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Transfer and Accumulation of Persistent Organochlorine Compounds from Bovine Dams to Newborn and Suckling Calves

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The maternal−fetal and neonatal transfers of polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and dioxin-like polychlorinated biphenyls (PCBs) were measured in the blood and milk of dams and in the blood of newborn and suckling calves. Calf blood toxic equivalent quantities (TEQs) were drastically increased by suckling. Blood concentrations of individual congeners were greater in suckling calves than in newborn calves, excluding octachlorodibenzo-*p*-dioxin (O_8CDD); O_8CDD did not readily transfer to milk but was readily absorbed by the gastrointestinal tract and remained in systemic circulation longer than other congeners. Congener concentrations in milk were correlated with maternal blood levels, and those in suckling calf blood were dependent on their concentrations in milk. These results suggest that neonatal calves absorb more lipophilic organochlorine compounds than prenatal fetuses, that those compounds that are transferred from dams to calves are associated not only with lipid transport but also with other carriers, and that the distribution of congeners is structure-dependent.

KEYWORDS: PCDD; PCDF; dioxin-like PCB; fetus; neonate; placental transport; suckling calf

INTRODUCTION

Chemicals with endocrine-disrupting properties have been shown to cause many adverse effects in various species including domestic animals and humans, ranging from effects on growth, reproduction, and brain development to induction of structural deformities, immune system deficits, and cancer (*1–4*). Pre- and neonatal exposure to these chemicals is thought to induce much stronger effects than exposure to the same compounds after maturation, because they are thought to interfere not only with the endocrine systems but also with the development of various organs (*5–7*). Therefore, the long-term and irreversible effects and the potential of transgenerational effects of those chemicals have raised serious public concern.

Persistent organochlorine compounds such as polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and dioxin-like polychlorinated biphenyls (PCBs), known as potent endocrine-disrupting chemicals, are unintentional byproducts of a variety of industrial and combustion processes involving chlorine-containing materials. In the last century, they had been extensively generated and released into the environment. Although the overall emission of these chemicals has been decreasing in industrialized nations owing to the regulation of waste incineration and the development of new technologies that avoid their syntheses and to reduce contamination, emission of these chemicals is still increasing in developing countries. Therefore, even at present, they are subtle but prevalent environmental pollutants.

In pastures contaminated with persistent organochlorine compounds, herbivores such as cattle may ingest a substantial amount of these compounds, because they have accumulated on the soil surface and onto forage. In contaminated areas, polychlorinated compounds may have accumulated on farmland during the past several decades, being byproducts of herbicides applied for weed control (*8*). Once ingested highly lipophilic chemicals are absorbed together with fat from the small intestine into systemic circulation and, then, accumulate in adipose tissue (*9*). In lactating cows, they are mobilized from body fat storage and concentrated in milk (*10, 11*). Consequently, when the dose is expressed on a body weight basis, suckling animals ingest much more organochlorine compounds than adult animals, a situation similar to that described for humans and their breastfed infants (*12, 13*).

In domestic animals, contamination with organochlorine compounds has been studied mainly to estimate human exposure levels (*14*). The distribution and accumulation of these chemicals have been well-studied in edible organs of adult animals (*9, 15, 16*). However, only a few studies have addressed organochlorine contamination in both fetal and infant animals, despite their high susceptibility (*17–19*).

The objectives of this study were to analyze the concentrations of 2,3,7,8-substituted PCDDs, PCDFs (PCDD/Fs), and dioxin-like PCBs in blood of newborn and suckling calves and to compare those concentrations with those in the blood and milk

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Table 1. TEQs of PCDD/Fs and Dioxin-Like PCBs, and the Lipid Content in Maternal Blood, Milk, and Calf Blood at Birth and 1 and 2 Months Postpartum (Mean ± SEM)[a]

	maternal blood (pg/g-lipid)			milk (pg/g-lipid)			calf blood (pg/g-lipid)		
	parturition	1 month	2 months	colostrum	1 month	2 months	birth	1 month	2 months
PCDDs	0.45 ± 0.13 *	0.41 ± 0.31 *	0.50 ± 0.22 *	2.57 ± 0.16	2.24 ± 0.21	2.27 ± 0.13	0.009 ± 0.004 a	3.06 ± 1.10 b	2.58 ± 0.43 b
PCDFs	1.15 ± 0.13 *	0.73 ± 0.15 *	1.69 ± 0.29 *	3.29 ± 0.29	2.14 ± 0.38	2.39 ± 0.23	0.43 ± 0.15 a	2.39 ± 0.37 b	2.61 ± 0.16 b
dioxin-like PCBs	1.51 ± 0.23 *	0.77 ± 0.25	1.81 ± 0.40	3.65 ± 0.34	2.89 ± 0.19	3.73 ± 0.36	0.17 ± 0.14 a	2.62 ± 0.58 b	2.11 ± 0.06 b
total TEQ	3.11 ± 0.35 *	1.91 ± 0.60 *	3.99 ± 0.81 *	9.50 ± 0.74	7.27 ± 0.68	8.39 ± 0.68	0.61 ± 0.23 a	8.06 ± 1.50 b	7.29 ± 0.32 b
lipid content (g/dL)	0.323 ± 0.016 *	0.354 ± 0.036	0.307 ± 0.026	5.77 ± 0.78 a	1.63 ± 0.32 b	2.87 ± 0.20 b	0.237 ± 0.019 a	0.344 ± 0.036 b	0.368 ± 0.034 b

