

A Congener Specific Evaluation of Transfer of Chlorinated Dibenzop-dioxins and Dibenzofurans to Milk of Cows following Ingestion of Pentachlorophenol-Treated Wood

GEORGE F. FRIES*

U.S. Department of Agriculture, 10300 Baltimore Avenue, Beltsville, Maryland 20705

DENNIS J. PAUSTENBACH

Exponent 149 Commonwealth Drive, Menlo Park, California 94025

DIPTI B. MATHER

Exponent 15375 SE 30th Place, Suite 250, Bellevue, Washington 98007

WILLIAM J. LUKSEMBURG

Alta Analytical Laboratory 5070 Robert J. Matthews Parkway, El Dorado Hills, California 95762

Pentachlorophenol (PCP) treated wood has been hypothesized as an important source of dioxins in milk and beef. This phase of studies to evaluate the hypothesis involved the administration of PCP-treated wood to cows and measurement of the transfer of polychlorinated dibenzo-*p*-dioxins (PCDD) and dibenzofurans (PCDF) to milk. The 3 g/day dose of ground wood was administered to four cows for 56 days. This dose provided a PCP intake of 4.8 mg/day and PCDD/F intakes in the range of 0.3 μ g/day for 2,3,7,8-TCDD to 17 000 μ g/day for 1,2,3,4,6,7,8,9-OCDD. Steady state with respect to excretion in milk was reached because concentrations of PCDD/Fs in milk fat were reasonably constant from day 28 through day 56. The PCDFs without chlorine in the 4 and 6 positions were metabolized and not transported to milk. The other PCDFs and all PCDDs had intake adjusted concentrations in milk that were inverse to the number of chlorines. Variations among cows in concentrations of specific congeners were small and were not related to body weight, dry matter intake, or production of milk and milk fat. The transfer coefficients calculated for the PCDD/Fs in this study provide tools for reducing uncertainty in risk assessments.

Introduction

The background concentrations of polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in foods are considered to be the primary source responsible for producing mean body burdens of 28 pg/g TCDD toxic equivalents (TEQ) in adipose tissue of the general population of the United States (1). Meat and dairy products are thought to provide as much as 90% of human exposure

from foods (2, 3). Forages and pastures contaminated by aerial deposition of PCDD/Fs from combustion sources are considered the major pathways of uptake in cattle. Papers and guidance documents for calculating estimated exposures by these pathways are available (2, 4, 5).

The most prevalent PCDD/Fs detected in surveys of the United States beef supply were 1,2,3,6,7,8-HxCDD, 1,2,3,4,6,7,8-HpCDD, 1,2,3,4,6,7,8-HpCDD, and OCDD (6, 7). High concentrations of PCDDs relative to PCDFs, and of 1,2,3,6,7,8-HxCDD relative to other HxCDDs, are typical of the contaminants of chlorophenol, whereas PCDFs are typically the dominant chemicals in combustion emissions (8). Animal confinement and housing facilities built before uses of pentachlorophenol (PCP) were restricted during the 1980s often contain PCP-treated wood (9). The importance of these facilities as reservoir sources of PCDD/Fs was suggested by finding PCP-treated wood in several facilities that produced beef with higher than average PCDD/F concentrations (10).

Reliable measurements of the transfer coefficients of PCDD/Fs from feed to milk and adipose tissue of cattle are required to produce dependable exposure assessments and to infer sources based on congener profiles found in meat and milk. Transfer of PCDD/Fs to milk and tissues of lactating cattle has been measured in studies that have one or more deficiencies (11-16). These deficiencies include inadequate replication, use of animals in nonrepresentative stages of lactation, failure to collect or report data on feed intake and production, and the failure to obtain data on the full range of toxic congeners.

This paper describes the first phase of studies designed to characterize the transport of PCDD/Fs to the milk and tissues of dairy cows that had been dosed with PCP-treated wood. An additional objective was to extend and improve the reliability of the database on the uptake and elimination of PCDD/Fs in milk and tissues. The transport of PCDD/Fs to milk is evaluated in this paper. Mass balance, depuration, tissue residues, and evaluation of the significance of PCP-treated wood as a PCDD/F source will be included in subsequent papers.

