14 Dioxins III. Relationship to Pre-Diabetes, Diabetes and Diabetic Nephropathy

C.J. Everett*
Medical University of South Carolina, Charleston, USA

14.1 Abstract

Toxic equivalency factors (TEFs) have been defined for seven polychlorinated dibenzo-*p*-dioxins, ten polychlorinated dibenzofurans and 12 polychlorinated biphenyls (van den Berg *et al.*, 2006). Twenty-three of the 29 dioxin-like chemicals were measured in human blood during the 1999–2004 National Health and Nutrition Examination Survey (NHANES) conducted in the USA (Everett and Thompson, 2012, 2014). Multiplying the concentration by the TEF for these 23 dioxin-like chemicals, and summing the products, yielded a measure called toxic equivalency (TEQ_{23}). In these investigations, pre-diabetes was defined as glycohaemoglobin (type A1c) 5.7–6.4%, diabetes was defined as diagnosed or A1c ≥ 6.5%, and nephropathy (kidney disease) as urinary albumin to creatinine ratio > 30 mg g^{-1} (microalbuminuria or macroalbuminuria). Expressed as a continuous variable, logarithm-transformed toxic equivalency ($\ln(TEQ_{23}+1)$) was associated with 'high' pre-diabetes (in the range of A1c 5.9–6.4%) with an odds ratio of 1.33 (95% confidence interval (CI): 1.03–1.72). Logarithm-transformed TEQ_{23} was also associated with diabetes (odds ratio 1.60, 95% CI: 1.22–2.09), diabetes without nephropathy (odds ratio 1.44, 95% CI: 1.11–1.87) and diabetic nephropathy (odds ratio 2.35, 95% CI: 1.57–3.52). Eight of the dioxin-like chemicals included in TEQ_{23} were most important in terms of the proportion of persons, included in the NHANES 1999–2004, with detectable values (> 25% above the maximum limit of detection). Of these eight dioxin-like chemicals, six were associated with diabetes, five associated with diabetes without nephropathy and seven associated with diabetic nephropathy. While 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin and PCB 126 were not associated with diabetes, they were associated with diabetes without nephropathy. The association of logarithm-transformed TEQ_{23} with diabetic nephropathy appears to be a case of reverse causality, or perhaps both due to reserve causality and a risk factor for the disease.

14.2 Introduction

Diabetes is a growing worldwide health problem. In the USA, the prevalence of diabetes was 12.3% (95% CI: 10.8–14.1%) in 2011–2012 (Menke *et al.*, 2015) and is projected to be 14.5% in 2031 (Mainous *et al.*, 2007). A diabetes risk score developed by Schmidt *et al.* (2005) utilized data from the Atherosclerosis Risk in Communities (ARIC) cohort. One point each was assigned

* E-mail address: everettc@musc.edu

for high waist circumference (women > 88 cm, men > 102 cm), high blood pressure (> 130/85 mmHg or using antihypertensive medication), low high-density lipoprotein (HDL) cholesterol (men < 1.03 mmol l^{-1}, women < 1.29 mmol l^{-1}), high triglycerides (> 1.7 mmol l^{-1}) and obesity (body mass index ≥ 30 kg m^{-2}); in addition, two points were assigned for fasting glucose ≥ 5.6 mmol l^{-1} or five points for fasting glucose ≥ 6.1 mmol l^{-1}. A score of 4 or more indicated a high risk of developing diabetes (sensitivity 68% and specificity 75%) with 32% of the ARIC sample being at high risk. While such a diabetes risk score is useful there is no consideration of the effect of environmental pollutants such as dioxin-like chemicals, which are the subject of this chapter.

Releases of dioxins and furans from industrial sources in the USA decreased by approximately 80% from the 1980s to 2005. Currently, release of these chemicals is due to open burning of household and municipal trash, landfill fires and agricultural and forest fires. Polychlorinated biphenyls (PCBs) were once used for electrical insulation and heat-exchange fluids. Their production peaked in the early 1970s and was banned in the USA after 1979 (CDC, 2005). Co-planar and mono-*ortho*-substituted PCBs are considered dioxin-like.