[a] Values with different letters differ significantly between stages in the same sample ($P < 0.05$). An asterisk indicates values differ significantly between maternal and calf blood samples at the same stage ($P < 0.05$).

of their dams. A subsequent objective was to demonstrate the maternal−fetal and maternal−neonatal transfer profiles of those chemicals.

MATERIALS AND METHODS

Animals. Eight Japanese Black and Holstein crossbred cows aged 3−11 years with 2−8 parities and their calves managed in the Nasu branch of the Institute (Nasushiobara, Japan) were used in this study. In order to reduce the influence of genetic factors, Japanese Black embryos recovered from superovulated donor cows on day 7 after insemination were nonsurgically transferred to recipient cows 7 or 8 days after standing estrus. The cows were allowed to graze perennial ryegrass-dominant pastures and were daily given 1 kg of concentrate (CP = 12%, TDN = 70% of DM) until 3 weeks before the expected day of delivery. Cows were subsequently kept in pens individually and were fed 1 kg of the concentrate and 5 kg (DM) of orchardgrass and Italian ryegrass twice daily. The amount of concentrate fed was increased to 1.5 kg at each feeding after parturition. Calves weighed 23−29 kg at birth and were reared with their surrogate dams until weaning. During the first month, each dam and calf pair was kept in the same pen as before the calf was born, then all pairs were kept together in a paddock. At 2 months postpartum, body weights of dams and calves ranged from 520 to 670 kg and from 75 to 105 kg, respectively.

Sample Collection. Maternal blood, calf blood, and colostrum were collected from all eight pairs of dams and calves at calf birth, before suckling of colostrum was allowed. Blood and milk samples were collected from four pairs of dams and calves when calves were 1 month (29−31 days) of age and from the other four pairs when calves were 2 months (59−64 days) of age, following a 6 h fast.

Sterile 50 mL polypropylene conical tubes and caps were prepared by washing several times with methanol and drying. One hundred milliliters of maternal blood was collected by jugular venipuncture into the 50 mL tubes, each containing 50 IU of heparin (1000 IU/mL sodium salt solution). Two hundred milliliters of colostrum and milk was gathered equally from four teats into the tubes after discarding the first 50 mL of colostrum or the first 100 mL of milk from each teat. Fifty milliliters of calf blood was bled from the jugular vein into heparinized 10 mL vacuum tubes. Samples were immediately cooled and shipped at 4 °C to the Institute of Environmental Ecology (Shizuoka, Japan). After a 1 day transit, samples were stored frozen at −70 °C until analyzed.

The experiment was performed during 2001−2003. All procedures on animal subjects were reviewed and approved by the Animal Care Committee of the National Institute of Livestock and Grassland Science prior to the start of the study. All animals received humane care following the Guide for the Care and Use of Experimental Animals at the National Institute of Livestock and Grassland Science.

Analyses of PCDD/Fs and Dioxin-Like PCBs. Extraction and measurements of PCDD/Fs and dioxin-like PCBs in bovine blood and milk, including colostrum, were performed according to the *Provisional Manual of Dioxin Analysis in Blood* (*20*) and the *Provisional Manual of Dioxin Analysis in Breast Milk* (*21*) established by the Ministry of Health, Labour and Welfare of Japan, respectively. Briefly, after being thawed, weighed, and homogenized, blood samples were mixed with saturated ammonium sulfate solution and ethanol, and milk samples were mixed with saturated sodium oxalate solution, diethyl ether, and ethanol, and then lipophilic substances were extracted three times with *n*-hexane. To measure the lipid content, the extract was weighed after evaporation of the solvent. Analytes were further purified using multilayered silica gel, activated charcoal, and silica gel columns. PCDD/Fs and dioxin-like PCBs in each sample were identified and quantified using high-resolution gas chromatography (HP 6890 series GC system, Agilent Technologies, Santa Clara, CA) with a fused-silica capillary column (30 m × 0.25 mm i.d., film thickness 0.25 mm; BPX5, SGE International, Melbourne, Australia) and high-resolution mass spectrometry (AutoSpec-Ultima NT, Micromass UK, Manchester, U.K.). A total of 7 PCDDs and 10 PCDFs with a 2,3,7,8-chlorine substitution, as well as 4 nonortho and 8 mono-ortho PCBs were quantified (*18, 19*). Recoveries and quantities of congeners were determined by spiking samples with ^{13}C-labeled internal standards (Wellington Laboratories, Guelph, Canada). The minimum quantification limits in blood and milk samples were 1 and 0.05, 2 and 0.1, 4 and 0.2, and 10 and 0.5 pg/g-lipid for PCDD/F congeners with 4−5, 6−7, 8 chlorine atoms, and dioxin-like PCBs, respectively. Toxic equivalent quantities (TEQs) of individual congeners were calculated using the human and mammalian toxic equivalency factors reevaluated by the World Health Organization in 2005 (*22*). The TEQs for PCDD/Fs and dioxin-like PCBs were calculated excluding congeners below the minimum quantification limits.

