ORGANOHALOGEN COMPOUNDS IN HISTORIC BUTTER

Jochen F. Müller[*], Miriam Jacobs[**], Adrian Covaci[***], Olaf Päpke[****]

[*] ENTOX, University of Queensland, 39 Kessels Rd., Coopers Plains, Qld 4108, Australia
[**] RVC, Univ. of London, Hawkeshead Lane, North Mymms,Hatfield, Herts AL9 7TA, UK
[***] Toxicological Center, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium
[****] ERGO Forschungsgesellschaft, Geierstrasse 1, 22305 Hamburg, Germany

Introduction

Organohalogen chemicals such as those classified as POPs, but also hexachlorohexanes and polybrominated diphenyl ethers (PBDEs) are persistent, hydrophobic and thus accumulate in the food chain. The relevance of dairy products as source for human contamination with pollutants is widely recognized and as a result, routine monitoring programs of dairy products are used to monitor exposure [1]. The accumulation of persistent hydrophobic chemicals in dairy products occurs usually via the atmosphere - plant - cow - milk pathway [2]. Firstly the extensive hydrophobic surface acts as a sink for the chemicals – similar to a solid phase extraction [3]. Then the chemicals are absorbed from the grass into the cow and during lactation these chemicals partition into the milk. Providing that the animals or the dairy product itself were not contaminated otherwise analysis of dairy products can thus provide a good indication of atmospheric concentrations of such chemicals in the area and for the period where the cows live(d). Analysis of butter samples has thus become a means to compare environmental/atmospheric levels of these pollutants in different regions or between countries[4]. To date very little information is available on historic levels of pollutants in the Australian environment, but analysis of a recently opened butter sample that was canned around the period of 1944 indicated that PCDD/Fs were present in the period around the Second World War [5]. Since then we have started a campaign to obtain historic butter samples and have analysed these as well as the sample from 1944 for various other pollutants in order to evaluate historic levels of pollutants in the environment.

Methods

Butter samples were obtained from the Australian War Memorial, tip sites near stations in the Australian Antarctic Territory. All samples were stored in tin-coated steel that had remained sealed since manufacture. For the early sample contact investigations with the dairy industry revealed that the sample was likely to be canned towards around 1944 and that the sample was the milk from which the butter was made would have been drawn from all over the Australian State of New South Wales which is located in the south-east of Australia. The other samples were originated mostly from Queensland the north-eastern state of Australia.

To access the butter, the cans of butter were heated in a water bath and two small holes were punctured into the cans. Then the butter was drained into a solvent washed glass jar, mixed and refrigerated until further use. For analysis a part of the drained butter was send to ERGO-Forschungsgesellschaft, Germany for analysis of dioxin-like chemicals and a second sample was

send to the Toxicological Center at the University of Antwerp, Belgium for analysis of organochlorine pesticides, PCBs (non-dioxin like PCBs) and PBDEs.

At ERGO sub-samples equivalent to about 10 g of lipid were homogenised in a water bath (40 °C) and the lipophilic phase was separated then filtered through anhydrous Na_2SO_4 after which the lipid content was determined gravimetrically. Then samples were spiked with ^{13}C-labelled PCDD/F and PCB standards, purified on an automated system using activated carbon followed by addition of H_2SO_4/SiO_2. Following several hours reaction time samples were filtered and concentrated and further purified using an acid/base activated silica gel column. The purified samples were concentrated to near dryness, transferred to vials and ^{13}C-labelled 1,2,3,4-TCDD recovery standard was added to the PCDD/F and co-planar PCBs fraction. Analysis of tetra- to octa-CDD/Fs, co-planar and mono-ortho PCBs was performed on a GC/MS (VG Autospec) (for details see [6].

