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REVIEWS

Dioxins in Food: A Modern Agricultural Perspective

JANICE K. HUWE†

Biosciences Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, P.O. Box 5674, Fargo, North Dakota 58105

This review attempts to cover and summarize the literature available on polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans in the environment with regard to problems of interest to agriculture. The coverage of the literature is extensive (120 references) but, by all means, not complete. Issues that are addressed in this review include a background summary of dioxins in the environment and their potential human health risks; current knowledge on the levels of dioxins in the U.S. food supply and comparisons to European data; descriptions of recent food contamination episodes; an evaluation of methods that may reduce incurred levels of dioxins in livestock and meats; and the status and limitations of dioxin analysis and rapid screening methods with regard to widespread monitoring programs. Research areas in agriculture where data and experimental results are scarce or nonexistent are also pointed out.

Keywords: Polychlorinated dibenzo-*p*-dioxins; polychlorinated dibenzofurans; agriculture; food

INTRODUCTION

Dioxins have been referred to as "the most toxic man-made compounds" and, therefore, have generated much concern over their potential health risks. Dioxins are generally thought of as an industrial problem because they are formed as byproducts of chlorine-containing manufacturing processes or from incineration. However, because the general population is exposed to dioxins almost entirely through the foods they consume, especially products containing animal fats, dioxins are also a concern for agriculture. The U.S. Department of Agriculture (USDA) established an ongoing dioxin research program as a response to the 1994 draft reassessment on dioxins issued by the U.S. EPA. At that time few data had been collected on levels of dioxins in U.S. foods, and the 1994 draft estimated human exposure levels based on 35 meat, 8 egg, 7 dairy, and 60 fish samples, which had been analyzed. Since then the USDA Food Safety Inspection Service (FSIS) and Agricultural Research Service (ARS) have been involved with collecting data on dioxin levels in foods to better estimate human dietary exposure.

A number of food contamination episodes in the past decade have also highlighted the need for improved monitoring of dioxins in the food supply and the need to better understand all sources that may contribute to the levels of dioxins in foods. This review covers areas of dioxin research that are important to agriculture, such as (1) reported levels in foods; (2) recent episodes of food contamination and the attributed sources; (3) strategies which can reduce dioxin levels in foods and food-producing animals, thereby reducing human exposure; and (4) the current state of analytical methods and feasibility of a widespread food monitoring program.

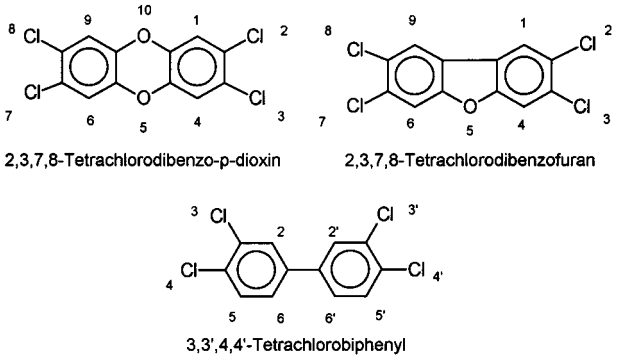

Figure 1. Structures and numbering of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, 2,3,7,8-tetrachlorodibenzofuran, and 3,3′,4,4′-tetrachlorobiphenyl.

THE DIOXIN PROBLEM

Polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs) are ubiquitous environmental contaminants, which persist and bioaccumulate through the food chain. They are often collectively referred to as "dioxins" because of their similar structures, chemical properties, and mechanism of action in biological systems. PCDDs, PCDFs, and the non-ortho- and mono-ortho-substituted PCBs are planar, highly lipophilic compounds (**Figure 1**). Although 210 different PCDDs and

† Telephone (701) 239-1288; fax (701) 239-1430; e-mail huwej@fargo.ars.usda.gov.

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Table 1. Comparison of the Most Common Toxic Equivalency Factors (TEFs) for PCDDs, PCDFs, and PCBs Used Prior to 1998 and the Most Recently Recommended[a]

PCDD/PCDF	1988 NATO TEF	1998 WHO TEF	PCB	1994 WHO TEF	1998 WHO TEF
2378-TCDD	1.0	1.0	non-ortho-PCBs		
12378-PeCDD	*0.5*	*1.0*	33′44′-TeCB	*0.0005*	*0.0001*
123478-HxCDD	0.1	0.1	344′5-TeCB		*0.0001*
123678-HxCDD	0.1	0.1	33′44′5-PeCB	0.1	0.1
123789-HxCDD	0.1	0.1	33′44′55′-HxCB	0.01	0.01
1234678-HpCDD	0.01	0.01	mono-ortho-PCBs		
OCDD	*0.001*	*0.0001*	233′44′-PeCB	0.0001	0.0001
2378-TCDF	0.1	0.1	2344′5-PeCB	0.0005	0.0005
12378-PeCDF	0.05	0.05	23′44′5-PeCB	0.0001	0.0001
23478-PeCDF	0.5	0.5	2′344′5-PeCB	0.0001	0.0001
123478-HxCDF	0.1	0.1	233′44′5-HxCB	0.0005	0.0005
123678-HxCDF	0.1	0.1	233′44′5′-HxCB	0.0005	0.0005
123789-HxCDF	0.1	0.1	23′44′55′-HxCB	0.00001	0.00001
234678-HxCDF	0.1	0.1	233′44′55′-HpCB	0.0001	0.0001
1234678-HpCDF	0.01	0.01	di-ortho-PCBs		
1234789-HpCDF	0.01	0.01	22′33′44′5-HpCB	*0.00001*	
OCDF	*0.001*	*0.0001*	22′3′44′55′-HpCB	*0.00001*	

[a] TEFs that differ are in italics.