Comparison of Distribution. Ratios of the respective concentrations of congeners in newborn calf blood and maternal blood at parturition, those in contemporaneous milk and maternal blood samples, and those in suckling calf blood and milk at 1 and 2 months postpartum were calculated on a lipid weight basis and compared. For calculation of the ratios between milk and maternal blood, milk samples collected at parturition were defined as colostrum and those collected at 1 and 2 months postpartum were defined as milk, because there were no significant differences between the latter stages. Similarly, data collected at 1 and 2 months were combined to calculate the ratio between calf blood and milk.

Statistics. Values are expressed as means ± SEM and all were subjected to repeated measures of ANOVA (StatView, SAS Institute, Cary, NC). Differences in concentrations of PCDD/Fs and dioxin-like PCBs between samples and growth stages of calves were analyzed using a Student's *t* test for paired data or nonpaired data. For all statistical comparisons, differences were considered significant at $P < 0.05$.

RESULTS

Lipid Content. As shown in **Table 1**, the lipid content of dam's blood was not different across sampling periods ($P > 0.20$). Lipid content of calf blood was greater at 1 and 2 months than at birth ($P < 0.05$) and was not different from the lipid content of dam's blood ($P > 0.20$) except at birth when dam's blood had a greater lipid content than calf blood ($P < 0.05$). Lipid content of colostrum was always greater than the lipid content of milk collected at 1 and 2 months after parturition.

TEQs. The TEQs of PCDD/Fs and dioxin-like PCBs expressed on a lipid weight basis are shown in **Table 1**. In maternal

Table 2. Concentrations of PCDD/F and Dioxin-Like PCB Congeners in Maternal Blood, Milk, and Calf Blood at Birth and 1 and 2 Months Postpartum (Mean ± SEM)[a]