14.3 Toxic Equivalency

The history of the toxic equivalency factor approach to assessing chlorinated dioxin-like chemicals was given by Haws *et al.* (2006) and covered the period 1984–2004. Haws *et al.* (2006) summarized relative effect potency (REP) data for 17 laterally substituted (2,3,7,8-substituted) polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PDCFs), and 12 PCBs). This database was focused on biological effects mediated by binding to and activating the aryl hydrocarbon (Ah) receptor in mammals. In humans, aryl hydrocarbon receptor binding has been hypothesized to antagonize the peroxisome proliferator-activated receptor (PPAR) and contribute to the pathophysiology of diabetes (Remillard and Bunce, 2002). The numbers of studies included in the REP database were 48 studies with *in vivo* data and 37 studies with *in vitro* data, the total being 83 studies. These investigations yielded 383 *in vivo* REPs and 251 *in vitro* REPs. Sixteen of the 29 dioxin-like chemicals in the database had ten or more REPs each, with PCB 126 (115 REPs) and 2,3,4,7,8-pentachlorodibenzofuran (99 REPs) being the best represented.

The work of Haws *et al.* (2006) was followed by a public session of the World Health Organization (WHO), working through the International Programme on Chemical Safety (IPCS), held in Geneva in June 2005 (van den Berg *et al.*, 2006). The purpose of the meeting was to re-evaluate toxic equivalency factors (TEFs) for the 29 dioxin-like chemicals. In this assessment, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) was assigned a TEF of 1. It was decided to assign TEFs using half-order-of-magnitude increments on a logarithmic scale of 0.01, 0.03, 0.1, 0.3, etc. and these are referred to as WHO 2005 TEFs. It is important to note that van den Berg *et al.* (2006) recommended caution when applying WHO 2005 TEFs to human tissue samples, because the concept is primarily designed for intake situations.

14.4 National Health and Nutrition Examination Survey

The National Health and Nutrition Examination Survey (NHANES) is a programme of studies conducted in the USA by the Centers for Disease Control and Prevention (CDC). The NHANES has been conducted on a continuing basis since 1999. Fifteen communities are visited each year and data released every 2 years. In the 1999–2004 NHANES dioxins, furans and PCBs in human blood were measured in a one-third subsample of participants. Twenty-three of the 29 dioxin-like chemicals having WHO 2005 TEFs were included in all three data releases of interest (1999–2000, 2001–2002 and 2003–2004). The concentrations and WHO 2005 TEFs of these 23 chemicals were used to calculate toxic equivalency by Everett and Thompson (2012, 2014) and are referred to here as TEQ_{23} (Table 14.1).

Whether or not a measurement was detectable was dependent on the concentration of each dioxin-like chemical, the amount of blood available for analysis and the survey year. Organochlorine pesticides were measured along with

Table 14.1. Twenty-nine dioxins and dioxin-like compounds having toxic equivalency factors (WHO 2005 TEF) and those included in TEQ_{23} and TEQ_{8} measures of toxic equivalency. Adapted from Everett and Thompson (2014) with permission. See van den Berg *et al*. (2006) for WHO 2005 TEF.