PCDFs are possible with one to eight chlorines, only 17 of these congeners are considered to be toxic. Toxicity and persistence are determined by structure, with lateral substitutions (positions 2, 3, 7, and 8) imparting the highest degree of toxicity. Of the 209 possible PCBs, only 12 have any dioxin-like toxicity. These are all non-ortho- and mono-ortho-substituted compounds. To define the relative potency of dioxin-like compounds, toxic equivalency factors (TEFs) based on in vivo and in vitro studies have been defined (*1*). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is the most toxic dioxin and has been assigned a TEF value of 1; TEFs for other dioxin-like compounds are based on activity relative to TCDD and have been updated as more experimental data are collected (*2*−*4*). **Table 1** summarizes the TEF values most commonly used for human risk assessment: the 1988 NATO/CCMS set, also called I-TEFs; the 1994 WHO set for PCBs (*3*); and the 1998 WHO set (*4*). The major change in the current 1998 TEFs is the increased weighting of PeCDD from 0.5 to 1.0. Using the TEF concept, toxicity equivalents (TEQs) can be calculated for any sample by summing the TEF-weighed concentrations of each dioxin-like compound.

Underlying the toxic equivalency theory is the assumption of a common mechanism of action for dioxin-like compounds in which toxic responses occur as a result of initial binding to the aryl hydrocarbon (Ah) receptor (*5*, 6). The cascade of events that follows is thought to produce multiple health effects such as carcinogenicity, endocrine disruption, developmental and reproductive problems, immunotoxicity, neurological alterations, chloracne, and, at high enough doses, wasting syndrome and death (*7*). The doses at which effects can be observed depend both on species and on endpoint. Lethal doses of TCDD range from micrograms per kilogram for guinea pigs, $LD_{50} = 0.6\ \mu g$/kg (*8*), to milligrams per kilogram for hamsters, $LD_{50} = 1160\ \mu g$/kg (*9*). Induction of cytochrome P-450 1A1 (CYP-1A1) mRNA is a particularly sensitive endpoint and is measurable after a single dose of 100 pg of TCDD/kg in rats (*10*). Immunological and developmental effects are other sensitive endpoints seen in laboratory animals. Mice, but not rats, showed a 50% decrease in antibody response after a single 0.7 μg/kg dose of TCDD (*11*). Monkeys, rabbits, and rats have shown increased prenatal mortality from multiple doses below 1 μg/kg of body weight (*12*). TCDD has also been shown to cause cancers in animals at chronic exposures as low as 1 ng/kg of body weight/day (*13*). Although measurable in animal studies, it is not certain what adverse effects some of these endpoints will produce in humans.

In humans, dioxin's effects have mainly been evaluated in occupationally or accidentally exposed cohorts. Pregnant women exposed to PCDFs by contaminated oil in Yu-Cheng, Taiwan, showed elevated CYP-1A1 enzyme levels compared to controls. The level of induction was similar to that cause by TCDD in rats (*14*). Children of these women exhibited developmental effects similar to the effects seen in mice and monkeys exposed to TCDD, including ectodermal dysplasia and delayed psychomotor and cognitive development (*12*). A number of epidemiology studies have shown overall increases in cancer mortality due to TCDD exposure (*13*), and recently the International Agency for Research on Cancer has named TCDD a known human carcinogen (*15*).

In light of continuing concerns about dioxin-related health risks, the U.S. EPA has been reassessing the impact that dioxins and related compounds have on society. The process has been ongoing since 1991 and has involved scientists from government, academia, industry, and public interest groups. The review of new data and research findings has been extensive and has delayed the completion of the document. A recent draft of the reassessment was released for comment in June 2000 but, because of its unfinalized nature, will be minimally referenced in this review. Thus far, no regulatory actions have been taken on the basis of the reassessment (*16*).

As part of the review on dioxins, the EPA and other groups have cataloged the primary sources of dioxins. Major sources are combustion and incineration processes, along with smelting operations and the pulp industry. In the early 1990s medical and municipal waste incinerators had estimated PCDD/PCDF emissions of 0.7−5 and 2−3 kg of TEQ/year, respectively (*17*). The EPA estimates that annual emissions have decreased from 13.5 to 2.8 kg of TEQ/year between 1987 and 1995 mainly due to improvements of incinerator performance and removal of highly polluting incinerators (*18*). Other regulations, including bans or restriction on the production and use of chemicals such as pentachlorophenol (PCP) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), the phase out of leaded gasoline, which contains halogenated additives, and the elimination of chlorine bleaching in the pulp industry have also contributed to reducing levels of PCDD/PCDFs.

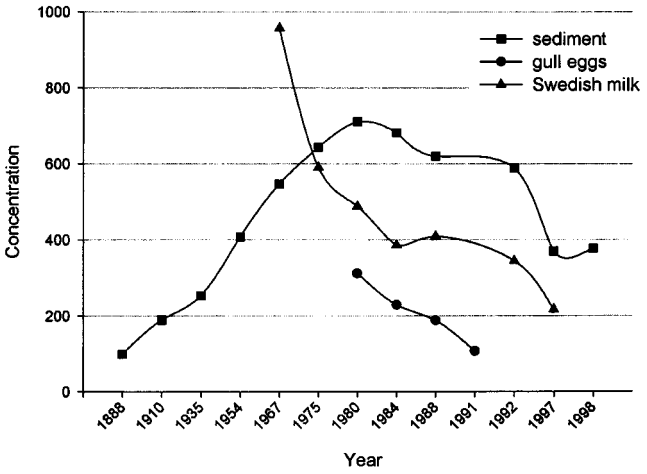

Figure 2. Temporal trends of PCDD/F levels in sediment cores, pg/g of dry weight (*26*), gull eggs, pg/g of wet weight (*27*), and Swedish human milk, pg/g of lipid (*37*). The gull egg data represent the sum of the five major PCDD/Fs for one colony (Channel/Shelter Island) presented in the reference.

In addition to the major sources, there are many diffuse sources that may be difficult, if not impossible, to regulate (i.e., home heating, diesel engines, forest and grass fires, agricultural and backyard burning). Backyard burning of household waste was recently shown to produce more PCDD/PCDFs per mass burned than a typical modern municipal waste incinerator (*19*) and was estimated to account for 22% of dioxin emissions in North America from 1996 to 1997 (*20*). Unidentified sources also remain as indicated by budget calculations on sources and deposition, which often show that depositions exceed known emissions (*21*, *22*). Baker and Hites (*23*) have proposed that reactions of atmospheric pentachlorophenol (PCP) contribute to the overall levels of dioxins. Another contribution may come from naturally formed PCDD/PCDFs, which have recently been suggested by findings in archived deep soils (*24*) and clays from the southern United States and Germany (*25*).