	maternal blood (pg/g-lipid)			milk (pg/g-lipid)			calf blood (pg/g-lipid)		
congeners	parturition	1 month	2 months	colostrum	1 month	2 months	birth	1 month	2 months
2,3,7,8-T_4CDD	ND	ND	ND	0.32 ± 0.01 (8)	0.44 ± 0.10 (4)	0.32 ± 0.02 (4)	ND	3.0 (1)	ND
1,2,3,7,8-P_5CDD	1.0 (1)[b]	1.0 (1)	ND	1.5 ± 0.12 (8)	1.5 ± 0.09 (4)	1.5 ± 0.09 (4)	ND	2.0 ± 0.0 (4)	2.0 ± 0.0 (4)
1,2,3,4,7,8-H_6CDD	ND	ND	ND	1.7 ± 0.12 a (8)	0.56 ± 0.24 b (4)	0.76 ± 0.05 b (4)	ND	ND	ND
1,2,3,6,7,8-H_6CDD	3.0 ± 0.31 (8)	3.0 ± 0.0 (2)	3.7 ± 1.2 (4)	3.6 ± 0.31 a (8)	2.2 ± 0.44 b (4)	2.5 ± 0.46 b (4)	ND	6.3 ± 0.63 (4)	7.0 ± 0.55 (4)
1,2,3,7,8,9-H_6CDD	ND	ND	3.0 (1)	1.5 ± 0.10 a (8)	0.90 ± 0.26 b (4)	0.80 ± 0.10 b (4)	ND	3.0 ± 0.0 (2)	4.0 ± 0.58 (3)
1,2,3,4,6,7,8-H_7CDD	2.0 ± 0.0 (3)	3.0 (1)	2.7 ± 0.33 (4)	5.9 ± 0.49 a (8)	1.8 ± 0.52 b (4)	1.50 ± 0.06 b (4)	2.0 ± 0.0 (2)	3.3 ± 0.25 (4)	3.6 ± 0.24 (4)
O_8CDD	5.3 ± 0.60 (8)	6.0 ± 1.0 (4)	6.3 ± 0.33 (4)	3.5 ± 0.55 a (8)	1.4 ± 0.15 b (4)	0.80 ± 0.04 c (4)	13 ± 1.9 (8)	8.8 ± 1.3 (4)	9.4 ± 1.1 (4)
2,3,7,8-T_4CDF	ND	ND	ND	0.08 ± 0.01 (4)	0.19 ± 0.12 (2)	0.07 ± 0.01 (2)	2.0 ± 0.50 (2)	ND	ND
1,2,3,7,8-P_5CDF	ND	ND	ND	0.11 ± 0.01 (4)	0.19 ± 0.07 (2)	0.08 ± 0.01 (4)	ND	ND	ND
2,3,4,7,8-P_5CDF	2.3 ± 0.18 (8)	1.8 ± 0.25 (4)	3.0 ± 0.58 (4)	5.0 ± 0.44 (8)	3.9 ± 0.31 (4)	4.9 ± 0.39 (4)	1.8 ± 0.37 (8)	4.0 ± 0.71 (4)	4.2 ± 0.37 (4)
1,2,3,4,7,8-H_6CDF	2.0 (1)	ND	2.0 (1)	5.0 ± 0.55 a (8)	2.4 ± 0.64 b (4)	2.5 ± 0.30 b (4)	ND	2.3 ± 0.33 (4)	2.8 ± 0.37 (4)
1,2,3,6,7,8-H_6CDF	2.4 ± 0.24 (6)	2.0 ± 0.0 (3)	3.7 ± 0.33 (4)	3.5 ± 0.36 a (8)	2.3 ± 0.63 b (4)	2.3 ± 0.29 b (4)	ND	4.8 ± 0.48 (4)	5.2 ± 0.20 (4)
1,2,3,7,8,9-H_6CDF	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,3,4,6,7,8-H_6CDF	2.6 ± 0.20 (8)	2.0 (1)	3.3 ± 0.67 (4)	8.9 ± 0.82 a (8)	4.8 ± 1.6 b (4)	4.4 ± 0.64 b (4)	ND	5.0 ± 0.71 (4)	5.0 ± 0.45 (4)
1,2,3,4,6,7,8-H_7CDF	3.0 (1)	2.0 (1)	3.0 ± 0.0 (2)	2.9 ± 0.30 a (8)	1.2 ± 0.40 b (4)	0.73 ± 0.07 b (4)	ND	3.5 ± 0.65 (4)	4.8 ± 0.80 (4)
1,2,3,4,7,8,9-H_7CDF	ND	ND	ND	0.40 ± 0.06 a (8)	0.19 ± 0.09 b (4)	0.13 ± 0.01 b (4)	ND	ND	ND
O_8CDF				0.37 ± 0.04 (3)	ND	ND	ND	ND	ND
3,3′,4,4′-T_4CB	ND	ND	ND	1.5 ± 0.20 (8)	9.0 ± 4.0 (4)	1.5 ± 0.15 (4)	10 (1)	ND	ND
3,4,4′,5-T_4CB	ND	ND	ND	2.3 ± 0.24 (8)	1.8 ± 0.71 (4)	3.1 ± 0.26 (4)	ND	ND	ND
3,3′,4,4′,5-P_5CB	14 ± 2.0 (8)	10 ± 0.0 (3)	17 ± 3.3 (4)	31 ± 2.5 (8)	26 ± 1.6 (4)	33 ± 2.6 (4)	10 (1)	25 ± 5.0 (4)	20 ± 0.0 (4)
3,3′,4,4′,5,5′-H_6CB	ND	ND	10 (1)	17 ± 3.1 (8)	8.6 ± 1.1 (4)	14 ± 3.3 (4)	ND	10 (1)	10 (1)
2,3,3′,4,4′-P_5CB	87 ± 11 (8)	68 ± 9.5 (4)	100 ± 5.8 (4)	170 ± 19 (8)	160 ± 13 (4)	190 ± 3.3 (4)	67 ± 7.1 a (8)	110 ± 16 b (4)	110 ± 12 b (4)
2,3,4,4′,5-P_5CB	19 ± 2.6 (8)	17 ± 3.3 (3)	23 ± 3.3 (4)	39 ± 5.1 (8)	33 ± 3.6 (4)	48 ± 6.9 (4)	13 ± 3.3 a (3)	30 ± 4.1 b (4)	30 ± 3.2 b (4)
2,3′,4,4′,5-P_5CB	1050 ± 140 (8)	600 ± 110 (4)	1040 ± 130 (4)	1180 ± 110 (8)	1130 ± 140 (4)	1370 ± 120 (4)	790 ± 65 a (8)	1030 ± 120 a b (4)	1200 ± 150 b (4)
2′,3,4,4′,5-P_5CB	20 ± 3.1 (8)	20 ± 0.0 (3)	20 ± 0.0 (4)	29 ± 3.9 (8)	25 ± 2.4 (4)	32 ± 2.5 (4)	15 ± 2.9 a (4)	23 ± 2.5 a b (4)	26 ± 2.4 b (4)
2,3,3′,4,4′,5-H_6CB	63 ± 10 (8)	40 ± 9.1 (4)	77 ± 12 (4)	130 ± 20 (8)	100 ± 12 (4)	140 ± 27 (4)	39 ± 5.1 a (8)	98 ± 18 b (4)	90 ± 13 b (4)
2,3,3′,4,4′,5′-H_6CB	31 ± 4.0 (8)	20 ± 5.8 (3)	30 ± 5.8 (4)	50 ± 7.0 (8)	36 ± 4.2 (4)	54 ± 11 (4)	20 ± 2.6 a (8)	40 ± 4.1 b (4)	40 ±5 b (4)
2,3′,4,4′,5,5′-H_6CB	114 ± 17 (8)	65 ± 18 (4)	90 ± 15 (4)	80 ± 12 (8)	53 ± 5.8 (4)	78 ± 11 (4)	81 ± 8.6 (8)	98 ± 13 (4)	82 ± 11 (4)
2,3,3′,4,4′,5,5′-H_7CB	15 ± 2.2 (7)	10 ± 0.0 (2)	20 ± 5.8 (4)	31 ± 5.5 (8)	14 ± 5.1 (4)	25 ± 6.4 (4)	10 (1)	20 ± 4.1 (4)	18 ± 4.8 (4)