	TEQ_{23}	TEQ_{8}	Toxic equivalency at maximum limit of detection (TEQ fg g^{-1})
Dioxins			
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD)	*		47.73
1,2,3,7,8-Pentachlorodibenzo-*p*-dioxin (PeCDD)	*		53.81
1,2,3,4,7,8- Hexachlorodibenzo-*p*-dioxin (HxCDD)			
1,2,3,6,7,8-Hexachlorodibenzo-*p*-dioxin (HxCDD)	*	*	8.86
1,2,3,7,8,9- Hexachlorodibenzo-*p*-dioxin (HxCDD)	*		8.15
1,2,3,4,6,7,8-Heptachlorodibenzo-*p*-dioxin (HpCDD)	*	*	1.57
1,2,3,4,6,7,8,9-Octachlorodibenzo-*p*-dioxin (OCDD)	*	*	0.58
Furans			
2,3,7,8-Tetrachlorodibenzofuran (TCDF)	*		5.19
1,2,3,7,8-Pentachlorodibenzofuran (PeCDF)	*		1.44
2,3,4,7,8-Pentachlorodibenzofuran (PeCDF)	*	*	15.34
1,2,3,4,7,8-Hexachlorodibenzofuran (HxCDF)	*		4.73
1,2,3,6,7,8-Hexachlorodibenzofuran (HxCDF)	*		4.93
1,2,3,7,8,9-Hexachlorodibenzofuran (HxCDF)	*		4.72
2,3,4,6,7,8-Hexachlorodibenzofuran (HxCDF)	*		5.52
1,2,3,4,6,7,8-Heptachlorodibenzofuran (HpCDF)	*		0.72
1,2,3,4,7,8,9- Heptachlorodibenzofuran (HpCDF)			
1,2,3,4,6,7,8,9-Octachlorodibenzofuran (OCDF)	*		0.050
PCBs			
3,3′,4,4′-Tetrachlorobiphenyl (PCB 77)			
3,4,4′,5-Tetrachlorobiphenyl (PCB 81)	*		0.082
3,3′,4,4′,5-Pentachlorobiphenyl (PCB 126)	*	*	8.65
3,3′,4,4′,5,5′-Hexachlorobiphenyl (PCB 169)	*	*	3.45
2,3,3′,4,4′-Pentachlorobiphenyl (PCB 105)	*		0.0014
2,3,4,4′,5-Pentachlorobiphenyl (PCB 114)			
2,3′,4,4′,5-Pentachlorobiphenyl (PCB 118)	*	*	0.0012
2′,3,4,4′,5-Pentachlorobiphenyl (PCB 123)			
2,3,3′,4,4′,5-Hexachlorobiphenyl (PCB 156)	*	*	0.0014
2,3,3′,4,4′,5′-Hexachlorobiphenyl (PCB 157)	*		0.0014
2,3′,4,4′,5,5′-Hexachlorobiphenyl (PCB 167)	*		0.0014
2,3,3′,4,4′,5,5′-Heptachlorobiphenyl (PCB 189)			

dioxins, furans and PCBs in the 1999–2000 and 2001–2002 survey years, but in a separate subsample in 2003–2004. Therefore there was more blood available for analysis in the 2003–2004 survey years. This resulted in a concentration range for a chemical where some measurements were detectable and some were not. To deal with this ambiguity the highest concentration that was not detectable was used as the limit of detection and is referred to as the maximum limit of detection (MLOD). The product of the WHO 2005 TEF multiplied by the MLOD is shown in Table 14.1. The toxic equivalency at the MLOD is high for 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD because the WHO 2005 TEF has a value of 1. Similarly, the toxic equivalency at the MLOD is low for PCB 105, PCB 118, PCB 156, PCB 157 and PCB 167 because the WHO 2005 TEF has a value of 0.00003. In between these extremes are cases where the toxic equivalencies at the MLOD, and the WHO 2005 TEF, have intermediate values. One example is PCB 126, which has a WHO 2005 TEF of 0.1 and toxic equivalency at the MLOD of 8.65. When all 23 dioxin-like

Table 14.2. Diabetes categories, number of compounds elevated and distribution of toxic equivalency (TEQ_{23}) in a representative sample of the US population, 1999–2004. Adapted from Everett and Thomson (2014), with permission.

