PCBs IN DAIRY COWS - METABOLISM AND BODY-BURDEN

G.O. Thomas, A.J. Sweetman and K.C. Jones

Department of Environmental Sciences, Lancaster University, Lancaster, LA1 4YQ, UK

INTRODUCTION

By far the greatest input of PCBs to the cow is through ingestion of fodder and concentrate feeds [1]. After ingestion PCBs are subjected to various chemical and physical processes which alter the congener patterns found in each compartment within the cow and in the excretion media (namely faeces and milk-fat). A long-term (four month) study on five lactating dairy cows fed 'naturally' contaminated background feed was carried out to investigate the input-output mass balance of PCBs and these processes.

MATERIALS AND METHODS

Controlled feeding trial. The controlled feeding study has been described elsewhere [1], but briefly consisted of the following. Five cows (between their 3rd and 5th lactation) were kept indoors on a farm, housed separately from the main herd, between late October and April. Feed intake and milk output was measured for each cow on a daily basis. The cows calved between 22 and 51 days before the start of the monitoring programme. The cows were weighed and assessed for condition on a weekly basis. One cow, which was ill for part of the study, showed a markedly different weight profile, data from this cow during this period was not used in the overall interpretation. The long-term sampling regime included samples of milk, faeces and feed taken on one day per week. Morning and evening samples were bulked on each sampling day.

Samples of blood and subcutaneous fat were taken from each cow on three occasions (before calving, at the beginning of the study and 3 weeks before the end of the study). Blood samples were taken from the neck, centrifuged within half an hour of collection and the serum and coagulate stored separately. After the study was complete one cow was slaughtered and various tissues sampled for PCB analysis including: hair, liver, kidney fat, kidney, heart fat, heart, diaphragm, leg muscle, lumbar fat, ventral abdominal fat and dorsal thoracic fat. Samples were all taken into hexane cleaned glass containers.

Analysis The analytical methods for feed, milk-fat and faeces [2] comprised extraction with hexane (milk-fat) or Soxhlet extraction of the sample (dried using sodium sulphate) with hexane/acetone (faeces and feed). Tissue samples were homogenised in hexane, with sodium sulphate added. Blood serum samples were modified with methanol, liquid-liquid extracted with a hexane:diethyl ether. Milk, blood and tissue extracts were cleaned up using acidified silica followed by basic alumina. Faeces and feed samples were cleaned up over silica and acidified silica followed by size exclusion chromatography using hexane/DCM. After the addition of internal standards extracts were analysed for 53 PCB congeners on a Fisons MD800 GC-MS equipped with a CPSil8 capillary column.

RESULTS AND DISCUSSION

PCBs in tissues. All fat and muscle tissues (except liver) gave similar total PCB concentrations (expressed on a fat weight basis) and congener profiles. Liver had elevated levels of PCBs which were entirely dominated by the hexa-chlorinated congeners 138 and 153, which may indicate congener-specific binding to components other than fat.

Similar congener patterns were found in body-fat and milk-fat (relatively metabolised) and between (the relatively un-metabolised) patterns of blood and faeces. Figure 1 shows the congener patterns found in blood, feed and body-fat. All congener patterns shown represent the average for all samples, throughout the study. Comparing the ratios of PCB 149 and 153 differences in the degree of metabolism in each matrix are apparent. PCB 149 was found in feed and faeces at approximately half the concentration of PCB 153, but is metabolised to a large degree within the cow, and was found at approximately ten times lower levels than PCB 153 in milk-fat and body-fat. In blood the ratio of the concentrations of PCB 149 to 153 was about 1:4. A possible explanation for this is that it may indicate that PCBs contained in the blood are not fully available for exchange with all tissues, perhaps because they are partly associated with blood components which are not directly utilised by adipose tissues, and perhaps partition relatively slowly between the various blood components. It is also possible that some metabolism is occurring within the adipose and udder tissues, although the time PCBs are within cells in the udder is likely to be relatively small, as fat is constantly being processed by these cells. Other studies in lactating cattle (for example by Klein *et al.* [3]) have shown that blood and milk-fat levels of unmetabolised PCBs correlate well which is not contradictory to the results presented here, but we know of no other study where metabolisable congeners have been analysed in both matrices. In the past, blood:tissue partition coefficients, calculated as the ratio of contaminant concentrations in the blood and the tissue of interest, have been used in pharmacokinetic models of the distribution of organochlorine contaminants within dairy cows [4]. From the data presented here it seems that such partition coefficients should be used with caution, since the contaminant concentration, especially of metabolisable congeners, found in the blood may not reflect that actually available for exchange.

Metabolism. Metabolism of each congener was estimated by comparing the concentration of each congener in the fat samples analysed (normalised to congener 153) to the daily absorbed concentration (i.e. the concentration in food multiplied by the fraction absorbed, also normalised to congener 153). This estimate will not discriminate between metabolism within the GI tract, metabolism within the animal after absorption into the bloodstream or storage in the body fat. There were obvious differences between congeners, so they were arranged into arbitrarily devised groups (shown in Table 1) designated: un-metabolised (normalised fat concentration:normalised absorbed concentration ratio of > 0.7); partially metabolised (fat:absorbed concentration ratio of 0.1-0.7); and largely metabolised (fat:absorbed concentration ratio < 0.1).

