KINETICS OF DIOXINS AND PCBs IN YOUNG SHEEP

Hoogenboom LAP[1]*, Zeilmaker MJ[2], Traag WA[1], Ten Dam G[1], Stark ML[3], Schafft H[3]

[1] RIKILT Wageningen UR, Akkermaalsbos 2, Wageningen, The Netherlands; [2] RIVM Institute of Public Health and the Environment, Bilthoven, The Netherlands; [3] Bundesanstalt für Risicobewertung, Berlin, Germany

Introduction

Dioxins and PCBs are a significant threat to the health of consumers, which to some extent still exceed the helath based guidance level (TWI). Since for most people food of animal origin is the most important source of these compounds, the EU has set maximum levels for such products according to the principle "strict but feasible" to further reduce the intake. It has been shown that sheep livers in most cases exceed the current maximum levels of 4.5 and 10 pg TEQ/g fat for respectively dioxins and the sum of dioxins and dioxin-like (dl) PCBs (EC, 2011). EFSA evaluated the levels reported by various member states showing median levels of 7.6 and 14.3 pg TEQ/g fat. Although based on TEFs from 1998 and thus somewhat higher than levels based on TEFs from 2005, these figures show that more than 50% of the sheep livers exceeds the MLs. EFSA also concluded that "the frequent consumption of sheep liver, particularly by women of child-bearing age and children, may be a potential health concern."

However, data also show that levels in fat and meat of sheep are much lower and in general below the MLs, indicating that these compounds accumulate specifically in the liver. The sequestration of dioxins and PCBs in livers is a well-known phenomenon in other species and has been ascribed to the binding of these compounds to cytochrome P450 enzymes of the 1A family rather than accumulation in the fat. Unfortunately, very little is known about the kinetics of dioxins and PCBs in sheep and the possible relation to the intake via grass and attached soil. For this reason, within the EU-project QSAFFE, a carry-over study was performed to obtain more insight in the behavior in sheep. Animals were exposed for up to 112 days to contaminated grass pellets obtained from a flood plain and exceeding the limit for dioxins by a factor of 2.5. Part of the animals were switched to clean grass after 56 days to study the effect of a period on clean feed, also to examine potential measures for reduction of the levels.

Materials and methods

Grass pellets were selected after a positive finding in the National Monitoring program on feed and feed ingredients in the Netherlands. The origin of the grass was located in the flood plain of the river IJssel and showed the presence of relatively high levels of contaminated soil. Part of the material as well as clean grass was bought for the study from the grass dryer and shipped to Berlin for the animal study. Straw was purchased by BfR from a local provider.

Young blackhead sheep were purchased by BfR and transferred to the animal facilities. The study protocol was approved by the Ethical Committee. After an initial period on the clean grass pellets, most sheep received the contaminated grass pellets, starting with about 0.5 kg and increasing to 1 kg at the end of the 112 days exposure period while control animals received clean grass pellets. Part of the animals fed with contaminated pellets were switched back to clean pellets after 56 days. In addition to the grass pellets animals also consumed part of the straw used as bedding in the stables. During this period animals doubled their body weight from about 20 kg to 40 kg.

From the animals fed with contaminated feed 4 animals were slaughtered after 8, 17, 29, 56 and 113 days to collect organ and tissue samples. In addition, from the animals switched to clean feed this was also performed at 8, 15, 36 and 57 days from the switch. Animals from the control group were slaughtered at day 0, 56 and 112. Samples were analysed for dioxins and PCBs using GC/HRMS by routine methods applied at RIKILT and has been described previously[3,4]. Raw kidney fat (2.5 grams) was extracted using a microwave while extraction of liver and muscle tissue was performed according to Smedes[5]. For this, 15 ml water saturated with sodium chloride, 80 ml 2-propanol and 120 ml cyclohexane was added to 45 grams of whole liver or 65 grams of muscle tissue and this mixture was blended using an ultraturrax for 2 min. Afterwards, 120 ml demineralized water was added and the mixture was blended for another 2 min. The mixture was then centrifuged for 10 min at 3000 r.p.m. and 10°C after which the upper layer of cyclohexane containing the fat was transferred over a funnel containing sodium sulphate into a flask. The extraction was repeated twice after adding 120 ml of cyclohexane after which the combined solvents were evaporated using a rotary evaporator from Buchi. A known amount of ^{13}C- isotope labelled internal standards was added to the extracted fat, and the samples were dissolved in 30 ml of hexane followed by an automated clean-up using an PowerPrep from FMS. The performance of the methods is regularly checked by participation in PT-tests.

