Minutes for an additional COT meeting on the EFSA draft opinion on PFAS currently out for public consultation

Thursday 9th April 2020 (via Teams meeting)

Attendees

COT Members:

Professor Alan Boobis (Chair)
Dr Phil Botham
Dr Stella Cochrane
Dr Caroline Harris
Dr René Crevel
Prof Gary Hutchison
Dr David Lovell
Dr Mac Provan
Ms Juliet Rix
Prof Faith Williams
Dr Michael Routledge
Dr Cheryl Scudamore
Dr John Foster
Prof Matthew Wright
Dr Gunter Kuhnle
Dr Sarah Judge

Invited Experts:

Dr George Loizou
Dr Andrew Povey

Secretariat:

Ms Cath Mulholland (FSA)
Ms Claire Potter (FSA)
Dr Olivia Osborne (FSA)
Ms Britta Gadeberg (PHE)

1. Professor Alan Boobis had been involved in the SETAC North America workshop on exposure and toxicity of perfluoroalkylated substances (PFASs) and in writing the report of the meeting. No other interests were declared.

2. Dr Andrew Povey, an epidemiologist from The University of Manchester and a Member of the Committee on Mutagenicity of Chemicals in Foods, Consumer Products and the Environment (COM) and Dr George Loizou, a biochemical and computational toxicologist, from the UK Health and Safety Executive, were in attendance to provide additional expertise.

3. Following on from Item 9 at the most recent COT meeting held on Tuesday 10th March 2020, the paper by Abraham *et al.* (2020)[1] had been provided to the secretariat, pertinent to the COT assessment of the EFSA PFAS opinion. Therefore, this additional COT meeting was held to discuss the new information.

4. EFSA had been asked, by the European Commission, to prepare an opinion on the risks to human health related to the presence of PFASs in food, and to consider existing hazard assessments and available occurrence data. This document had been published for public consultation.

5. In the draft opinion, the EFSA panel assessed 27 PFASs. They decided to use a mixtures approach and have established a Tolerable Weekly Intake (TWI) for the sum of four PFAS (perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA) and perfluorohexane sulfonic acid (PFHxS)). These are currently the PFASs which contribute most to the levels observed in human serum, they share toxicokinetic properties in humans and show similar toxicological profiles. Although some other PFASs like perfluorobutanoic acid (PFBA) and perfluorohexanoic acid (PFHxA) also contribute significantly to the exposure, these compounds have much shorter half-lives in humans.

6. EFSA decided to base their PFASs assessment on the effects on the immune system, specifically on a decrease in vaccination response. A TWI had been established from serum levels of the four PFASs in a human study. A no observed adverse effect concentration (NOAEC) of 31.9 ng/mL was taken from the Abraham et al. (2020) study for the sum of the four PFASs. Pharmacologically-based pharmacokinetic (PBPK) modelling was then used, taking into account 12 months of breastfeeding by the mother, to calculate an estimated intake by the mother of 1.16 ng/kg bw per day for the sum of the four PFASs. This value was multiplied by 7 to calculate the TWI (1.16 x 7 = 8 ng/kg bw per week).

7. EFSA had summarised EU exposures in the draft opinion. Weekly exposures were calculated for the UK population taken from the data in the EFSA Opinion.

8. UK Lower bound (LB) mean exposures for adolescents and older subjects were below the TWI. Exposures for infants and toddlers exceed the TWI. All LB 95th percentile and upper bound mean and 95th percentile exposures exceed the TWI from < 2-fold to >100-fold.

9. Comments were made on the Abraham *et al.* 2020 paper. This study was on 101 one-year old children and the levels of PFOA (but not PFOS) were statistically

[1] Abraham K, Mielke H, Fromme H, Völkel W, Menzel J, Peiser M, Zepp F, Willich SN, Weikert C. (2020). Internal exposure to perfluoroalkyl substances (PFASs) and biological markers in 101 healthy 1-year-old children: associations between levels of perfluorooctanoic acid (PFOA) and vaccine response. Arch Toxicol. [in press]

significantly associated with the response to some vaccines. The relationship with vaccination antibodies and PFOA was not linear, but there was a significant decrease. This was a cross-sectional study and therefore the ability to establish cause and effect was limited. The study population was not representative, it was from a dioxin hotspot just outside of Berlin. The strict requirements for study entry also meant that the study was not representative of the population.

10. Conclusions on PFOS and PFOA exposures from the Abraham et al. (2020) and Grandjean et al. (2012)[2] study were inconsistent.

11. In the Abraham paper, the main exposure source for a population of this age is breast milk, which also contains other persistent organic pollutants (POPs) such as dioxin-like substances. A previous paper had shown that there was no association with other POPs therefore it was assumed that the effect in this instance was due to PFOA.

12. In the study there were very large differences between formula-fed and breastfed children. The original study was carried out in the 1990's and the samples were more than 20 years old when they were analysed recently for this publication. Adjustments had been made to take into account the number of vaccinations received by the children. It was unclear how the data were handled because 80 children had very high levels and 20 children had very low levels of PFOA. These were put together in the analyses and adjustment was made for the time and number of vaccinations.

13. Members discussed the strengths of both the Abraham *et al.* (2020) and the Grandjean *et al.* (2012) studies. The Grandjean study was better designed and the Abraham study made use of samples that were already available. The original study had been designed to look at high levels of POPs in breast milk.

14. Members queried the adsorption of PFASs onto the plastics that had been used for storing the samples at -80 ˚C for a prolonged period and assumed that the different PFAS substances would probably have different adsorption rates and parameters.

15. The levels of PFOA/PFASs correlated with duration of breastfeeding. Breastfeeding confers immune protection in the offspring. If the PFASs are antagonising this are they obscuring the magnitude of the effect? It was unclear whether breastfeeding duration had been corrected for in estimating the PFAS effect on immunity.