	Unweighted *N*	Population estimate	Proportion (%)
Diabetes Category			
Normal A1c (< 5.7%)			
- Without nephropathy	1788	89,282,573	75.9
- With nephropathy	156	6,497,735	5.5
Pre-diabetes (A1c 5.7–6.4%)			
- Without nephropathy	312	11,463,344	9.7
- With nephropathy	50	1,585,421	1.3
Pre-diabetes			
- A1c 5.7–5.8%	191	6,914,475	5.9
- A1c 5.9–6.4%	171	6,134,289	5.2
Total diabetes (Diagnosed or A1c ≥ 6.5%)			
- Without nephropathy	184	6,143,212	5.2
- With nephropathy	98	2,686,073	2.3
Number of compounds elevated[a]			
0	946	47,558,488	40.4
1	335	16,809,962	14.3
2	235	11,035,270	9.4
3	163	7,372,429	6.3
4–8	519	20,513,596	17.4
9–13	317	11,828,906	10.0
≥ 14	73	2,539,706	2.2
Toxic equivalency (TEQ_{23} fg g^{-1})			
< 13.87	593	29,382,702	25.0
13.87–34.53	585	29,417,231	25.0
34.54–81.65	615	29,400,551	25.0
≥ 81.66	795	29,457,873	25.0
Total sample	2588	117,658,357	100

[a]Only 11.9% of the sample (unweighted *N* = 284) had no compounds above the maximum limit of detection. The number of compounds elevated does not include values above the maximum limit of detection, and below the 75th percentile, for the eight chemicals included in TEQ_8. For the other 15 chemicals, values above the maximum limits of detection are included.

chemicals for an individual are summed, the resulting TEQ_{23} is a function of both the concentrations and the sensitivity of the measurements as indicated by the toxic equivalency at the MLOD. When the toxic equivalency at the MLOD is high, the measurement is not particularly sensitive, but it does substantially influence the final summed TEQ_{23} result if the concentration of the chemical is above the MLOD.

Only 11.9% of the US population had none of the 23 dioxin-like chemicals above their respective MLODs and 59.6% had one or more of the compounds substantially elevated (Table 14.2). In terms of TEQ_{23}, 25% of the US population had summed TEQ_{23} < 13.87 fg g^{-1} and 25% had summed TEQ_{23} ≥ 81.66 fg g^{-1}, making the interquartile range 67.79 fg g^{-1}. The proportion of the US population having diabetes was 7.5% and the proportion having nephropathy (kidney disease) was 9.1% (Table 14.2). In Everett and Thompson (2014), diabetes was defined as diagnosed or glycohaemoglobin (A1c) ≥ 6.5%, and nephropathy defined as urinary albumin to creatinine ratio > 30 mg g^{-1} (microalbuminuria or macroalbuminuria). In addition, 11% of the US population had pre-diabetes, defined by

Everett and Thompson (2012, 2014) as A1c 5.7–6.4% (Table 14.2).

There are eight dioxin-like chemicals in human blood that are most important in terms of the proportion of persons, included in the NHANES 1999–2004, with detectable values (> 25% above the MLOD). Three are dioxins, one is a furan and four are PCBs and are referred to as TEQ_8 (Table 14.1). The relationship between the summed TEQ_{23} and the summed TEQ_8 is given by the following equation:

$$TEQ_{23} = 1.56 * TEQ_8 - 11.86 \; r^2 = 0.92 \quad \text{(Eqn 14.1)}$$

The 75th percentile of TEQ_{23} reported in Everett and Thompson (2014) was 81.66 fg g^{-1}. Using the above equation, the comparable level of TEQ_8 is 59.95 fg g^{-1}, which is 73.4% of the TEQ_{23} value (Everett and Thompson, 2016). Odds ratios for these eight dioxin-like chemicals and diabetes with or without nephropathy (kidney disease) are shown in Fig. 14.1. When considering concentrations above the 75th percentile, 12 of 16 of the relationships have odds ratios significantly greater than 1.00 (Everett and Thompson, 2014).

14.5 Pre-Diabetes

Pre-diabetes is a state that precedes diabetes. What is not clear is if a single dioxin-like chemical being elevated is most important for the relationship with pre-diabetes, or if the toxic equivalency is most important for the relationship. There is evidence to support both lines of reasoning, but only 14.3% of the US population have a single dioxin-like chemical substantially elevated and 45.3% have two or more elevated. What is meant by substantially elevated is that the concentration of one of the chemicals included in TEQ_8 is above the 75th percentile, or the concentration of one of the other 15 chemicals included in TEQ_{23} is above the MLOD (Table 14.2). When one or more of the 23 dioxin-like chemicals is substantially elevated, the odds ratio for pre-diabetes with nephropathy is 4.70 (95% CI: 1.17–18.92) and the odds ratio for pre-diabetes without nephropathy is 1.54 (95% CI: 1.04–2.26) (Everett, 2014). The proportion of the US population having pre-diabetes with nephropathy is only 1.3%, while the proportion having pre-diabetes without nephropathy is 9.7% (Table 14.2).

