Pergamon

Chemosphere, Vol. 28, No. 6, pp. 1049-1067, 1994
Elsevier Science Ltd
Printed in Great Britain
0045-6535/94 $6.00+0.00

0045-6535(94)E0070-A

TOXIC EQUIVALENCY FACTORS FOR DIOXIN-LIKE PCBs

Report on a WHO-ECEH and IPCS consultation, December 1993

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(Received in Germany 10 February 1994; accepted 16 February 1994)

ABSTRACT

The WHO-European Centre for Environment and Health (WHO-ECEH) and the International Programme on Chemical Safety (IPCS), have initiated a project to create a data base containing information relevant to the setting of Toxic Equivalency Factors (TEFs), and, based on the available information, to assess the relative potencies and to derive consensus TEFs for PCDDs, PCDFs and dioxin-like PCBs. Available data on the relative toxicities of dioxin-like PCBs with respect to a number of endpoints were collected and analyzed. A consultation was held at the WHO-European Centre for Environment and Health in Bilthoven, the Netherlands, at which the available data were discussed to derive TEFs for dioxin-like PCBs. TEFs were recommended for 3 non-*ortho*-, 8 mono-*ortho*- and 2 di-*ortho*-substituted PCBs. The consultation recommended that the project should be extended to include PCDDs and PCDFs and other dioxin-like halogenated environmental pollutants. It was also recommended that the possibilities of separate TEFs for body burdens and ecotoxicology should be explored.

INTRODUCTION

Polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs), as well as other related halogenated aromatic compounds, constitute a group of lipophilic, chemically stable environmental contaminants with low volatility which have been identified in fatty tissues of animals and humans. Several PCDDs and PCDFs, as well as a few (dioxin-like) PCBs have been shown to exert a number of common toxic responses similar to those observed for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). These include dermal toxicity, immunotoxicity, reproductive deficits, teratogenicity, endocrine toxicity and carcinogenicity/tumour promotion. There is strong evidence suggesting a common mechanism of action of 2,3,7,8-TCDD and related compounds, based on a binding of these compounds to a specific receptor (the Ah-receptor).

Due to the fact that dioxin-like compounds normally exist in environmental and biological samples as complex mixtures of congeners, the concept of toxic equivalents (TEQs) has been introduced to simplify risk assessment and regulatory control. In applying this concept, relative toxicities of dioxin-like compounds in relation to 2,3,7,8-TCDD (i.e. toxic equivalency factors, TEFs) are determined based on *in vitro* and *in vivo* studies. This approach is based on the evidence that there is a common, receptor-mediated mechanism of action for these compounds, but it has its limitations due to a number of simplifications. The most important limitation is that the combined toxic effects of the components of a given mixture would be additive, neglecting possible synergism or antagonism. Furthermore, pharmacokinetics is not always taken into account.

A number of different TEF-schemes have been developed for PCDDs and PCDFs (Switzerland 1982, Denmark 1984, FRG 1985, Ontario 1985, USEPA 1987, 1989, Ahlborg *et al.* (Nordic) 1988, CCMS/NATO (I-TEFs) 1988 and van Zorge *et al.* (Dutch) 1989), as well as for dioxin-like PCBs (Safe 1990, 1994, Ahlborg *et al.* 1992a). Recognizing the necessity for a more consistent approach towards setting internationally agreed TEFs, the WHO-European Centre for Environment and Health (WHO-ECEH) and the International Programme on Chemical Safety (IPCS), initiated a project to create a data base containing information relevant to the setting of TEFs, and, based on the available information, to assess the relative potencies and to derive consensus TEFs for PCDDs, PCDFs and dioxin-like PCBs.

In an initial stage, available data on the relative toxicities of dioxin-like PCBs with respect to a number of endpoints were collected and analyzed by Professor Ulf G. Ahlborg and his collaborators at the Karolinska Institute in Stockholm, Sweden. Following this data collection exercise, a consultation was held at the WHO-European Centre for Environment and Health in Bilthoven, the Netherlands, at which the available data were discussed. The ultimate goal of the consultation was to analyze the data base in order to define general criteria for further development of a more comprehensive TEF approach and to derive TEFs for dioxin-like PCBs. The consultation was attended by 12 experts from 8 countries, two observers, and WHO staff. Professor Ulf G. Ahlborg was selected chairman, and Dr Linda S. Birnbaum rapporteur.

