Distribution of PCDDs/PCDFs into milk and organs of Egyptian Baladi goats after oral supplementation of dioxins

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Abstract

From a herd of five Egyptian Baladi lactating goats four had been orally dosed with 6.9, 3.4, 2.3 and 1.1 µg WHO-(PCDD/F)-TEQ. The dose contained 17 congeners of both ^{13}C-labelled and native dioxins and furans. The fifth goat was kept as control. The goats stayed alive for 16 days after oral supplementation. During this time samples of milk and serum were taken. After 16 days two of the goats were slaughtered and samples of liver, kidney, kidney fat, brain, spleen, heart, lung, mammary gland, uterus and foetus were taken for analysis of PCDD/Fs. The results indicated that the percentage of excretion of the PCDD/Fs with the milk was 0.0015 % while in the case of liver, kidney, musculature, mammary gland and kidney fat the transfer was 0.013, 0.0012, 0.0007, 0.0012 and 0.0011 % respectively. On the other hand the average content of pg WHO-TEQ for PCDDs and PCDFs in brain was 6.75 per g on fat base. This was the lowest value in organs. The highest value of WHO-TEQ was in the liver with 693.62 pg/g fat. Average concentrations of WHO-TEQ/g fat in lung, spleen, heart, foetus and uterus were 107.78, 57.12, 51.12, 33.96 and 32.77 pg respectively. The milk samples from untreated goats showed similar levels as the milk samples from goats which were given oral doses. But in the case of liver, kidney, spleen, heart and other organs of control the observed concentrations showed higher levels of dioxins.

1. Introduction

Polychlorinated dibenzodioxins (PCDDs) and dibenzofurans (PCDFs) are an abundant group of organic compounds. Of the 210 compounds 17 congeners are toxicologically important and substituted with chlorine at least in the 2,3,7,8 position of the molecule. They are formed mainly as a result of pyrolytic processes. Especially at incomplete combustion of organic materials during industrial and other human activities such as the processing of coal and crude oil, the combustion of natural gas, traffic, cooking and smoking of tobacco (1). Human exposure to these compounds occurs mainly through food (2). Due to their physical and chemical properties they migrate through the food chain into lipophilic compartments. Thus they accumulate in lipids at the end of the food chain

(3, 4). The knowledge of transfer pathways through the food chain is an important issue in food safety. It is known that dioxins are transferred from feed to milk after oral ingestion by lactating ruminants (5).

Therefore the aim of this study was to indicate the transfer of PCDDs and PCDFs into milk and organs of 4 lactating Egyptian Baladi goats after oral administration of 6.9, 3.4, 2.3 and 1.1 µg WHO-(PCDD/F)–TEQ respectively to the individual animals. Expressed in picograms the doses were 69 x 10^5, 34 x 10^5, 23 x 10^5 and 11 x 10^5 to facilitate the comparison with the results given in Tables 2-12 and Figures 1 and 2.

2. Materials and Method

2.1 Dioxin standard

The stock standard solution contained each 17 native and ^{13}C-labelled 2,3,7,8-substituted PCDD/F congeners with the concentrations given in Table 1. It was obtained from Chemisches und Veterinäruntersuchungsamt Freiburg, Germany (6). The display with four digits after decimal point is according to the selected computation programme of the mass spectrometer and does not indicate the measurable concentration below the femtogram-range (100 attograms). Therefore in the following the results (given in pg/g material) are rounded to the second digit after the decimal point.

Tab. 1: PCDD and PCDF concentrations in the standard stocksolution

Compounds	^{13}C-labelled (pg/µl)	Native (pg/µl)
2378TCDD	9.123	9.3950
2378TCDF	7.0695	6.9550
12378PeCDF	7.5200	7.8100
12378PeCDD	7.9460	8.7380
23478PeCDF	9.4400	9.2000
123478HxCDF	6.2000	6.3580
123678HxCDF	8.2210	8.7070
123478HxCDD	9.7330	8.5260
123678HxCDD	7.0700	7.4600
12378HxCDD	6.2440	6.0800
234678HxCDF	6.7740	7.0000
123789HxCDF	9.6750	8.5400
1234678HpCDF	7.9610	7.0250
1234678HpCDD	9.6950	8.4940
1234789HpCDF	7.1450	6.8640
OCDD	6.3890	7.4100
OCDF	7.2670	7.2100
pg WHO –TEQ /µl value of standard	**28.5191**	**29.2635**

