Levels of PCDD/Fs and PCBs in goat's milk exposed to a chronic intake of contaminated hay

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Introduction

Lactating ruminants may participate to the transfer of persistent semi-volatile organic contaminants into the human food chain by consuming herbage or silage. Most of the studies on contaminated animals are carried out giving them spiked feeding stuffs with one or few compounds, which is very different to a natural contamination[1]. The aim of the study was to feed three lactating goats with naturally contaminated hay and to collect their milk to determine how long it should take to exceed PCDD/F maximum tolerable limit for milk. Another objective of the experiment was to establish, in controlled conditions (i.e. feed intake and milk output), the carry over rate of 17 PCDD/Fs and 18 PCBs from contaminated hay to milk at steady state. This part of the study is described elsewhere[2].

Material and methods

Animals and feeding

Three lactating Alpine goats from the experimental station (ENSAIA) were used for this study. Before the study, they were fed with controlled hay. After a 10-day period to get adapted to the experimental facilities, goats were fed daily with 800 g of naturally contaminated hay. This contaminated hay had been collected in the vicinity of a hazardous municipal waste incinerator several years ago. The animals also received a daily additional ration composed by dehydrated sugar beet pulps (800 g), crushed maize (400 g), soybean meal (200 g) and mineral salt (10 g). Controlled hay and contaminated hay samples as well as additional rations were checked for PCDD/F and PCB content. Five hundred milliliter of milk samples were collected and analyzed individually at the morning milking, prior to the experiment and at day 8, 15, 22, 29, 36, 43, 57 and 71.

Extraction, purification and detection

Milk samples were extracted with a liquid/liquid method while Accelerated Solvent Extraction was used for all the feeding stuff samples. Purification included 3 chromatographic steps with successively silica, Florisil and carbon columns. PCDD/F and PCB analyses were performed by GC-HRMS. The mass spectrometer (JMS 700D, Jeol, Tokyo, Japan) was operating at a resolution of 10,000 in electron ionization mode (38 and 42 eV electron energy for PCDD/Fs and PCBs respectively). Analyses were in accordance with the guidelines of Directive 2002/69/EC and 2002/70/EC laying down the sampling methods and the methods of analysis (isotopic dilution) for the official control of dioxins and the determination of DL-PCBs in food and feed.

Results and discussion

PCDD/Fs and PCBs in feedstuffs

Table 1 indicates the levels of the 17 PCDD/F congeners and the 18 PCB congeners analyzed in all the feeding stuffs. It reveals that the contaminated hay collected in the vicinity of a waste incinerator was the main source of PCDD/Fs and PCBs. When comparing the PCDD/F concentrations in the different feeding stuffs, all the congeners in the contaminated hay presented higher values than in the control hay, and the additional ration brought no extra contamination. In the case of the PCBs, the additional ration concentrations were also very low. Measured values for the control hay and the contaminated hay were similar, especially for marker PCBs.

Table 1: Mean concentrations in pg/g dried matrix of 17 PCDD/Fs and 18 PCBs congeners

PCDD/Fs	Control hay	Contaminated hay	Additional ration
2.3.7.8 – TCDD	0.04	0.07	0.02
1.2.3.7.8 - PeCDD	0.01	0.60	0.02
1.2.3.4.7.8 - HxCDD	0.02	0.60	0.02
1.2.3.6.7.8 - HxCDD	0.11	0.72	0.01
1.2.3.7.8.9 - HxCDD	0.04	0.63	0.01
1.2.3.4.6.7.8- HpCDD	1.92	4.67	0.19
OCDD	6.71	10.56	0.82
2.3.7.8 - TCDF	0.06	0.35	0.05
1.2.3.7.8 - PeCDF	0.04	0.60	0.02
2.3.4.7.8 - PeCDF	0.04	0.98	0.03
1.2.3.4.7.8 - HxCDF	0.05	1.13	0.02
1.2.3.6.7.8 - HxCDF	0.05	1.35	0.02
1.2.3.7.8.9 - HxCDF	0.05	0.10	0.02
2.3.4.6.7.8 - HxCDF	0.05	1.64	0.02
1.2.3.4.6.7.8 -HpCDF	0.36	4.24	0.05
1.2.3.4.7.8.9 -HpCDF	0.10	0.31	0.04
OCDF	0.40	1.35	0.06
PCBs	**Control hay**	**Contaminated hay**	**Additional ration**
77	3.86	10.43	0.72
81	0.14	0.59	0.05
126	0.63	3.02	0.14
169	0.12	1.03	0.03
105	19.45	42.42	6.53
114	1.27	2.49	0.40
118	63.69	126.70	18.94
123	2.23	5.00	0.99
156	6.68	13.82	2.56
157	1.17	2.87	0.49
167	4.57	8.26	1.54
189	0.90	2.22	0.03
28	57.00	66.76	21.29
52	46.00	68.53	20.26