Materials and Methods

Four Holstein cows in mid to late lactation were used in the study (Table 1). The work, conducted at the Beltsville Agricultural Research Center (BARC), followed a protocol approved by the Beltsville Area Animal Use and Care Committee. The design and methods are described in more detail in the Supporting Information on the ES&T Web site.

The housing facilities for the cows contained no PCP-treated wood, and the animals had no known past exposure to treated wood. The cows were offered a complete mixed diet that was typical of diets for lactating cows at BARC (Table S.1, Supporting Information section). Feed intake was measured from day 54 through day 58 of dosing. The PCDD/F analysis of the feed is shown in Table 2. Milk production was measured daily, and fat content was determined monthly as part of the routine record keeping at BARC.

A 3.0 g/day dose of ground PCP-treated wood was administered to each cow for 58 days. The treated wood was obtained from an unused structure at BARC. The date of treatment of the wood and the manufacturer of the PCP are not known. The analysis of a composite wood sample is shown in Table 2.

Milk samples for this phase of the study were collected from each cow at a single milking at 14-day intervals. Samples of the bulk milk from the BARC herd were obtained at the

* Corresponding author phone: (301)504-9198; fax: (301)504-8438; e-mail: fries@lpsi.barc.usda.gov.

The average values of the transfer coefficients are presented in Table 3. The concentrations of 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD in feed were below the detection limits. Concentrations of half the detection limits were used for feed when calculating the coefficients listed for these PCDDs.

where C_{MF} is the concentration in milk fat (pg/g) and C_{diet} is the concentration in dry matter of the diet (pg/g) and C_{milk} is the concentration in whole milk (pg/kg) and I is the intake (mg/day), and

where C_{milk} is the concentration in whole milk (pg/kg) and I is the intake (mg/day), and

$$CR = 100 \cdot A_{milk} / I \quad (3)$$

where C_{milk} is the concentration in whole milk (pg/kg) and I is the intake (mg/day), and

$$BTF = C_{milk} / I \quad (2)$$

where C_{MF} is the concentration in milk fat (pg/g) and C_{diet} is the concentration in dry matter of the diet (pg/g)

FIGURE 2. Concentrations of 2,3,7,8-substituted furans in the milk fat of cows administered 3 g/day pentachlorophenol-treated wood for 58 days. Each point is the mean and standard deviation of four cows. The 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF, and 1,2,3,7,8-HxCDF congeners are not shown because occurrence of residues at concentrations greater than the 0.01 to 0.02 pg/g detection limits were inconsistent.

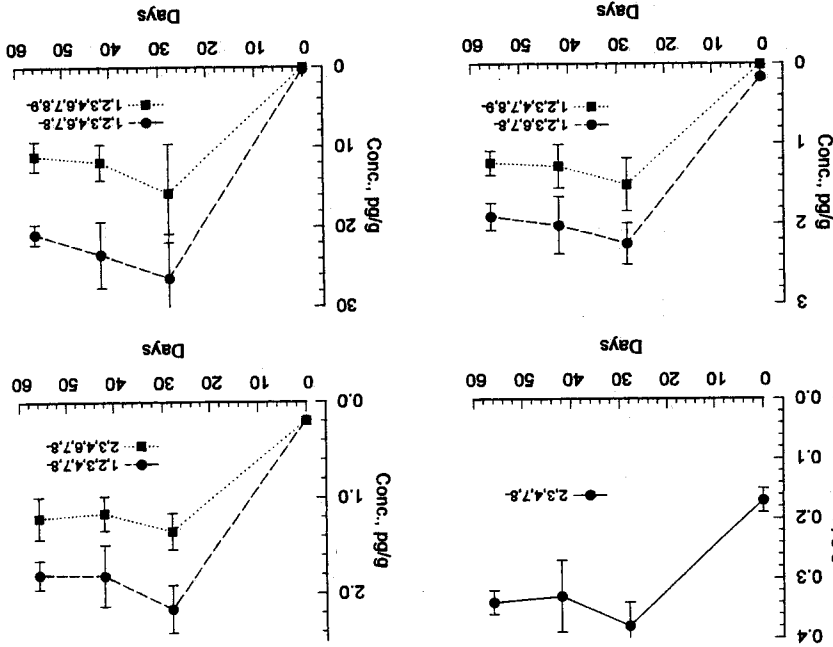


FIGURE 1. Concentrations of 2,3,7,8-substituted dioxins in the milk fat of cows administered 3 g/day pentachlorophenol-treated wood for 58 days. Each point is the mean and standard deviation of four cows.