2.2 Animals and supplemented doses

For the experiment five Baladi goats from Abou Rawash experimental farm, National Research Centre – Egypt, were used with a mean body weight of 30 ± 5 kg and an age of 3 years ± 6 months. One of them was pregnant (3 months). The lactating goats were milked by hand. The average milk production during the week before the experiment started was 50 ± 10 ml/day. The doses were calculated allowing for the weight of goats and the LD_{50} for guinea-pig (0.6 µg/kg body weight). The amounts given to the goats were 6.9, 3.4, 2.3 and 1.1 mg which represent 0.23, 0.11, 0.077, 0.037 µg/body weight and equal 1/3, 1/6, 1/13, 1/27 of the LD_{50} for the guinea-pig.

2.3 Dioxin administration and sampling

At the first day of the experiment after morning milking the oral application of the dioxin stock standard solution (concentrations and doses see above) diluted in 5 ml water was performed by direct injection into the mouth of the animals with a sterile syringe. The application was repeated every two days for two of the animals three times. The two other animals were dosed once only. Milk and blood samples were collected daily. Blood samples (7 ml) were immediately centrifuged (3000 R /15 min) to obtain plasma. Total milk production was collected and stored at -20°C. After 16 days two of the goats were slaughtered and samples of liver, kidney, kidney fat, brain, spleen, heart, lung, mammary gland, uterus and foetus were taken for analysis of PCDD and PCDF residues.

2.4 Method

2.4.1 Analytical procedures

Fat is extracted with acetone/petroleum benzene and submitted to gel permeation chromatography to separate fat and fat solubles for the first crude extract. The following clean up of the extracts is performed with florisil and aluminium oxide. The dioxins are determined by GC/HRMS (method in a modified version according to Fürst et. al., (7)).

2.4.2 GC/HRMS

Finnigan MAT 95/HP Series 5890. Conditions: Injector 280°C ; column: DB5 60 m, 0.25 µ film thickness, 0.25 mm ID ; temperature programme: 1 min at 140°C, in 15 min to 240°C, in 3.5 min to 300°C, 15 min at 300°C; carrier gas : helium, 4 ml/min ; SIM: 305.90-471.78 ; scan time: 0.2 s; SEM: 1.6kV; ion source pressure: 3×10^{-7} pa; system pressure: 1×10^{-10} pa; transfer line temperature: 280°C; working resolution between 6000 and 8000 (10% valley).

2.4.3 Validation and detection limit

The method was validated by the Fraunhofer Institut für Verfahrenstechnik und Verpackung, Munich Germany. The detection limit for all compounds determined is 0.003 pg/g fat.

3. Results and discussion

The excretion of PCDDs and PCDFs into milk of the goats 16 days after oral supplementation was as shown in Table 2. According to this the levels of dioxins were 82.61 (goat 259), 85.08 (goat 234), 30.20 (goat 270), 29.47 (goat 272) and 30.53 pg WHO-TEQ/g milk fat for untreated goats. The average percentage of excretion of dioxins with the milk was 0.0015 pg WHO-TEQ/g milk fat.

Tab. 2: Excretion of PCDDs and PCDFs with the milk of lactating goats 16 days after oral supplementation

Item	Animals				
	Untreated goats	goat 259	goat 234	goat 270	goat 272
	Oral doses of µg WHO-(PCDD/F)-TEQ per animal				
		6.9	3.4	2.3	1.1
	Excretion with the milk after 16 days				
pg WHO-(PCCDD/F)-TEQ/g milk fat	82.61	85.08	30.20	29.47	30.53
% excretion of dioxins with the milk fat	-	0.0012	0.0008	0.0012	0.0027

Table 3 indicates the distribution of dioxins in the liver of the goats after 16 days. Concentrations of PCDD/Fs in the liver were 498.26, 693.62 and 618.33 pg WHO-TEQ (PCDD/F)/g fat for untreated goats , goat 259 and goat 234 respectively.

