GERMAN-FRENCH JOINT PROJECT: PCDD/F IN FOOD SAMPLES FROM UPPER RHINE RIVER VALLEY

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Introduction

In 1998, the Ministry of Agriculture of the German state of Baden-Württemberg (south-western Germany) and the Ministry of Agriculture and Fisheries - Directorate General for Food (DGAL) of France signed a German-French contract to launch a joint programme. This project should determine the levels of PCDD/F in different categories of food which is produced in the upper River Rhine valley between Basel and Karlsruhe. On the German and on the French side of this valley 4 areas were selected to collect food samples. The samples of the German side of the upper Rhine valley were analysed by the Chemische Landesuntersuchungsanstalt (CLUA) Freiburg, the samples of the French side were analyzed by CARSO laboratory. The food samples of each area included milk, meat, eggs, fish and vegetable. The results of this surveillance study are presented in this paper. Further, on the French side, the results should contribute to a first assessment of the daily intake of PCDD/F

Materials and methods

The areas for sampling in France were The Vosges, Mulhouse area, Strasbourg area and North-East of Hagenau. The areas in Germany were the Black Forest, Basel area, Kehl area and Karlsruhe area. The following food categories should have been analyzed:

- cow's milk: three samples per area (one pooled milk sample from a tanker tour and two farm's milk samples),
- cow's meat: two samples per area,
- eggs: samples from two farms per area ,
- vegetable: two samples per area,
- fish: five samples of trouts of the whole area on each side of the Rhine River and three samples of fish of the Rhine River.

Overall, each laboratory should have analyzed 44 food samples. Additionally, both parties agreed to include a quality control programme. Besides a reciprocal visit in each laboratory, 18 samples were exchanged to be analyzed in both laboratories.The CLUA Freiburg applied methods which performed well in collaborative studies for determination of PCDD/Fs in eggs [1], kale [2] and milk [3] with optimisation of the extraction for each sort of food. Briefly, eggs, meat and fish samples were freeze-dried and the fat extracted with cyclohexane/toluene in a Soxhlet extractor. Milk samples were centrifuged, the upper cream layer freeze dried and extracted with hexane. An aliquot of the extracted fat was spiked with all 2,3,7,8-substituted $^{13}C_{12}$-labelled PCDD/F

congeners. Vegetable samples were spiked with all 2,3,7,8-substituted $^{13}C_{12}$-labelled PCDD/F congeners, freeze-dried and extracted with ethanol/toluene in a Twisselmann extractor. The clean-up procedure included gel chromatography, chromatography on a sulfuric acid- and NaOH-impregnated silica column, on a florisil column and on a Carbopack B/Celite column. As a recovery standard, $^{13}C_{12}$-labelled 1,2,3,4-TCDD was used. Final volume was 20 µl of toluene. GC/MS-detection was performed on a VG Autospec at 10,000 resolution using a 60 m DB-5MS column. The A200S autosampler injected 5 µl into the Multinjector of a Carlo Erba Mega GC. Usually, a 5 point-calibration curve was acquired in duplicate. CARSO used a method described by Liem et al [4]. Shortly, samples were fortified with sixteen 2,3,7,8-substituted $^{13}C_{12}$-labelled PCDD/F congeners. The milk fat fraction was extracted by the appropriate method using organic solvents. After drying and determination of the fat content, the clean up-steps included Carbosphere column chromatography and alumina column chromatography. The finally purified extract was concentrated to 25 µl of dodecane containing two internal standards ($^{13}C_{12}$-labelled 1,2,3,4-TCDD and $^{13}C_{12}$-labelled 1,2,3,7,8,9-HxCDD). A volume of 1.5 µl was injected for HRGC/HRMS quantification. The resolution of the mass spectrometer (Ultima Micromass) was set at 10,000 resolution.

Results and discussion

The quality control study proved that the results of both laboratories were comparable (deviation of I-TEQ-value in the range of +/-10 % of the mean for all samples of animal origin).

In general, the dioxin contamination of the milk and meat samples is low and in the range of the German [5, 6] and French average (ARILAIT 1998 study stating an average of 0.65 pg I-TEQ/g fat on 148 samples). However, a few samples collected in Germany had elevated PCDD/F levels as result of a new dioxin source which was detected in 1998: the use of contaminated citrus pulp from Brazil in feedingstuffs for beef and dairy cattle. As a result, in 1998 many milk, beef and veal samples contained significantly elevated PCDD/F concentrations [7].

In 1993 the Federal Health Office (formerly the Bundesgesundheitsamt) and Federal Office for the Environment (Umweltbundesamt) recommended guidelines and action limits for the PCDD/F content of milk and milk products [8]. These orientation values should guarantee a harmonized valuation of all dioxin results for these products in Germany.

