The Belgian PCB/Dioxin Incident: Analysis of the Food Chain Contamination and Health Risk Evaluation

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The Belgian PCB incident occurred at the end of January 1999 when a mixture of polychlorinated biphenyls (PCBs) contaminated with dioxins was accidentally added to a stock of recycled fat used in the production of animal feeds. Although signs of poultry poisoning were noticed by February, 1999, the source and the extent of the contamination were discovered only in May 1999, when it appeared that more than 2500 farms could have been supplied with contaminated feeds. This resulted in a major food crisis, which rapidly extended to the whole country and could be resolved only by the implementation of a large PCB/dioxin food monitoring program. Screening for PCB contamination was based on the determination of the seven PCB markers. When PCB concentrations exceeded the tolerance levels of 0.1 (milk), 0.2 (poultry, bovine, and pig meat), or 1 (animal feed) µg/g fat, dioxins (17 PCDD/Fs congeners) were also determined. At the end of December 1999, the database contained the results of more than 55,000 PCB and 500 dioxin analyses. The study of PCB levels and profiles in contaminated feeds delivered to poultry or pig farms confirmed that the Belgian PCB incident was due to a single source of PCB oil introduced into the food chain at the end of January 1999. This PCB oil had a congeners pattern closely matched to a mixture of Aroclor 1260/1254 in the proportion 75/25. The total amount of PCBs added to recycled fats was estimated at 50 kg (sum of the seven markers) or approximately 150 kg total PCBs, which corresponds to about 100 liters of PCB oil. This PCB mixture contained about 1 g TEQ dioxins (more than 90% contributed by PCDFs) and about 2 g TEQ dioxin-like PCBs. The proportions of PCB 52 and 101 congeners were fairly constant in animal feeds, excluding the possibility of secondary contamination due to fat recycling from contaminated animals. The highest concentrations of PCBs and dioxins were found in poultry and especially in the reproduction animals (hens and chicks), which showed the classical manifestations of chick edema disease. The pigs were also affected but to a lesser extent and no sign of intoxication was observed. The study of PCB/dioxin patterns and of the PCB:dioxin ratios revealed major differences in the metabolism of these compounds by farm animals. Whereas the PCBs:dioxins ratio was fairly constant in all poultry products with a mean value similar to that found in contaminated feeds (50,000), in pigs this ratio was both much higher and more variable (values up to 10,000,000), reflecting a faster elimination of dioxins than PCBs in these animals. These metabolic differences also emerged from the PCB and dioxin patterns which were altered much more in pigs than in poultry. Although the most contaminated food products (chicken meat) had PCB and dioxin levels more than 100 times above maximal recommended values, it is unlikely that this incident could have caused adverse effects in the general population of Belgium. A doubling of the PCB and dioxin burden of the young adult population would require the consumption of, respectively, 10 and 20 highly contaminated meals. In view of the very limited proportion of the poultry chain effectively contaminated during the incident (around 2%), such an extreme scenario was quite improbable for the general population except perhaps for farmers consuming their own products. But even in that case, it would have meant going back to the levels in the 1980s or attaining the body burden of subjects regularly eating contaminated seafood. © 2002 Elsevier Science

Key Words: PCB; dioxin; PCDD/Fs; food safety; food chain; chick edema disease; food contamination; risk assessment.

TABLE 1
Concentrations of PCBs and Dioxins (PCDD/Fs) in PCB-Positive Samples Originating from Farms Suspected of Having Received Contaminated Feeds during the Belgian PCB Incident

Type of sample	N (PCBs)	PCBs (ng/g fat) [range]	N (PCDD/Fs)	PCDD/Fs (pg TEQ/g fat) [range]
Pig feed	11	4,258 [809–14,154]	3	180.2 [73–301.4]
Poultry feed	20	14,996 [832–452,836]	12	231.5 [20.1–11,143]
Chicken	15	3,409 [1,010–56,856]	4	255.4 [15.8–2,613]
Laying hen	14	889 [234–3,868]	3	3.7 [2.6–6.95]
Chick	5	8,160 [2,721–47,101]	5	170.5 [54.4–965.4]
Hen	13	5,434 [2,549–22,637]	1	463.3
Brooded eggs	23	2,852 [510–38,890]	9	44.9 [1.0–713.1]
Whole Eggs	23	839 [515–1,631	17	3.8 [1.2–19.6]
Pig	94	2,928 [1,188–15,080]	48	0.9 [0.03–36.25]
Young pig	9	2,957 [1,040–25,472]	4	0.8 [0.38–2.7]
Saw	60	6,688 [654–17,271]	40	2.1 [0.08–23.82]
Bovine[a]	12	487 [246–1,060]	9	5.81 [3.6–13.2]
Milk	55	25 [6–160]	54	2.07 [1.09–6.0]
Total	355		209	

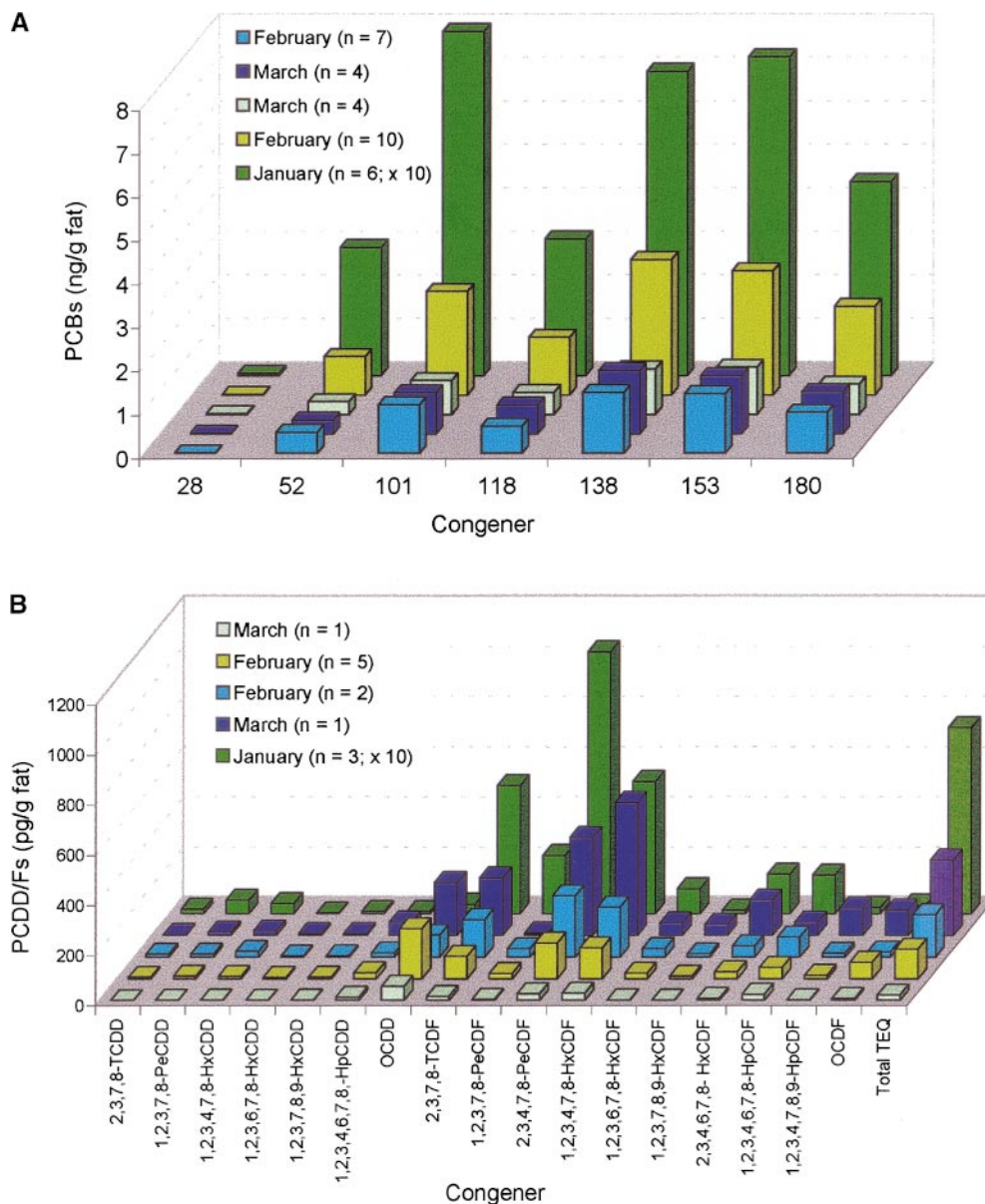[a] Includes dairy and culled cows.
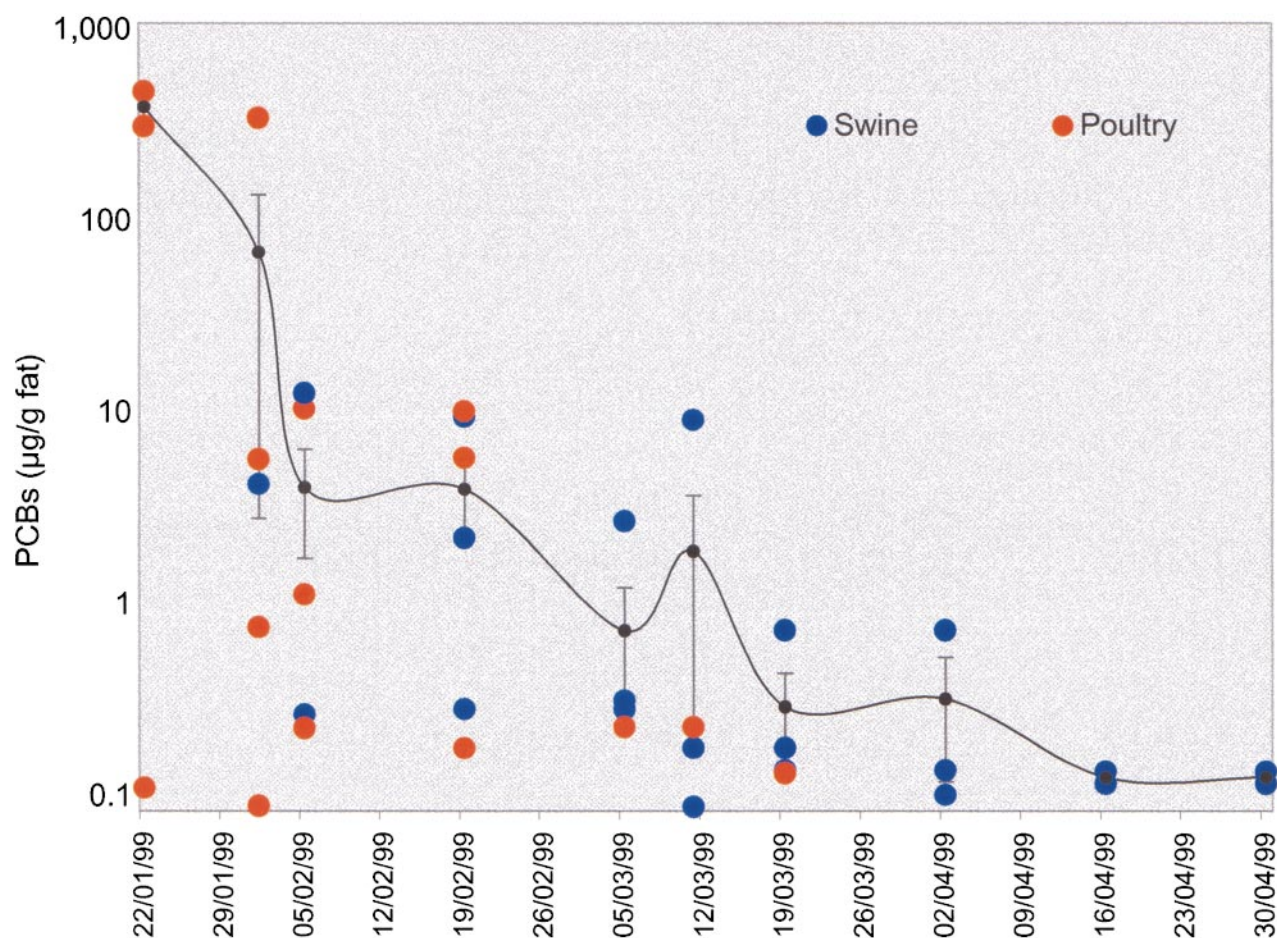
FIG. 1. Time course of PCB (sum of seven markers) concentrations in poultry and pig feeds from farms affected by the PCB incident. The mean value of January 22, 1999 has been calculated by exclusion of the negative sample (sample containing about 0.1 μg PCBs/g fat). Each point represents one to three values.