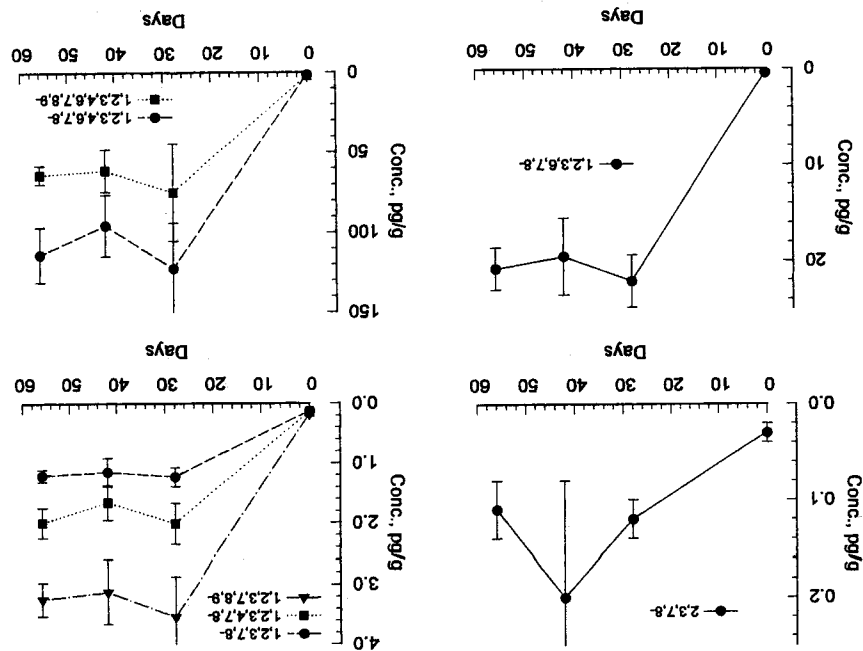


TABLE 3. Mean Bioconcentration Factors (BCFs), Biotransfer Factors (BTFs), and Carry-Over Rates (CR) of PCDD and PCDF Congeners of Four Cows Dosed with PCP-Treated Wood*

Congener	BCF		BTF, (pg/kg)/(ng/d)		CR, %	
	mean ± SD	CV	mean ± SD	CV	mean ± SD	CV
2,3,7,8-PCDDs	7.1 ± 4.2	58	14 ± 7.5	52	35 ± 25	73
1,2,3,7,8-PCDD	5.0 ± 1.2	24	10 ± 1.0	10	24 ± 10	42
1,2,3,4,7,8-PCDD	3.1 ± 0.8	26	6.2 ± 0.8	13	15 ± 6	42
1,2,3,6,7,8-PCDD	3.7 ± 0.9	24	7.4 ± 0.8	10	18 ± 7	41
1,2,3,7,8,9-PCDD	2.6 ± 0.7	26	5.2 ± 0.6	11	13 ± 6	43
1,2,3,4,6,7,8-PCDD	0.68 ± 0.22	32	1.4 ± 0.3	18	3.3 ± 1.6	45
1,2,3,4,6,7,8,9-PCDD	0.08 ± 0.03	40	0.16 ± 0.04	27	0.39 ± 0.22	57
2,3,7,8-PCDFs	<0.01		<0.1		<0.1	
1,2,3,7,8-PCDF	3.5 ± 0.7	19	7.2 ± 1.1	15	17 ± 6	37
1,2,3,4,7,8-PCDF	3.0 ± 0.7	23	6.0 ± 0.7	12	14 ± 6	41
1,2,3,6,7,8-PCDF	3.1 ± 0.8	24	6.3 ± 0.7	10	15 ± 6	42
2,3,4,6,7,8-PCDF	1.9 ± 0.5	26	3.7 ± 0.5	13	8.9 ± 3.9	43
1,2,3,7,8,9-PCDF	<0.01		<0.10		<0.1	
1,2,3,4,6,7,8-PCDF	0.72 ± 0.21	30	1.4 ± 0.2	16	3.5 ± 1.7	47
1,2,3,4,6,7,8-PCDF	0.87 ± 0.27	31	1.7 ± 0.3	16	4.3 ± 2.1	48
1,2,3,4,6,7,8,9-PCDF	0.07 ± 0.03	35	0.14 ± 0.04	31	0.33 ± 0.21	63