Considering the relationship of toxic equivalency with pre-diabetes, one has to divide the

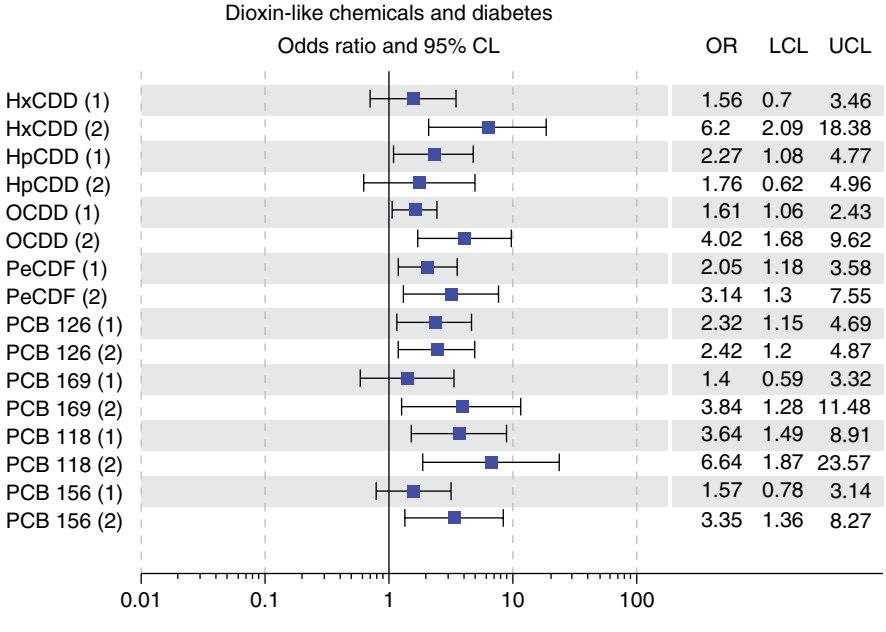

Fig. 14.1. Association of eight dioxin-like chemicals, used in TEQ_8, with (1) diabetes without nephropathy and (2) diabetic nephropathy. Adapted from Everett and Thompson (2014) with permission.

data based on glycohaemoglobin (A1c) concentration in blood (Table 14.2). Approximately half of those with pre-diabetes had A1c 5.7–5.8% (5.9% of the US population) and half had A1c 5.9–6.4% (5.2% of the US population). In Everett and Thompson (2012), the relationship of toxic equivalency with pre-diabetes was investigated using quartiles of TEQ_{23}; however, here we report relationships with continuous logarithm-transformed toxic equivalency ($\ln(TEQ_{23}+1)$). Those persons having A1c 5.7–5.8% do not have an association of logarithm-transformed TEQ_{23} with pre-diabetes (odds ratio: 1.06; 95% CI: 0.87–1.30), while those having A1c 5.9–6.4% do have an association of logarithm-transformed TEQ_{23} with pre-diabetes (odds ratio: 1.33; 95% CI: 1.03–1.72). The reference category for these logistic regressions was the group with normal A1c (< 5.7%) without nephropathy (Table 14.2).