101	169.00	215.57	51.04
138	176.00	283.42	52.41
153	319.00	457.98	93.02
180	68.00	126.45	24.66

On one hand, measured values for the contaminated hay were elevated for dioxins (Table 2), e.g. 2.5 times higher than the maximum tolerable limit fixed at 0.75 pg WHO-TEQ/g 12 % moisture. On the other hand, low concentrations were found for PCBs.

Howether the daily ration concentrations given to animals (contaminated hay and additional ration) were also calculated and the value obtained for PCDD/Fs was below the authorized limit and for this daily ration, PCB concentrations were very low.

Table 2: Measured concentrations in feeding stuff (pg WHO-TEQ/g 12 % moisture for PCDD/Fs and DL-PCBs and ng/g 12 % moisture for Marker PCBs)

	PCDD/Fs	**Dioxin-like PCBs**	**Marker PCBs**
Contaminated hay	1.78	0.33	0.88
Daily ration	**0.66**	0.12	0.75

PCDD/F and PCB excretion in milk

In this experiment, the major aim was to study the excretion of the contaminants in milk. Figure 1 presents the PCDD/F level in the 3 goat milks during the study. The same kinetics were observed for the three goats: the increase of PCDD/F concentrations was very fast during the first two weeks and reached a plateau at the end of the study period. After one week of experiment, the maximum EU authorized value of 3 pg WHO-TEQ/g fat in milk was already reached although the dioxin concentration in daily ration given to the goats was below 0.75 pg WHO-TEQ/g matrix at 12 % moisture. This has already been observed for dioxin concentration in eggs[3]. PCBs were also analyzed. When adding the dioxin-like PCBs to the PCDD/Fs, the total WHO-TEQ value in the milk was above 5 pg WHO-TEQ/g fat after the first week (one of the two proposed future limits for total WHO-TEQ in dairy products).

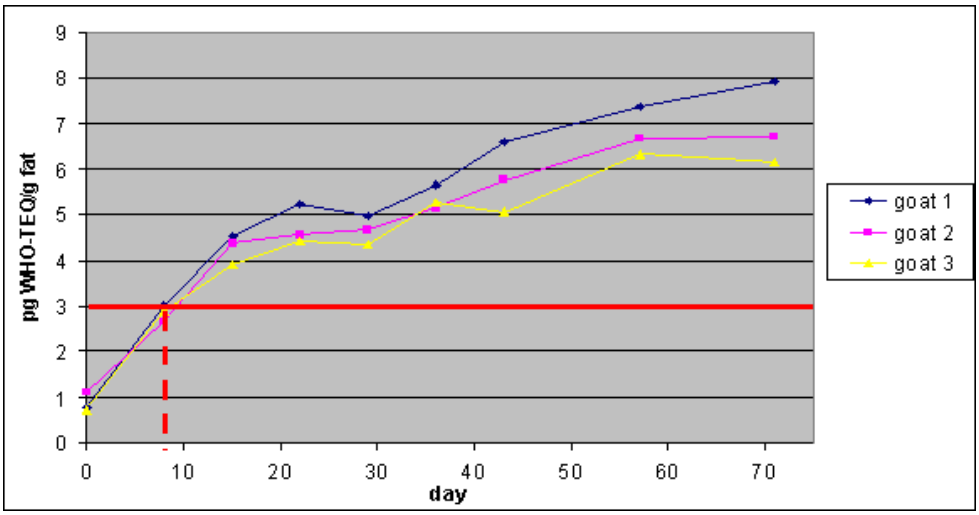

Figure 1: kinetic of PCDD/F contamination in milk

The ratio between dioxin-like PCB and PCDD/F concentrations is presented in Figure 2. The ratio observed between the contaminated hay and the daily ration is similar with PCDD/Fs representing 80% of the sum total. The