INTRODUCTION

In January 1999, Belgium was the location of an unprecedented food crisis caused by the contamination of animal feeds with polychlorobiphenyls (PCBs) and dioxins (PCDD/Fs). The first signs of contamination were observed early February 1999 in several poultry farms. The first symptom was a sudden drop in egg production, followed a few weeks later by a marked reduction in egg hatchability, a reduced weight gain, and an increased mortality of chicks. These birds presented ascites,

subcutaneous edema of the neck, and neurological disturbances (ataxia). Histology revealed degenerative changes of the skeletal and cardiac muscles. Such lesions resembled the classical manifestations of the "chick-edema disease" which was described in the 1950s–1970s in outbreaks of poultry poisoning by polyhalogenated hydrocarbons (Bernard *et al.*, 1999; Report of the Belgian Parliament, 2000; Gilbertson *et al.*, 1991).

These observations led to suspicion of dioxins as the causative agent, an hypothesis which was confirmed in April 1999 when exceptionally high levels

FIG. 2. Patterns of PCB (A) and dioxin (B) congeners in contaminated feeds delivered to pig (blue bars) and poultry (green bars) farms in January, February, and March, 1999. Values in poultry feeds in January have been divided by 10 (PCBs) or 20 (dioxins) for the ease of representation.
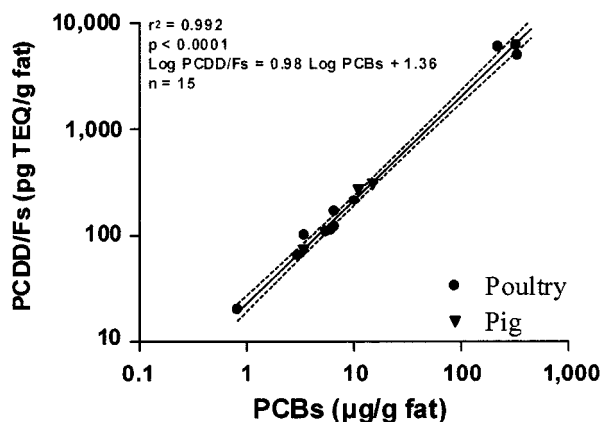
FIG. 3. Correlation between dioxins and PCBs in contaminated feeds delivered to pig and chicken farms.

MATERIALS AND METHODS

Samples. All the samples that could be retrieved from poultry farms where chick oedema disease had broken out in early February were analyzed for both dioxins and PCBs. In the PCB food monitoring program launched later (the end of June), the sampling was performed on a random basis at a frequency that depended on the homogeneity of the animal lots and on whether the farms were suspected of having received contaminated feeds. At suspected farms, when animal lots could be considered as homogeneous, i.e., animals bred in the same farm with feed from the same manufacturer, a minimum of two to five samples were collected according to the size of the lots (two below 1000, three above 1000, and four above 10,000). When lots could not considered homogeneous, the minimum number of samples collected was set at seven was for bovine herds (when herd contained fewer than 8 animals, all had to be sampled) and at three for lots of pig and poultry animals. At unsuspected farms, a minimum of three samples were collected for all species irrespective of the size of the lots. Participation in the monitoring program was obligatory for all farms.

For each animal, a minimum of 100 g of fat was sampled in the subcutaneous area, except for bovines, for which peritoneal fat was sometimes taken. All poultry were sacrificed for the sampling. For pigs and bovines, samples were obtained either from sacrificed animals or from live animals after local anesthesia. For small animals (chicks), samples were obtained by pooling fat from a maximum of three animals. Egg samples were obtained by mixing a minimum of 100 g of yolk. For milk, fat was extracted from a minimum of 1 liter of milk from the same cow.

Analysis of PCBs and dioxins. Analysis of PCBs was based on the determination of the seven PCB markers (IUPAC Nos. 28, 52, 101, 118, 138, 153, and 180) measured by high-resolution gas chromatography/mass spectrometry or electron capture detection. These markers were measured on the lipid fraction and all results were expressed on a fat basis. Only a few laboratories in Europe were measuring these seven markers when the dioxin crisis broke out. To process the huge number of samples to be analyzed daily, the Belgian authorities asked several private or official laboratories to rapidly set up a method for measuring these seven markers. An intercomparison program was then organized to ensure the comparability of the results obtained from the different laboratories (Beernaert and De Poorter, 1999). With the exception of milk, dioxins were

of dioxins (more than 1000 pg TEQ/g fat) were found in feed, meat, and eggs of affected poultry. In June 1999, additional analyses revealed that these dioxins, as suggested by their patterns, originated from a contamination by PCB oil accidentally introduced in a tank of recycled fats used in the manufacture of animal feeds. When it appeared that the contaminated fat could have been sold to nine manufacturers of animal feeds which in turn supplied a total of 2500 farms, it became impossible to trace the contamination in the Belgian food chain. This prompted the Belgian authorities to implement a large PCB monitoring program to identify contaminated foods. Initially this program was intended to detect contaminated products from suspected farms but it was rapidly extended to all farms over the country as a result of the embargo imposed on all Belgian food products. At the end of 1999, the database contained the results of more than 50,000 PCB and 500 dioxin analyses performed on virtually all types of food products.

In the present study, we analyzed the levels and the patterns of PCBs and dioxins in animal feeds and in food products that were effectively contaminated (poultry, pigs) or suspected of having been contaminated by the incident (bovines). Using the PCB/dioxin fingerprints and the PCB:dioxin ratio to trace the contamination in the food chain, we determined the time course of the incident, the source and amount of PCB oil, and the transfer of the contamination into farm animals and their products. By estimating the proportion of the food chain actually affected by the contamination, we then assessed the likelihood that this incident may have increased the body burden of the general population of Belgium.

measured only when PCB concentrations exceeded the tolerance levels of 0.1 (milk and derivatives), 0.2 (poultry, bovine, and pig meat), or 1 (animal feed) ng/g fat. However, when PCB concentrations were above 1 µg/g fat, the animals were immediately destroyed, with the explanation that for these cases dioxins could not be systematically analyzed. Determination of the 17 2,3,7,8-substituted congeners of PCDDs and PCDFs was achieved by high-resolution mass spectrometry. The total dioxin activity was calculated with the international toxic equivalent factors (TEFs) of WHO, 1998 (TEQ) (Van Leeuwen and Younes, 1998; Van den Berg *et al.*, 1998). The dioxin-like PCBs were determined by measuring non-*ortho*-PCBs (IUPAC Nos. 77, 126, and 169) and mono-*ortho*-PCBs (IUPAC Nos. 105, 114, 118, 123, 156, 157, 167, and 189). The dioxin-like activity of these congeners was also calculated with the WHO TEFs (Van Leeuwen and Younes 1998; Van den Berg *et al.*, 1998). Dioxin concentrations were also expressed on a fat basis (pg TEQ/g fat). For studying the PCDD/Fs patterns, however, we used the absolute concentrations of PCDD/Fs congeners without normalization to equivalents of TCDD (pg/g fat). These analyses were performed by more than 15 different official laboratories, mostly located in Belgium. Because all these laboratories were certified laboratories regularly participating in international quality control programs, no intercomparison was specifically organized during the dioxin crisis for the determination of dioxins.