Unlike PCDD/PCDFs, PCBs were intentionally produced as industrial fluids and plasticizers. Hundreds of thousands of metric tons were produced in the United States until 1977, when production was banned. As a result of their chemical stability and the reservoirs that still exist, PCBs are ubiquitous in the environment and, like PCDD/PCDFs, bioaccumulate and bioconcentrate in the food chain.

Following the trend of reducing PCDD/PCDF/PCB sources, the levels in the environment have decreased as well (**Figure 2**). Sediment cores from Lake Siskiwit (a lake on Isle Royale in Lake Superior) were analyzed for PCDD/PCDFs for the time period 1888−1998 (*26*). PCDD/PCDF levels peaked in the early to mid 1980s and declined 50% by 1998. Herring gull and guillemot eggs from the Great Lakes and Baltic regions, respectively, have shown similar decreases in PCDD/PCDF and PCB levels (*27*, *28*).

Along with the decline in environmental levels, background human exposure to dioxin-like compounds has also declined. In general, humans are exposed to dioxins mainly through their diet, which accounts for >90% of their exposure (*29*−*32*). Meats, fish, and dairy products have been estimated to account for nearly equal shares of the dietary intake (*18*, *29*, *30*). Estimates of dietary exposure in Europe have shown that daily I-TEQ intakes decreased by ∼60% from the 1980s to the mid 1990s (*32*−*34*). Although some of this decrease was attributed to changing dietary intakes, much of it was due to decreasing levels of PCDD/PCDFs in the food supply. The current European daily intakes were estimated to be 1−3 pg of I-TEQ/kg of body weight, which is comparable to the range of 0.3−3.0 pg of I-TEQ/kg of body weight estimated in a study of the U.S. population (*35*). The decline in body burden is also indicated by human blood and milk monitoring (**Figure 2**). In Germany the average blood level of PCDD/PCDFs decreased by 50% to 20.7 pg of I-TEQ/g of lipid from 1989 to 1998 (*36*); human milk levels decreased by 30% to 20.7 pg of I-TEQ/g of lipid from 1987 to 1992 (*29*). Swedish human milk showed a 70% decline in PCDD/PCDFs and PCBs from 1972 to 1997, reaching 26.4 pg of I-TEQ/g of lipid; PCBs accounted for 52% of the TEQ (*37*). Body burdens in the United States have not shown a consistent trend. Perhaps due to the small numbers of sample sets analyzed, blood levels of PCDD/PCDFs appeared to remain steady at ∼27 pg of I-TEQ/g of lipid from the 1980s to 1996, whereas human milk showed a decline from 20 to 8.2 pg of I-TEQ/g of lipid during this time (*38*).

Due to the risks associated with dioxin exposure, the World Health Organization (WHO) has recommended a total daily intake of 1−4 pg of I-TEQ/kg of body weight (*39*). The U.S. EPA has set a one/million cancer risk level at 0.006 pg of I-TEQ/kg of body weight/day (*16*). On the basis of the current estimates of daily intake in the United States, the general population falls within the recommended guideline of WHO but is well above the virtually safe dose set by the EPA. Because dioxins can show measurable biological effects at extremely low levels (ppt or ppq), there is concern at the EPA that our current intakes and resulting body burdens may produce subtle adverse effects in the population and especially in subsets of the population who may be most sensitive to dioxins' effects.

LEVELS IN FOODS

Because fish and animal products are the predominant source of human intake and exposure, several models have been developed to predict the accumulation of dioxins in livestock (*40*−*42*) and fish (*43*, *44*). For livestock, air deposition of dioxins onto forage and, to a lesser extent, soils is assumed to be the major route of exposure. Although the models predicted beef and milk dioxin levels in fairly good agreement with observed values, several deficiencies should be noted. Congener-specific data for rural air levels are minimal, as are data on the actual levels in beef and other livestock. The models relied on one early study to provide bioavailability and bioconcentration factors for multiple PCDD/PCDFs, and that study had been conducted with one lactating cow (*45*). No studies have been done to evaluate specific practices such as feedlot fattening, which may affect dioxin levels in beef before slaughter. More research in all of these areas is needed to validate the models and strengthen their predictive capabilities. It should also be noted that these models predict background dioxin exposure from air deposition, not contaminations arising from other sources.

For fish and aquatic species, the models include discharges of dioxins into water in addition to atmospheric depositions. These models have also shown good agreement between predicted and observed data, although the number of measured samples is small and limited to a few species. Other limitations of these models include assumptions on the composition of fish diets, estimates of actual water concentrations and its homogeneity, and estimates on the bioavailability and metabolic transformation of PCDD/Fs in various species. In relation to human exposures, these models would best be applied and validated for those species most commonly consumed as foods.

Table 2. Dioxin and Furan Toxicity Equivalents (TEQs) on a Lipid Weight Basis in Food Items (ppt)[a]

	MS, U.S. (*64*)	across U.S. (*63*)	Germany (*119*)	Germany (*34*)	The Netherlands (*65*)
beef	0.77 (0.67)	2.16 (1.90)	2.53 (1.69)	(0.71)	(1.75)
pork	0.75 (0.74)	2.61 (2.29)	0.64 (0.4)	(0.31)	(0.43)
chicken	0.78 (0.70)	3.90 (3.0)	1.89 (1.41)	(0.62)	(1.65)
dairy	0.96 (0.77)	2.69 (2.15)	1.69 (1.32)	0.80 (0.69)	(1.59)
eggs	0.29 (0.23)	2.11 (2.11)		2.38 (2.10)	(2.0)
farm-raised fish	27.1 (20.5)			(7.44)	
other fish	19.4 (15.6)	18.17 (15.88)	19.26 (14.87)	(37.54)	(19.23)
vegetable products		2.76 (2.21)	>0.6 (<0.4)		(0.02)
		vegan diet	salad oil		vegetable oils
nondetects	= LOQ/2	= LOD/2	= LOD	not reported	not reported

[a] 1998 WHO TEFs were used to calculate TEQs, and nondetects were treated as indicated in the last row. Values in parentheses are original data calculated using earlier TEFs.