[a] Values with different letters differ significantly between stages in the same sample ($P < 0.05$). ND indicates not detected or below the minimum quantification limit. [b] The number in parentheses indicates the number of samples in which the congener was detected at or above the minimum quantification limit.

blood, TEQ levels for all analytes remained almost constant during the experiment. The TEQs were also constant in milk at any stage on a lipid weight basis but were significantly greater in colostrum than in milk when expressed on a total weight basis ($P < 0.001$). The TEQs in calf blood increased after birth ($P < 0.001$). At 1 month of age, total blood TEQs were more than 10 times greater than those at birth. However, blood TEQ levels in suckling calves were not different between samples collected at 1 and 2 months postpartum ($P = 0.59$) and were approximately the same as TEQ levels in milk on a lipid weight basis. Therefore, blood TEQ levels in newborn and suckling calves were significantly less and greater than those in their dams, respectively ($P < 0.01$), except for dioxin-like PCBs in suckling calves. Nevertheless, blood TEQ levels in newborn calves were well-correlated with those in their dams at parturition ($R = 0.78$, $P < 0.05$), whereas those in suckling calves were correlated with TEQ levels in milk collected at the same time ($R = 0.69$, $P = 0.06$).

Concentrations of Individual Congeners. Concentrations of individual congeners of PCDD/Fs and dioxin-like PCBs in maternal blood and milk and in calf blood are shown in **Table 2**. Congeners are expressed as tetra (T_4), penta (P_5), hexa (H_6), hepta (H_7), or octa (O_8) CDD, CDF, or CB according to the number of chlorine atoms in the molecule. Although 1,2,3,7,8,9-H_6CDF was not detected in any sample, the remaining 28 of the 29 congeners analyzed were detected in one or more samples. The largest number of congeners was detected in milk. Excluding 2,3,7,8-T_4CDF, 1,2,3,7,8-P_5CDF, and O_8CDF, 25 congeners were detected in both colostrum and milk of all cows. In contrast, the smallest number of congeners was detected in newborn calf blood. Seven congeners, O_8CDD, 2,3,4,7,8-P_5CDF, and five mono-ortho PCBs were detected in all newborn calves. The number of congeners detected in blood of all calves increased to 18 at 1 month of age.

Concentrations of PCDD congeners in maternal blood were almost constant during the experiment, but those in milk decreased as time passed. As chlorine substitution of the congener increased, its concentration in milk tended to decrease. In calf blood, 1,2,3,4,6,7,8-H_7CDD and O_8CDD were detected at birth, whereas five congeners were detected at both 1 and 2 months of age. The concentrations of PCDD congeners detected in calf blood were almost constant regardless of the growth stage.

Profiles of PCDF congeners were similar to those of PCDDs. In maternal blood, the concentrations of PCDF congeners were typically constant regardless of sampling time. As shown in **Table 2**, chlorine substitution in PCDF congeners increased, a decrease in milk concentration tended to occur, similar to the PCDDs. In calf blood, 2,3,7,8-T_4CDF and 2,3,4,7,8-P_5CDF were detected at birth, whereas five congeners were detected in all samples at both 1 and 2 months of age. Furthermore, blood concentrations of 2,3,4,7,8-P_5CDF increased in suckling calves compared with newborn calves ($P < 0.05$). Nevertheless, the concentrations of PCDF congeners detected in suckling calf blood were almost constant irrespective of the growth stage.