Data base description

From about 1200 articles on PCBs, 146 were selected which were considered useful for developing a data base for determining TEFs. These articles were analyzed and the data included in the final data base were selected using the following criteria:

1. At least one PCB congener studied
2. TCDD or a PCB-reference (PCB 77, 126 or 169) also studied in the same experiment
   or
   TCDD or a PCB-reference (PCB 77, 126 or 169) studied with the same experimental design and by the same authors in another experiment
3. Endpoints were affected by TCDD or the PCB-reference (PCB 77, 126 or 169)

Criteria No 3 was included so that only dioxin-specific endpoints would be included in the data base. However, it is recognized that some endpoints, such as liver weight increase and tumour promotion are affected by both dioxin-like and nondioxin-like halogenated aryl hydrocarbons.

This process resulted in the selection of 57 articles/manuscripts, and three more were included at the consultation (see: References included in the data base). Of these articles, 12 concerned *in vitro* and 48 *in vivo* studies.

Calculation of TEFs from the reported data

The following methods were used to calculate TEFs.

1. TEFs were used as reported in the article. If experimental data were also reported, these were used to calculate the TEFs, using one of the methods below.
2. TEFs were calculated from dose-response curves using linear interpolation of log-doses, comparing the same effect level. If necessary, corrections were made for different control levels.
3. TEFs were determined from ratios of ED_{50}-, LD_{50}-, EC_{50}-, ED_{25}- or ED_{12}-values or ratios of no or lowest observed effect levels (NOEL, LOEL) or minimum detectable concentration values.
4. TEFs were also calculated from ratios of tumour promotion indexes (as described by Dragan and Pitot 1992), maximal induction levels (mainly CYP1A1-related), ≥80% effect levels, K_d-values for Ah-receptor binding or directly estimated from graphs.

Methods 2 and 3 were most frequently used for determining TEFs.

The main problems encountered with the reported data for calculating TEFs were the following:

1. In several studies (particularly concerning enzyme induction), different compounds caused different maximal effect levels. When using ED_{50}-values to calculate TEFs, this resulted in different effect levels being compared.
2. Only one dose level was reported (if this value was not in between the dose-response curve of the reference compound, this resulted in < or >-values as TEFs).
3. Data were presented only in graphical form.
4. Only a few, high dose levels were studied (these may have reached maximal effect).
5. There was a confusion with the dose units (e.g. 0.14 mmol and 140 nmol reported as the same dose!).
6. Congeners were not correctly identified (e.g. PCB 156 was reported to be 2,3,3',4,4',5'-hexachlorobiphenyl, which is PCB 157)

All the data were compiled into a computerized spreadsheet format. The program used was Quattro Pro® (ver. 5.0) for Windows (Borland). The spreadsheet format was selected because it is easy to use and is very flexible. The present data base consists of more than 900 entries and the number of variables (e.g. congener, effect, doses used, estimated TEF, etc) is about 50. The size of the present file is about 800 kb.
The data base can easily be translated from Quattro Pro® for Windows to other spreadsheet formats (123®, Excel®, Visicalc®), to data base-formats (Reflex®, Paradox®, DBASEII-IV®) or tab-delimited text-formats.

The contents of the data base are illustrated in Figures 1-4. From these figures it is clearly apparent that the available information is quite uneven among different congeners. For some congeners the datapoints are very limited.

In Figure 1, all *in vivo* and *in vitro* entries for individual PCB congeners are shown, where it was possible to calculate TEFs relative to TCDD. However, for several entries in the data base, comparisons were made to PCB 126 or PCBs 77/169. Note the wide range of TEF-values reported for most individual PCB congeners. The number of articles (A) and entries (B) in the data base for which TEFs could be set relative to TCDD, PCB 126 or PCBs 77/169 are shown in Figure 2. Note the differences in the number of entries per article for different PCB congeners. In Figure 3, entries for individual PCB congeners are separated on the basis of acute, subacute and subchronic/chronic exposure. There are apparent differences between acute and subchronic/chronic exposures for some congeners, e.g. PCB 77 and 105, due to their relatively rapid metabolism and/or elimination. In Figure 4, the number of entries are sorted by endpoint. It is obvious that the majority of the entries are derived from CYP1A1-related enzyme induction studies and body and organ weight effects.