Results and discussion

Analysis of the grass pellets prepared from grass collected on a river flood plain showed levels of 1.71 ng TEQ dioxins, 0.32 ng TEQ/kg dioxin-like PCBs and 2.33 µg/kg of non-dioxin-like PCBs (sum of 6), while the clean grass contained 0.27 ng TEQ dioxins, 0.06 ng TEQ/kg dioxin-like PCBs and 0.45 µg/kg of non-dioxin-like PCBs.

After a period on clean grass, sheep were fed with these materials for a period up to 112 days. Levels of dioxins, dl-PCBs and ndl-PCBs in livers of animals killed before administration of contaminated grass were already quite high and exceeded the current MLs. However, levels increased rapidly in livers of exposed animals (Fig 1). Dioxin levels in livers of exposed animals at days 56 and 112 were respectively 51 and 58 pg TEQ/g fat, as compared to 12 and 13 pg TEQ/g fat in control animals (Fig 1A). TEQ-based levels of dl-PCBs also increased but remained a bit lower than dioxins and showed a different profile (Fig. 1B). Also control animals killed on day 112 showed an increased dl-PCB level. Total TEQ levels raised to about 80 pg TEQ/g fat (Fig. 1C). Levels of ndl-PCBs clearly increased 2.5-fold in exposed animals and also exceeded the ML.

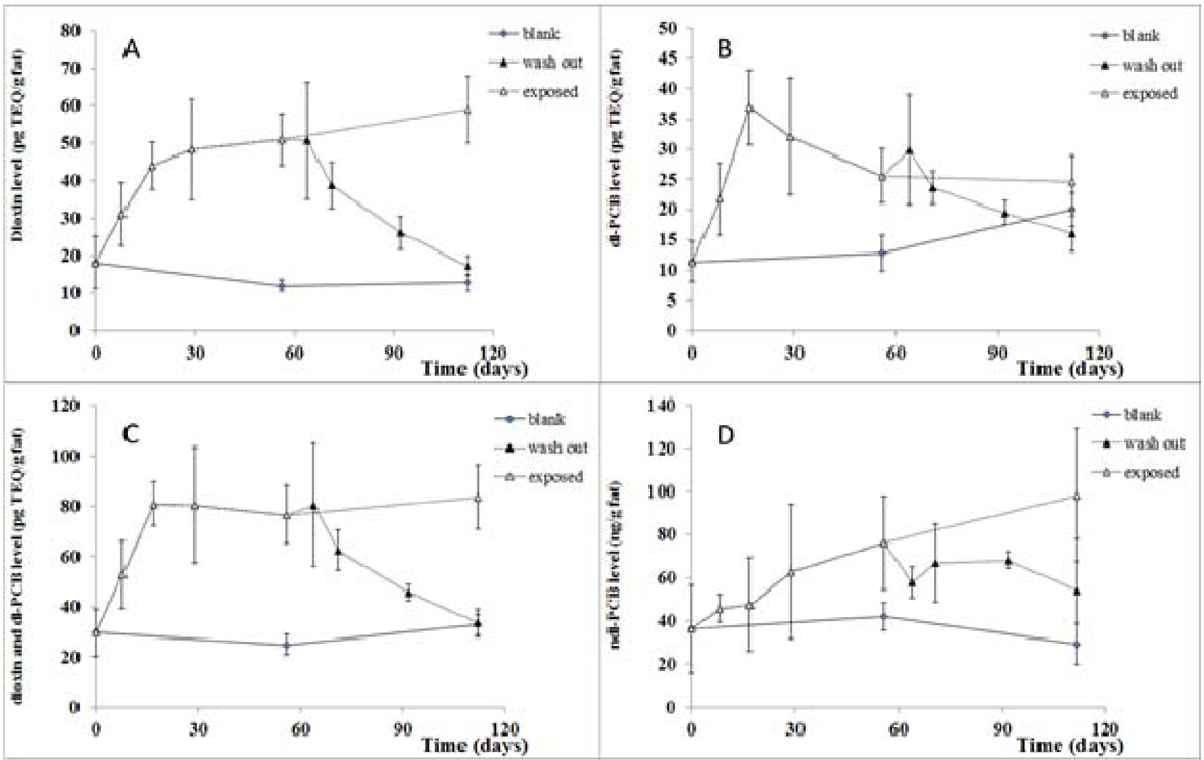

Figure 1. Levels of dioxins (A), dl-PCBs (B), sum of dioxins and dl-PCBs (C) and ndl-PCBs (D) in livers of sheep. Current MLs in the EU are 4.5 and 10.0 pg TEQ/g fat for dioxins and the sum of dioxins and dl-PCBs and 40 ng/g fat for ndl-PCBs.