Regarding dioxin-like PCBs, concentrations of individual congeners in maternal blood and milk were almost constant regardless of sampling time. The profiles of dioxin-like PCB concentrations in milk accordingly differed from those of PCDDs and PCDFs. Five mono-ortho PCB congeners were detected in all samples of calf blood at birth. In the blood of suckling calves, one of the nonortho and all of the mono-ortho congeners analyzed were detected in all samples. As shown in **Table 2**, blood concentrations were typically greater in suckling

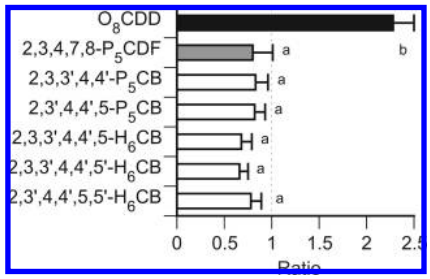

Figure 1. Ratios of major congener concentrations in calf blood compared to those in maternal blood at birth (lipid weight basis; mean + SEM; $N = 8$). Values with different superscripts a and b differ between congeners ($P < 0.01$).

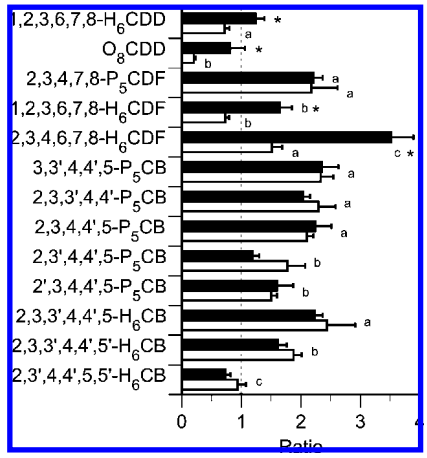

Figure 2. Ratios of major congener concentrations in colostrum (solid) and milk (open) to those in maternal blood collected at the same time (lipid weight basis; mean + SEM; $N = 8$). Values with different superscripts a, b, and c differ between congeners of the same compound in the same sample ($P < 0.05$). An asterisk indicates values differ between colostrum and milk ($P < 0.05$).

calves than in newborns ($P < 0.05$). Concentrations of dioxin-like PCB congeners detected in suckling calf blood were almost constant, as were those of both PCDDs and PCDFs.

Distribution of Major Congeners. As shown in **Figure 1**, in comparison of the ratios of concentrations of eight major congeners in newborn calf blood to those in maternal blood at parturition, O_8CDD had a significantly higher ratio (2.3 ± 0.2) than the other congeners ($P < 0.01$), whereas the ratios for the other seven congeners were almost constant within the range of 0.66 ± 0.09 and 0.83 ± 0.13. Furthermore, blood levels of these congeners in newborn calves were well-correlated with those in their dams ($R = 0.89$ for O_8CDD and 0.92 for the others, $P < 0.01$).

The ratios of individual congener concentrations in milk to those in maternal blood at the same stage are shown in **Figure 2**. Ratios for highly substituted PCDD/F congeners in milk were declined compared with colostrum, whereas those for 2,3,4,7,8-P_5CDF and dioxin-like PCB congeners were almost constant between colostrum and milk. Ratios for O_8CDD (0.81 ± 0.25 and 0.20 ± 0.04 in colostrum and milk, respectively) were considerably lower than for the other congeners, whereas the ratio for 2,3,4,6,7,8-H_6CDF in colostrum (3.5 ± 0.4) was significantly higher than for the other congeners ($P < 0.05$). Levels of 2,3′,4,4′,5,5′-H_6CB were roughly equivalent between milk and maternal blood. The concentrations of the other PCB congeners in milk were higher than those in maternal blood, with their ratios ranging from 1.2 ± 0.1 to 2.4 ± 0.5.

The ratio for O_8CDD was very much higher than for the other

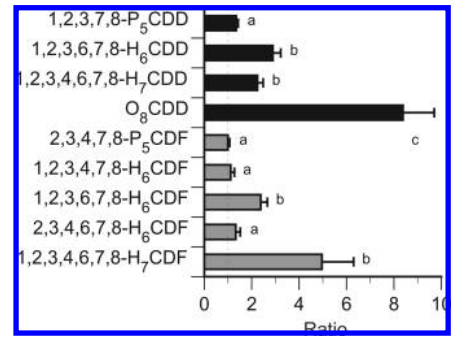

Figure 3. Ratios of major PCDD/F congener concentrations in calf blood to those in milk collected at the same time (lipid weight basis; mean + SEM; $N = 8$). Values with different superscripts a, b, and c differ between congeners of the same compound ($P < 0.05$).