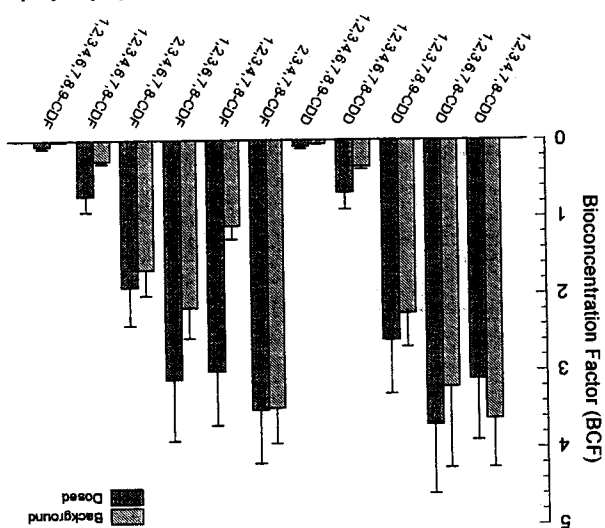
* Values are the mean ± standard deviation (SD) of 12 observations involving four cows at three times. CV is the coefficient of variation (%). Concentrations of 2,3,7,8- and 1,2,3,7,8-PCDD in feed were below the limit of quantitation, and half of the detection limit was used in calculations for these congeners. Detection of 2,3,7,8- and 1,2,3,7,8-PCDF was inconsistent, and only the nominal upper limits based on the detection limits are shown.

This assumption is not serious in the case of 1,2,3,7,8-PCDD because approximately 95% of the estimated intake was from PCP-treated wood. In the case of 2,3,7,8-TCDD, however, less than 50% of the estimated intake was from wood and the coefficients could be in serious error if the actual concentration in feed was markedly above or below half the detection limit.

The differences in transfer coefficients among the three sampling periods were not significant. The greatest source of variation among the transfer coefficients for individual congeners was attributed to differences among cows. The coefficients of variation (CVs) were calculated to determine which of the methods of expressing transfer provided the least variation among animals. The BTFs, which essentially are PCDD/F concentrations in whole milk normalized to a unit of intake (eq 2), had lower CVs than other measures of biotransfer. The biological basis for this finding is not clear. The PCDD/Fs are fat soluble and are transported through biological compartments by processes dependent on concentration gradients in the lipid fractions. Thus, the comparability of the concentrations in whole milk, which is largely water, is somewhat unexpected. On the other hand, the low variation in BTFs may be an artifact related to the small variation in the fat concentrations in the milk (Table 1).

The carry-over rates were the most variable of the coefficients (Table 3). The large variations in CRs mainly reflect the 3-fold variation in milk production among the cows (Table 1). Because of the influence of the amount of milk and fat produced on CRs, the use of CRs in exposure assessments requires adjustment for any differences in milk production between the experimental cows used to determine the CRs and cow population in the field situation. The greater variability of BCFs than BTFs was related to the differences in dry matter intake (Table 1). Intake of PCDD/Fs was essentially the same for all cows with the result that calculated concentrations in the diet were inverse to dry matter intake. **Transfer Rates at Background Intake.** Concentrations of PCDD/Fs in milk fat reached a stable level within 28 days of the initiation of dosing, but it cannot be inferred that body burdens were at steady state. Longer times would be required to perfuse PCDD/Fs to deep fat stores than the time required