Description of the database. At the end of December 1999, the database contained the results of 55,205 PCB analyses performed on feed or food samples produced by farms all over the country. Of these samples, 20,460 were from farms suspected of having received delivery of contaminated feeds. The PCB tolerance levels were exceeded in 11.1% of these samples. The highest prevalence of positive samples was observed in pigs (1890/7528; 25.1%), followed by eggs (50/312; 16%), poultry meat (147/1735; 8.5%), bovine meat (12/513; 2%), and milk (3/932; 0.3%). These values, however, should be interpreted with caution since they probably reflect the differences in life cycle of these animals more than the proportions of livestock actually affected by the contamination. For instance, the much higher proportion of positive samples in pig is simply due to the fact that these animals or their offspring were still alive when the monitoring program was launched in May 1999 and this is in contrast to chickens, which had already been sacrificed and consumed (with the exception of laying hens). (Fries *et al.*,

1977). For purpose of exportation, PCBs on a total of 26,979 samples taken from nonsuspected farms across the whole territory were also analyzed. Depending on the animal species and the type of food, 0.0 to 2.8% of these samples exceeded the PCB tolerance levels (a total of 0.83% samples were positive). With the exception of bovine products, the patterns of PCBs and dioxins were studied only in samples from suspected farms presenting the highest levels of PCB contamination. This selection was deliberately made to preserve the original fingerprints characterizing the incident and to study its evolution in the food chain. Indeed, with PCB tolerance levels as low as 0.1–0.2 µg/g fat, there is an important risk of confounding contamination cases caused by the incident with cases resulting from sporadic variations in the background pollution by PCBs/dioxins.

Statistical analyses. The results were expressed as geometric means and ranges. The profiles of PCB and dioxin congeners in the different types of feed or food were studied by calculating the geometric mean of individual absolute values for each congener. All statistical tests were applied on log transformed data. We studied the associations between dioxins and PCBs by calculating the Pearson's correlation coefficients. The relationships between dioxins and PCBs were fitted by linear regression analysis, except for pigs, for which data could be fitted only by a curve with a shape similar to that of saturation kinetics. The level of statistical difference was set at $P < 0.05$.

RESULTS

Levels of PCBs and PCDD/Fs in the contaminated food chain. Table 1 gives the geometric means and ranges of PCB and dioxin concentrations in the most contaminated samples discovered after the PCB incident. All these samples originated from farms that received feed from the manufacturers affected by the incident. Samples of chicken feed produced at the end of January 1999 showed the highest levels of contamination, with PCB and dioxin concentrations reaching 450 µg/g fat and 11,140 pg TEQ/g fat, respectively. The PCB concentrations in poultry products were at least one order of magnitude lower, with means ranging from 0.84 to 8.2 µg/g fat and maximal values between 1.63 and 56.8 µg/g fat. The mean dioxin concentrations in poultry products ranged from 3.8 to 255 pg TEQ/g fat and the maximal values from 19.6 to 2613 pg TEQ/g fat. The highest concentrations of PCBs (>50 µg/g fat) and dioxins (>1000 pg TEQ/g fat) were found in birds with signs
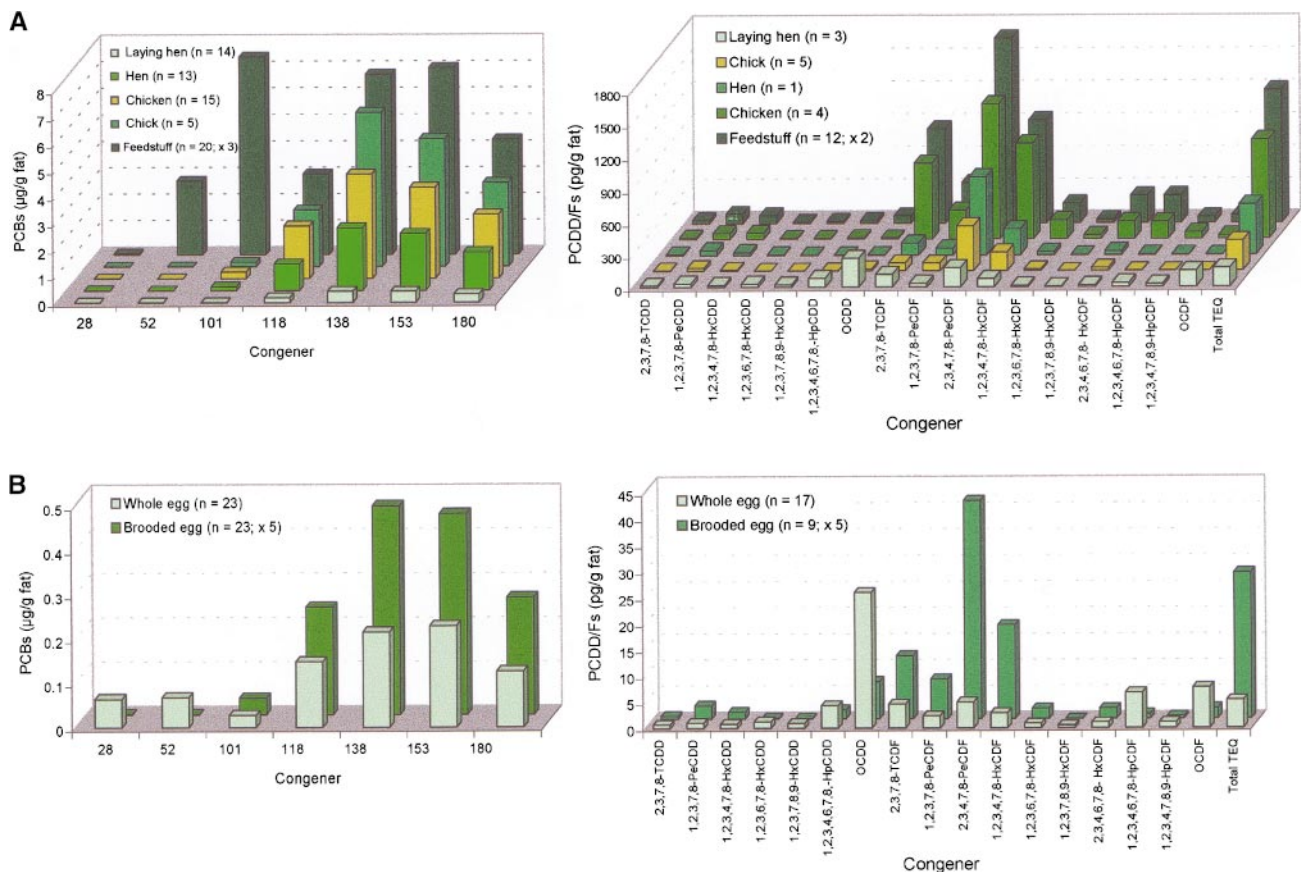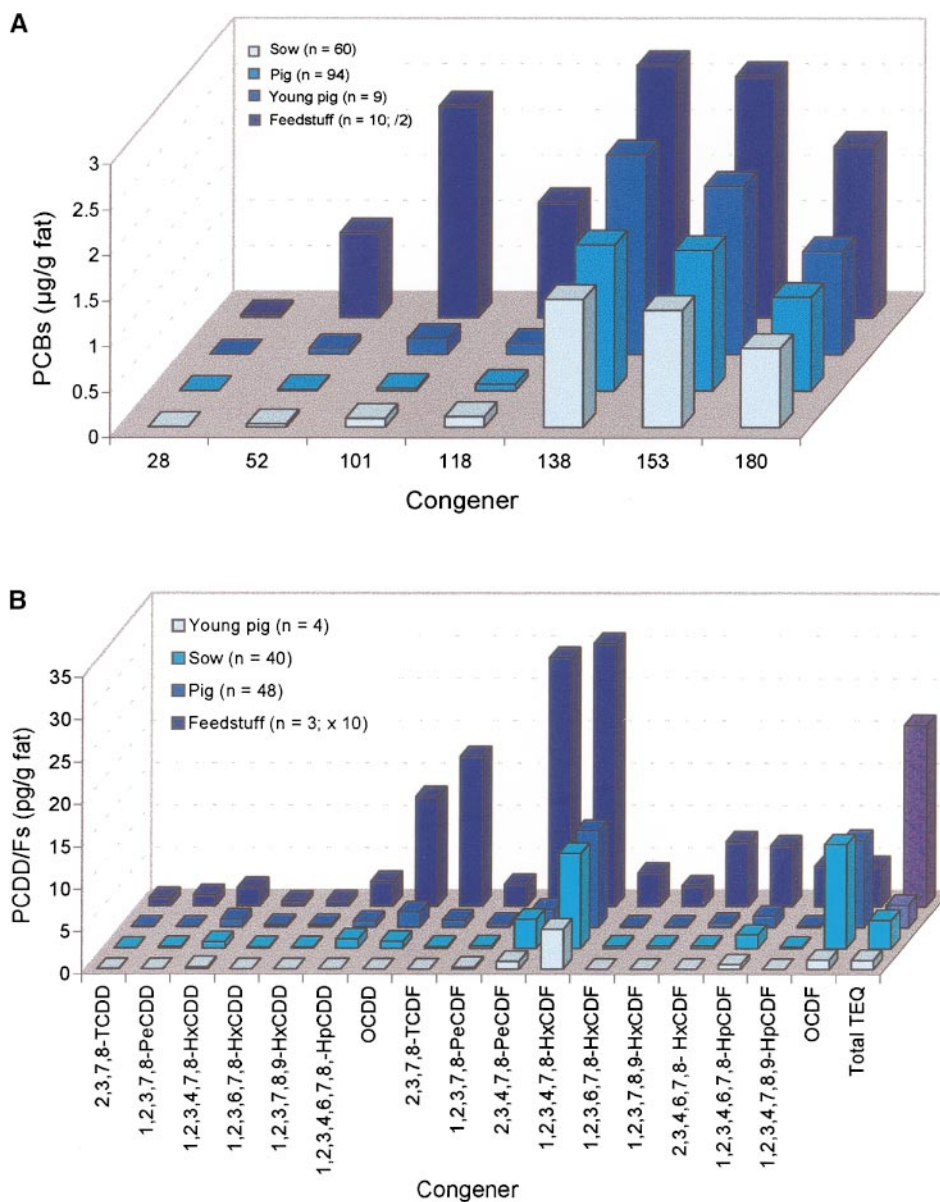
FIG. 4. Patterns of PCB (A) and dioxin (B) congeners in contaminated poultry feed, poultry meat, and eggs. Patterns are presented separately for chickens, laying hens, consumption and brooded eggs. For ease of representation values in feeds have been divided by 3 (PCBs) or 2 (dioxins) and values in brooded eggs by 5.