Tab. 3: Distribution of PCDD/Fs in the liver of Egyptian Baladi lactating goats 16 days after oral supplementation

Item	Animals		
	Untreated goats	goat 259	goat 234
	Oral doses in µg WHO-(PCDD/F)-TEQ per animal		
		6.9	3.4
	Concentration of PCDD/Fs found in the liver after 16 days		
pg WHO-(PCDD/F)-TEQ/g fat in the liver	498.26	693.62	618.33
% of dose found in the liver	-	0.010	0.017

Table 4 indicates the distribution of dioxins in the kidney of the goats after 16 days from first dosing. Concentrations of PCDD/Fs in the kidney were 113.54 (untreated goats), 81.15 (goat 259) and 45.59 (goat 234) pg WHO-TEQ (PCDD/F)/g fat respectively.

Table 5 indicates the distribution of dioxins in the pectoral muscles of the goats after 16 days from first dosing. Concentrations of PCDD/Fs in the muscles were 39.68 (untreated goats), 54.90 (goat 259) and 29.75 (goat 234) pg WHO-TEQ (PCDD/F)/g fat respectively.

Table 6 indicates the distribution of dioxins in the mammary gland of the goats after 16 days from first dosing. Concentrations of PCDD/Fs in the mammary gland were 85.66 and 40.34 pg WHO-(PCDD/F)-TEQ /g fat for goat 259 and goat 234 respectively.

Tab. 4: Distribution of PCDD/Fs in the kidney of Egyptian Baladi lactating goats 16 days after oral supplementation

Item	Animals		
	Untreated goats	goat 259	goat 234
	Oral doses in µg WHO-(PCDD/F)-TEQ per animal		
		6.9	3.4
	Concentration of PCDD/Fs found in the kidney after 16 days		
pg WHO-(PCDD/F)-TEQ/g fat in the kidney	113.54	81.15	45.59
% storage of dioxins in the kidney	-	0.0011	0.0013

Tab. 5: Distribution of PCDD/Fs in the pectoral muscles of Egyptian Baladi lactating goats 16 days after oral supplementation

Item	Animals		
	Untreated goats	goat 259	goat 234
	Oral doses in µg WHO-(PCDD/F)-TEQ per animal		
		6.9	3.4
	Concentration of PCDD/Fs found in the pectoral muscles after 16 days		
pg WHO-(PCDD/F)-TEQ/g fat in the pectoral muscles	39.68	54.90	29.75
% storage of dioxins in the pectoral muscles	-	0.0007	0.0008

Tab. 6: Distribution of PCDD/Fs in the mammary gland of Egyptian Baladi lactating goats after 16 days of single oral dioxin dose

Item	Animals	
	goat 259	goat 234
	Oral doses in µg WHO-(PCDD/F)-TEQ per animal	
	6.9	3.4
	Concentration of PCDD/Fs found in the mammary gland after 16 days	
pg WHO-(PCDD/F)-TEQ/g fat in the mammary gland	85.66	40.34
% storage of dioxins in the mammary gland	0.0012	0.0011

Table 7 indicates the distribution of dioxins in kidney fat of the goats after 16 days from first dosing. Concentrations of PCDD/Fs in the kidney fat were 191.61, 54.61 and 54.97 pg WHO-(PCDD/F) -TEQ/g fat for untreated goats , goat 259 and goat 234 respectively.

Tab. 7: Distribution of PCDD/Fs in the kidney fat of Egyptian Baladi lactating goats 16 days after oral supplementation

Item	Animals		
	Untreated goats	goat 1	goat 2
	Oral doses in µg WHO-(PCDD/F)-TEQ per animal		
		6.9	3.4
	Concentration of PCDD/Fs found in the kidney fat after 16 days		
pg WHO-(PCDD/F)-TEQ/g kidney fat	191.61	54.61	54.97
% storage of dioxins in the kidney fat	-	0.0007	0.0015

Table 8 indicates the distribution of dioxins in the brain of the goats after 16 days from first dosing. Concentrations of PCDD/Fs in the brain were 19.26, 6.85, 6.65 pg WHO-(PCDD/F)-TEQ/g fat for untreated goats, goat 259 and goat 234 respectively.