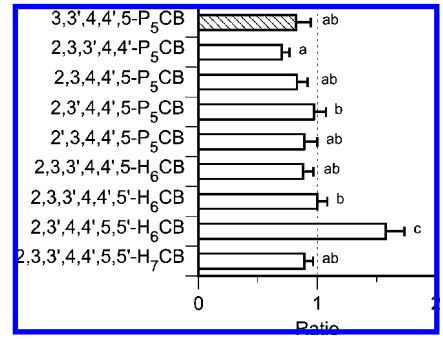

Figure 4. Ratios of major dioxin-like PCB congener concentrations in calf blood to those in milk collected at the same time (lipid weight basis; mean + SEM; $N = 8$). Values with different superscripts a, b, and c differ between congeners ($P < 0.05$).

congeners in the comparison of concentrations between calf blood and milk ($P < 0.05$) (**Figure 3**). Ratios for 1,2,3,6,7,8-H_6CDD/F and 1,2,3,4,6,7,8-H_7CDD/F were also higher than the equivalent level ($P < 0.01$). As shown in **Figure 4**, the range of the ratios for dioxin-like PCBs (0.70 ± 0.07 to 1.6 ± 0.2) was smaller compared with PCDD/Fs (1.4 ± 0.1 to 8.4 ± 1.3 and 0.99 ± 0.07 to 5.0 ± 1.3, respectively). Among dioxin-like PCB congeners, the ratio for 2,3′,4,4′,5,5′-H_6CB was higher than those for the other congeners ($P < 0.05$), which were approximately the same as the equivalent level. Furthermore, blood levels of PCB congeners in suckling calves were well-correlated with those in milk ($R = 0.96$, $P < 0.001$).

DISCUSSION

Exposure of mammals to lipophilic organochlorine compounds begins before birth by transfer from maternal blood to the fetus across the placenta (*23*). After parturition, these compounds continue to be mobilized from maternal fat to be released into milk during lactation, although the concentrations in milk decline substantially over the duration of lactation, presumably reflecting the gradual depletion of maternal stores (*24*). In general, greater quantities are transferred to neonates via suckling than are transferred to fetuses via the placenta (*25–28*). Nevertheless, their adverse physiological effects appear to be greater with transplacental transfer than lactational exposure in both humans and animals (*2, 3, 6, 7*).

Umbilical cord blood of newborn infants is typically analyzed to estimate fetal exposure to organochlorines in humans (*25–28*). Similarly, levels of persistent contaminants in the blood of newborn calves before colostrum ingestion can be regarded to reflect those in the fetal circulation after placental formation.

The ratios for major congeners in newborn and maternal blood determined in this study were indeed consistent with those in fetal and maternal blood at late gestation as shown in our previous report (*18*). Therefore, the present results provide useful information to estimate fetal exposure in cattle and may be applied to humans as well.

Organochlorine compounds are environmentally stable and have extremely long biological half-lives in the body. Their blood half-lives, especially of highly substituted PCDD/F congeners, are considerably longer in comparison with feeding or grazing intervals (*16*). Therefore, the absorption and excretion of those compounds are considered in equilibrium and they remain in the blood circulation at relatively constant concentrations. Their concentrations in fetal and maternal blood are also in equilibrium because mammalian fetuses continuously exchange substances exclusively with maternal blood through the placenta. In this study, blood levels of major congeners in newborn calves were correlated with those in their dams, similar to reports in humans (*27*). Therefore, the ratios of concentrations of organochlorine compounds in the blood of newborns and their dams can be assumed as distribution coefficients of those compounds between bovine fetuses and dams.

O_8CDD concentrations in the blood of newborn calves were several times greater than those in their dams, indicating that O_8CDD readily accumulates in the fetus after crossing the placenta. Highly substituted congeners except for O_8CDD were hardly detected in newborns, so their distribution coefficients between dams and fetuses were not assumed. Blood half-lives of organochlorine congeners tend to lengthen as the number of chlorine substitutions increases. Thus, highly substituted PCDD/F congeners presumably have distribution coefficients roughly within the range of 1.0−2.5. On the other hand, highly toxic congeners with four to five chlorine substitutions were just above detection limits in blood of both dams and calves but were readily detected in milk samples. Because all contaminants in milk must be transferred from maternal blood, these congeners are considered to be present in blood at concentrations lower than the minimum quantification limit. Considering the ratios for other congeners, their distribution coefficients can be presumed to be less than 1.0. At any rate, these results suggest that a reduction in fetal exposure can be achieved by decreasing the contamination of maternal blood with those chemicals.

Lipid content gradually increases from foremilk to hindmilk during a suckling bout. In this study, foremilk was used for analysis, because concentrations of organochlorine compounds in milk had been shown to be stable during a breastfeeding session on a lipid weight basis (*29, 30*). Lipid content of milk shown in **Table 1** was accordingly lower than the ordinary milk level. In a previous report using the same herd as the current study, milk fat levels were 3.3−3.6% in whole milk and approximately 1% higher than these levels in hindmilk (*31*). Considering the lipid content of whole milk, daily intake of dioxins and dioxin-like compounds by bovine calves estimated from the results in this study was 5−10 times higher than the maximum tolerable daily intake (4 pg-TEQ/kg-body weight) for humans established by the World Health Organization in 1998 (*32*). These estimates were almost consistent with the case of human breast milk feeding (*30, 33, 34*). Despite exceeding the intake limit, breast milk feeding is recommended for newborns because of its great benefits and the short exposure period compared with the human life span. Bovine colostrum contains many beneficial substances for growth and health of calves, as does human colostrum for infants. These substances include indispensable components and essential nutrients, such as growth promoting factors, immunoglobulins, lactoferrins, various carbohydrates, lipids, peptides, vitamins, and minerals (*35–37*). Therefore, bovine colostrum should be given to newborn calves as recommended in humans, even if contamination of the colostrum with dioxins and related compounds is not negligible. However, in a highly polluted area, colostrum substitutes or early weaning may be necessary to reduce contamination of calves with those chemicals.