Dioxin levels in kidney fat showed similar tendencies as in liver (Figure 2), but levels were much lower than in livers and did not exceed the MLs. Levels in meat (M. longissimus dorsi) were in general even lower than those in kidney fat (both expressed on fat weight). This is of interest regarding indications in other animal species that levels in meat are often higher than in fat, potentially due to a poorer distribution to the fat tissue.

Switching of animals to clean grass pellets resulted in a decrease of the levels almost back to those observed in control animals, both in livers and kidney fat. This decrease in liver did not result in a clear redistribution to the fat or meat. Theoretically, part of the decrease could be explained by the further growth of the animals and an increase in liver weights and fat content. However, the increase in liver weights from around 440 g at day 0 to 565 g at day 112, and the fat content from 4.2% on day 0 to 4.9% on day 112 shows that these effects play a minor role (max 1.5 decrease). In general, fat levels in livers of various animals showed very low variation, also due to the applied method for lipid extraction. The transfer of animals to clean grass and soil may offer a potential solution of reducing levels and thus the exposure of consumers.

Dioxins and PCBs in straw
The relatively small difference in the dl-PCB levels between exposed and control animals might be explained by the presence of PCBs in the straw which was partly consumed by the animals and shown to contain levels of 0.24 ng TEQ dioxins, 0.38 ng TEQ/kg dioxin-like PCBs and 1.38 µg/kg of non-dioxin-like PCBs. As a result the difference in exposure between treated and control animals became smaller, especially for PCBs. Based on an estimated daily intake of 0.5 kg, the straw could also have contributed to the relatively high starting levels in liver. Modeling of the data and further analysis of the straw given at different time points will have to give more insight into this issue.

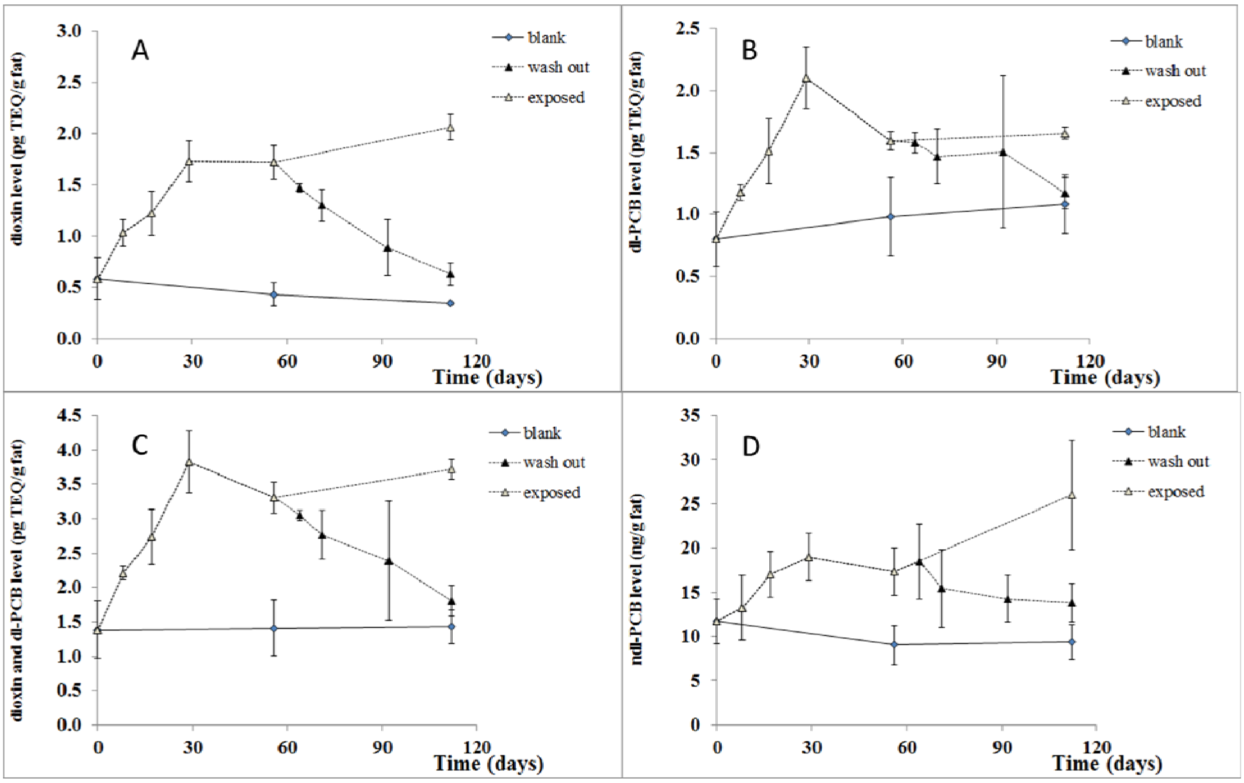

Figure 2. Levels of dioxins (A), dl-PCBs (B), sum of dioxins and dl-PCBs (C) and ndl-PCBs (D) in kidney fat of sheep. Current MLs in the EU are 2.5 and 4.0 pg TEQ/g fat for dioxins and the sum of dioxins and dl-PCBs and 40 ng/g fat for ndl-PCBs.