of intoxication (reduced fertility and deformed chicks). In pig farms no sample of the originally contaminated feed manufactured in January 1999 could be retrieved, explaining why in Table 1 concentrations of PCBs appear lower in pig than in poultry feed. Pig farms, however, have presumably received feeds as contaminated as feeds delivered to chicken farms, as evidenced by the PCB concentrations in pig and poultry animals. Surprisingly, despite similar levels of PCBs, pigs showed much lower dioxin concentrations than chickens, the highest values not exceeding 40 pg TEQ/g. No abnormality which could be related to PCB contamination was observed in pig farms. In the bovine livestock, the few samples positive for the PCB test corresponded to meat of dairy and culled cows. The concentrations of PCBs and dioxins in these samples were, however, much lower than those in contaminated pig and poultry animals. In milk, PCB and dioxin concentrations were still lower and were within the range of normal background levels in Belgium and Europe (European

Commission, 1999; Van Cleuvenbergen *et al.*, 1994a,b). These observations indicate thus that dairy cows and dairy products were spared by the incident.

The concentrations of dioxin-like PCBs were determined in five contaminated samples, two of eggs (dioxin values: 53 and 18 pg TEQ/g fat) and three of chicken feed (dioxin values: 301, 288, and 315 pg TEQ/g fat). The addition to dioxin-like PCBs increases the TEQ values of the egg samples on average by 5.3 (4.6, 5.9) and that of feeds by 2.9 (2.7, 2.9, and 3.0).

PCBs and PCDD/Fs in animal feeds. Figure 1 shows the time course of PCB concentrations in the contaminated feed samples that could be retrieved in farms affected by the incident. These samples originated only from poultry and pig farms, as no sample of feed positive for the PCB test was found in bovine farms. The PCB contamination peaked in feeds produced at the end of January 1999, attaining a mean

FIG. 6. Patterns of PCB (A) and dioxin (B) congeners in contaminated pig feed and in meat from sows, pigs, and piglets. Concentrations of PCBs in feeds have been multiplied by 2 and those of dioxins divided by 10 for the ease of representation.

PCB level of about 300 μg/g fat. Between February and March 1999, PCB level in feed rapidly dropped and mid-April 1999, no sample of feed exceeded the PCB tolerance level of 1 μg/g fat. During this period, PCB and dioxin profiles in all contaminated feeds delivered to chicken or pig farms were remarkably similar (Fig. 2). It is also interesting to note that the proportions of the PCB 52 and 101 congeners were fairly constant. Since these two congeners are labile in the livestock, this allowed us to rule out the possibility of secondary contamination due to the recycling of fats from contaminated animals. The concentrations of dioxins and PCBs in feeds were very closely correlated, with an average PCBs:dioxins ratio of about 50,000 (Fig. 3). No difference was found between poultry and pig feeds in their mean PCBs:dioxins ratio (poultry feed, ratio = 46,537, $n = 12$; pig feed, ratio = 45,763, $n = 3$). The PCB profiles in poultry and pig feeds were matched to a mixture of Aroclors 1260/1254 (or of commercial PCBs with similar compositions) in the proportion of 75/25 (ATSDR, 1997; IPCS, 1993). Because of its high content in penta- and hexachlorinated congeners (more than 60%), this mixture should have
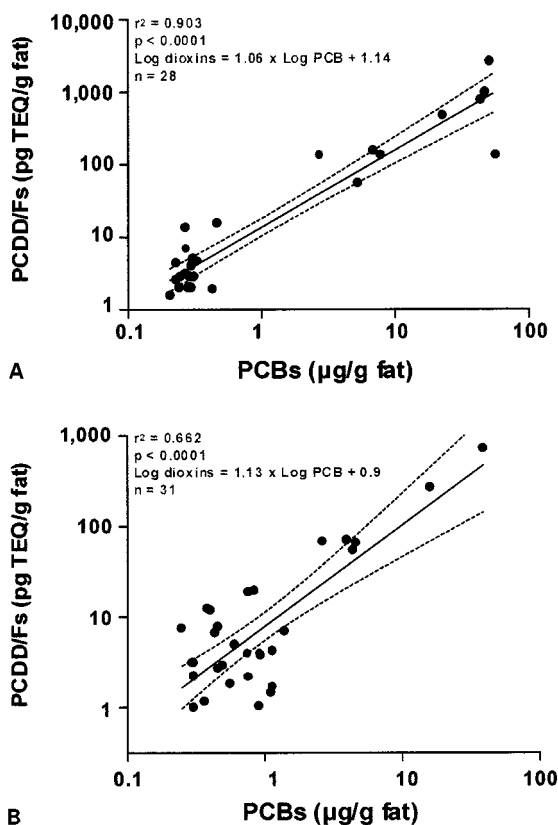
FIG. 5. Correlation between PCBs and dioxins in poultry meat (A) and eggs (B). The correlations have been calculated on samples positive for the PCB test originating from suspected farms (15 meat and 26 egg samples) or from unsuspected farms (15 meat and 5 egg samples). In suspected farms, PCB concentrations ranged from 0.23 to 56.8 μg/g fat in meat and from 0.36 to 38.9 μg/g fat in eggs. In unsuspected farms PCB concentrations ranged from 0.21 to 0.44 μg/g fat in meat and from 0.25 to 0.31 μg/g fat in eggs. Meat samples originated from chickens ($n = 12$), laying hens ($n = 10$), hen ($n = 1$), and chicks ($n = 5$). Egg samples originated from laying hens ($n = 8$) and nonlaying hens ($n = 23$).

a resinous/waxy appearance, similar to that of a frying oil.

Amounts of PCBs and dioxins at the origin of the contamination. The total amounts of PCBs (sum of seven markers), dioxins (17 PCDD/Fs congeners), and dioxin-like PCBs accidentally mixed with the recycled fat in January 1999 were estimated at about 50 kg, 1 g TEQ, and 2 g TEQ, respectively. These values were obtained by extrapolation of the mean concentration of PCBs and dioxins in the fat of the most contaminated poultry feeds (end of January 1999; Fig. 1) to the total volume of contaminated fat (60 tons), assuming a twofold dilution of the original PCB and dioxin concentrations with the vegetable fat used in the production of feeds. For estimating

the amount of dioxin-like PCBs, we used the ratio of three as derived from the analysis of three contaminated feed samples (see above). Since the sum of the seven PCB markers represents theoretically 30% of the total weight of all PCB congeners in a 75/25 mixture of Aroclors 1260/1254 (or of commercial PCBs with similar compositions), we estimated the total amount of PCBs at the origin of the incident at approximately 150 kg (sum of all congeners), which corresponds to a volume of about 100 liters of PCB oil (density 1.60 g/cm^3) (IPCS, 1993).

Poultry. Patterns of PCBs and dioxins in poultry products are presented in Fig. 4. The patterns of PCBs were very consistent in all poultry products, the fingerprint being transferred almost unchanged from hens to eggs and from eggs to chicks (Fig. 4A). Compared to the original pattern in feed, the patterns in poultry were characterized by the disappearance of the PCB 52 and 101 congeners as a result of the preferential biotransformation of these lower chlorinated congeners. The PCDD/Fs patterns in poultry feed, chickens, hens, chicks, and brooded eggs were almost indistinguishable, indicating a very limited biotransformation of dioxins in these birds (Figs. 4A and 4B). As for PCBs, the dioxin patterns were transferred almost intact from the hens to the eggs and then to the chicks. Laying hens and consumption eggs, in contrast, presented quite different dioxin profiles, with a predominance of OCDD and OCDF. These profiles, especially those in consumption eggs, exhibited a typically environmental fingerprint which was unaltered by the PCB incident (Liem and Theelen, 1997). These observations are in accordance with the low concentrations of dioxins found in these products (Table 1). The concentrations of dioxins and PCBs in poultry meat (Fig. 5A) and in eggs (Fig. 5B) were highly correlated, with slopes or PCBs:dioxins ratios almost identical to those in feeds. These correlations allowed validation of the PCB test used to trace the contamination in the food chain.