Tab. 8: Distribution of PCDD/Fs in the brain of Egyptian Baladi lactating goats 16 days after oral supplementation

Item	Animals		
	Untreated goats	goat 259	goat 234
	Oral doses in µg WHO-(PCDD/F)-TEQ per animal		
		6.9	3.4
	Concentration of PCDD/Fs found in thebrain after 16 days		
pg WHO-(PCDD/F)-TEQ/g fat in the brain	19.26	6.85	6.65
% storage of dioxins in the brain	-	0.000009	0.00019

Table 9 indicates the distribution of dioxins in the lung of the goats after 16 days from first dosing. Concentrations of PCDD/Fs in lung were 38.19, 138.94 and 76.61pg WHO-(PCDD/F)-TEQ/g fat for untreated goats, goat 259 and goat 234 respectively.

Tab. 9: Distribution of PCDD/Fs in the lung of Egyptian Baladi lactating goats 16 days after oral supplementation

Item	Animals		
	Untreated goats	goat 259	goat 234
	Oral doses in µg WHO-(PCDD/F)-TEQ per animal		
		6.9	3.4
	Concentration of PCDD/Fs found in the lung after 16 days		
pg WHO-(PCDD/F)-TEQ/g fat in the lung	38.19	138.94	76.61
% storage of dioxins in the lung	-	0.002	0.0022

Table 10 indicates the distribution of dioxins in the spleen of the goats after 16 days from first dosing. Concentrations of PCDD/Fs in spleen were 113.73, 68.64 and 45.60 pg WHO-(PCDD/F)-TEQ /g fat for untreated goats, goat 259 and goat 234 respectively.

Tab. 10: Distribution of PCDD/Fs in the spleen of Egyptian Baladi lactating goats 16 days after oral supplementation

Item	Animals		
	Untreated goats	goat 259	goat 234
	Oral doses in µg WHO-(PCDD/F)-TEQ per animal		
		6.9	3.4
	Concentration of PCDD/Fs found in the spleen after 16 days		
pg WHO-(PCDD/F)-TEQ/g fat in the spleen	113.73	68.64	45.60
% storage of dioxins in the spleen	-	0.0009	0.0013

Table 11 indicates the distribution of dioxins in the heart of the goats after 16 days from first dosing. Concentrations of PCDD/Fs in heart were 78.69, 65.78 and 36.46 pg WHO-(PCDD/F)-TEQ/g fat for untreated goats, goat 259 and goat 234 respectively.

Tab. 11: Distribution of PCDD/Fs in the heart of Egyptian Baladi lactating goats 16 days after oral supplementation

Item	Animals		
	Untreated goats	goat 259	goat 234
	Oral doses in µg WHO-(PCDD/F)-TEQ per animal		
		6.9	3.4
	Concentration of PCDD/Fs found in the heart after 16 days		
pg WHO-(PCDD/F)-TEQ/g fat in the heart	78.69	65.78	36.46
% storage of dioxins in the heart	-	0.0009	0.0010

Table 12 indicates the transfer of dioxins into the foetus and uterus of the goats after 16 days from first dosing. Concentration of PCDD/Fs in the foetus was 33.96 pg WHO-(PCDD/F)-TEQ/g fat for goat 259 and in the uterus 32.77 pg WHO-(PCDD/F)-TEQ/g fat for goat 234 .

Tab. 12: Transfer of PCDD/Fs into the foetus and uterus of Egyptian Baladi lactating goats 16 days after oral application

Item	Animals	
	goat 259	goat 234
	Oral doses in µg WHO-(PCDD/F)-TEQ per animal	
	6.9	3.4
	Concentration of PCDD/Fs found in foetus and uterus after 16 days	
pg WHO-(PCDD/F)-TEQ/g fat in the foetus and uterus	33.9627	32.7667
% storage of dioxins in the foetus and uterus	0.0004	0.0009

Figure1 shows the transfer of PCDD/Fs into milk and organs of goat 259 (high dose) 16 days after first oral application of dioxins and furans while Figure 2 shows the transfer of PCDD/Fs into milk and organs of goat 234 (low dose) after the same dosing regime.