FIGURE 3. Comparison of bioconcentration factors calculated using data from cows exposed to background PCDD/F sources only (day 0) and with the same cows dosed with PCP-treated wood (days 28, 42, and 56). Only the congeners consistently occurring at concentrations above background are shown. Error bars represent the standard deviations.



to reach stable concentrations in milk fat (20). The fortuitous finding that background concentrations in milk fat were relatively stable (Figure S.1 of the Supporting Information) suggested a stable background concentration in feed with that the cows at day 0 were probably near steady state with their environment. Because changes in daily dry matter intake over a 56-day period would not be great (24), it is reasonable to calculate the transfer coefficients for day 0 using the intake and analyses values from day 56 (Tables 1 and 2). The BCFs calculated for day 0 and those calculated for the dosing periods are compared in Figure 3. The BCFs were comparable for many congeners, but the background values were lower in the case of several PCDFs. The differences were relatively large for 1,2,3,4,7,8-HxCDF and 1,2,3,4,6,7,8-HxCDF and somewhat smaller for 1,2,3,6,7,8-HxCDF. The

(1) Orban, J. E.; Stanley, J. S.; Schembarger, J. G.; Remmers, J. C. *Am. J. Public Health* 1994, 84, 439-445.
 (2) U.S. Environmental Protection Agency. *Estimating Exposure to Dioxin-like Compounds*. Report No. EPA/600/6-88/005a-c. Office of Research and Development. Washington, DC, 1994.
 (3) Fries, G. F. *J. Anim. Sci.* 1995, 73, 1639-1650.
 (4) Fries, G. F.; Paustendach, D. J. *J. Toxicol. Environ. Health* 1990, 29, 1-43.

Literature Cited

Animal facilities and care, analytical methods, table of daily feed allowances for each cow, and figures of background concentrations of dioxin and furan congeners, effect of mammary gland infection on the concentrations of selected PCDD/Fs in the milk fat of a cow, and relationship of bioconcentration factors to log K_{ow} values and the degree of chlorination. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Supporting Information Available

Animal facilities and care, analytical methods, table of daily feed allowances for each cow, and figures of background concentrations of dioxin and furan congeners, effect of mammary gland infection on the concentrations of selected PCDD/Fs in the milk fat of a cow, and relationship of bioconcentration factors to log K_{ow} values and the degree of chlorination. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Animal facilities and care, analytical methods, table of daily feed allowances for each cow, and figures of background concentrations of dioxin and furan congeners, effect of mammary gland infection on the concentrations of selected PCDD/Fs in the milk fat of a cow, and relationship of bioconcentration factors to log K_{ow} values and the degree of chlorination. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Animal facilities and care, analytical methods, table of daily feed allowances for each cow, and figures of background concentrations of dioxin and furan congeners, effect of mammary gland infection on the concentrations of selected PCDD/Fs in the milk fat of a cow, and relationship of bioconcentration factors to log K_{ow} values and the degree of chlorination. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Animal facilities and care, analytical methods, table of daily feed allowances for each cow, and figures of background concentrations of dioxin and furan congeners, effect of mammary gland infection on the concentrations of selected PCDD/Fs in the milk fat of a cow, and relationship of bioconcentration factors to log K_{ow} values and the degree of chlorination. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Animal facilities and care, analytical methods, table of daily feed allowances for each cow, and figures of background concentrations of dioxin and furan congeners, effect of mammary gland infection on the concentrations of selected PCDD/Fs in the milk fat of a cow, and relationship of bioconcentration factors to log K_{ow} values and the degree of chlorination. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Animal facilities and care, analytical methods, table of daily feed allowances for each cow, and figures of background concentrations of dioxin and furan congeners, effect of mammary gland infection on the concentrations of selected PCDD/Fs in the milk fat of a cow, and relationship of bioconcentration factors to log K_{ow} values and the degree of chlorination. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Animal facilities and care, analytical methods, table of daily feed allowances for each cow, and figures of background concentrations of dioxin and furan congeners, effect of mammary gland infection on the concentrations of selected PCDD/Fs in the milk fat of a cow, and relationship of bioconcentration factors to log K_{ow} values and the degree of chlorination. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Animal facilities and care, analytical methods, table of daily feed allowances for each cow, and figures of background concentrations of dioxin and furan congeners, effect of mammary gland infection on the concentrations of selected PCDD/Fs in the milk fat of a cow, and relationship of bioconcentration factors to log K_{ow} values and the degree of chlorination. This material is available free of charge via the Internet at <http://pubs.acs.org>.