It is possible to convert the odds ratio (OR_1) for continuous logarithm-transformed toxic equivalency to a more easily understood odds ratio (OR_2) comparing the 75th percentile of TEQ_{23} to the 25th percentile, i.e. over the interquartile range (IQR). This relationship can be expressed by the following equation:

$$OR_2 = \left((OR_1 - 1) * \ln(IQR)\right) + 1 \quad \text{(Eqn 14.2)}$$

Given OR_1 = 1.33, IQR = 67.79 fg g^{-1} and ln(IQR) = 4.216, then OR_2 = 2.39. Similarly, the 95% confidence limits can be calculated. In this example, the association of A1c 5.9–6.4% with toxic equivalency over the interquartile range has an odds ratio of 2.39 (95% CI: 1.13–4.04). While this odds ratio applies to persons 20 years of age and older (Everett and Thompson, 2014), there is no evidence of a relationship of pre-diabetes with dioxin-like chemicals before 30 years of age (Everett and Thompson, 2016).

14.6 Diabetes

There are a few published meta-analyses on the association of diabetes with dioxins, furans and PCBs (Henley *et al.*, 2012; Wu *et al.*, 2013; Tang *et al.*, 2014; Song *et al.*, 2016). A meta-analysis of four studies on occupational exposures to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and diabetes had a summary odds ratio of 1.48 (95% CI: 1.10–1.98) for a comparison of the highest category with the lowest category (Henley *et al.*, 2012). Two studies of exposure to TCDD due to an industrial accident had a summary odds ratio of 0.45 (95% CI: 0.39–0.52), indicating a lower prevalence of diabetes. Hence, repeated exposure to TCDD appears to be more important when considering associations with diabetes. A meta-analysis of six studies on dioxins (and furans) and diabetes produced a summary relative risk of 1.91 (95% CI: 1.44–2.54) for the highest category compared with the lowest category (Song *et al.*, 2016).

There are several meta-analyses on PCBs, but most are focused on non-dioxin-like PCBs, or include both dioxin-like PCBs and non-dioxin-like PCBs. Thirteen cross-sectional studies of PCBs and diabetes had a summary relative risk of 2.90 (95% CI: 2.14–3.92) and eight prospective studies produced a summary relative risk of 1.65 (95% CI: 1.16–2.34) (Song *et al.*, 2016). Tang *et al.* (2014) used ten studies (eight cross-sectional and two case-control) and also found an association of PCBs with diabetes. The summary odds ratio for the meta-analysis was 2.36 (95% CI: 1.64–3.41). Looking specifically at dioxin-like PCB 118, Wu *et al.* (2013) conducted a meta-analysis of four prospective studies that yielded a summary odds ratio of 1.20 (95% CI: 0.73–1.96). It is common in the literature on persistent organic pollutants for prospective studies to have lower odds ratios, or relative risks, than cross-sectional studies. This is likely due to the inclusion of persons having complications of diabetes in cross-sectional studies. In prospective studies, new cases of diabetes are identified and while some cases are complex, there are fewer such instances.

When considering associations between dioxin-like chemicals and diabetes, there are two opposing factors. The first is the size of the group that has diabetes, which in the case of Everett and Thompson (2014) would have been 7.5% of the US population (Table 14.2). The second factor is how well-defined subgroups of diabetes are. Looking at diabetes with and without nephropathy gives a more detailed view of the associations with dioxin-like chemicals but requires analysis of smaller groups. Therefore the logistic regressions for these subgroups may have wider confidence intervals solely due to the smaller size of the groups involved. As shown in Table 14.2,

diabetes without nephropathy was 5.2% of the US population and diabetic nephropathy was 2.3%. It is appropriate to consider both approaches to the problem.

In Everett and Thompson (2012), the fourth quartile of TEQ_{23} compared with the first quartile had an odds ratio of 3.08 (95% CI: 1.20–7.90) for diabetes, and six of the eight dioxin-like chemicals included in TEQ_8 showed associations with diabetes. The two that were not associated were 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin and PCB 126. However, these two dioxin-like chemicals had significant associations in Everett and Thompson (2014) for diabetes without nephropathy, indicating that the better-defined subgroup was more appropriate in the case of these two chemicals.

