Draft document commenting the Scientific Opinion on the Risk for animal and human health related to the presence of dioxins and dioxin-like PCBs in feed and food.

1. Foreword

We have examined the "Scientific Opinion on the Risk for animal and human health related to the presence of dioxins and dioxin-like PCBs in feed and food", the most relevant literature cited in the Scientific Opinion (Minguez-Alarcón et al., 2017; Mocarelli et al., 2008; Mocarelli et al., 2011), relevant literature cited by the paper cited in the Opinion (Russ-Hauser et al., 2008; Williams et al., 2010; Korrick et al, 2011; Burns et al., 2016), and some other publications on related topics (Wang et al., 2016; Pant et al., 2007; Swan et al., 2003; Mumford et al., 2015).

In the paper providing the primary data for a revision of the TWI (Minguez-Alarcón et al., 2017), there are several potential sources of bias that have not been satisfactorily clarified and that need clarification.

2. The Russian children's study, the cohort analyzed, the features of their area of origin, and potential sources of bias

The Russian Children's Study is a longitudinal cohort study of peripubertal boys, residents of Chapaevsk (Russia), where several persistent environmental contaminants were produced and deposited in the environment. Before 1949, in the area, extensive manufacturing of chemical warfare agents was carried out. Subsequently, chlorine-containing industrial and agricultural chemicals were produced (e.g., hexachlorocyclohexane, hexachlorobenzene, and other OCPs). The manufacturing process and waste incineration also resulted in widespread contamination by PCDD/Fs and metals, including lead (text of the Opinion, page 77).

For most of these substances, a toxic effect on the human and animal reproductive system has been described in the scientific literature. Therefore, a proper analysis of the correlation between some specific substances and a set of adverse effects on reproduction requires careful control of the sources of bias related to the possible summation of the effects of these toxic substances. The summation of the effects of the different substances can also occur in different time windows. The possibility of the summation of the effects in different time windows is clearly shown in the paper by Minguez-Alarcón et al., 2017. Two possible confounding factors evidenced by the authors of the Russian Children's cohort study were the consumption of alcohol and tobacco smoking during the year previous to the collection of semen, several years after the exposure to the dioxins.

The objective of the study was to assess the association between the exposure of male subjects to dioxins, furans, and dioxin-like PCB during the puberty and long-term damage to the

reproductive system measured by the quality of the semen produced when the subjects were young adults.
Now let's consider individually the various toxics released along time in the study area and detected at puberty in the blood serum of the subjects investigated.

2.1 TCDD
TCDD is the most toxic of the substances of the group of dioxins, furans, and dioxin-like PCBs, and was the main target of the Russian Children's Study (Minguez-Alarcón et al., 2017). It was detected in concentrations between 0.35 and 12.1 (median 2.9) pg/g lipid in the blood serum of the children at enrollment (8-9 years of age) (see Table 2 in Minguez-Alarcón et al., 2017).

The concentration of TCDD was significantly negatively correlated with the sperm concentration in million per ml (p=0.005), the total sperm counts in millions (p=0.02), not with the motile sperm in percent (p=0.11). The association between TCDD and total sperm counts led to an association of TCDD to the total motile sperm count (p=0.02) despite the lack of association with the sperm motility. All sperm parameter had a decreasing trend in relation to the TCDD concentration (See Table S1 in Supplementary material to the paper by Minguez-Alarcón et al., 2017).

2.2 PCDD
PCDD is a group of 7 congeners including TCDD and very similar to the TCDD. The toxicity of these congeners is expressed (by calculating their equivalence to TCDD) as pg TEQ /g and indicates the concentration of pure TCDD able to give the same toxic effect of the mixture analyzed.

The concentrations of PCDD varied between a minimum of 0.95 and a maximum of 36 (median 8.7) pg TEQ /g lipid in the blood serum of the children at enrollment (Table 2).