Dioxin levels have been surveyed in foods in the United States only on a limited basis because of the costs associated with the analysis. Statistically designed surveys of beef, swine, and poultry were conducted by the USDA and EPA in the mid 1990s and involved 60−80 samples from federally inspected slaughterhouses (*46*−*48*). Using the 1998 TEFs and nondetects set equal to half the limits of detection, beef back fat had an average PCDD/PCDF level of 1.08 ppt of TEQ, pork belly fat averaged 1.48 ppt of TEQ, and poultry abdominal fat averaged 0.83 ppt of TEQ. The dioxin-like PCBs contributed another 0.47, 0.06, and 0.36 ppt of TEQ to beef, pork, and poultry, respectively. In each survey, adipose tissue was sampled and assumed to give a good measure of the levels that would be found in retail meats because dioxins are thought to be distributed equally into lipid compartments. A few studies have been done to investigate this assumption. Ferrario and Bryne (*49*) analyzed various chicken samples and compared dioxin levels in breast and thigh meat to adipose tissue on a lipid-adjusted basis. All matrices were equivalent. In cattle, however, intramuscular lipids (i.e., ribeye) may contain more of the higher chlorinated dioxins and furans and more of certain PCBs than the subcutaneous fat or perirenal fat (*50*, *51*), especially if steady state equilibrium has not been reached. The use of subcutaneous fat as a sampling matrix could then lead to underestimates of the dioxin levels in edible meats. The most toxic congeners (tetras and pentas), however, have been shown to distribute equally into adipose and muscle tissues on a lipid basis (*50*, *52*).

In a geographical survey designed by the USDA-ARS, >160 beef samples from 13 states across the United States were analyzed for PCDD/PCDFs to determine regional variations in the background levels of dioxins. To obtain samples from cattle that were raised and fed in a given location and to increase compliance with the experimental sampling protocols, samples were collected from state and federal experiment stations. Although data from this survey have not been fully evaluated, several locations produced animals with noticeably high levels of dioxins, 7.8−52.9 pg of I-TEQ/g of lipid (*53*), compared to the mean and median, 2.62 and 0.92 pg of I-TEQ/g of lipid, respectively (nondetects = 0). Investigations of these sites found that the animal feeds and local soils had nondetectable levels of almost all dioxins; however, wood from posts and feed bunks at the facilities had TEQ levels up to 5 orders of magnitude higher than the other environmental samples (*54*). The pattern of dioxins found in the wood indicated that pentachlorophenol (PCP) may have been used as a wood preservative; PCP was also identified in these samples (*54*). The PCDD/F congener pattern and TEQ amounts found in these animals were similar to those of cows fed PCP-treated wood (*55*) and of control steers accidentally exposed to PCP-treated wood during a controlled feeding study (*51*).

Although the use of PCP was restricted in the 1980s, PCP was heavily used on farms as a wood preservative in the late 1970s (*56*). Because PCP is a very effective preservative, treated wood can last for decades with most of the original PCP remaining in the wood even after 25 years (www.awpi.org/pentacouncil). The extent of buildings or fences that may contain PCP-treated wood and the associated dioxin contaminants is, to our knowledge, not known today. Therefore, the impact of PCP-treated wood on dioxin levels in meat and dairy products cannot be adequately evaluated at this time.

Reported dioxin levels in U.S. dairy products and farm-raised fish have been minimal. An EPA survey of milk in the U.S. showed an average of 0.82 pg of I-TEQ/g of lipid due to PCDD/PCDFs and another 0.50 pg of I-TEQ/g of lipid due to PCBs (nondetects = LOD/2) (*57*). Geographically, the southwestern United States appeared to have the lowest dioxin levels and the southeast the highest (av = 0.51 and 1.13 pg of I-TEQ/g of lipid, respectively). The FDA recently reported a mean of 0.12 pg of TEQ_{WHO}/g of wet weight in milk or 3.05 ppt on a lipid basis, assuming 4% fat content and nondetects = LOD/2 (*58*). Other dairy products in this survey averaged from 0.08 to 0.31 pg of TEQ_{WHO}/g of wet weight.

Reported dioxin levels in farm-raised catfish in the United States have mainly stemmed from a contaminated feed incident caused by the use of a dioxin-containing ball clay additive in the soybean meal component of the diet. Lipid weight concentrations in these catfish were 6.5−44.9 pg of I-TEQ/g, with PCDDs accounting for 75−95% of the TEQ (*59*). Catfish raised on diets that did not contain the contaminated ball clay had TCDD levels about one-fifth those of the contaminated fish, 0.12 versus 0.7 pg of TCDD/g of wet weight (*60*), and average TEQs about one-tenth those of the contaminated fish, 0.31 versus 3.27 ppt wet weight (*58*). As a comparison, wild fish in the United States have been monitored for TCDD by the Food and Drug Administration (FDA) and EPA for the past 15 years (*61*, *62*). Fish collected in the late 1980s had an average TCDD level of 6.9 ppt wet weight, whereas by the mid 1990s TCDD was not detected in most fish (LOD = 1−2 ppt wet weight).