congener	Fries' wood'	Mel' Oiling'	Slob' Tuins'	Fries'
milk, kg/day	26	25	28	23
milk fat, kg/day	1.1	1.0	1.4	1.2
2,3,7,8-CDD	35	35	30	34
1,2,3,7,8-CDD	24	33	28	28
1,2,3,4,7,8-CDD	18	15	17	5,6
1,2,3,6,7,8-CDD	16	18	14	6,4
1,2,3,7,8-CDD	12	13	3	3,1
1,2,3,4,6,7,8-CDD	1.8	3.3	3	0,6
1,2,3,4,6,7,8,9-CDD	0.3	0.4	4	0,1
2,3,4,7,8-CDF	18	17	25	36
2,3,4,7,8-CDF	5,7	14	18	4,3
1,2,3,6,7,8-CDF	11	15	16	3,6
2,3,4,6,7,8-CDF	8,4	9,0	14	4,2
1,2,3,4,6,7,8-CDF	1,4	3,5	3	0,4
1,2,3,6,7,8-CDF	4,3	8	8	0,5
1,2,3,4,6,7,8,9-CDF	0.1	0.3	1	0,1

TABLE 4. Comparison of Carry-Over Rates (%) Reported in This Study and in Previous Studies*

* This study. The value is based on the mean of four cows. Intake and milk fat and concentrations of PCDD/Fs were measured. Mean of three estimates on each of four cows made after dosing for 28, 42, and 56 days. Intake of PCDD/Fs primarily from PCP-treated wood but background sources from feed also were included. Production of milk and milk fat and concentrations of PCDD/Fs were measured. Production of milk and milk fat and concentrations of PCDD/Fs were measured. Values were obtained from the original paper. Values were obtained during mass balance measurements in a single cow in midlactation with all PCDD/F input from normal environmental sources. Oiling et al. (15). Values were obtained from the original paper. No data on milk and fat production was provided. Four cows were given a single ¹⁴C-labeled dose of PCDD/Fs. Carry-over was estimated as the percent of dose excreted in milk over an infinite time period. Slob et al. (25). Values were calculated from data presented in the original paper. Data were derived by measuring environmental concentrations of PCDD/Fs in feed and composite milk fat on two farms. Intake of 16 kg/day dry matter and fat production of 1.03 kg/ were assumed. Fat content of 3.6% was assumed to derive milk production value in this table. Tuinstra et al. (16) and Roos et al. (26). Values were based on measurement in one cow that was dosed during the dry period with concentrations in milk fat measured for 100 days post-calving. For this table, carry-over was estimated as the percent of dose excreted in milk over an infinite time period. Friesstone et al. (12). Values were calculated from data in the original paper. Values are an average of three cows that were dosed with technical-grade pentachlorophenol. Input, concentrations of PCDD/Fs in milk fat, and milk production are from that paper. Fat content of milk was assumed to be 3.6% when calculating carry-over. The congeners 2,3,7,8-, 1,2,3,7,8-, and 1,2,3,7,8,9-CDF are not included because most values are below the limit of detection.