Figure 1

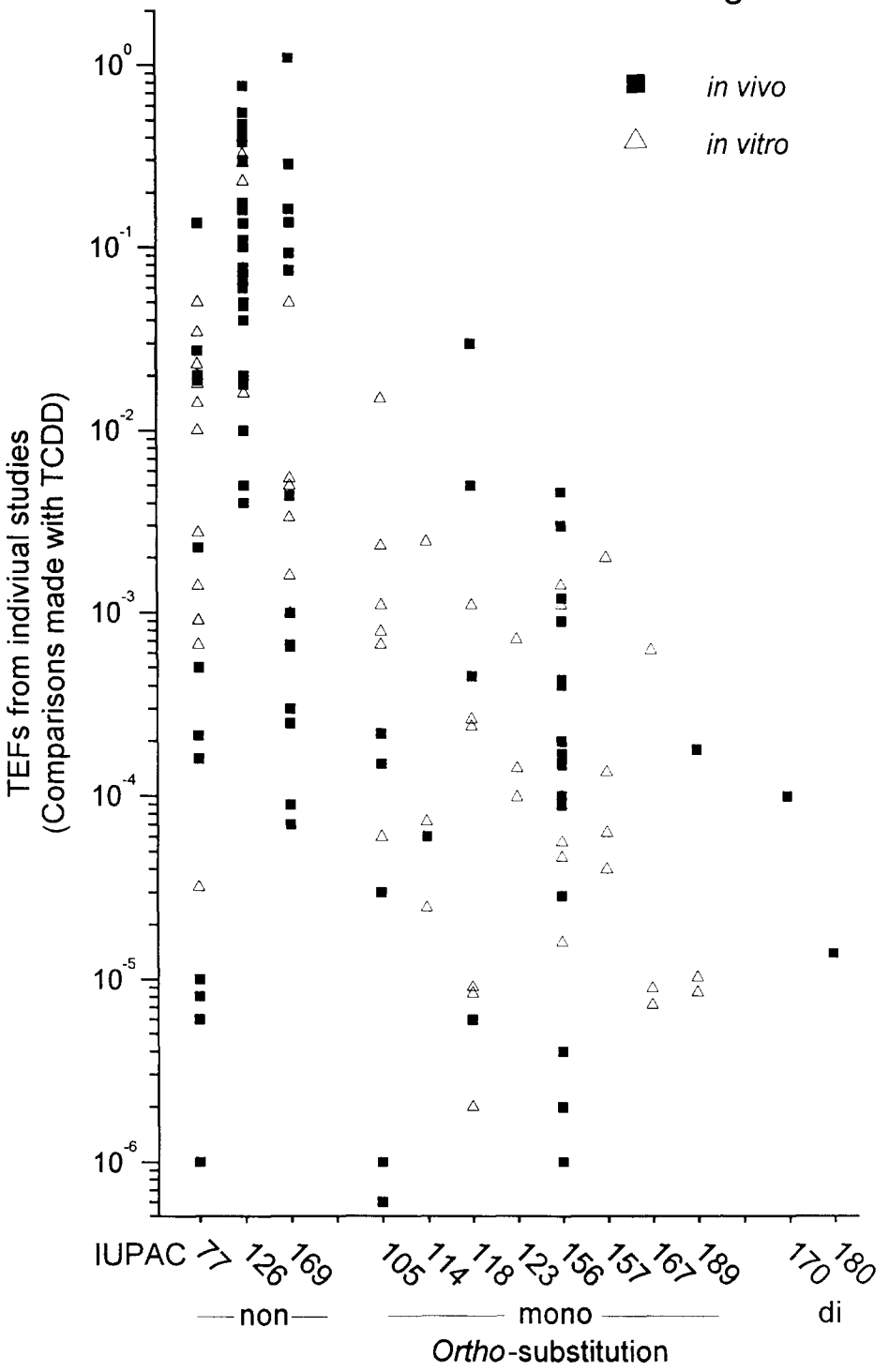

in vivo
in vitro
TEFs from indiviual studies
(Comparisons made with TCDD)
10^{0}
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
10^{-6}
IUPAC 77 126 169 105 114 118 123 156 157 167 189 170 180
non
mono
di
Ortho-substitution