Pig products. In comparison with the pattern in the contaminated feed, the metabolic alteration of PCB patterns in pigs was still more pronounced than that in poultry, leading to the disappearance of PCB 118 in addition to the PCB 52 and PCB 101 congeners (Fig. 6A). Of the seven PCB markers measured, only the three most chlorinated congeners were still present in significant proportions in pig meat. The dioxin patterns in pigs were very different from those in feeds, containing only a few persistent congeners (OCDD and 1, 2, 3, 4, 7, 8-HxCDF) (Fig. 6B). If one compares these profiles with those

TABLE 2

**Estimation of the Total PCDD/Fs TEQ Intake during the PCB Incident on the Basis of the Average
Consumption of Foodstuffs in Belgium**

Type of food	Consumption (g/day)	% fat	Fat intake (g fat/day)	PCDD/Fs (pg TEQ/g fat)	TEQ (pg/day)
Pig meat	76	15	5.7[a]	20	114
Chicken meat	44	10	2.2[a]	1000	220
Beef meat	63	10	3.15[a]	15	47
Eggs	38	32	12.2	200	2440
Milk	287	4.5	12.9	15	194
Butter	14	82	11.5	15	173
Cheese	28	31	8.7	15	131
Total TEQ/day					5300

[a]Assuming a 50% loss during cooking (Schecter *et al.*, 1998). The PCDD/Fs concentrations correspond to the arithmetic mean of the three highest values of the database of Table 1.

reported in the literature for pigs exposed to the background pollution (Liem and Theelen, 1997), the only reminiscence of a PCB contamination was the presence of HxCDF in relatively high proportions. The concentrations of dioxins and PCBs in pigs were significantly correlated but formed a nonlinear relationship contrasting sharply with the perfectly linear relationships observed in contaminated feeds and in poultry products (Fig. 7). The concentration of dioxins in pig meat increased with that of PCB up to a PCB level of 1 μg/g fat. Above this value, the dioxin concentrations leveled off despite very high concentrations of PCBs. As a corollary, the PCBs:dioxins ratio in pigs showed extremely variable values extending from 50,000 up to 10,000,000.

Bovine products. The patterns of PCBs in the contaminated bovine meat (dairy or culled cows) from suspected farms were similar to those found in PCB-positive samples of bovine meat (dairy cows) originating from unsuspected farms (Fig. 8A). Dioxin patterns in dairy cow meat were not different in suspected and unsuspected farms and they exhibited fingerprints corresponding to the background environmental contamination (Fig. 8B). The dioxin profile characterizing the PCB incident was found only in a few meat samples from culled cows. These cows also had the highest levels of PCBs, presumably because of the lack of milk production (Olling *et al.*, 1991) (Table 1). The PCB patterns in the few milk samples exceeding the tolerance level were indistinguishable from the patterns in samples with normal PCB levels and from the patterns reported previously for human and cow milk in Belgium (Van Cleuvenbergen *et al.*, 1994a, b) (Fig. 9). The proportion of PCDFs in these samples was increased but

this change cannot be interpreted as a reflection of PCB contamination since the same profile was found also in about 60% of PCB-negative milk samples. Although the dioxin and PCB concentrations were weakly correlated in these milk samples ($r^2 = 0.081$, $P = 0.04$, $n = 54$), the proportions of PCDFs (values in pg/g fat expressed as a percentage) in milk samples were not correlated with the PCB concentrations ($r^2 = 0.006$, $P > 0.5$). With the exception of culled cow meat, the bovine livestock appears thus to have been largely spared by the PCB incident since the levels and patterns of PCBs in bovine products from suspected farms were not different from those associated with the background environmental pollution by these substances. This limited impact of the incident on the bovine livestock can be explained
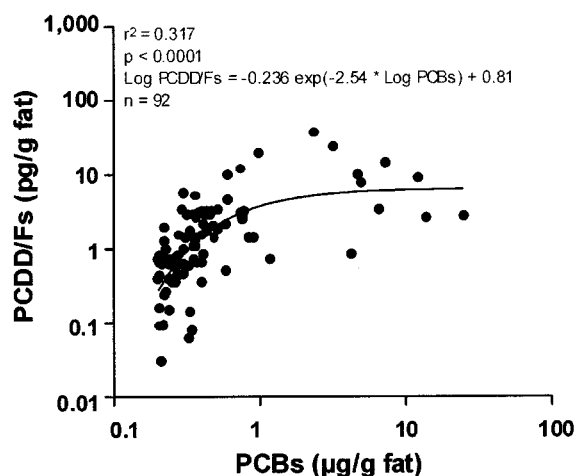
FIG. 7. Nonlinear relationship between PCBs and dioxins in samples of pig meat positive for the PCB test. The samples originated from pigs ($n = 48$), young pigs ($n = 4$), and sows ($n = 40$).
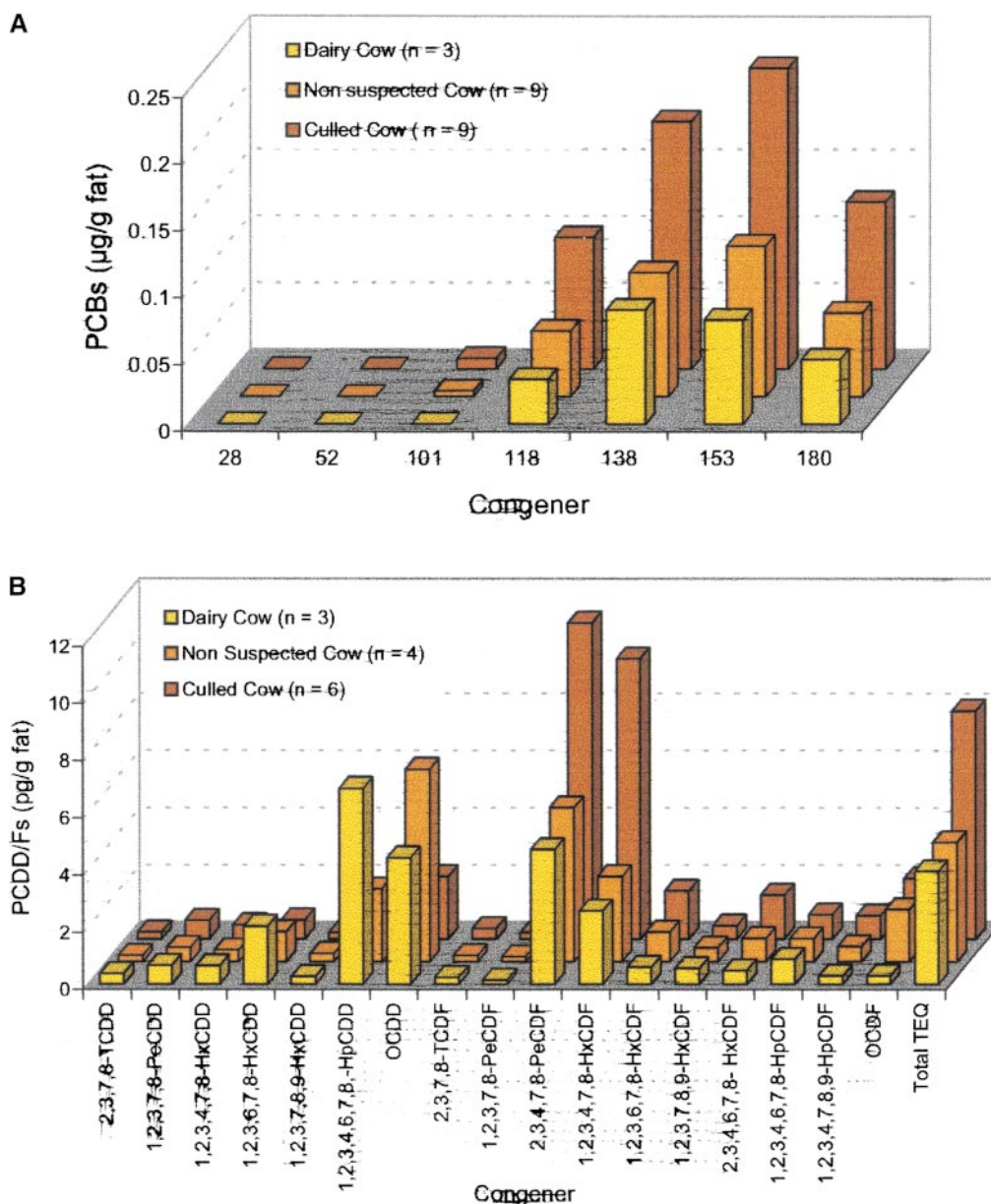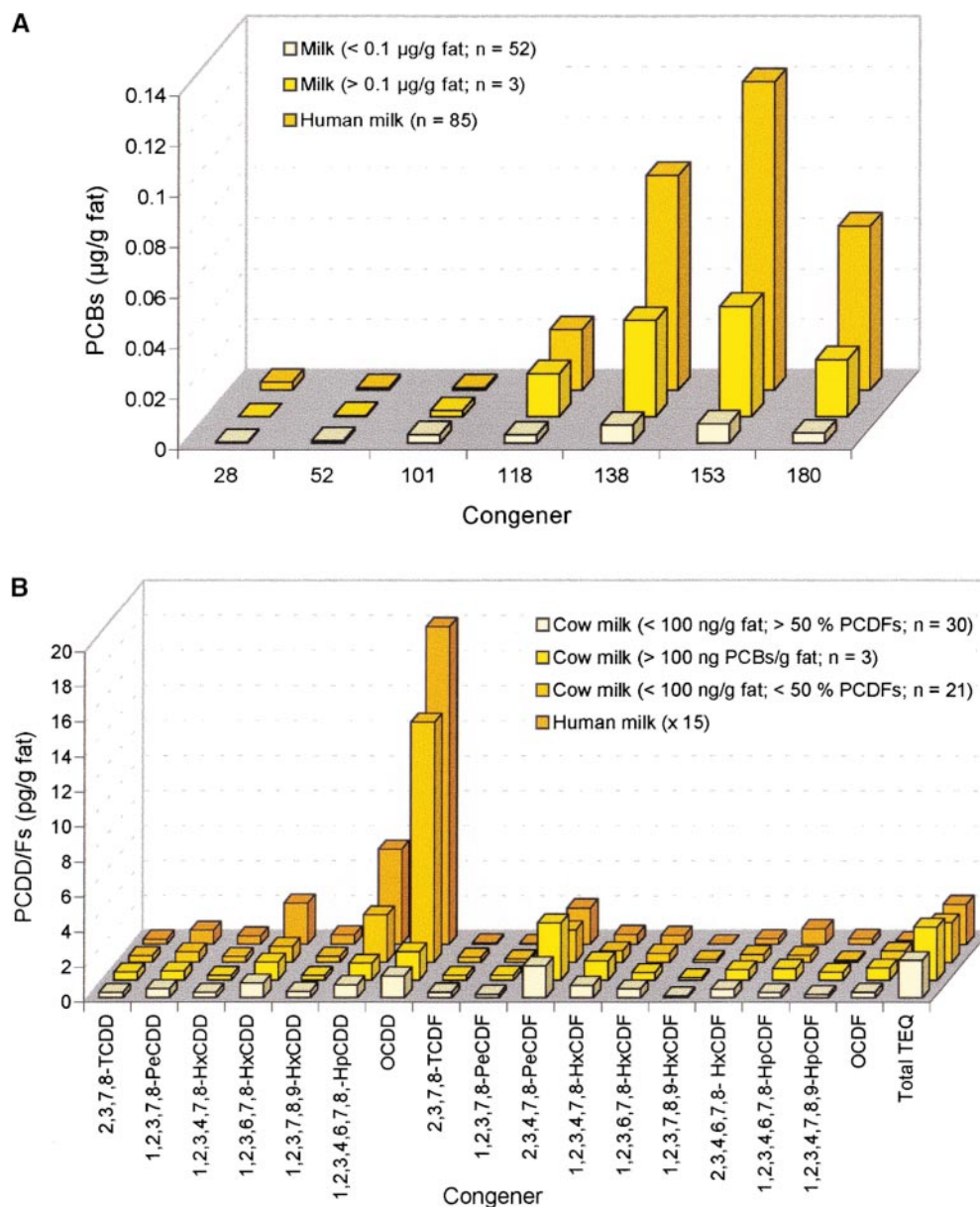
FIG. 8. Patterns of PCB (A) and dioxin (B) congeners in samples of meat from dairy cows or culled cows from suspected farms and from dairy cows from unsuspected farms.

