles reported. How did EFSA determine that four quantiles are sufficient to derive a reliable NOAEL? Has EFSA considered organising the data in deciles?

To evaluate animal toxicity, only studies using pure TCDD have been taken into account because of uncertainty about the TEF factors (3.1.7.3).

Question 11: Could EFSA explain the reasons for not applying this approach for the human studies, other than wishing not to limit the availability of human studies?

Derivation of the TWI

TEF

The TWI is based on findings on PCDD/F-TEQ only (3.1.8.3, page 154), because according to EFSA there are strong indications that the DL-PCB-TEFs may be seriously overestimated (3.1.7.2.3, page 143).

Question 12: Leaving out the DL-PCBs altogether results in an overly conservative TWI. Could the TWI also be derived including the DL-PCBs? This would provide the reader to some insight in the level of uncertainty involved in deciding to leave out the DL-PCBs.

Binding to the aryl hydrocarbon receptor (AhR) is the molecular initiating event of the toxicities of PCDD/Fs and DL-PCBs: interspecies differences in the structure of this receptor are contributed to the sensitivity to TCDD toxicity. Human AHR affinity to TCDD is lower compared to rat and mouse, this may however differ for other substances.
Whether semen quality is also AhR-mediated is unclear. The previous TWI has been derived from a rat study using TCDD only, and no other AhR binding compounds. If a different mechanism of action (MoA) is causing these semen quality effect the TEFs may be not applicable.

Question 13: What is the Mode of Action for the critical effect semen quality? Can EFSA explain why it is valid to apply TEFs on the selected critical effect (semen quality) in the derivation of the TWI while the basis for the TEF scheme is mainly based on AhR mediated toxicity.

Model parameters

3.1.1.3 half-lives in human

The breast feeding phase has a significant impact on total body burden at the critical age. The following assumptions need to be reflected:

- Half-lives are estimated as between 6.1 and 11.3 years, decreased at higher levels in the body, varies from 4 years at high serum levels to more than 10 years at background levels. Half-lives are very similar for most PCDDs but higher for PeCDD and 1,2,3,4,5,6,7,-HcCDD: PCDD was estimated to be 8.7 years. The whole body half-life in infants, based on stillborn children and non-breastfed sudden death infants is< 26 weeks, the modelled half-life is 0.32 years for TCDD (page 42).
- For elimination rate constants (ke) of hydrophobic (or lipophilic) substances, usually there is an allometric and a lipid content-related dependency, i.e. inversely related to fat content and related to weight to the power 1/3. A 70 kg adult would thus have a 2.4-fold lower ke than an infant weighing 5 kg, and a 1.9-fold lower ke than an infant weighing 10 kg. These ke-values in the adult would even be lower if their lipid content would be higher than the infant. Inversely, a 5 kg infant adult would have a 2.4-fold higher ke than an adult weighing 70 kg,

and a 10 kg infant would have a 1.9-fold higher ke than an adult weighing 70 kg. These ke-values in the infants would even be higher if their lipid content would be lower than the adult. The factor for a 39 kg 9-year old compared to the 70 kg is 0.82-fold (not taking into account differences in lipid content).

- Exposure from milk during breastfeeding is assumed constant over time, where milk intake = 800 mL/ day (which is very high for a new-born baby), fat content in milk is constant at 3.5%, and daily intake of dioxins. Cited from the WHO report: "Compared to adults, the daily intake of PCDDs/PCDFs and PCBs for breast fed babies is still 1-2 orders of magnitude higher on a per body weight basis (based on 10-35 pg/g milk fat in industrialized countries, this value decreased significantly since the WHO evaluation).
- Constant exposure via breast feeding (mother is releasing dioxins, changing fat composition of the child, constant volume of breast milk). In addition the following assumptions have been made: equilibrium between maternal fat concentrations and breast milk, equilibrium between maternal and foetal fat concentrations.

EFSA explains shortly some of these choices (see section 3.1.8.2, page 151), but these explanations are not fully self-explanatory.

Question 14: As half-lives are dependent on, i.e.
- *concentration,*
- *age (or weight)*
- *constant milk supply,*
- *daily intake,*
- *and lipid content dependent,*

how is this incorporated in the model?

In section 3.1.8.2. the duration of breastfeeding is not well explained, while its effect is shown in Table 15 (page 153). Figure 14 shows that serum levels increase during breastfeeding and then decline during the first 9 years of age. If duration of breastfeeding would be shorter, lower levels are achieved in the 9-year old infant. On the other hand, if breastfeeding would be longer, higher levels are achieved in the 9-year old infant.

Question 15: What is the basis for taking a duration of 12 months of breastfeeding? In addition, should an indication be given to the TWI that it is only protective when not breastfeeding for longer than 12 months?

For the determination of the body burden in 9-year old infants, an oral exposure of twice that of the mother is assumed, based on energy intake. If exposure of the sons would be different, the body burden of the 9-year old son would be different.