16. The Committee discussed the 'knee-bend' response and agreed that although it was an entirely empirical way of analysing the data with no underlying *a priori*

[2] Grandjean P, Andersen EW, Budtz-Jorgensen E, Nielsen F, Molbak K, Weihe P and Heilmann C. (2012). Serum Vaccine antibody concentrations in children exposed to perfluorinated compounds. *JAMA.* **307:** 391-397.

statistical or scientific bias, it was a pragmatic solution and had previously been used for example in analysing cadmium data in humans.

17. The authors of the Abraham study acknowledged that there were limitations of their study and that more, better designed studies were needed to assess the effect on PFASs on vaccination response.

18. The levels of PFOS in the plasma were reported to have no relationship with vaccine response in the Abraham study but at the same and even lower levels were associated with an effect in the Grandjean study.

19. Members noted the measuring at different time points after vaccination could be an explanation for the difference in potency between the compounds.

20. When looking at the animal data, it was noted that there was a very wide range of points of departure (PODs) in the studies. If the lowest value, from a mouse study, is used for establishing a health-based guidance value (HBGV), the TWI would be lower than that from use of human data.

21. The Grandjean and Abraham studies also looked at the antibody levels at different time points post-vaccination. Members were unsure of the correlation between 1, 3, 5 and 7 years. For children that were breastfed the impact of the mother's transfer of PFASs will be in the first year. Therefore, the data from the first year may not be as robust as data in the 3-7 years age groups. Members suggest preference should be given to using the data from the older children.

22. Members agreed that it was difficult to prove causation, but there is biological plausibility because immune effects have been observed in both animal and human studies in relation to (very low) levels of PFASs. However, the effects on the immune system observed are not necessarily the same for experimental animals and humans.

23. It would have been possible to use a weight of evidence approach from the experimental and human studies, but Members agreed that as human data were available it was appropriate to use this preferentially.

24. Either the Abraham or Grandjean study should be used as the critical study as these are the best currently available. The mechanism of action is not known and there are inconsistencies between the studies. More insights into the mechanism of action are needed.

25. In reality the NOAELs from the studies are not too far apart. Given the broad similarity in effects and sensitivity of the two studies, it was probably easier to determine a reference point from the Abraham study and was presumably why it was selected by EFSA as the critical study.

26. Members had some concerns about the differences in potencies and exposure levels of the two key compounds (PFOS and PFOA) on the additive approach taken. Whilst overall the amounts seem similar, when broken down to weekly intake it is hard to have confidence that this is correct. The methodology used seems to give a reasonable approximation of co-occurrence. If the exposures were below the HBGV there would not be an issue, but as some of the exposures exceed the HBGV the modelling needs to be more accurate. Currently, a lower bound versus upper bound approach is taken, but the reality will lie somewhere between the two. More monitoring data could provide a more robust data set, along with better sensitivity and specificity of the analytical assays. A probabilistic approach with independent distributions could be used. If sufficient iterations were carried out, a better estimate of exposure would be obtained. In terms of toxicity, it is not unreasonable to add the four PFASs.

27. Comments were made on the modelling approach taken. It had been possible to reconstruct the Worsley model, which was a later version of the Loccisano model, but with the addition of the liver. It had been possible to reproduce the graphs in the Worsley paper. It is assumed that EFSA used this model as the backbone of their model with the addition of breast milk levels. There did not appear to be any information on the evaluation of the EFSA model, although the backbone of the model is the Worsley model, which has been published and reasonably predicts PFAS levels.

28. The data for the transporters and associated parameters used in the models came from *in vitro* data.

29. The model has also been modified to take into account changes in body weight for very young children and they modelled the mother to 35 years before birth and twelve months of breastfeeding. Placental transfer was also modelled, therefore the TWI also applies to women who will become pregnant.

30. The Grandjean study grouped data into quintiles but some significant effects were seen at levels below the NOAEL. These were not consistent and perhaps this informed the identification of the NOAEL. The Grandjean data had to be grouped into quintiles. One of the strengths of the Abraham study was that the data did not need to be grouped.

31. Benchmark modelling did not work on these data sets as there is no zero value. There is a semi-linear response. There is an increase at all PFAS levels and if the data were analysed for a trend it would be significant.

32. Compared to the 2018 EFSA opinion on PFOS and PFOA there was not much discussion about the uncertainty around the modelling in this draft opinion. There were a number of caveats about the modelling in the 2018 opinion.

33. Breastfed infants in the UK could be receiving levels of up to approximately 100-fold the TWI. However, these are environmental contaminants that cannot be removed from the diet. A reduction in antibody response would have serious health consequences, but it is not known what the threshold is for this effect and there does not appear to be any evidence in the general population that vaccine efficacy is being impaired in the UK. Indeed, vaccination programmes have led to the successful eradication of some diseases.

34. One area of concern is the analysis of PFASs in food and the large number of non-detects.

35. In summary, immune effects have been seen in both experimental animal and human studies. The Grandjean and Abraham studies are the only ones suitable for determining a POD. However, Members felt that they were still less than ideal and it would be helpful to have a a more robust POD. The modelling used seems to take account of the critical toxicokinetic effects. The pathological consequences of the reduction in vaccine response in these children are unknown. It is unknown how this effect relates to the TWI. A one hundred-fold exceedance of the TWI does not necessarily mean that there will be one hundred times greater risk.

36. Whilst the COT are unable to suggest an alternative TWI at this time, there will need to be strong caveats explaining the exposure estimates versus TWI relative to exposures and these would need to be considered carefully to avoid miscommunication of the data.