In addition to analyzing samples from production sites, several studies have analyzed goods purchased at local retail stores for PCDD/PCDF levels. These results are shown in **Tables 2** and **3** on a lipid weight basis and a whole weight basis and compared to values reported in several European countries. Considering the different methods used to report nondetect values, data among the different studies in Europe and the United States show similar levels. The levels found by Schecter et al. (*63*) in samples from a few sites across the United States appear to be somewhat higher than those found in Mississippi (*64*) on a lipid weight basis and may reflect regional variations in dioxin levels. Comparison of these two data sets shows the importance of

Table 3. Dioxin and Furan TEQs on a Whole Weight Basis in Food Items (ppt)[a]

	MS, U.S. (*64*)	across U.S. (*63*)	Germany (*34*)	U.K. (*120*)
meats (general)			0.14 (0.12)	0.79 (0.68)
beef	0.22 (0.19)	0.28 (0.25)		
pork	0.24 (0.23)	0.24 (0.21)		
chicken	0.07 (0.06)	0.21 (0.18)		0.37 (0.33)
milk	0.03 (0.02)	0.13 (0.10)	0.03 (0.02)	0.26 (0.21)
cheeses	0.33 (0.26)	0.33 (0.27)		0.18 (0.16)
butter	0.76 (0.61)	0.47 (0.52)	0.61 (0.53)	1.27 (1.07)
eggs	0.03 (0.02)	0.31 (0.31)	0.23 (0.20)	0.22 (0.19)
farm-raised fish	2.90 (2.19)		(0.33)	
other fish	0.35 (0.28)	0.55 (0.47)	(0.60)	0.57 (0.47)
vegetable products		0.08 (0.06)	(0.015)	(0.05)
		vegan diet	vegetables	vegetables
nondetects	= LOQ/2	= LOD/2	not reported	= LOD

[a] 1998 WHO TEFs were used to calculate TEQs, and nondetects were treated as indicated in the last row. Values in parentheses are original data calculated using earlier TEFs.

reporting levels on a lipid weight basis for sample-to-sample comparison and on a wet weight basis for calculating actual consumption amounts. A food item with relatively high dioxin concentrations on a lipid basis may have a much lower value when converted to a wet weight or serving portion size, if the fat content is low. In the Schecter study, meat and cheese samples all had lower fat content than those sampled by Fiedler, resulting in similar concentrations of dioxins on a whole weight basis but not on a lipid weight basis. **Tables 2** and **3** also point out the impact of switching to the newest set of TEFs. For food samples almost all TEQs increased due to the increased contribution of 1,2,3,7,8-PeCDD (TEF changed from 0.5 to 1.0). In the two surveys in which PCBs were measured, the PCB TEQ averaged 50% of the dioxin TEQ in the United States (*63*) and was equivalent to the dioxin TEQ in The Netherlands with the exception of Dutch fish, which had almost 300% more PCB TEQ than dioxin TEQ (*65*).

Although many of the surveys have shown low background levels of dioxins, occasionally a highly contaminated sample was found. In the ARS geographical survey, high levels of dioxins were strongly correlated to PCP-treated wood used at the rearing facilities (*54*, *66*). In the EPA survey on poultry, two chicken samples were found with levels well above the average (22 and 26 ppt I-TEQ, lipid weight basis). The origin of this contamination was ball clay, which had been added as an anticaking agent to soy meal in the feed (*49*). This same contaminated feed was also used by the catfish industry and resulted in lipid dioxin levels of ~40 ppt I-TEQ in catfish from Arkansas, as described above (*59*). The unique congener pattern identified in the ball clay was not similar to any known anthropogenic source and led to speculation that the dioxins in the clay were naturally formed (*67*, *68*).

Several dioxin contaminations have occurred recently in Europe. In 1998 during routine monitoring, dairy products were identified that had dioxin levels that were 2−4 times higher than normal. The source of the contamination was traced to citrus pulp used as a cattle feed component (*69*). The citrus pulp and contaminated feeds were immediately removed from the market. In another incident, PCB/PCDD/PCDF-contaminated oil was added to recycled fat used as an additive in animal feeds (*70*, *71*). The tainted feeds contaminated Belgian poultry, dairy, and meat and were discovered only after toxic effects were seen in chickens. Animals and products were quarantined, recalled, and eventually destroyed. The incident led to international recalls and bans against Belgian products. All of these contamination episodes point out not only the importance of regular monitoring of the food supply for dioxins but also our lack of understanding of all dioxin sources. Ball clay and citrus pulp were not obvious matrices in which to find dioxins—one being a predominantly inorganic material and the other of modified plant origin.

The unique congener patterns (fingerprints) found in some of the contamination incidents helped point to the sources responsible. For example, PCP-treated wood and ball clay have characteristic fingerprints, which carried over, to a great extent, to the animal tissues (**Figure 3**). Distinctive fingerprints have been cataloged for most major dioxin sources (*72*); however, data on the biotransformations of these patterns in the animal system are lacking. A few studies have reported bioavailability and bioconcentration factors for multiple PCDD/PCDFs in dairy cattle (*55*, *73*) and even fewer in beef cattle (*51*) and chickens (*74*). A better understanding of these transformation factors may facilitate source attribution.

METHODS TO REDUCE EXPOSURE

Once animals have been contaminated with dioxins, no practical methods are available to quickly reduce the body burdens. Therefore, in a contamination incident, products are removed from the market and animals may even be destroyed. One common strategy to reduce levels of undesirable compounds in exposed animals is by depuration. For example, drugs or therapeutics used in animal care are withheld for a given period of time before animals or products are ready for market to ensure that the compound has been cleared from the animal's system and provides no exposure risk to consumers. In the case of dioxins, long half-lives in the animals require long withdrawal periods. Estimates of the half-lives of PCDD/Fs in milk range from 40 to 190 days (*75*−*77*). In beef cattle adipose tissues, these half-lives are even longer at 100−200 days (*78*, *79*). Limited data have shown dioxin half-lives in chickens to be 25−60 days in adipose and eggs (*74*). Depuration together with increasing carcass and lipid weights to dilute the dioxin concentration are the only means to reduce body burdens but may prove to be uneconomical in many situations due to the length of time required.