For analysis of PCBs, organochlorine pesticides and PBDEs at the Toxicology Center, 1.0 - 1.5 g of sample was homogenized with anhydrous Na_2SO_4, spiked with known quantities of internal standard (PCB 46 & PCB 143, ε-HCH, BB 103 & BB 155) and Soxhlet extracted using hexane:acetone (3:1). A small aliquote was used to quantify the lipid content whereas the reminder was subjected to clean-up on a cartridge containing ~8g silica impregnated with concentrated sulphuric acid (1/1, w/w). PCBs, pesticides and PBDEs were eluted with 15 ml hexane followed by 10 ml dichloromethane. The final eluate was concentrated to near dryness then taken up in 80 µl iso-octane. PCBs and DDTs were analysed on a GC equipped with a µ–ECD, while PBDEs and other organochlorine pesticides were analysed using GC/MS operated in NCI mode (SIM) (for details see [7]).

Both laboratories use a range of standard QC/QA procedures with clearly defined techniques that assure appropriate recognition of peaks, laboratory blanks, QC samples, detection limits and participation in interlaboratory calibration studies.

Results and Discussion

DDTs and HCHs

Organochlorine pesticides and dioxin-like chemicals were detectable in all samples covering butter samples from the last 60 years. For example, HCHs were detectable in all samples ranging from less than 2 ng/g lipid in butter from the late 1980s to about 530 ng/g lipid in the late 1950s. Similarly the concentrations of DDTs ranged from about 20 ng/g lipid in the most recent samples from the late 80s to several µg/g lipid in samples from the 1950s and 1960s. For both DDTs and HCHs, the ratios of individual isomers (HCHs) or metabolites (DDTs) revealed an interesting pattern. β-HCH, the most stable isomer of HCHs, and p'p-DDE, a key stable metabolite of DDT, dominated the sample from 1944 which may indicate that this sample may have been subject to more extended metabolic activities. It is noteworthy that in contrast to the other samples this sample was kept at room temperature (i.e. private home and museum). The HCH profile in the samples from the 1950s and 1960 were dominated by α-HCH, which reflects the use of technical mixtures of HCH at the time. Similarly, for DDTs, the samples from the 50s and 60s are dominated by the parent compound, p,p'-DDT, that was widely used at the time. In contrast to the older samples, γ-HCH was the dominant HCH isomer detected in the sample from 1987 which

reflects a change in the 80s towards usage of the active ingredient of HCH. For DDTs we observe a shift back to dominance of p,p'-DDE in the most recent sample. This is likely to reflect the ban of DDT within Australia and consequently contamination related to aging and metabolism of the DDT during this period (i.e. aged DDT).

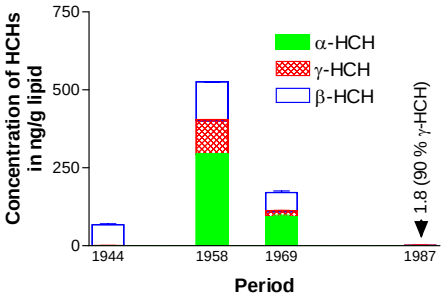

Figure 1: Concentration and isomer distribution of HCHs in historical butter samples.

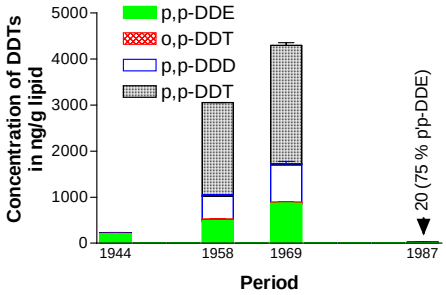

Figure 2: Concentration and distribution of composition of isomers and metabolites of DDT in historical butter samples.

PCBs

PCBs were analysed in two laboratories. Combining the two data-sets obtained from the two different laboratories demonstrates that levels of PCBs were lowest in the oldest sample (~1944) and highest in the 50s and 60s. However in contrast to DDTs and HCH the concentration decrease of PCBs in the 80s compared to the 70s and 60s was far less which is certainly related to the continued presence of PCBs.

