Bull. Environ. Contam. Toxicol. (1988) 41:63–70

Bulletin of Environmental Contamination and Toxicology

Polychlorinated Biphenyls and Polychlorinated Dibenzofurans in the Tissues of Patients with Yusho or Yu-Chen: Total Toxicity

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There is increasing concern over the potentially adverse health effects associated with human exposure to polychlorinated biphenyls (PCBs), polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzo-p-dioxins (PCDDs), in view of the high toxicity associated with certain of their congeners and isomers. The most serious incident of PCB and PCDF poisoning in humans, referred to as Yusho, occurred in Western Japan in 1968 and involved the ingestion of rice oil contaminated with these compounds (Nagayama, 1976). A second similar mass food poisoning, Yu-Chen, took place in central Taiwan in 1979. Since the occurrence of these events, continued attention has been given to the disposition of these toxic substances, in the tissues of the victims, with the passage of time.

High resolution capillary gas chromatographic (GC) analysis has provided an accurate quantitative assessment of the individual toxic congeners and isomers. However, it is presently impossible to assess, directly from the GC data, the total toxicity resulting from a mixture of these toxic substances in a particular tissue. It is the objective of this study to provide a method for determining the relative toxicity of each of these congeners and isomers in terms of an equivalent toxic concentration of TCDD so that the overall toxicity of the mixture may be evaluated. The applicability of this approach will then be demonstrated by the direct conversion of available Yusho and Yu-Chen GC data to total equivalent toxicities of TCDD.

Kuroki and Masuda (1978) analyzed the adipose tissue and liver of deceased Yusho patients for PCDFs by gas chromatography with electron capture detection following separation of the PCBs and PCDFs by column chromatography on alumina. These fractions were subsequently examined by GC and mass spectrometry utilizing more efficient columns for separating the PCDF congeners (Rappe 1979) to yield the concentration of the toxic congener 2,3,7,8-tetrachlorodibenzofuran, the two isomeric 1,2,4,7,8- and 2,3,4,7,8- pentachlorodibenzofurans as well as a mixture of the 1,2,3,4,7,8- and 1,2,3,6,7,8- hexachlorodibenzofurans. These

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workers also carried out a congeneric analysis of the same tissues from a baby who was a victim of 'Yusho'.

Chen and Hites (1983) determined the concentrations of PCB and PCDF congeners in the tissues of a deceased patient with Yu-Chen in Taiwan. These congeneric fractions were obtained with a HP-5730A GC equipped with an electron capture detector and utilizing DB-5 silica columns.

RESULTS AND DISCUSSION

Animal studies carried out with PCBs, PCDFs, and PCDDs have clearly established that the relative toxicities of congeners and isomers of these polychloroaromatics are definitely structure dependent (Poland and Knutsen 1982; Parkinson and Safe 1981; Safe 1982) and that the most toxic polychloroaromatic compound is 2,3,7,8- tetrachlorodibenzo-p-dioxin (TCDD). Molecular orbital calculations (Miller 1977) have established that TCDD can act as an electron acceptor and that halogenation in the 2,3,7, or 8 positions results in considerable structural stabilization of the added charge. Furthermore these calculations also indicate that the greater the degree of halogenation in these positions, the stronger the electron transfer complex formed with a specific electron donor such as the cytosolic protein in the liver. The resulting complex of protein and ligand translocates to the cell nucleus where the ligand, in some unknown manner, initiates transcription and translation of the gene(s) which code(s) for P-448 (aryl hydrocarbon hydroxylase (AHH) activity), the enzyme which participates in the metabolism of many lipophilic compounds to more polar metabolies, which may be eliminated from the system (Poland 1979; Okey 1979).

Specific structural requirements for PCB congener induction of P-448 have been investigated (Parkinson 1980; Poland and Glover 1977). Those congeners which induce P-448 as measured by the induction of AHH, must be able to assume a planar conformation and be substituted in both para and at least two meta positions of the biphenyl ring systems (Andres 1983; Poland 1980; Safe 1982). These compounds are isostereomers of TCDD. One or two additional chlorine substituents tend to reduce but not eliminate the AHH inducing activity.

In an effort to establish a direct relationship between AHH activity and toxic responses, Safe and coworkers (Mason 1985; Sawyer 1982; Sawyer 1985) examined the in vivo quantitative structure activity relationships (QSARs) of several individual toxic congeners and isomers present in PCDFs. An excellent linear correlation was established between - log EC_{50} (EC_{50} being the effective concentration required to produce 50% of the

maximum value) for in vitro induction of AHH activity in rat hepatoma H-4-II E cells and the corresponding - log ED_{50} values for several mediated toxic responses such as thymic atrophy and body weight loss in immature Wistar rats (Safe 1987). The existence of such a direct correlation between PCDF congener structure, toxicity and AHH activity has permitted the in vitro enzyme induction data to be used in the present study to quantitatively estimate the relative toxicity of PCB and PCDF congeners. The in vitro induction potencies of several of these congeners have been determined by Safe (1987) and are listed in Table 1. In view of the reciprocal relationship between the concentration of a congener necessary to induce AHH activity (the AHH induction value) and toxicity, it is possible to calculate the toxic equivalent factors (Table 1) required to convert the concentrations of toxic PDCF and PCB congener, obtained via high resolution gas chromatography, into equivalent toxic concentrations of TCDD

$$\text{T.E.F}_{\text{Toxic Congener}} = \frac{\text{AHH induction Value}_{\text{TCDD}}}{\text{AHH induction Value}_{\text{Toxic Congeners}}}$$

Table 1. TEFs for converting GC concentrations of PDCFs into equivalent toxic conc. of TCDD.