Several methods have recently been reported that may increase clearance of dioxins from animal systems. The use of a leanness-enhancing agent, clenbuterol, was investigated in rats as a means to reduce dioxin body burdens after an acute exposure. Compared to controls, rats fed clenbuterol-supplemented feed for 10 days after a dioxin exposure had 30% less fat and 30% less total dioxin burden (*80*). In other studies, the addition of dietary fibers, chlorophyll, or an insoluble evacuation substance (chlorophyllin-chitosan) to the feed was studied in

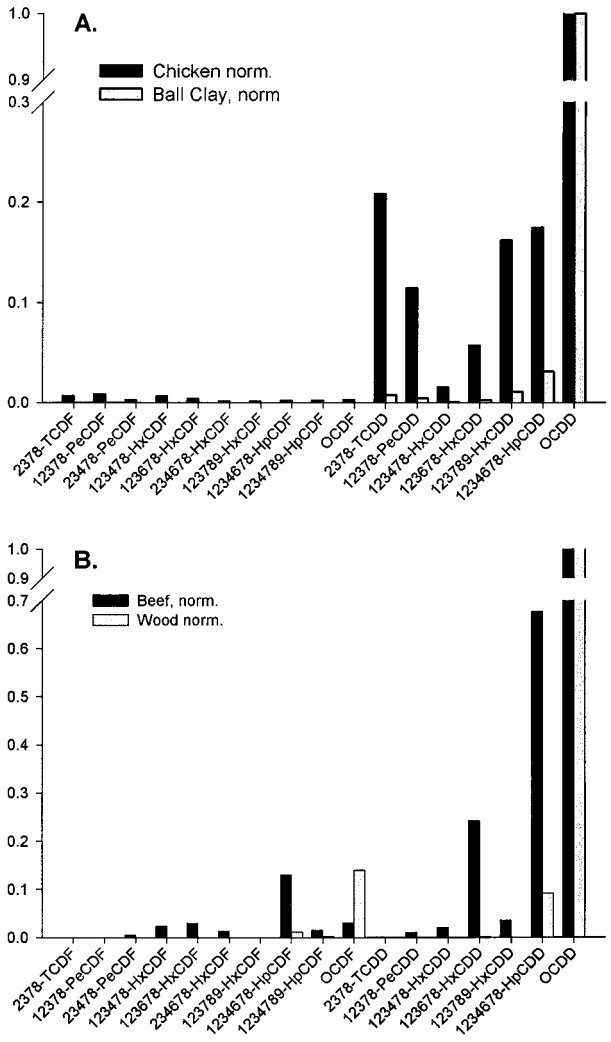

Figure 3. Comparison of PCDD/F congener patterns in (A) ball clay and chickens fed ball clay and (B) pentachlorophenol-treated wood and cattle exposed to pentachlorophenol-treated wood. Values have been normalized to OCDD.

rats and mice as a means of promoting dioxin excretion (*81*, *82*). Diets containing 10% added fiber or 0.02% chlorophyll significantly increased the excretion of fed PCDDs and PCDFs by 40−1000% in rats. Diets containing 0.02−0.5% chlorophyll also reduced the total TEQ body burden in rats fed PCDD/PCDFs by 10−45%. A diet containing 1% chlorophyllin-chitosan was fed to mice after a single oral dose of HxCDD and increased excretion of HxCDD by 350% and decreased deposition into adipose tissue by 30% compared to controls. Approaches such as these may eventually prove to be practical and economical, if the necessary supplements are inexpensive, cause no adverse growth or health effects, and are easily incorporated into animal husbandry routines. All of these strategies are still in the research stage and a long way from implementation.

At present the best way to reduce dioxin levels in livestock is to minimize exposure. Known sources of dioxin such as PCP-treated wood should be identified and removed from areas where animals may come into contact with them. Feed and feed components that have been identified as contaminated should be removed from all markets. A few studies have shown that feed ingredients from plant origin are generally lower in dioxins than those of animal origin. Rappe et al. (*68*) found an average of 142 pg of TEQ/kg of dry weight for plant feed materials (excluding soymeal contaminated by ball clay) but 615 pg of TEQ/kg of dry weight for animal meals. Animal and fish lipids used in feeds had even higher levels, 1040 and 2750 pg of TEQ/kg, respectively (*83*, *84*). The substitution of plant meals, for example, soy, for animal or fish meals may prove to be an effective means to lowering dioxin intake in livestock and aquaculture; however, further research is needed to confirm and optimize this strategy.

Other agricultural practices that have not been evaluated with respect to their impact on dioxin levels in livestock include the application of sewage sludges to pasture lands, the burning of pesticide-laden crop lands, forest and range fires, and differences between grazing and pen-fed animals. One study in Germany showed the potential for increased PCDD/PCDF/PCB levels in milk from dairy cattle raised on a farm where sludge had been applied to the fields (*85*). In the United States the proposed level of dioxin-like compounds allowed in sewage sludge used for field application is 300 ppt TEQ on a dry weight basis (amendment to 40 CFR 503).

The agricultural industries have already made progress toward reducing dioxin levels by the production of leaner beef and pork. Because dioxins accumulate in fat stores, the production of leaner meats should decrease the overall dioxin body burdens in these animals, although controlled studies have not been carried out to confirm this hypothesis. As a consumer, maintaining a low-fat diet and trimming excess fats are ways to further diminish dioxin intake. Following the USDA recommended dietary guidelines should provide one means to lower dioxin exposure: grains and rice should constitute the primary base of a diet, five servings of fruits and vegetables a day should be eaten, calories from fat should be limited to 30% of the total, and calories from saturated fats should be limited to $\leq$10%.

Another method for consumers to reduce dioxin intake is through cooking practices. Several studies on the effects of cooking on dioxin levels in foods have been published. In all cases the amounts of dioxins in a serving portion were decreased by 30−70% on average by various cooking methods. Stachiw et al. (*86*) used restructured carp fillets containing 50−100 ppt of 2,3,7,8-TCDD to evaluate roasting and charbroiling. The largest reductions in TCDD (59−70%) were found for well-done fillets. Zabik and Zabik (*87*) found baking, pan frying, and deep fat frying of fish fillets similar to broiling in the amounts of TCDD removed. Removal of the skin from fillets also increased TCDD losses during cooking. The amounts of TCDD present in the original fillets had little effect on the percentage loss in these studies (TCDD range = 0.5−100 ppt).

Other dioxin, furan, and PCB congeners have been shown to decrease by a percentage similar to TCDD in broiled hamburger, bacon, and catfish (*88*) or in pan-fried hamburger (*89*). In hamburger, levels of each congener decreased 30−50% while the total TEQ decreased 48%. Decreases correlated well with the loss of lipids during cooking, which averaged 42 and 48%, respectively, in each study. Bacon and catfish lost an even higher percentage of congeners due to the higher percent of lipids cooked out: TEQ was down 56% in bacon with a concomitant loss of 75% lipids; TEQ was down 58% in cooked catfish with 62% of the lipids removed. In the mass balance study by Petroske et al. (*89*), the fats and juices cooked out of the hamburger contained the balance of dioxins and furans. Total recoveries were 82−99% for all congeners, indicating no formation from the frying process and little thermal degradation of any congeners. Rose et al. (*90*) have also demonstrated mass balances for five PCDD/PCDFs with fried, grilled, barbequed, roasted, and stewed beef.