evolution of the ratio is also presented on this graph for the milk from one goat. At the beginning of the experiment, PCDD/Fs represent 35 % of the total. This ratio is due to the feedingstuff eaten before the start of the experiment. This ratio has been already observed in another project realised in LABERCA dedicated to cow's milk. After one week, this ratio was almost inverted: 60 % for PCDD/Fs and 40 % for PCBs. During the experiment it tended to reach the contaminated hay ratio and consequently the daily ration ratio. This means that due to the rapid transfer of those contaminants, the ratio found in excreted milk tends to be similar to the feed given to animals.

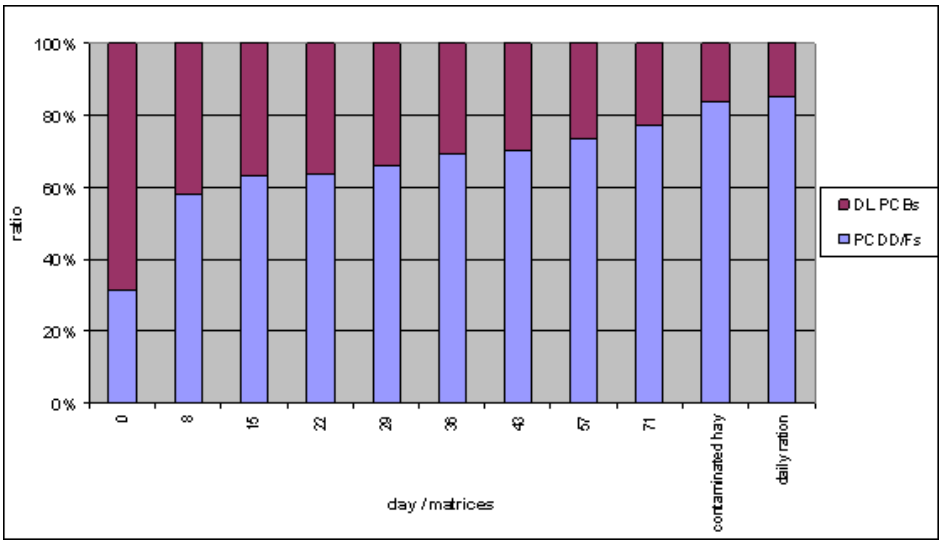

Figure 2: Evolution of DL PCBs/dioxins ratio in the milk of one goat along the experiment and the same ratio in contaminated hay and daily ration.

Conclusion

The major aim of this experiment was to study the feed to milk transfer of PCDD/Fs and PCBs. Three goats were fed with hay naturally contaminated in PCDD/Fs but not in PCBs. The goat daily ration was below the maximal tolerable value for dioxins in feeding stuff. The study of the excretion of the contaminants in milk underlined the quick increase of the PCDD/F concentration. After the first experimental week, PCDD/F WHO-TEQ was already found to be higher than 3 pg/g of milk fat.These data indicate the rapidity of feed to milk transfer of these molecules and therefore their strong affinity to milk fat. Examining the ratio between dioxin-like PCB and PCDD/F concentrations: the ratio calculated in the milk tended to be greatest for the contaminated hay.

References

1. Slob, W., Olling, M., Derks, H.J.G.M., & de Jong, A.P.J.M. (1995). Congener-specific bioavailability of PCDD/Fs and coplanar PCBs in cows: Laboratory and field measurements. *Chemosphere,* 31, 3827-3838.

2. Costera A., Feidt C., Marchand P., Le Bizec B., Rychen G. PCDD/F and PCB transfer to milk in goats exposed to a chronic intake of contaminated hay. International Dairy Journal, *submitted*.

3. Traag W., Kan K., Zeilmaker M., Hoogenboom R. Carry-over of dioxins and PCBs from feed to eggs at low contamination levels. (2003) Organohalogen Compounds, 61, 381-384.