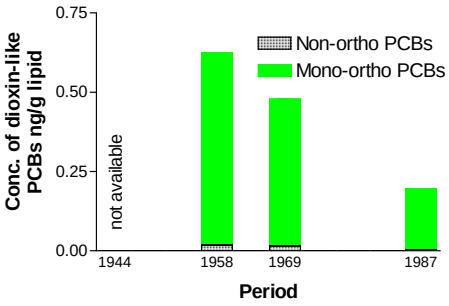

Figure 3: Concentration of non-ortho and mono-ortho PCBs in historical butter samples.

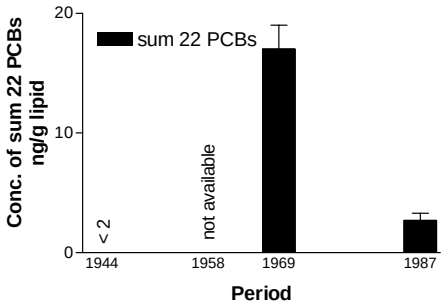

Figure 4: Concentration of sum of 22 PCBs in historical butter samples.

Dioxins

Dioxins were detectable in all samples from the 1940s to the 1980s and for comparison we also included the values for average concentrations in butter in Australia sampled in 2000. In fact as reported previously the sample of butter from the early 1940s showed highest levels of OCDD which is a key contributor to the sum of PCDDs (hence highest ∑PCDD/F concentrations in

1944). Similarly OCDD levels were high throughout the samples in the 1950s and 1960 and as reported we even found some elevated OCDD levels in samples collected in 2000 although none of them were in the range of those found in the samples of the 40s-60s samples.

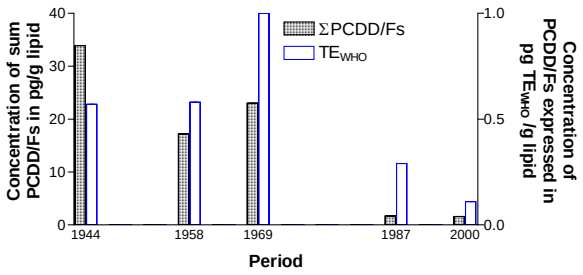

Figure 5: Concentrations of PCDD/Fs expressed as ∑2,3,7,8 PCDD/Fs (Y1 axis) and TE_{WHO} (Y2 axis) in historical butter samples from Australia.

PBDEs

PBDE concentration in the butter samples were consistently below the limit of detection and the sum of 8 PBDEs was less than 0.20 ng/g lipid.

Conclusions

Analysis of sealed butter samples provides a good indication for historic contamination of the Australian Environment with persistent organohalogen compounds. These results indicate that for organochlorines that were intentionally produced (PCBs and organochlorine pesticides) levels increased rapidly following their introduction, peaked in the 50s and 60s and potentially 70s, but strongly decreased again in the 80s. The data can provide an indication of the potential cumulative exposure of humans.

Acknowledgment

The authors would like to acknowledge the Australian War Memorial and staff of the Australian Antarctic Division for providing butter sample, Dr Keith Horsley (DVA) for his enthusiasm in getting the first sample and staff at Entox, ERGO and the Tox. Center for support with the study. NRCET is co-funded by Queensland Health.

References

1. Malisch, R. *Chemosphere* **40**, 1041-1053 (2000).
2. McLachlan, M.S. *Environ. Sci. Technol.* **30**, 252-259 (1996).
3. Müller, J.F., McLachlan, M.S., Hawker, D.W. & Connell, D.W. in *Ecoogical Issues and Environmental Impact Assessment* (ed. Cheremisinoff, P. N.) 421-442 (Gulf Publishing Company, Houston, Texas, USA, 1997).
4. Santillo, D. et al. *Food Add. Contam.* **20**, 281-290 (2003).
5. Müller, J. F., Prange, J. A., Gaus, C., Paepke, O. & Horsley, K. *Organohalogen Compounds* **57**, 233-237 (2002).
6. Müller, J. F., Prange, J. A., Gaus, C., Moore, M. R. & Paepke, O. *Environmental Science and Pollution Research* **8**, 7-10 (2001).
7. Jacobs, M., Covaci, A. & Schepens, P. *Environ. Sci. Technol.* **36**, 2797-2805 (2002).