by the longer life-cycle of these animals and their less dependency on manufactured feeds.

PCBs:dioxins ratio in the Belgian food chain. The PCBs:dioxins ratio in the PCB oil at the origin of the Belgian incident has been estimated at 50,000. This is a relatively low value, reflecting an important thermal degradation of the oil. This ratio was found in all contaminated feeds delivered to pig or poultry farms, suggesting a unique source of contamination since, of course, biotransformation is unlikely to occur in recycled fat. In contrast, as shown

in Fig. 10, the PCBs:dioxins ratio presented substantial variations among animal species. The ratio was fairly constant in most contaminated poultry products, varying between 47,848 in chicks and 71,563 in brooded eggs. A somewhat higher ratio was observed in consumption eggs (182,238), probably because these products were unaffected by the incident (see Table 2). In all pigs, in contrast, the PCBs:dioxins ratio was dramatically increased, reaching values more than 100 times higher than those found in the feed. In bovine products, the PCBs:dioxins ratio was either close to (meat) or

FIG. 9. Comparison of the patterns of PCB (A) and dioxin (B) congeners in milk from dairy cows according to the level of PCBs (above or below the tolerance level of 0.1 μg/g fat) and the proportion of PCDFs in the dioxin pattern.

much lower than (milk) that characterizing the PCB incident but no conclusion can be drawn from this comparison since the bovine livestock was spared by the incident.

Risk assessment. PCBs and dioxins are cumulative toxins which may produce toxic effects when critical concentrations are reached in target tissues (Van Leeuwen and Younes, 1998; Van den Berg *et al.*, 1998; Walker, 1999). To assess the potential health risks, we have thus calculated the increase in

body burden that might result from the consumption of the most contaminated products observed during the incident. Within the whole database, the highest PCB concentrations in foodstuffs were found in samples of poultry, in particular in chicken meat (56.9 μg/g fat) and in chicks with edema disease (47.1 μg/g fat) (Table 1, Fig. 4). These values were found in animals with the clinical manifestations of poisoning and even in animals found dead (chicks). It is thus quite unlikely that the Belgian population could had consumed food still more contaminated.

The worst case scenario would thus consist of regular consumption of foods with these extreme levels of contamination. For a reliable estimate, we used the arithmetic means of the three highest concentrations of dioxins and PCBs found in chicken meat (50 μg/g fat and 1000 pg TEQ/g fat, respectively). If one considers the case of a person eating 200 g of chicken meat (5% fat, taking into account the loss of fat during cooking), this leads to an intake of about 500 μg PCBs and 10,000 pg TEQ dioxins. Assuming that the average body burden of PCBs and dioxins by the young adult population in Belgium is, respectively, around 5 mg and 200,000 pg TEQ (WHO, 1996), this intake would result in a doubling of the PCB and dioxin body burden after 10 and 20 contaminated meals, respectively. Another way to estimate the rise in body burden that may have resulted from the incident is to calculate the total dioxin intake on the basis of the standard Belgian diet, assuming again a daily consumption of the most contaminated foodstuffs over 2 months (Table 2). For this calculation, we also used the highest values of dioxins and PCBs found during the incident. For poultry meat, we used the same values as above. For eggs, we assumed that the values found in brooded eggs with reduced hatchability and giving birth to abnormal chicks were representative of the maximal concentrations that may have occurred in eggs sold in Feburary and March. For bovine products, in view of the rapid clearance by lactating cows, we assumed that the highest values found in culled cow meat were representative of the maximal levels attainable in dairy cows, and thus in dairy products such as meat, milk, butter, and cheese. For pigs, the values found after June which were still very high were considered representative of the values in early 1999 considering the slow accumulation of PCBs and dioxins in these fatty animals. As shown in Table 2, this scenario results in a daily intake of about 5000 pg TEQ dioxins, which leads also to a doubling of the dioxin body burden after approximately 2 months. The same calculation with PCBs leads to a doubling of the body burden much earlier, after about 2 weeks. Such a doubling of the body burden would approximate the levels of PCBs and dioxins that occurred in the 1980s (Liem and Theelen, 1997; Papke, 1998; Wittsiepe et al., 2000; WHO, 1996) and a further increase by a factor of 3 to 4 would bring the body burden to the levels probably prevailing in the 1970s or to the levels of populations regularly eating contaminated fish from polluted seas (Ryan et al., 1997; Kiviranta et al., 2000).

The probability that some individuals could have experienced this worst-case scenario depends both on the duration of the contamination episode and on the proportion of the food chain contaminated during that period. As shown by the time course of PCB levels in animal feeds, the contamination has been limited to the period of January 20 to March 15, 1999. After that time, the most contaminated part of the food chain (chickens) had probably been consumed and only pigs or their offspring could still contain elevated levels of PCBs (but not of dioxins, which had been largely eliminated). According to the veterinary inspector (Dr. Destickere), about 40,000 reproduction hens and 1,000,000 chickens were contaminated in farms affected by the incident (Report of the Belgian Parliament, 2000). This estimate is in good accordance with the maximal number of chickens (also around one million) that theoretically can be contaminated to a PCB level of 50 μg/g fat and a dioxin level of 1000 pg TEQ/g fat with a total of 50 kg PCBs (seven markers) and 1 g dioxins (of which 80% were delivered to chicken farms). These numbers represent 2% of the total number of chickens produced in Belgium in February–March 1999, an estimate that fits well with the proportion of chicken farms where samples positive for the PCB test were discovered (1.98%). Under these conditions, it appears extremely improbable that a person could have consumed these most-contaminated chickens a sufficient number of times to significantly increase his or her body burden. The only situation in which such a scenario could be conceivable is that of individuals who would have consumed contaminated products from a single farm, but, even in that case, the time factor allows us to exclude any serious health effects, even in pregnant women, who are the subjects most at risk.

DISCUSSION

The Belgian PCB incident was almost an exact replica of the poultry poisoning episodes that repeatedly occurred in the 1950s and 1960s in the United States and Japan (Bernard et al., 1999; Gilbertson et al., 1991). As in these earlier incidents, the problem was noticed first in laying hens, which showed a sudden drop in egg production, and a few weeks later in the chicks, which developed clinical manifestations of the chick edema disease. The incident most probably would have never been detected if the contaminated fat had been used only in the production of feeds for pigs or bovines. These animals indeed did not show any sign of intoxication and the levels of dioxin in cow's milk (the only foodstuff regularly monitored for dioxins in Belgium at that time) were not increased by the incident. The

possibility of a dioxin contamination was envisaged only in March 1999 after a series of other hypotheses were tested and rejected. In April 1999, when high concentrations of dioxins were discovered in dead birds and their feed, most of the contaminated poultry had already been destroyed or consumed. At the end of May 1999, when the incident was revealed to the public, triggering a major political and food crisis, sows and their offspring were the only animals still to contain elevated levels of PCBs. These animals, however, had already eliminated most of their dioxin body burden. Paradoxically, thus, the dioxin contamination was almost over when the dioxin-contaminated food scare broke out in Belgium and spread all over Europe and even the world (Report of the Belgian Parliament, 2000).