These results agree with Grova et al. (8). They reported that a small amount of 2,3,7,8-TCDD transfers into milk of lactating goats after a single oral ingestion and a large part of 2,3,7,8-TCDD ingested remained in the organs. Also Ruoff (9) reported about the transfer of polychlorinated dibenzodioxins and -furans (PCDD/Fs) into the milk and

organs of lactating cows after oral supplementation. The results indicated that under tissue distribution the liver is a prominent depot in most cases and the central nervous system seems to be un-effected by PCDD/Fs.

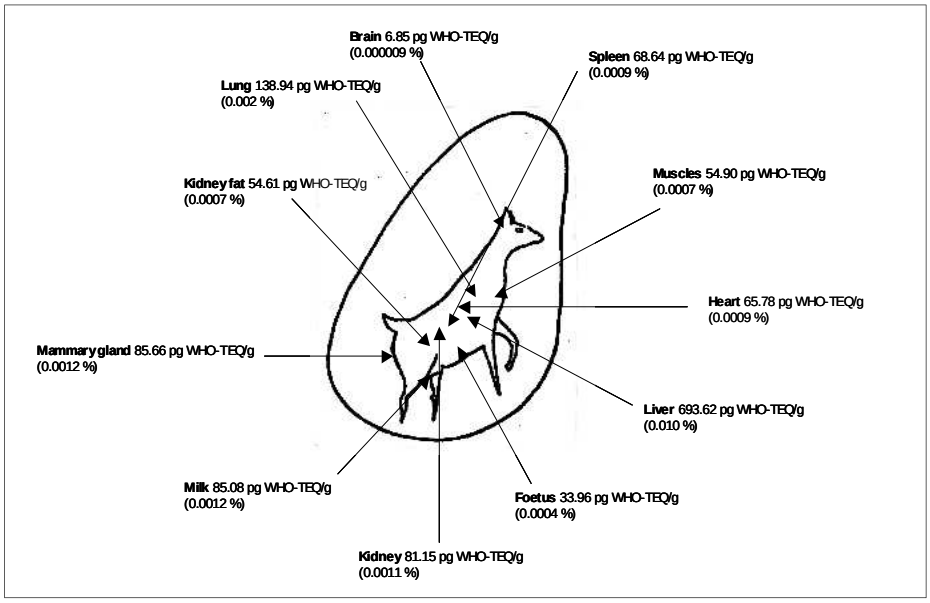

Fig. 1: Transfer of PCDD/Fs into milk and organs on fat base of goat 259 (high dose) 16 days after first oral application