PCDF Congener	AHH Induction Value	Toxic Equivalent Factor (TEF)
2,3,4,7,8-Pentachloro-	2.56×10^{-10}	2.83×10^{-1}
1,2,3,4,7,8-Hexachloro-	3.56×10^{-10}	2.03×10^{-1}
1,2,3,7,8-Pentachloro-	2.54×10^{-9}	2.85×10^{-2}
2,3,4,6,7,8-Hexachloro-	6.87×10^{-10}	1.05×10^{-1}
1,2,3,6,7,8-Hexachloro-	1.47×10^{-9}	3.67×10^{-2}
2,3,7,8-Tetrachloro-	3.91×10^{-9}	1.85×10^{-2}
1,3,4,7,8-Pentachloro-	1.60×10^{-9}	4.52×10^{-2}
2,3,4,7,9-Pentachloro-	7.90×10^{-9}	0.915×10^{-2}
2,3,4,7-Tetrachloro-	1.79×10^{-8}	4.04×10^{-3}
1,2,3,7,9-Pentachloro-	8.60×10^{-8}	0.841×10^{-3}
1,2,4,7,8-Pentachloro-	1.06×10^{-7}	6.82×10^{-4}
1,2,3,7-Tetrachloro-	2.70×10^{-5}	2.68×10^{-6}
2,3,4,8-Tetrachloro-	4.14×10^{-8}	1.75×10^{-3}
1,2,4,6,7-Pentachloro-	1.00×10^{-5}	7.23×10^{-6}
1,2,3,6-Tetrachloro-	$>10^{-4}$	$<10^{-7}$
2,3,7,8-TCDD	7.23×10^{-11}	

The structures of PCDF congeners which favor induction of AHH and exhibit toxic properties are also the structures which tend to resist metabolism. The major pathway for metabolism of PCDF congeners in mammals has been well established as hydroxylation of aromatic systems via involvement of arene oxide formation

(Sundstrom 1979). Halogenation of the dibenzofuran molecule can, depending on the degree and position of substitution, cause marked and varied effects on its metabolism and disposition. Resistance to oxidative metabolism is generally favored by higher levels of chlorine substitution and the absence of adjacent unsubstituted carbon atoms, especially those meta and para to the bridge positions between the aromatic rings and the furan nucleus (Matthews 1980). These are the dibenzofuran congeners found in the tissues of deceased victims of Yusho.

Kuroki (1978) and Rappe (1979) determined the concentrations of PCDF congeners in the liver and adipose tissues of Yusho patients at the time of death. The toxic congeners present included, 2,3,7,8- tetrachlorodibenzofuran, the 1,2,4,7,8- and 2,3,4,7,8-pentachloro dibenzofurans as well as a mixture of 1,2,3,4,7,8- and 1,2,3,6,7,8- hexachlorodibenzofurans. To permit inclusion of the latter isomers, in the calculation of total toxicity, the concentrations of each isomer in the mixture was determined in terms of the relative peak heights obtained from the published chromatogram (Masuda, 1983). Utilizing the appropriate TEFs, the concentrations of the toxic TCDFS were converted into toxic equivalents of TCDD which were then added to yield the total toxic equivalent of TCDD (Table 2).

Table 2. Concentration of toxic PCDF congeners in liver (L) and adipose (A) tissues of Yusho patients at the time of death: Expressed as toxic equivalents of TCDD (ppb x 10^{-2}).

Conc. PCDF Congeners in Toxic Equiv TCDD (ppb x 10^{-2})

	2,3,7,8-	1,2,4,7,8-	2,3,4,7,8-	1,2,3,4,7,8-	1,2,3,6,7
TEF	1.85×10^{-2}	6.82×10^{-4}	2.83×10^{-1}	2.03×10^{-1}	3.67×10^{-2}
July '69					
(L)	0.555	0.484	195	38.4	237
July '69					
(L)	0.037	0.0273	34.0	4.43	38.8
(A)	0.550	0.068	161	25.2	183
May '72					
(L)	0.0185	0.661	8.49	0.442	8.99
(A)	ND	0.0136	22.6	2.95	26.4
April '75					
(L)	ND	0.0546	2.83	7.38	10.8
March '77					
(L)	ND	ND	2.83	0.590	3.47
(A)	ND	0.0136	14.1	0.0738	14.2

Table 2 Con't

Sept. 77					
(L)	ND	ND	0.141	0.0738	0.229
(A)	ND	ND	0.141	0.0738	0.229

where ND = <0.00925

Total Toxic Equivalents of TCDD (ppb x 10^{-2})