Congener patterns

In Figure 3 a comparison is made of the dioxin and PCB congener patterns observed in livers and kidney fat at day 56 and 112 for the exposed animals with those in the contaminated grass pellets. When comparing livers and fat it is evident that especially the higher chlorinated PCDD/Fs are more retained in the liver with e.g. ratios in liver to kidney fat at days 56 and 112 for OCDD of 128 and 97, and for OCDF of 326 and 269, as compared to 4.4 and 4.6 for TCDD and 7.8 and 8.2 for TCDF. For PCBs the ratios were in general much lower, being 3.9 and 3.4 for the total sum of all 18 PCBs determined. However, liver to kidney ratios between the different groups of PCBs were diverse, for example the ratio for PCB 126 was much higher (19.0 and 18.6), whereas for most mono-ortho PCBs the levels in fat were higher than in liver. This shows a clearly different behavior between the various dioxin and PCB congeners.

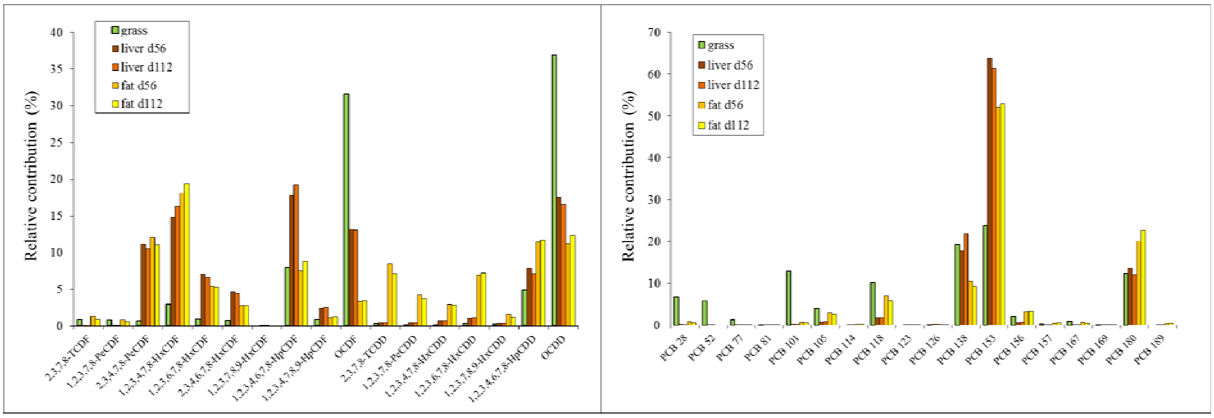

Figure 3. Dioxin and PCB-congener patterns in contaminated grass pellets and livers and kidney fats of animals exposed for 56 or 112 days to these pellets. Both based on absolute amounts.

Conclusions

The present data show that relatively low levels in grass result in high levels in livers of sheep. Although such levels are unusual in summer time in most areas, in winter time, when sheep also graze outside, such levels are rather common. In addition, slightly elevated levels in soil, as observed in industrial areas and flood plains, may strongly contribute to such levels. The current study also clearly show that levels may be strongly reduced when putting sheep on clean grass or soil. The potential contamination of straw is another matter of concern that may deserve further attention.

Acknowledgements

The study was financed by the EU (QSAFFE-project), the Dutch Ministry of Economics and the BfR. The authors would like to acknowledge the help of the animal care takers at BfR Berlin and technicians at RIKILT Wageningen.

References

1. EC (2011) *Official J. Eur. Union*, L 320, 18-23.
2. EFSA (2011) *EFSA Journal* 9: 2297, 1-71.
3. Tuinstra L G M.Th., Traag W A, Van Rhijn J A, Van de Spreng P F (1994) *Chemosphere* 29, 1859-75.
4. Hoogenboom L A P, Van Eijkeren J C H, Zeilmaker M J, Mengelers M J B, Herbes R, Immerzeel J and Traag W A (2007) *Chemosphere*, 68, 814-23.
5. Smedes F (1999) *Analyst* 124, 1711-18.