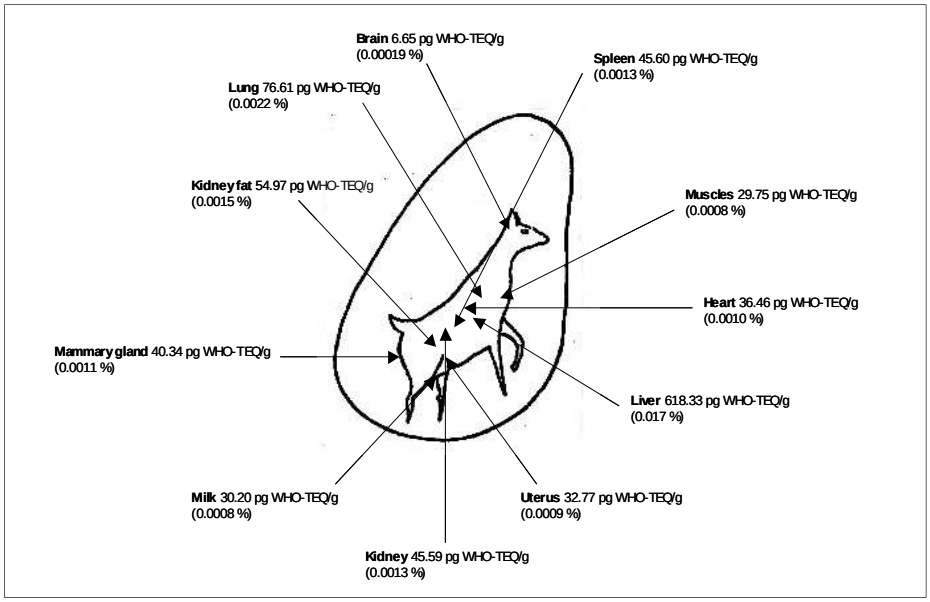

Fig. 2: Transfer of PCDD/Fs into milk and organs on fat base of goat 234 (low dose) 16 days after first oral application

4. Conclusions

The results presented in this paper contribute to new insights on evaluation of PCDD/Fs (dioxins) excretion in lactating goats. They show the percentage of PCDD/Fs transfer into milk and organs after oral dose. Also they indicate the selectivity of the intestinal or mammary epithelial barrier for PCDD/Fs in lactating goats or their capacity to metabolize them (depending on their physical and chemical properties). This may explain the differential behaviour within the compounds.

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6. Summary

Fouzy, S.M., Ruoff, U.: **Distribution of PCDDs/PCDFs in milk and organs of Egyptian Baladi goats after oral supplementation of dioxins**. Kieler Milchwirtschaftliche Forschungsberichte **58** (1) 5-15 (2006)

06 Food safety (PCDD/Fs, goat milk and organs, carry over)

It has been known for years that 17 of the 210 PCDD/Fs are toxicologically relevant for humans. The mechanisms of the development of PCDD/Fs are known, too. Whereas the concentrations in German food are at a very low level in the meantime the concentrations in food from Egypt are significantly higher.

In Egypt the goat is an important animal for food production. Therefore feeding experiments concerning the carry over of the PCDD/Fs into milk and different organs were carried out

Zusammenfassung

Fouzy, S.M., Ruoff, U.: **Verteilung von PCDD/PCDF in der Milch und den Organen der ägyptischen Ziege Baladi nach Verabreichung von Dioxinen**. Kieler Milchwirtschaftliche Forschungsberichte **58** (1) 5-15 (2006)

06 Produktsicherheit (PCDD/F, Ziegenmilch und Organe, Carry over)

Seit Jahren ist bekannt, dass von den 210 Verbindungen der PCDD/F 17 für den Menschen toxikologisch relevant sind. Auch die Mechanismen ihres Entstehens sind geklärt. Während in Deutschland durch administrative und technische Maßnahmen die Konzentrationen in Lebensmitteln inzwischen auf ein sehr niedriges Niveau abgesunken sind, liegen die Konzentrationen in ägyptischen Lebensmitteln deutlich höher.

Da die Ziege in Ägypten ein wichtiges lebensmittellieferndes Tier ist, wurden mit 4 Ziegen Fütterungsversuche zum Übergang der PCDD/F in Milch und verschiedene Organe durchgeführt.

Résumé

Fouzy, S.M., Ruoff, U. **Distribution des PCDD/PCDF dans le lait et les organes de la brebis égyptienne Baladi après l'administration orale de dioxines**. Kieler Milchwirtschaftliche Forschungsberichte **58** (1) 5-15 (2006))

06 Sécurité alimentaire (PCDD/F, lait de brebis et organes, carry over)

Il est connu depuis des années que 17 des 210 PCDD/F peuvent avoir des effets toxicologiques sur les humains. De même, les mécanismes de leur origine sont connus. Tandis qu'en Allemagne, grâce des mesures administratives et techniques, les concentrations de PCDD/F ont pu être réduites à un niveau très bas, les concentrations dans des aliments d'origine égyptienne sont nettement plus élevées.

Comme la brebis est un animal important dans la production alimentaire en Egypte, des essais alimentaires sur le carry over des PCDD/F dans le lait et différents organes ont été réalisés dans 4 brebis.