Similarly, the relationship between diabetes and continuous logarithm-transformed toxic equivalency ($\ln(TEQ_{23}+1)$) can be looked at in two ways. For diabetes and logarithm-transformed TEQ_{23}, the odds ratio is 1.60 (95% CI: 1.22–2.09); and for diabetes without nephropathy and logarithm-transformed TEQ_{23}, the odds ratio is 1.44 (95% CI: 1.11–1.87). The reference category for both of these logistic regressions was the normal A1c (< 5.7%) without nephropathy group (Table 14.2). As the diabetes without nephropathy category has a more conservative odds ratio, the association for that group is considered the best measure of the effect. Expressed in another way, the odds ratio for diabetes without nephropathy and toxic equivalency over the interquartile range is 2.86 (95% CI: 1.46–4.67).

14.7 Diabetic Nephropathy

Seven of the eight dioxin-like chemicals included in TEQ_8 are associated with diabetic nephropathy in spite of the fact that only 2.3% of the US population is involved (Everett and Thompson, 2014). Logarithm-transformed toxic equivalency is also associated with diabetic nephropathy, having an odds ratio of 2.35 (95% CI: 1.57–3.52). The odds ratio for diabetic nephropathy and toxic equivalency over the interquartile range is 6.69 (95% CI: 3.40–11.62). Given this very high odds ratio, the relationship between toxic equivalency and diabetic nephropathy appears to be a case of reverse causality. Reverse causality, in this case, is when the disease precedes the rise in the chemical concentration in blood.

However, the relationship between TEQ_{23} and diabetic nephropathy may be more complicated. High levels of dioxin-like chemicals may be both a case of reverse causality and a risk factor for diabetic nephropathy. A negative feedback loop may exist, with a rise in dioxin-like chemicals in blood causing the beginnings of diabetic nephropathy, followed by a build-up of more dioxin-like chemicals as the kidneys become less efficient at removing toxins from the blood (Everett and Thompson, 2014). Notably, dioxin-like chemicals cannot be detected in urine, which suggests another organ is responsible for metabolism of these compounds (D.O. Carpenter, New York, 2015, personal communication). Lee *et al.* (2006) found fatty liver and gamma-glutamyltransferase (GGT) to be associated with elevated levels of dioxins and furans, which suggests liver function may be involved.

14.8 Conclusions

TEFs have been defined for seven polychlorinated dibenzo-*p*-dioxins, ten polychlorinated dibenzofurans and 12 polychlorinated biphenyls (van den Berg *et al.*, 2006). Concentrations of these 29 dioxin-like chemicals can be multiplied by their respective TEFs and summed to yield a measure called toxic equivalency (TEQ). Toxic equivalency provides a way to quantify the overall toxicity of this class of chemicals. The NHANES 1999–2004, conducted in the USA, has been used to assess toxic equivalency in human blood. Two summary measures have been defined. The first uses 23 of the 29 dioxin-like chemicals and is referred to as TEQ_{23}. The second measure, TEQ_8, uses the eight dioxin-like chemicals that have the highest proportion of persons with detectable levels of the compounds. TEQ_8 includes three dioxins, one furan and four PCBs.

There are significant associations of toxic equivalency with pre-diabetes, diabetes and diabetic nephropathy in the NHANES 1999–2004. Expressed as a continuous variable, logarithm-transformed toxic equivalency ($\ln(TEQ_{23}+1)$) was associated with 'high' pre-diabetes (in the range of A1c 5.9–6.4%) with an odds ratio of 1.33 (95% CI: 1.03–1.72). Logarithm-transformed TEQ_{23} was

also associated with diabetes (odds ratio: 1.60; 95% CI: 1.22–2.09), diabetes without nephropathy (odds ratio 1.44; 95% CI 1.11–1.87) and diabetic nephropathy (odds ratio: 2.35; 95% CI: 1.57–3.52). In cross-sectional studies it is more appropriate to exclude persons having diabetic nephropathy when considering the association with diabetes. Therefore, the best estimate of the effect size is the odds ratio for diabetes without nephropathy. The association of logarithm-transformed TEQ_{23} with diabetic nephropathy appears to be a case of reverse causality, or perhaps both due to reserve causality and a risk factor for the disease. The odds ratio of 2.86 (95% CI: 1.46–4.67) for diabetes without nephropathy and toxic equivalency over the interquartile range can be used for comparisons to other studies on dioxin-like chemicals and diabetes.