From these studies, cooking appears to be a reliable way to reduce dioxin levels in meats provided the fats and juices are discarded. The removal of skin from fish fillets is another means of enhancing the loss of dioxins through cooking. No studies have yet been reported that assess the effects of the use of contaminated oil or grease to fry meats or other food products. In mass balance studies PCDD/PCDFs do not appear to be destroyed or formed; however, further studies using different cooking methods may be warranted to definitively rule out formation of dioxins or furans by cooking processes.

MONITORING

In Europe recommended levels have been established for dioxins in foods and feedstuffs. Germany and The Netherlands have limited the levels of dioxins acceptable in dairy products: 5 and 6 pg of I-TEQ/g of lipid, respectively (*91*). Tolerance levels of 500 pg/kg have recently been set for certain feed additives in Europe (*84*). In July 2002 new European Union regulations will lower the accepted limits of dioxins in meats to 1−3 pg of TEQ/g of lipid and include limits for other feedstuffs (Amendments of Commission Regulation EC/466/2001 and Council Directive 1999/29/EC). In the United States a temporary action level of 1 ppt TCDD whole weight was set by the FSIS and FDA during a 1997 chicken contamination crisis.

One way to ensure that foods are low in dioxins and remain low is through routine monitoring. Monitoring programs provide an estimate of the background levels found in different food groups. Once a baseline has been established, following trends in dioxin levels can indicate problems or progress. Decreasing dioxin levels would indicate the effectiveness of regulations on point sources and of improved agricultural practices. Increasing dioxin levels would alert agencies to a contamination or to an unidentified source, and remediation steps could be quickly taken. Because livestock are mainly exposed to dioxins through their diet, feeds and feed ingredients are practical monitoring points. Dioxin analyses of feed ingredients are often less expensive than for food products because the lower lipid content of feed ingredients may require less rigorous cleanup methods. Difficulties in monitoring feedstuffs may arise from the numerous feed sources and ingredients utilized and the low lipid contents, which result in low PCDD/PCDF levels. For grazing animals, feed sources are not easily controlled or defined. Therefore, animal products may remain the most logical samples for monitoring. The EPA, FDA, and FSIS continue to build databases on dioxin levels in foods and animal feeds; however, the expense and time required for dioxin analyses limit the number of samples that can be assayed.

To make routine screening for dioxins feasible and faster, less expensive methods of analysis must be found. The current cost of a PCDD/F analysis is $600−1200 per sample. Because dioxins are present at extremely low levels, that is, part per trillion (ppt) or even part per quadrillion (ppq), samples require extensive cleanup before detection; this contributes to the cost and also the time required for the analysis. An established cleanup method for adipose tissue includes a sulfuric acid treatment to digest the fats followed by multiple chromatography steps using acid, basic, and neutral silica gel, basic alumina, and carbon columns. In our laboratory this procedure takes 1.5 days and consumes 2.2 L of organic solvents and ∼40 mL of sulfuric acid per sample. The other cost factor in dioxin analyses involves detection of multiple PCDD/PCDF congeners at the ppt level for which isotope dilution techniques utilizing high-resolution gas chromatography−high-resolution mass spectrometry (HRGC-HRMS) (*92*, *93*) are currently the recommended methods for food samples (*94*). Mass spectrometers capable of meeting the HRGC-HRMS performance criteria are expensive (∼$500,000) and require an experienced, full-time operator to maintain and operate the system. The need for isotopically labeled standards also adds to the analysis cost.

HRMS was first applied to the analysis of TCDD in fish in 1973 (*95*). Limits of detection were >3 ppt. Since then improvements in mass spectrometers, the use of high-resolution capillary GC columns, and the availability of high-purity chemical standards have allowed the quantitation of all PCDD/Fs in the sub-ppt range [see reviews by Crummett (*96*) and Buser (*97*)]. Recently, reported limits of detection for TCDD using HRGC-HRMS are as follows: in animal tissue, 0.05−0.3 ppt (*49*, *51*); in feeds, <0.02 ppt (*68*); in fish, <0.03 ppt (*60*); and in eggs, 0.02 ppt (*60*).

Improvements, which provide either faster, more efficient cleanup procedures or rapid, inexpensive screening assays, will help to lower costs of the analysis but at the same time must maintain reasonable detection limits. Although the basic sample cleanup has not changed much from the method first reported by Smith et al. (*98*), automation has decreased the sample preparation time. The Centers for Disease Control and Prevention (CDC) along with Fluid Management Systems (FMS) have automated the procedure to reduce personnel time and solvent usage (*99*−*101*). Recently, FMS has introduced high-capacity silica cartridges, which replace the manual processing steps needed to remove lipids from samples (*102*). With the most recent improvements, no direct handling of concentrated sulfuric acid is required, and the number of samples processed in one day by our laboratory will potentially double.

Immunoaffinity chromatography (IAC) is one approach that has been investigated to simplify dioxin cleanup. Immunoaffinity columns have been generated from anti-dioxin antibodies and shown to selectively bind dioxins from serum and milk samples (*103*, *104*). Although milk samples required a delipidation step prior to IAC, serum was directly applied to the immunoaffinity columns. A monoclonal antibody column showed acceptable recoveries and reliable quantitation for five of the most toxic dioxins and furans in a serum matrix at the sub-ppt level (*105*). These five dioxins and furans represented 70% of the total TEQ in the samples. The procedure required <2 h for the entire cleanup, used <10 mL of organic solvent, and showed promise as a high-throughput, environmentally friendly, inexpensive method of dioxin cleanup. The application of IAC to PCB analysis has shown similar potential in a preliminary study (*106*). Limitations of IAC include its incompatibility with high fat matrices and the lack of selectivity for all 17 toxic congeners. These problems may be overcome by using delipidation steps prior to chromatography and by incorporating new antibodies into the column, which have specificity toward other PCDD/PCDF/PCB congeners.