The concentration of PCDD was significantly associated with the sperm concentration in millions per ml (p=0.02), the total sperm counts in millions (p=0.02), not with the motile sperm in percent (p=0.44). The association between PCDD and total sperm counts led to an association of PCDD with the total motile sperm count (p=0.02) despite the lack of association with the sperm motility. All sperm parameter had a decreasing trend to the PCDD concentration (Table S1).

2.3 PCDF
PCDF is a group of 10 congeners with a toxic effect generally similar to that of PCDD. Similarly to PCDD, the toxicity of these congeners is expressed (by calculating their equivalence to TCDD) as pg TEQ /g and indicates the concentration of pure TCDD able to give the same toxic effect of the mixture analyzed.

The concentrations of PCDF varied between a minimum of 0.55 and a maximum of 50.6 (median 4.8) pg TEQ /g lipid in the blood serum of the children at enrollment (Table 2).

The concentration of PCDF was not significantly associated with any of the sperm parameters considered (p values varying between 0.57 and 0.76)(Table S1).

2.4 DL-PCBs

DL-PCBs is a group of 12 congeners with a toxic effect generally similar to that of PCDD/F. Similarly to PCDD, the toxicity of these congeners is expressed (by calculating their equivalence to TCDD) as pg TEQ /g and indicates the concentration of pure TCDD able to give the same toxic effect of the mixture analyzed.

The Russian Children's Study (Minguez-Alarcón et al., 2017) considered only the four most toxic congeners of this group of substances (coplanar PCBs, co-PCBs).

The concentrations of co-PCBs varied between a minimum of 0.52 and a maximum of 67.2 (median 6.9) pg TEQ /g lipid in the blood serum of the children at enrollment (Table 2).

The concentration of co-PCBs was not significantly associated with any of the sperm parameters considered (p values varying between 0.40 and 0.76) (Table S1).

Given that the risk management of dioxin toxicity is based on a TWI for the Total TEQ originated by the sum of PCDD/F and DL-PCBs, it is worthy to note that **no significant association was detected when considering the Total TEQ vs. the sperm parameters**.

2.5 Potential confounding factors related to the life styles at the moment of semen collection

Two potential confounding factors related to the lifestyles were considered in the Russian Children's Study at the moment of semen collection:

1. the consumption of alcohol, and
2. the smoke of tobacco.

Information on the presence of these two confounding was collected by interview at the time of semen collection. The question asked were respectively:

1. "Have you drunk alcohol in the last year, including beer?"
2. "Have you smoked a cigarette, even a few puffs, within the past year?"

The possible answers to both questions were "yes" or "no."

Unfortunately, the two questions have a very high sensitivity in the detection of the two behaviors at risk, but also have a very low discrimination power, i.e., they do not address the issue of heavy consumption of these two substances, which is what most likely may be linked to an adverse (therefore confounding) effect on the semen quality.

2.6 Lead

Lead is reported in the scientific literature as associated with the reproductive parameters. Investigations performed on the same Russian Children's cohort used for dioxins was able to

identify a significant association between the concentration of lead in the serum of children at enrollment and a delay in the onset of puberty (Hauser et al., 2008; Williams et al., 2010).

No specific study is reported in the Russian Children's cohort aimed at assessing the association of the exposure to lead and the semen quality.

An investigation performed in Mexico (Morán-Martínez et al., 2013) was able to show an association between the chronic exposure to lead in adults and the sperm concentration ($p<0.01$), the percentage of mobile spermatozoa ($p<0.05$), the viability of spermatozoa ($p<0.05$). The association between exposure and the percentage of abnormality was not significant.

Since the Russian cohort study was performed analyzing the concentration of toxics at 8-9 years of age and the quality of semen collected about ten years later, this means that the lack of association between lead at 8-9 years of age and semen quality at 18-19 years of age does not exclude that lead could be a confounder. It remains possible that the effect of chronic lead exposure after puberty may express itself through a lowering of the intercept of the correlation, without changing the slope of the relation between PCDD and semen quality parameters.

Proper control of this potential confounder would have required the analysis also of blood samples at the moment of semen collection.