The presence of adjacent meta- and para- hydrogen atoms and, to a lesser degree, adjacent ortho- and meta- hydrogen atoms [5] appears to explain the metabolism patterns observed in this study while ortho-chlorine substitution or ortho-meta-para chlorine substitution does not. A new approach was developed to predict the metabolism of congeners based on assigning a 'metabolism score' to the ortho-meta and the meta-para adjacent hydrogen features. A score of 1 was given for each ortho-meta pair of hydrogen atoms present, and a score of 3 was given for each meta-para pair. A sum of 2 is taken as predicting partial metabolism, a sum of >2 predicting the congener to be largely metabolised, and a sum of <2 predicting unmetabolised congeners. Only the metabolism of PCBs 141 and 187 were found to be poorly predicted by this approach, congener 141 was

predicted to be largely metabolised but was found to be un-metabolised, and congener 187 was predicted to be un-metabolised, but was found to be partially metabolised based on our criteria. A system for predicting metabolism in the cow presented by McLachlan [6], based on 4,4' and 2,3,5 substitution patterns also performed quite well on this data set, although it underpredicts the degree of metabolism found in this study for eight congeners.

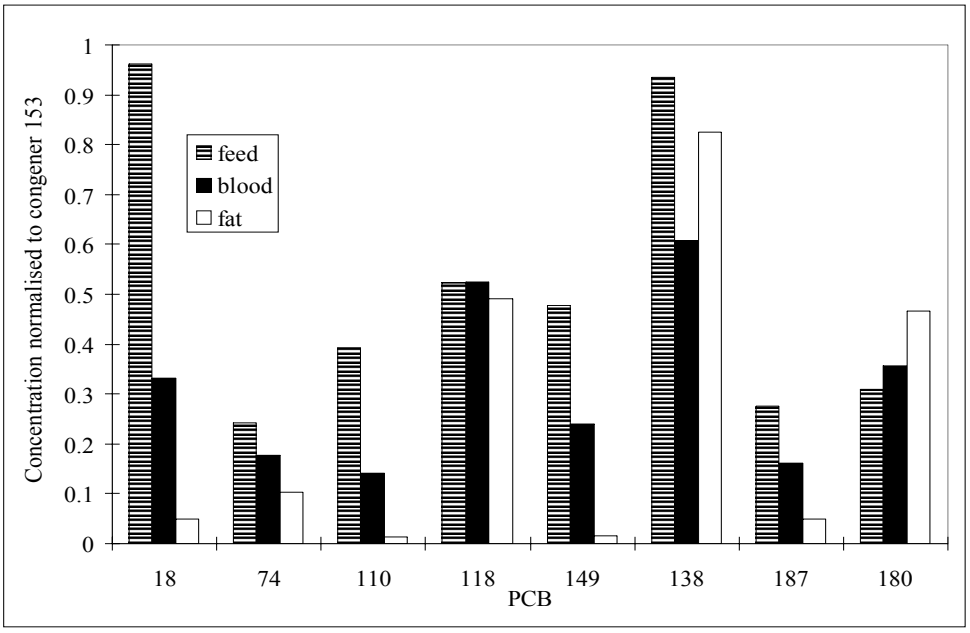

Figure 1 - Congener profiles of feed, faeces, blood, fat and milk-fat normalised to congener 153

Body burden and residence times. Using tissue weights as a percentage of total liveweight for an average lactating cow [7] a total PCB body burden was calculated for the cows in this study. Fat was by far the dominant compartment, and the total average body burden was 450 µg total PCB. The average feed intake flux of 54 µg total PCB per day measured in the study [1] represents ~12 % of the total body burden (although the congener pattern differences between stored and input PCBs make this a poor estimation for individual congeners).

Table 1 - Groups assigned for the estimated degree of metabolism

	Largely metabolised	Partially metabolised	Un-metabolised
PCB	18, 28, 31, 33, 37, 44, 49, 52, 60, 87, 101, 110, 149, 151	47, 61, 66, 105, 128, 187	118, 138, 141, 153, 156, 170, 180, 183, 194

A summary of the input, output and within cow fluxes for selected congeners, averaged throughout the study, is shown in Table 2 together with the relationship between PCB intake and the total body burden. The 'residence time' of PCBs within the cow (calculated as the body burden of each congener divided by the amount absorbed per day) is also shown. The table shows clear

differences in residence times for persistent and metabolised congeners, with a general increase with degree of chlorination.

Table 2 - Summary of the input, output and within cow fluxes, averaged throughout the study

PCB	Intake Flux ($\mu g\ d^{-1}$)	Amount Absorbed ($\mu g\ d^{-1}$)	Body Burden* (μg)	Intake Flux as % of Body Burden	Residence Time † (days)	Milk Flux ($\mu g\ d^{-1}$)	Faeces Flux ($\mu g\ d^{-1}$)	Milk Carry-over Rate**
18	1.53	1.24	5.9	26	5	0.03	0.28	0.00
47	0.26	0.20	4.7	5.5	24	0.06	0.06	0.01
52	0.53	0.41	1.2	43	3	0.00	0.12	0.00
74	0.38	0.31	12.2	3.1	39	0.14	0.07	0.05
101	0.75	0.37	2.3	32	6	0.03	0.38	0.00
105	0.25	0.16	13.5	1.8	84	0.00	0.09	0.00
110	0.62	0.36	1.5	41	4	0.01	0.26	0.00
118	0.83	0.54	57.7	1.4	106	0.78	0.29	0.56
138	1.48	0.62	96.9	1.5	157	1.02	0.87	0.68
149	0.76	0.19	1.9	39	10	0.03	0.56	0.00
153	1.58	0.65	117.4	1.4	180	1.19	0.93	0.85
170	0.24	0.04	24.8	1.0	659	0.14	0.20	0.14
180	0.49	0.13	54.8	0.9	416	0.31	0.36	0.34
183	0.14	0.03	15.3	0.9	528	0.09	0.11	0.10
187	0.44	0.21	5.9	7.4	28	0.04	0.22	0.01
194	0.09	0.03	8.4	1.0	256	0.00	0.05	0.00

* - based on body-fat at 15 % of liveweight
† - defined as body burden divided by amount absorbed
** - defined as milk flux divided by intake flux

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