In this study, levels of dioxins and dioxin-like compounds in bovine colostrum and milk were correlated with those in blood, excluding highly substituted PCDD/Fs, which were more concentrated in colostrum than in milk. Highly substituted congeners remain longer in the circulation than congeners having lesser chlorine substitution because they bind to and are transported by various plasma proteins besides lipoproteins (*38*). These carrier proteins are thought to play important roles for the transfer of highly substituted congeners from blood into the mammary gland and/or their excretion in milk. As described above, colostrum is compositionally more complex than milk. Composition differences other than fatty acid composition between colostrum and milk are likely a major cause of the reduced concentrations of highly polychlorinated organics in milk relative to colostrum when expressed on a lipid weight basis. Furthermore, concentrations of highly substituted PCDD/F congeners in milk collected 2 months postpartum tended to be lower than those at 1 month, whereas dioxin-like PCBs remained nearly constant at both sampling times. The affinity of highly substituted PCDD/F congeners for adipose tissue is considerably lower than that of the other congeners, although they have a higher affinity for the liver than other congeners (*15*). In addition, the mobility of highly substituted PCDD/F congeners accumulated by the liver and other tissues is extremely low due to their low affinity for lipoproteins (*38*). Thus, regardless of the total body burden and blood concentrations, highly substituted congeners bound to lipoproteins in the blood and readily transferred to milk may steadily decrease as the duration of lactation is prolonged.

Contamination of breast milk with organochlorine compounds has been extensively studied in humans (*30, 33*). Wittsiepe et al. (*34*) found good correlations of contaminant levels between maternal blood and breast milk and reported that higher chlorinated PCDD/F and PCB congeners were present in 2−4-fold higher concentrations in blood relative to milk and that the concentrations of lower chlorinated PCB congeners were up to 2-fold higher in milk in relation to blood. Data generated in this study are remarkably consistent with the report of Wittsiepe et al. (*34*). This consistency suggests that to characterize blood-to-milk transfer of organochlorine compounds in cattle provides helpful information to clarify the profiles of those chemicals in humans.

As shown in **Tables 1** and **2**, blood levels of PCDD/Fs and dioxin-like PCBs in bovine calves were elevated significantly in the 1−2 month period after birth, similar to in humans and other animals (*5, 7, 39*). The increase in blood PCDD/Fs and dioxin-like PCBs had to be derived from contaminated milk, because calves predominantly suckled from their dams and ingested little to no grain or forage during the 2 month duration of this study. Indeed, as shown in **Table 1**, the total TEQ levels in the blood of suckling calves were almost the same as those in milk collected at the same time but more than twice as high as those in the blood of their dams. Because the lipid content in blood was nearly the same between dams and suckling calves, these results suggested that susceptible organs in the neonates

were constantly exposed to dioxin and dioxin-like compounds at much higher concentrations than those in adult animals.

Highly substituted PCDD/F congeners were more concentrated in calf blood than in the milk of their dams on a lipid weight basis (**Figure 3**), whereas levels of dioxin-like PCB congeners in both samples were almost equivalent, excluding 2,3′,4,4′,5,5′-H_6CB, whose distribution among the blood of dams, milk, and the blood of calves resembled that of PCDD/Fs rather than the other PCBs. These results suggest that in suckling calves the gastrointestinal absorption of PCDD/Fs is dependent on the number of chlorine atoms in the molecule, whereas the absorption of most dioxin-like PCBs is dependent on the absorption of lipid, regardless of the degree of chlorination.

In conclusion, it was demonstrated that neonatal calves absorbed considerably higher amounts of lipophilic organochlorine compounds from maternal milk through the digestive tract compared with those absorbed by prenatal fetuses through the placenta. Notwithstanding this, bovine fetuses were regarded as continuously exposed to substantial levels of those chemicals considering how vulnerable the fetuses were. Contamination levels of bovine milk with those chemicals were highly correlated with blood levels of the same compounds, which implied that the levels of those chemicals in milk basically reflected contamination of foodstuff before and during lactation. Furthermore, highly substituted PCDD/Fs, especially O_8CDD, remained in the circulation longer and did not readily transfer to milk, whereas they were readily transferred to and accumulated in fetuses and calves. These results suggest that those compounds are transferred from dams to fetuses through the placenta and to calves by suckling associated not only with lipid transport but with other carriers. In contrast, lipid absorption and transport systems play major role in the transfer of PCBs.

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