- (5) Fuerst P.; Kruse, G. H. M.; Heim, D.; Delachen, T.; Wilmers, K. *Chemosphere* 1993, 27, 1349-1357.
- (6) Fell, V. J.; Davison, K. L.; Larsen, G. L.; Tieman, T. O. *Organohalogen Compd.* 1995, 26, 117-120.
- (7) Winters D.; Cleverly, D.; Mater, K.; Dupuy, A.; Byrne, C.; Deyrup, C.; Ellis, R.; Ferrario, J.; Hartless, R.; Leese, W.; Lorber, M.; McDaniel, D.; Schaum, J.; Walcott, J. *Chemosphere* 1996, 32, 469-478.
- (8) Hagemater, H.; Lindig, C.; She, J. *Chemosphere* 1994, 29, 2163-2174.
- (9) Shull, L. R.; Foss, M.; Anderson, C. R.; Feighner, K. *Bull. Environ. Contam. Toxicol.* 1981, 26, 561-566.
- (10) Fries, G. F.; Fell, V. J.; Davison, K. L. *Organohalogen Compd.* 1996, 28, 156-159.
- (11) Derks, H. J. G. M.; Berender, P. L. M.; Olling, M.; Liem, A. K. D.; de Jong, A. P. J. M. *Chemosphere* 1994, 28, 711-715.
- (12) Frestione, D.; Clower, M.; Borsetti, A. P.; Teske, R. H.; Long, P. E. *J. Agric. Food Chem.* 1979, 27, 1171-1177.
- (13) Jensen, D. J.; Hummel, R. A. *Bull. Environ. Contam. Toxicol.* 1982, 29, 440-446.
- (14) McLachlan, M. S.; Thoma, H.; Reisinger, M.; Huizinger, O. *Chemosphere* 1990, 20, 1013-1020.
- (15) Olling, M.; Derks, H. J. G. M.; Berender, P. L. M.; Liem, A. K. D.; de Jong, A. P. J. M. *Chemosphere* 1991, 23, 1377-1385.
- (16) Tuinstra, L. G. M. T.; Roos, A. H.; Menglers, M. J. B. *J. Agric. Food Chem.* 1992, 40, 1772-1776.
- (17) Smith, L. M.; Stalling, D. L.; Johnson, J. L. *Anal. Chem.* 1984, 56, 1830-1842.
- (18) Lorber, N. M.; Winters, D. L.; Griggs, J.; Cook, R.; Baker, R.; Baker, S.; Ferrario, J.; Byrne, C.; Dupuy, A.; Schaum, J. *Organohalogen Compd.* 1998, 38, 125-130.
- (19) U. S. Environmental Protection Agency. *Health Assessment Document for 2,3,7,8-Tetrachlorodibenzo-p-dioxin and Related Compounds*. Report No. EPA/600/BP-92/001a, Office of Research and Development; Washington, DC, 1994.
- (20) Fries, G. F. In *Fate of Pesticides in the Large Animal*; Ivie, G. W., Dorough, H. H., Eds.; Academic Press: New York, 1977; pp 159-173.
- (21) Paape, M. J.; Capuco, A. V.; Guldry, A. J.; Burenich, C. J. *Anim. Sci.* 1995, 73(Suppl. 2), 1-17.
- (22) McLachlan, M. S. *J. Agric. Food Chem.* 1993, 41, 474-480.
- (23) Travis, C. C.; Arms, A. D. *Environ. Sci. Technol.* 1988, 22, 271-274.
- (24) Subcommittee on Feed Intake. *Predicting Feed Intake of Food-Producing Animals*. National Academy Press: Washington, DC, 1987; pp 48-55.
- (25) Slob, W.; Olling, M.; Derks, H. J. G. M.; de Jong, A. P. J. M. *Chemosphere* 1995, 31, 1625-1635.
- (26) Roos, A. H.; Berende, P. L. M.; Traag, W. A.; van Rhijn, J. A.; Menglers, M. J. B.; Tuinstra, L. G. M. T. *Onderzoek naar het voorkomen, gehalte, stapeling en uitscheiding van polygechlorideerdibenzo-p-dioxinen en furanen*. RIKILT report 91.01, State Institute for Quality Control of Agricultural Products: Wageningen, The Netherlands, 1991.
- (27) Shiu, W. Y.; Doucette, W.; Gobas, F. A. P. C.; Andren, A.; Mackay, D. *Environ. Sci. Technol.* 1988, 22, 651-658.
- (28) Govers, H. A. J.; Krop, H. B. *Organohalogen Compd.* 1996, 26, 5-10.

Received for review November 9, 1998. Revised manuscript received January 25, 1999. Accepted February 4, 1999.

ES981153D