Question 16: Could EFSA explain if other assumptions on the exposure of the sons have been considered?

Table 41 (page 177) present data on levels of PCDD/Fs in human milk, based on the WHO survey. It shows that levels have declined, they were higher in the past and lower at present. The information on page 179 shows that levels of PCDD/Fs decline over the length of the nursing period and with increasing number of breastfed children per mother.

Question 17: What is the basis for the selection of the level of 10 pg/g fat in breastmilk and how did the model take into account declining levels?

The models are based on the properties of TCDD, not on other congeners.

Question 18: Can EFSA explain what the uncertainty is not taken into account the properties of the other congeners in the model calculations?

Interpretation of the TWI

In Section 3.4 it is stated *'Breastfed infants are known to have a higher exposure than Toddlers and Other Children. The exposure of breastfed infants should not be compared to the TWI. The reason is that the TWI was set to prevent a level in breast milk that would result in serum levels in children that have been associated with adverse effects.'*

Indeed the TWI describes a level of exposure for mother-to-be that would not result in plasma TEQ levels (actually plasma PCDD/PCDF-TEQ levels) that could give rise to sperm quality effects in their sons. However, for the sons, it is completely irrelevant whether they are exposed to TEQ via their

mothers, or via other sources (read: infant formulae). If their exposure to TEQ would lead to too high plasma levels at the age of say 9 years old, no-one can see where that TEQ came from and the development of adversities is not dependent on the source either.
Therefore, the statement quoted above only makes sense if it is explicitly declared that the TWI is only intended to protect the children of mothers-to-be, and not suitable for the safety assessment for other sub-populations. However, in the opinion it is also stated that the TWI is also protective for all other effects of TEQ.

Question 19: Can EFSA provide a more thorough reasoning why exposure of breast-fed children should be interpreted on a different basis than exposure of infant-formulae fed children, and how this should be done (i.e., to what should the exposure of breast-fed infants be compared)?

In the same section, it is stated '*The factor of 2 higher exposure in Toddlers and and Other children, that was accounted for in the toxicokinetic modelling (see Section 3.1.8.2), needs to be considered when comparing their exposure to the TWI*'.

Question 20: Could EFSA explain how this should be done?

Further on in the section it is stated 'For Toddlers and Other Children, the exceedances are approximately a factor of 2 higher than in the older age groups. But since higher exposure at young age was taken into account when deriving the TWI, the exceedances are in a similar range to the older age groups.'

Question 21: Does EFSA mean with this that for Toddlers and other children actually a twice higher TWI should be applied?

The current TWI describes a level of exposure for mother-to-be that would not result in plasma PCDD/PCDF-TEQ levels that could give rise to sperm quality effects in their sons. If this TWI is exceeded, it only indicates a risk for the sons of the exposed mothers. In case of high exposures (e.g. in case of accidents), risks for other subpopulations cannot be determined with this TWI.

Question 22: Has EFSA considered deriving HBGVs for effects that are not mediated by exposure of the mother?

Exposure results
The exposure results per survey are only presented in Figures 21 and 22 for the older age groups.

Question 23: Can EFSA include an Annex in the opinion reporting on the exposure results per survey, as well as the main food groups contributing to the exposure?

The long-term exposure is calculated based on the average intake over the days present in the consumption survey. This approach is known to overestimate the exposure in the right tail of the exposure distribution as also stated in Table 56

Question 24: Could EFSA explain why the chronic exposure has not been assessed using statistical models? As the estimated exposures to dioxins exceed almost all the proposed TWI, this would have been a logical step to refine the assessment.

The long-term exposure is calculated based on FoodEx1, which resulted in several uncertainties that can either have resulted in an over- and underestimation of the exposure.

Question 25: Can EFSA explain why, given the high exposures, FoodEx2 was not used in the opinion to refine the linkage between the foods analysed and those consumed? Such a refined linkage would very likely have resulted in lower estimates of the exposure.

Table 56 present the uncertainties of the different inputs of the risk assessment.
The uncertainty 'inclusion of consumption surveys covering only few days to estimate high percentiles of chronic exposure' relates to the methodology used by the Panel to estimate the long-term exposure, and not to the underlying data. With the use of statistical models, this type of data would not necessarily need to result in an overestimation (+) of the long-term exposure.

Question 26: Would EFSA consider rephrasing this uncertainty as 'methodology used to assess long-term exposure based on consumption surveys covering two or more days'.

Overall uncertainty
Given the aforementioned comments and questions, in addition to those already included in the opinion, it would be helpful to indicate uncertainty and uncertainty levels given the variation of the various parameters.

Question 27: Can EFSA provide uncertainty and uncertainty levels when addressing the variation of the various parameters in the model calculations?

Table 56 combines the uncertainty of the exposure via food and feed.

Question 28: Would EFSA therefore consider separating uncertainties to make clear they refer to different assessments?