July '69	(L)	237	(A)	-
July '69	(L)	38.8	(A)	188
May '72	(L)	8.99	(A)	26.4
April '75		-	(A)	10.8
Mar '77	(L)	3.47	(A)	14.2
Sept '77	(L)	0.229	(A)	0.229

These data show the dominant contribution of 2,3,4,7,8-pentachlorodibenzofuran to the overall toxicity, the inverse relationship between total toxicity vs life expectancy following exposure to PCDFs and the distinct preference of toxic congeners to reside in the adipose tissue rather than in the liver. In contrast to the latter preference, toxic PCDF congeners favored residence in the liver of the Yu-Chen infant (Table 3).

Table 3 Concentration of PCDF Congeners in Liver (L) and Adipose (A) Tissues of a Yu-Chen Infant: Expressed in Toxic Equivalents of TCDD (TE TCDD)

	Conc. PCDF Congeners in Toxic Equiv. TCDD (ppt 10^{-1})				
	2,3,7,8-	1,2,4,7,8-	1,2,3,7,8-	2,3,4,7,8	1,2,3,4,7,8-
TEFS	1.85×10^{-2}	6.82×10^{-4}	2.85×10^{-2}	2.83×10^{-1}	2.83×10^{-1}
(A)	3.14×10^{-1}	0.0955×10^{-1}	12.5×10^{-1}	192×10^{-1}	178×10^{-1}
(L)	11.1×10^{-1}	0.286×10^{-1}	55.3×10^{-1}	258×10^{-1}	392×10^{-1}

Total Toxic Equivalents of TCDD in Adipose Tissue = 38.6 ppt.
Total Toxic Equivalents of TCDD in Liver = 71.7 ppt.

Chen and Hites (1983) determined the concentrations of polychlorinated biphenyls and dibenzofurans retained in the tissues of a deceased Yu-Chen patient. The TEFs listed in Table 4 were used to convert the concentrations of the toxic TCDF congeners to equivalent concentrations of TCDD. The TEF necessary for making a similar conversion of the toxic PCB

congener, 3,4,2',3',4',5'-hexachlorobiphenyl, had previously been determined (Olafsson 1987).

Table 4 The Concentrations of Toxic PCB and PCDF Congeners in the Tissues of a Deceased Patient with Yu-Chen in Taiwan:Expressed in Total Toxic equivalents (TE) of TCDD (ppb)

Tissue	PCB Congener conc. in TE. TCDD. ppb x 10^{-1}	PCDF Congener Conc. in T.E. TCDD. 1,2,4,7,8- ppb x 10^{-4}	2,3,4,7,8- ppb x 10^{-1}	1,2,3,4,7,8- ppb x 10^{-1}
Liver	0.135	23.2	17.8	51.6
Intestinal Fat	2.12	6.14	11.3	15.8
Bronchus	1.16	2.73	5.09	6.50
Large Intestine	0.635	2.05	3.40	4.67
Heart	0.513	1.36	2.26	2.84
Stomach	0.122	0.341	0.65	0.812
Small Intestine	0.054	0.341	0.594	0.690
Kidney	0.054	0.273	0.509	0.650
Lung	0.041	0.0682	0.170	0.305
Brain	0.135	0.0682	0.170	0.305
Spleen	0.135	0.0682	0.226	0.203

Total Toxic Equivalents TCDD (ppb)

Liver	6.95	Small Intestine	0.134
Intestinal Fat	2.92	Kidney	0.121
Bronchus	1.28	Lung	0.0616
Large Intestine	0.871	Brain	0.0610
Heart	0.561	Spleen	0.0564
Stomach	0.159		

These data reveal that the toxic potency of the PCDF resides in the liver whereas that of the PCBs is dominant in the intestinal fat with disposition in the bronchus, large intestine and heart also being preferred over the liver. It is also apparent that 3,4,2',3',4',5'-hexachlorobiphenyl contributes almost a thousand times greater contribution to the overall toxicity than does 1,2,4,7,8-pentachlorodipbenzofuran.

Due to the wide variation of toxicity residing in a limited number of PCB and PCDF congeners and isomers, it is evident that risk assessment should be based on the concentrations of these compounds, weighted in terms of relative toxicity. The relationship between structure and toxicity has been established so that the toxic components can be identified. The high resolution gas chromatographic systems necessary to accomplish their separation and quantitation are available. A method has

now been provided to assess the total toxicity of a sample by converting the concentration of each toxic PCB congener and isomer to a toxic equivalent concentration of TCDD so as to permit summation. In the present study this approach has been extended to permit computation of the total toxicity in matrices containing PCBs and PCDFs. Furthermore, toxicities obtained in this manner are directly comparable with similar data obtained via in vitro AHH induction assays. This program of research, centering on risk assessment via congener specific analysis involving high resolution capillary gas chromatography, is presently being extended to include mixtures containing PCDDs as well as PCBs and PCDFs.

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Received December 17, 1987; accepted January 9, 1988.