The development of screening assays for dioxins to complement expensive HRGC-HRMS is another way to reduce monitoring costs. Inexpensive initial screens could be used to analyze a large number of samples; only those samples that had levels of dioxins above a threshold value would be more rigorously analyzed by HRGC-HRMS to determine congener patterns and exact TEQ. Two types of screening techniques being explored are in vitro Ah receptor-based assays and immunoassays. A typical receptor-based system is the chemically activated luciferase expression (CALUX) bioassay, which utilizes a recombinant cell line (*107*). The CALUX assay produces a luminescent response when compounds actively bind

to the Ah receptor, which can be measured with a commonly available spectrometer. Because dioxin-like compounds bind to the Ah receptor as the first step in toxicity, compounds that produce a response in the CALUX bioassay are considered to be dioxin-like. The assay's response is interpreted as a CALUX TEQ by correlation to a TCDD standard response curve. The CALUX assay has been used to measure dioxin-like compounds in plasma, milk, animal fats, feeds, soils, and ash (*108*−*111*). The current limit of detection is ∼50 fg of TCDD; in actual biological matrices, the LOD has been reported at 0.5 ppt with some cleanup needed to remove lipids before analysis (*112*). When compared to HRGC-HRMS-calculated TEQs, CALUX TEQs correlated only on a logarithmic basis ($r = 0.82-0.97$), generally overestimated the actual TEQs, and gave 1−4% false negatives.

Another approach to screening is with immunoassays. Polyclonal and monoclonal anti-dioxin antibodies have been used to develop radioimmunoassays (*113*) and enzyme immunoassays, such as ELISAs (*114*−*116*). Although detection limits for these assays were in the 10−25 pg of TEQ range with standards, with actual sample matrices, the limits were >100 ppt TEQ even after extensive sample cleanup. Recent improvements have demonstrated the potential for detection down to the 5 ppt level in fat and milk matrices by immunoassays (*117*, *118*). In these studies, correlation of the ELISA-calculated TEQs to the HRGC-HRMS-calculated TEQs was good ($r > 0.9$); however, TEQs were underestimated by half or more in most cases.

Screening assays have made rapid progress in recent years but still present a number of challenges for dioxin analysis. Because these assays are aqueous-based systems, lipid removal and solubility are critical to allowing interactions between the binding sites and dioxins. Matrix interferences will differ from sample to sample. No internal standards are employed to determine recoveries from cleanup procedures, which can lead to underestimated TEQ values. Other complications arise due to the nature of the binding events. Receptor-based assays detect all compounds that activate the receptor, not just the dioxin-like ones, which tends to overestimate TEQs. The antibodies currently used for ELISAs are limited in the number of congeners that they recognize, tending to underestimate TEQs. Despite these problems, recent results have demonstrated that these assays can function as inexpensive screening tools if care is taken to control standards and matrix interferences.

CONCLUSIONS

Levels of dioxins in the environment and food supply have been declining since the 1980s, so that the current average daily intake for Europeans and Americans appears to be within the WHO recommended values of 1−4 pg of I-TEQ/kg of body weight. However, most of the data establishing declining trends in foods are from European countries with little supporting evidence from North America. In the United States, surveys of samples from slaughtering plants and grocery stores have shown generally low background levels of dioxins (<3 ppt lipid weight) in food products. The USDA, together with the EPA and FDA, need to continue to collect such survey data on raw commodities, feeds, processed and prepared goods from the marketplace, and fully cooked items to determine the impact of typical practices on dioxin levels in a farm-to-table continuum. These data will establish the baseline levels of dioxins in U.S. foods and identify practices that may add to those levels. As screening assays become more reliable and costs of analyses decrease, routine monitoring of dioxins will become more feasible and result in an even safer food supply. Samples that show elevated dioxins will quickly be identified, removed from the market, if necessary, and investigated in trace-back studies to discover the source of contamination.

As the major sources of dioxins are regulated and controlled (i.e., incinerators), other minor sources begin to predominate in their contribution to total environmental dioxins. Several agricultural practices that need to be evaluated as potential contributors to the dioxin pool include the use of sewage sludge for fertilizing fields, agricultural burning practices, and animal husbandry issues. Included in the latter category are selections of certain feed components (e.g., animal and fish byproducts), grazing styles that may result in larger intakes of contaminated soils by livestock, and the use of PCP-treated wood in barn facilities. One way to obtain information on the extent of PCP usage on farms may be through National Animal Health Monitoring System (NAHMS) questionnaires, which are periodically sent to farmers by APHIS (personal communication with Judy Akkina). If PCP-treated wood was found to be quite prevalent, educational materials or programs emphasizing the potential risk of dioxin contamination from PCP could be distributed or initiated. Investigations should also continue into the possible in vivo and in vitro formation of dioxins from precursors such as PCP or predioxins, the occurrence of dioxins from natural sources (i.e., ball clays), and currently unrecognized sources of dioxins. Reservoirs and routes of exposure for the dioxin-like PCBs should also be investigated.

Basic research into the biotransformations of dioxins and PCBs in animal systems is an area that needs more attention. Knowledge of absorption, disposition, metabolism, and excretion parameters could facilitate trace-back studies by providing a means to recognize patterns that may be attributed to specific sources. Few data are currently available on the biological fate of dioxin-like compounds in livestock animals or fish. Studies into the mechanisms of absorption and deposition may point to new techniques that can help to remediate body burdens after an exposure or prevent uptake in the first place. In preliminary studies, leanness-enhancing agents and chitosan supplements have shown some promise as methods to decrease dioxin body burdens in laboratory animals. Other possible avenues to investigate are enzyme systems, which may be induced to increase metabolism and excretion of dioxin-like compounds, or microflora, which could be exploited to degrade these compounds to less toxic compounds.

In the meantime, producing high-quality lean meats and encouraging consumers to eat low-fat healthy diets will help to keep dioxin intake low and allow the U.S. food supply to remain the safest and most economical in the world.

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