Table 1: Proposed guidelines for PCDD/F in milk fat and recommended actions in Germany

PCDD/F-contamination in pg I-TEQ/g milk fat	recommended action
< 0.9	Target value to be met. Only to be achieved by long-term reduction of PCDD/F release into the environment.
> 3.0	Investigate sources and start measures to minimize release. If no short term measures to reduce emissions are possible, it is recommended to the farm to change pattern of land use. Recommendation not to distribute milk and dairy products directly to the consumer.
> 5.0	Milk and dairy products no longer marketable

On March 17, 1998, the CSHPF (French Superior Counsel of Public Health) issued a statement specifying the following guidance limits for PCDD/F concentrations in milk:

- 5 pg I-TEQ/g fat is the limit for trade of milk and dairy products,
- 3 pg I-TEQ/g fat is the value which generates a search of PCDD/F sources and steps to rapidly reduce these sources,
- < 1 pg I-TEQ/g fat is the target to be met for all milk and dairy products of large consumption.

Although these guidelines do not strictly apply to meat samples, it can be assumed for cow's meat that the levels of PCDD/Fs in milk fat are in the same range as those in other body fats (and thus in meat) under steady state conditions. Thus, these milk guidelines can give a first orientation for beef and with some precaution for meat of other breeding animals, as well, to find out whether a sample is probably low or possibly high contaminated. Data from 313 meat samples from breeding animals and game were summarized to help to recommend guidelines and action limits for different sorts of meat samples. [9].
The determination of PCDD/F after oral administration in milk and meat samples proved a wide range of contamination for different congeners in different edible parts [10]. As example, the following levels of 2,3,7,8-TCDD were reported (in pg/g fat): fillet steak 3.01, shoulder not detectable, adipose tissue 17.3, heart 16.1, liver 12.5, thigh not detectable. Thus, when the joint programme was planned, it was feared that the influence of the kind of meat was more important than the origin. Therefore, the CLUA Freiburg analyzed altogether 16 meat samples from different parts from two cows (leg, shoulder, neck, belly, flank, chop, kidney, heart, liver). As a result, besides liver all other samples had very homogenous PCDD/F levels (range 0.55 to 0.66 pg I-TEQ/g fat for cow no. 1, range 0.48 to 0.74 pg I-TEQ/g fat for cow no. 2; liver for cow no. 2: 2.24 pg I-TEQ/g fat.).
The PCDD/F content in egg samples ranges from 0.24 to 5.68 pg I-TEQ/g fat. Fürst et al. showed that the dioxin contamination in commercial chicken eggs depends on the type of housing [11]. The CLUA Freiburg analyzed 374 egg samples between 1993 and 1998. As a result, the median contamination of eggs from laying hens housed in elevated wire cages was 0.70 pg I-TEQ/g fat, from hens kept on the ground 0.98 pg I-TEQ/g fat and from foraging chickens 1.90 pg I-TEQ/g fat. These data are lower than those resulting from analyses between 1993 and 1996 [5] proving a gradual decrease over years. Thus, a contamination below 1 pg I-TEQ/g fat is considered to be background. A content above 3 pg I-TEQ/g fat is a clear indication for an influence of a PCDD/F source. The highest value relates to a location near to the South Mulhouse MSW incinerator. Two samples of the Black Forest region which were expected to have background contamination had elevated dioxin levels with a PCP pattern. Additional investigations of the CLUA Freiburg proved that the hen-house was treated with PCP.The PCDD/F content of fish (trout category) varied between 2.19 and 11.3 pg I-TEQ/g fat (0.074 and 0.99 pg I-TEQ/g fresh weight). These values are considered to be the background level for this type of food [5]. Three different species of fish from the River Rhine had up to 70.9 pg I-TEQ/g fat (roach) or 11.2 pg I-TEQ/g fresh weight (eel). These findings confirm that the PCDD/F level is strongly dependent on the fat content of the fish which varies considerably (between 0.4 % for roach and 27.5 % for eel). Because of the accumulation of PCDD/F in adipose tissue the extreme different fat amounts can cause extreme different dioxin levels when correlated to fresh weight or fat base.

The content of most vegetable samples (all besides one lettuce; one rhubarb) ranges between 67 and about 300 pg I-TEQ/kg dry matter which is considered to be background contamination. Two

salad samples on the French side were contaminated by soil and thus taken out of the survey. As a general conclusion, the PCDD/F content of the most milk, eggs, fish, meat and vegetable samples collected on both sides of the Rhine River was in the range of background contamination. Some samples indicated an influence of specific sources (such as use of contaminated citrus pulp as feedingstuff, of former use PCP for painting of hen-houses or of the municipal waste incineration); however the resulting dioxin levels didn't achieve any action limits.

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