2.7 OCPs

OCPs are reported in the scientific literature as associated with reproductive effects, including semen parameters (Wang et al., 2016). Pant et al., 2007, reported significantly lower sperm counts following to the exposure to □-HCH ($p<0.002$), □-HCH ($p<0.05$), pp'DDE ($p<0.005$), and pp'DDD ($p<0.01$). The study was performed as a case-control study, collecting semen from infertile and fertile men and analyzing the semen for quality parameters and the presence of pesticides. The associations detected, therefore, are between recent exposure to pesticides and semen quality. These results are similar to what observed by De Jager et al., 2006 (association between DDE and motility) and by Ayotte et al., 2001 (association of serum pp'DDE concentration with semen volume and semen counts).

The Russian cohort study was performed analyzing the concentration of toxics at 8-9 years of age and the quality of semen collected about ten years late. On the other hand, the listed studies on OCPs considered toxic concentrations measured concomitantly with the assessment of semen. The Russian cohort study apparently did not collect data useful to control the possible bias of a summation of effects. Like in the case of the lead, we could have that the effect of chronic OCPs exposure after puberty may lead to a lowering of the intercept of the correlation, without changing the slope of the relation between PCDD and semen quality parameters. In this case, also, proper control of this potential confounder would have required the analysis also of blood samples at the moment of semen collection.

3. The Seveso study.

The Seveso study (Mocarelli et al., 2008) is a very articulate study which also includes the assessment of the effect of exposure to TCDD during infancy and puberty on the quality of semen 22 years after. Mocarelli et al. observed a significantly decreased sperm concentration ($p=0.025$), motility ($p=0.001$), total motile sperm count ($p=0.018$) in the exposed group compared to the control group. The exposed group had a median serum TCDD concentration level of 210 pg/g of serum lipids, while the control group had a concentration of ≤15 pg/g of lipids. However, the concentrations observed in the Seveso study, especially that in the control group, were too high to be used to calculate/modify the existing TWI for dioxins.

4. Conclusions of the Scientific Opinion and new TWI values proposed.

Based on the results obtained in the Russian Children's study, the EFSA Panel estimated a new value for the NOAEL and suggested new values for the total TWI.

5. Problems with the newly proposed TWI

The total TWI refers to the total sum of PCDD/F +DL-PCBs. The management of the risk due to these substances does not consider a separation between PCDD, PCDF, and DL-PCB, but the maximum allowable intake is always referred to the overall group. Furthermore, the TWI is defined without considering the different categories of consumers. Therefore, the final proposals do not take into consideration the specific characteristics of the results of the Russian Children's study:

1. The effects on semen quality of exposure to these OC substances during puberty are only linked to TCDD and PCDD. Furans and DL-PCB did not show a correlation with the doses of exposure. Since the sources of DL-PCB and PCDD/F contaminations are distinct, frequently we observe contamination by DL-PCB virtually in the absence of PCDD/F. In such cases, the application of a TWI due mainly to the effects of PCDD/F would be inappropriate. To be consistent with the results of the study, any suggestion of the modification of TWI, therefore, should refer specifically to PCDD/F.
2. The problem evidenced by the Russian Children's study concerns a very narrow age window (pre-puberty and puberty) while the TWI, according to the rules in force, apply to all consumer categories and age classes. To be consistent with the results of the Study, any new TWI should refer only to the specific category at risk.

6. Suggested further studies

In conclusion, we can make a few suggestions:

1. Given the uncertainties evidenced about the control of bias in the Russian Children's study, any modification of TWI based on this study should be considered as a precautionary approach, pending a more in-depth analysis of the data available and possibly the collection of further data from the population resident in the city of Chapaevsk.

2. This provisional adoption of a precautionary value for the TWI should make the distinctions concerning the different groups of OC toxic and should also differentiate the various age-sex classes of consumers.
3. A set of risk-benefit analyses for each of the main groups of diets and alimentary habits/traditions in Europe should be performed to better define the criteria for the application of the newly proposed TWI.