The PCB oil at the origin of the accident was inadvertently introduced into the food chain via the recycling of oils and fats collected in public container parks, a practice that was forbidden in Belgium in June 1999. The total volume and the characteristics of this PCB oil could be determined with a certain precision due to the analysis of originally contaminated feed samples which could be retrieved from chicken farms. By extrapolating the PCB and dioxin concentrations in these samples to the volume of the contaminated tanks, we estimated the total amount of PCBs mixed with animal feeds to be about 50 kg PCBs (seven markers) or about 150 kg total PCBs, which corresponds to about 100 liters of PCB oil. The pattern of PCB congeners in the contaminated fat was matched to a mixture of Aroclors 1260/1254 (or of similar commercial PCBs) in the proportions of 75/25. In view of the widespread use in the past of a few commercial PCB mixtures, one can conceive that different sources of PCB oil could by chance present the same profiles of PCBs but it is extremely unlikely that these sources could also present exactly the same extent of thermal degradation as reflected by the PCBs:dioxins ratio. The consistency of both the PCB profiles and the PCBs:dioxins ratio in all contaminated feeds allowed us to conclude that the incident was caused by a unique source of PCB oil that peaked at the end of January and was progressively resolved by mid March 1999. The relatively slow disappearance of PCBs from animal feeds most likely resulted from the well-known phenomena of carry over and memory effect in the transportation (trucks) and production (tanks) facilities. Assuming that all contaminated animal feeds have been captured by the monitoring program, the possibility that the contamination was perpetuated by the recycling of fats from contaminated animals can be excluded on the basis of the PCBs patterns observed in contaminated feeds, which showed fairly constant proportions of the labile PCB 52 and 101 congeners during the whole contamination period.

The food monitoring program implemented during the Belgian crisis is probably one of the largest PCB/dioxin food surveys ever undertaken over such a limited period of time. Since there was no official norm for PCB levels in food when the crisis broke out, the first difficulty for the Belgian authorities was to rapidly set up a tolerance level for PCBs in foodstuffs which would be accepted by regulatory agencies of the economic partners of Belgium. A meeting of international experts convened by the Belgian authorities decided in June 1999 to adopt for poultry meat, eggs, and derived food products the value of 0.2 µg/g fat PCB (seven markers). This value was derived from the relationship between dioxins and PCBs in poultry as the PCB level corresponding to a dioxin concentration of 5 pg TEQ/g fat (Fig. 5). It was later decided to apply the same tolerance level to pig and bovine products despite the fact that these species exhibited very different PCBs/dioxins relationships. For milk, the tolerance level was set at a value of 0.1 µg/g fat. These tolerance levels were among the lowest foods standards ever recommended to trace PCB contamination. For instance, the action level recommended by FDA for bovine or poultry meat in 3 µg/g fat for total PCBs (Boyer et al., 1991), which corresponds to about 1 µg/g fat for the seven PCB markers, a value five times higher than that adopted during the Belgian incident. Most standards applied to PCB levels in seafood are largely above 0.5 µg/g fat for seven PCB markers (the FDA tolerance level in edible portion of fish and shellfish is 2 ppm for total PCBs). The tolerance levels adopted by Belgium were even lower than the mean concentration of PCBs in the serum of the general population (WHO, 1996; Pauwels et al., 2000). If such low tolerance levels offer a very large margin of safety, in the case of ubiquitous pollutants such as dioxins and PCBs, they present, however, a risk of confusion between the true cases of contamination due to the incident and the cases resulting from variations in the background environmental contamination. This is precisely what occurred in Belgium in September 1999 when the bovine livestock was suspected of having been contaminated on the basis of three milk samples with PCB concentrations slightly above the tolerance level of 0.1 µg/g fat. The survey conducted later over the whole country confirmed that these values were still in the range of the normal background levels of PCBs in the Belgian and probably also the European livestock (0.9% of
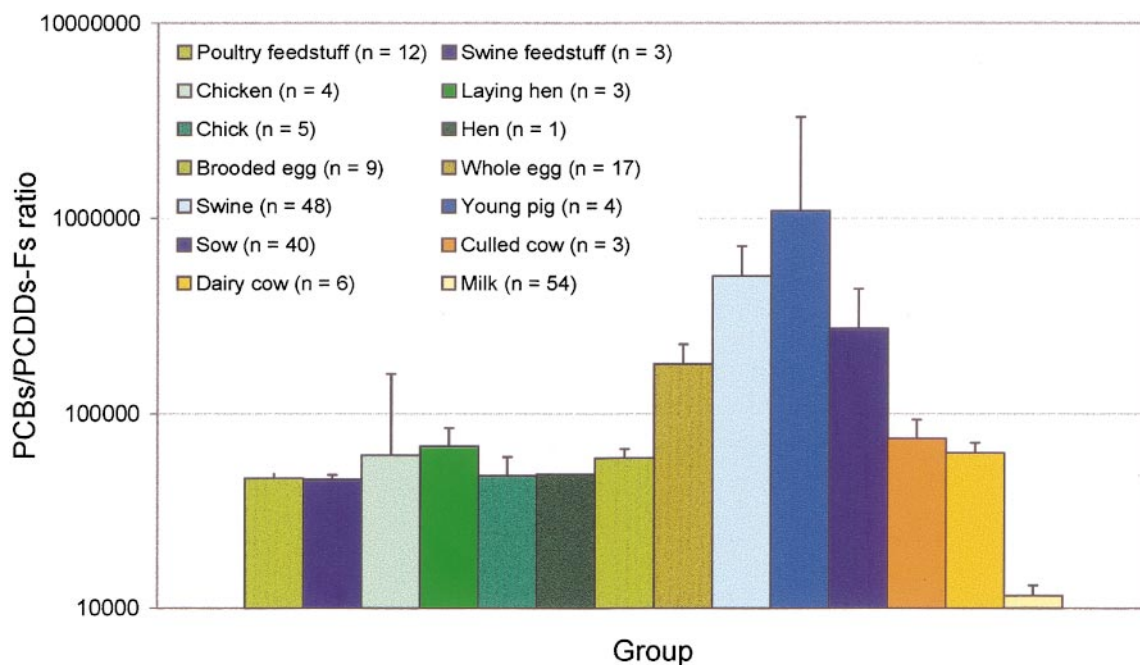
FIG. 10. PCBs : dioxins ratio in contaminated samples analyzed after the Belgian PCB incident. The results are presented as the geometric mean plus one SE.

bovine or poultry samples from unsuspected farms in Belgium have a PCB concentration exceeding 0.2 μg/g fat; Table 1).

The study of PCB and dioxin patterns revealed interesting differences in the metabolism and elimination of these compounds by farm animals. The patterns of both contaminants were remarkably preserved all along the poultry chain from the hens to the eggs and from the eggs to the chicks. The inability of birds to metabolize these polyhalogenated hydrocarbons probably explains the great sensitivity of their embryos which during brooding are exposed to increasing concentrations of these lipophilic pollutants. The most interesting observation, however, was made when the fates of PCBs and dioxins in pigs were compared. These animals, which were killed more than 6 months after the incident, had still elevated levels of PCBs but surprisingly were almost free of dioxins. Since the PCB fingerprints and the PCBs:dioxins ratios in pig feeds were identical to those in poultry feeds, the only logical way to explain this discrepancy was to postulate a faster elimination of dioxins by the pigs, which could manifest itself more easily as these animals or their offspring were sacrificed several months after the peak of exposure. Several observations support this interpretation. (i) The relationships between PCBs and dioxins in the two species have different forms. In pigs, the PCBs/dioxins rela-

tionship was not linear but evoked a saturation kinetics highly suggestive of a faster elimination of dioxins than of PCBs. Since PCB mixtures (e.g., Aroclors 1254 and 1260) are classical inducers of xenobiotic biotransformation and in particular of glucuroconjugation (Connor *et al.*, 1995), one attractive hypothesis would be that the biotransformation of dioxins in pigs has been progressively induced by the rise of the PCB body burden, explaining the shape of the relationship between both contaminants. (ii) As reported by Liem and Theelen (1997), the liver to fat ratio of dioxins is about one order of magnitude higher in pigs than in chickens, suggesting also a more efficient hepatic metabolism of dioxins in pigs compared to chickens. (iii) The alterations of patterns of PCBs and dioxins were much more pronounced in pigs than in chickens, confirming again the greater metabolic potential of mammals compared to birds.