References

CDC (2005) *Third National Report on Human Exposure to Environmental Chemicals*. National Center for Environmental Health Pub. No. 05-0570. Centers for Disease Control and Prevention, Atlanta, Georgia.

Everett, C.J. (2014) Commentary on nephropathy and longitudinal studies of diabetes and dioxins, furans, and dioxin-like PCBs. *Environmental Research* 134, 8.

Everett, C.J. and Thompson, O.M. (2012) Associations of dioxins, furans and dioxin-like PCBs with diabetes and pre-diabetes: is the toxic equivalency approach useful? *Environmental Research* 118, 107–111.

Everett, C.J. and Thompson, O.M. (2014) Dioxins, furans and dioxin-like PCBs in human blood: causes or consequences of diabetic nephropathy? *Environmental Research* 132, 126–131.

Everett, C.J. and Thompson, O.M. (2016) Association of dioxins, furans and dioxin-like PCBs in human blood with nephropathy among US teens and young adults. *Reviews on Environmental Health* 31, 195–201.

Haws, L.C., Su, S.H., Harris, M., DeVito, M.J., Walker, N.J. *et al*. (2006) Development of a refined database of mammalian relative potency estimates for dioxin-like compounds. *Toxicological Sciences* 89, 4–30.

Henley, P., Hill, J., Moretti, M.E., Jahedmotlagh, Z., Schoeman, K., Koren, G. and Bend, J.R. (2012) Relationships between exposure to polyhalogenated aromatic hydrocarbons and organochlorine pesticides and the risk for developing type 2 diabetes: a systematic review and a meta-analysis of exposures to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). *Toxicology and Environmental Chemistry* 94, 814–845.

Lee, C.C., Yao, Y.J., Chen, H.L., Guo, Y.L. and Su, H.J. (2006) Fatty liver and hepatic function for residents with markedly high serum PCDD/Fs levels in Taiwan. *Journal of Toxicology and Environmental Health, Part A* 69, 367–380.

Mainous, A.G. 3rd, Baker, R., Koopman, R.J., Saxena, S., Diaz, V.A., Everett, C.J. and Majeed, A. (2007) Impact of population at risk of diabetes on projections of diabetes burden in the United States: an epidemic on the way. *Diabetologia* 50, 934–940.

Menke, A., Casagrande, S., Geiss, L. and Cowie, C.C. (2015) Prevalence of and trends in diabetes among adults in the United States, 1988–2012. *Journal of the American Medical Association* 314, 1021–1029.

Remillard, R.B. and Bunce, N.J. (2002) Linking dioxins to diabetes: epidemiology and biologic plausibility. *Environmental Health Perspectives* 110, 853–858.

Schmidt, M.I., Duncan, B.B., Bang, H., Pankow, J.S., Ballantyne, C.M. *et al*. (2005) Identifying individuals at high risk for diabetes. *Diabetes Care* 28, 2013–2018.

Song, Y., Chou, E.L., Baecker, A., You, N.C.Y., Song, Y., Sun, Q. and Liu, S. (2016) Endocrine-disrupting chemicals, risk of type 2 diabetes, and diabetes-related metabolic traits: a systematic review and meta-analysis. *Journal of Diabetes* 8, 516–532.

Tang, M., Chen, K., Yang, F. and Liu, W. (2014) Exposure to organochlorine pollutants and type 2 diabetes: a systematic review and meta-analysis. *PLoS ONE* 9, 10.

van den Berg, M., Birnbaum, L.S., Denison, M., De Vito, M., Farland, W. *et al*. (2006) The 2005 World Health Organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. *Toxicological Sciences* 93, 223–241.

Wu, H., Bertrand, K.A., Choi, A.L., Hu, F.B., Laden, F., Grandjean, P. and Sun, Q. (2013) Persistent organic pollutants and type 2 diabetes: a prospective analysis in the nurses' health study and meta-analysis. *Environmental Health Perspectives* 121, 153–161.