The health risk has been a matter of hot debate in Belgium during the crisis and even after when several research teams proposed to the authorities to undertake large scale epidemiological studies on the possible health outcomes of the incident (Van Oyen, 1999). The concern was particularly focused on pregnant women in view of the poorly characterized developmental effects of dioxin-like PCBs. The present analysis indicates that the contamination has not only been limited in time but also has affected a very

small fraction of the food chain. This dispersion of the contamination in the whole food chain has made almost impossible the identification of individuals that have consumed contaminated foods. The risk assessment is also further complicated by the lack of information on the PCB and dioxin body burden of the general population of Belgium before the incident. In view of these uncertainties, we have limited the risk assessment to the estimate of the likelihood that some individuals could have increased their PCB and dioxin body burden by consuming the most contaminated products. We calculated that, in the worst-case scenario, a doubling of the PCB and dioxin body burden could have been reached after consumption of, respectively, 10 and 20 meals of the most contaminated chickens. This conclusion is reached whether the total intake is calculated by assuming a regular consumption of the most contaminated chickens (at least twice a week for 2 months) or on the basis of the average consumption by a standard adult in Belgium of the most contaminated foodstuffs. In the case of PCBs, this estimate is valid whether PCBs are expressed as total PCBs, the seven PCB markers, or dioxin-like PCBs. It is important to stress that this risk assessment is based on the maximal values of PCBs and dioxins found in poultry products in January. Because of the rapid disappearance of PCBs and dioxins in laying hens and their eggs (Fries et al., 1977), values observed in eggs in June were no longer representative of the initial contamination. For bovine products, the maximal values in culled cow meat were considered representative of the maximal values which could have been attained in all dairy products. In pigs the levels of PCBs observed after June 1999 were still sufficiently elevated to be considered representative of the peak of contamination early 1999. The dioxin intake in pigs is more difficult to evaluate because if these animals can quickly get rid of dioxins, this results in relatively high levels in the liver. This uncertainly, however, concerns only a small fraction of the total intake in view of the mean consumption of foodstuffs derived from pig liver. In proceeding with the analysis, we realize now that this scenario, which was envisaged at the heart of the dioxin crisis, was quite improbable at the population level in view of the proportion of chickens on the food market that really could have been contaminated (around 2%). Such a scenario was conceivable only for individuals such as farmers who would have consumed contaminated products from a single farm. But even in this case, a doubling of their PCB or dioxin body burden would have increased their contamination levels to levels similar to those of fish consumers or

those of people who were living in the 1980s. This conclusion is in accordance with other evaluations and with the view shared by most scientists and international organizations that, for cumulative toxins such as dioxins or PCBs, a short excursion above the TDI like that observed in Belgium is not consequential provided that the integrated dose remains largely below the critical body burden (Walker, 1999; WHO European Center for Environment and Health, 1999; Conseil Supérieur d'Hygiene Publique de France, 1999; Tuomisto et al., 1999; Van Leeuwen, 2000).

Although most experts agreed that the Belgian PCB incident was too limited in time and scale to have affected public health, some scientists were much more pessimistic in their conclusions. In particular, Van Larebeke and Hens estimated that this incident could cause between 40 and 8000 additional cases of cancer in Belgium (Report of the Belgian Parliament, 2000). These estimates were made using the lifetime cancer risk estimates derived by EPA (Schecter and Olson, 1997) or by Becher et al. (1998) and assuming that the contamination had been uniformly distributed in the food chain. The present study indicates that the latter assumption was unrealistic in view of the limited part of the food chain affected by the incident. Such an extrapolation is also not in accordance with the epidemiological studies on industrial workers, which altogether indicate that TCDD increases the risk of all cancers combined at exposure levels one to two orders of magnitude above the maximal levels the Belgian population could have attained. This extrapolation is also difficult to conciliate with findings in fishers who, despite dioxin body burden up to 5 times that of the rest of the population, show no clear evidence of an increased risk of cancer (Kiviranta et al., 2000). For these reasons, we think that the risk assessment should be focused on reproductive and developmental effects which are more likely to occur at the exposure levels encountered by the general population.

The worst case scenario envisaged in this study leads, in one meal, to a dioxin intake up to 10,000 pg TEQ (PCDD/Fs). If one adds to this value the activity of dioxin-like PCBs, this results in a total intake of 30,000 pg TEQ in one meal. This value (around 500 pg TEQ/kg) exceeds the upper limit of the TDI recommended by WHO by a factor of 100. Although very high, such an intake, remains at least two orders of magnitude below doses that have produced clinical manifestations of toxicity in adults or newborns in industrial accidents (Seveso) or in previous episodes of food poisoning (Yusho). It is also unlikely

that such an acute exposure causes subclinical adverse effects in adults or children since a similar exceedance of the TDI occurs normally during breastfeeding with so far no evidence of adverse effects on newborns. The only time at which an acute exposure of that magnitude could lead to developmental effects is during pregnancy. Such a possibility is suggested by epidemiological studies associating subtle developmental effects of *in utero* exposure with backgrounds levels of these pollutants (Van Leeuwen and Younes, 1998). However, it is difficult to evaluate to what extent these effects observed in the context of chronic exposures can be extrapolated to the peaks of exposure that the Belgian population might have experienced. Animals data are not very helpful in that respect. Acute developmental effects have been induced in the offspring of rats by a single administration of TCDD but at doses at least 100 times higher than that attainable during the Belgian incident in a single meal (64,000 pg/kg) (Gray *et al.*, 1997). A learning deficit has also been described in the offspring of monkeys given a daily TCDD dose in the range of the Belgian incident (160 pg TCDD/kg) but these effects were reported after a continuous administration of 4 years, leading to a body burden about 10 times higher than that of the young adult population (Schantz and Bowman, 1989). Theoretically, the risk of subclinical developmental effects cannot be excluded after the Belgian incident, but the detection of these effects would have required an epidemiological study at the end of 1999 of women who were pregnant between February and April 1999. But even if the Belgian authorities had supported such a large-scale study, the investigators would have been faced with the almost impossible task of identifying pregnant women who had consumed contaminated food. These women could have been found among farmers consuming their own products, but the size of the cohort would have been a limiting factor in that case. Another major difficulty with which investigators would have been confronted is how to differentiate the increase of PCB/dioxin body burden caused by the incident from that resulting from seafood, which at the peak of dioxin crisis was almost the sole source of meat in Belgium.

The current approaches used to quantify contamination by dioxins and PCBs rely on measurements primarily intended to assess the risks of the congener mixtures, which require a selection of some congeners and possibly an adjustment for their variable potency. For dioxins and dioxin-like PCBs, the existence of a common mechanism has led to the development of the toxic equivalency concept which despite some uncertainties is presently regarded the most reliable and pragmatic approach for risk assessment purposes (Van den Berg *et al.*, 2000). For non-dioxin-like PCBs, the sum of the seven markers is also a rather reliable index of the health risks since it integrates some of the most persistent PCBs in humans. It is important to note, however, that these measurements do not reflect the true profiles of the congeners. With the application of TEFs, the congeners profile is shifted toward congeners with the highest TEF values, which results in the elimination of the congeners with the lowest TEF values, i.e., precisely those congeners that are the most persistent and usually predominate in the original patterns (e.g., OCDD). Likewise, the sum of the seven PCB markers represents only 10 to 30% of the total congeners of commercial PCBs, depending on their degree of chlorination. This means that the pattern of these seven markers represents also a very small proportion of the original fingerprint. Therefore, when a dioxin contamination occurs upstream in the food chain in animal feeds or in any inert material entering the food system, we think that the analysis of the original congeners pattern is equally if not more important than the determination of the total TEQ value. The congeners profile can indeed provide key information for determining the source of contamination. Dioxins derived from combustion processes (incinerators, smelters) are characterized by profiles containing detectable levels of most PCDD/Fs congeners with, along the food chain, a progressive shift of distribution to higher chlorinated compounds and to PCDDs relative to PCDFs. In contrast, dioxins formed as impurities in chemical processes, notably during the synthesis of polychlorophenols (PCPs) or of polychlorobiphenyls, feature profiles dominated by fewer congeners consisting predominantly either of PCDDs (PCPs) or of PCDFs (PCBs) (Zook and Rappe, 1994). If in addition a close correlation can be demonstrated between the levels of dioxins and those of the primary contaminant (PCPs or PCBs), this provides a sound basis for identification of the source of the contamination. If such analysis had been done in April 1999 on the first results of dioxins, the Belgian incident would have been correctly diagnosed as a primary PCB contamination in view of the perfect matching of the dioxin pattern with that of the classical Yusho PCB incident (Bernard *et al.*, 1999). There is little doubt that under these conditions the perception and management of risks would have been very different. In the case of a PCB contamination, the analysis of the congeners pattern allows evaluation of the transmissibility of the contamination along the food

chain since the persistence of PCB mixtures is directly related to their degree of chlorination. The Belgian incident was caused by a mixture of the most chlorinated commercial PCBs (containing more than 50% chlorine). It can be estimated from our observations than more than 80% of the PCBs in this mixture were persistent in the livestock and transmissible to man (more 80% of these PCBs had a chlorination degree higher than that of PCB 138). Conversely, it can be inferred from our analysis that, in the case of a contamination by less chlorinated PCB mixtures (i.e., mixtures containing less than 50% of chlorine, such as Aroclors 1242, 1232, 1221, or 1021), less than 10% of all PCB congeners persist in farm animals and are transmissible to humans (except of course if the animals are killed shortly after having consumed the contaminated feed).

After the development of the bovine spongiform encephalopathy in the United Kingdom, the PCB/dioxin contamination of poultry and pig products in Belgium is the second major food crisis in Europe due to recycling practices upstream in the food chains. If these practices are beneficial for the environment and for the energetic yield of agriculture, they may accidentally or chronically contaminate the food chains by a variety of polyhalogenated hydrocarbons and other persistent lipophilic contaminants. There is thus an urgent need for food safety and public health organizations to carefully evaluate the risks inherent in such practices and to implement monitoring programs for animal feeds. Another important lesson learned from the Belgian incident is the need to carefully analyze the fingerprints of dioxins and PCBs when a case of contamination is discovered. This analysis may indeed provide crucial information for the identification of the source of the contamination and for the prediction of its transmission along the food chain and its possible impact on public health.

ACKNOWLEDGMENTS

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