Figure 2

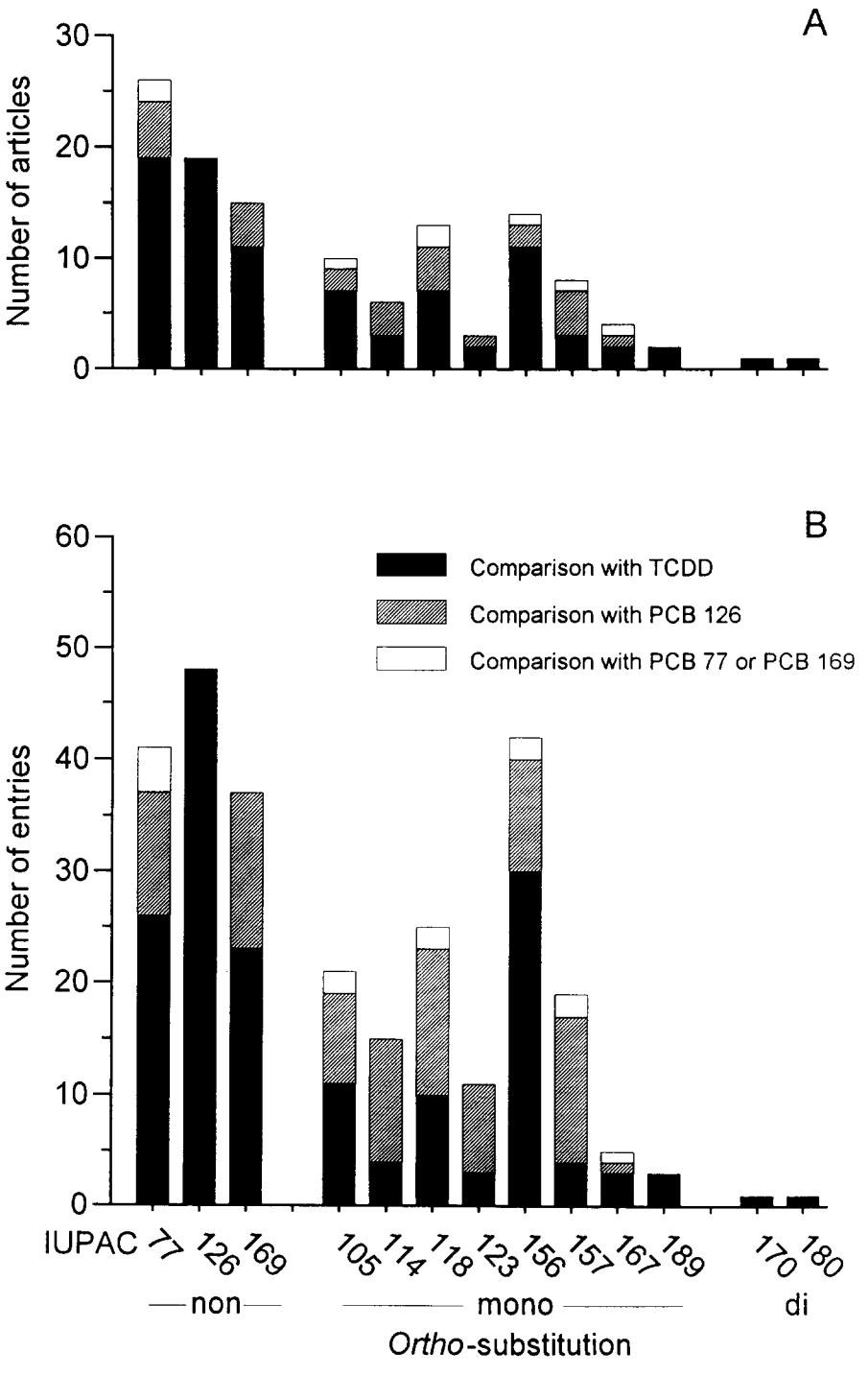

A
Number of articles
30
20
10
0
B
Number of entries
60
50
40
30
20
10
0
Comparison with TCDD
Comparison with PCB 126
Comparison with PCB 77 or PCB 169
IUPAC 77 126 169 105 114 118 123 156 157 167 189 170 180
non
mono
di
Ortho-substitution

Figure 3

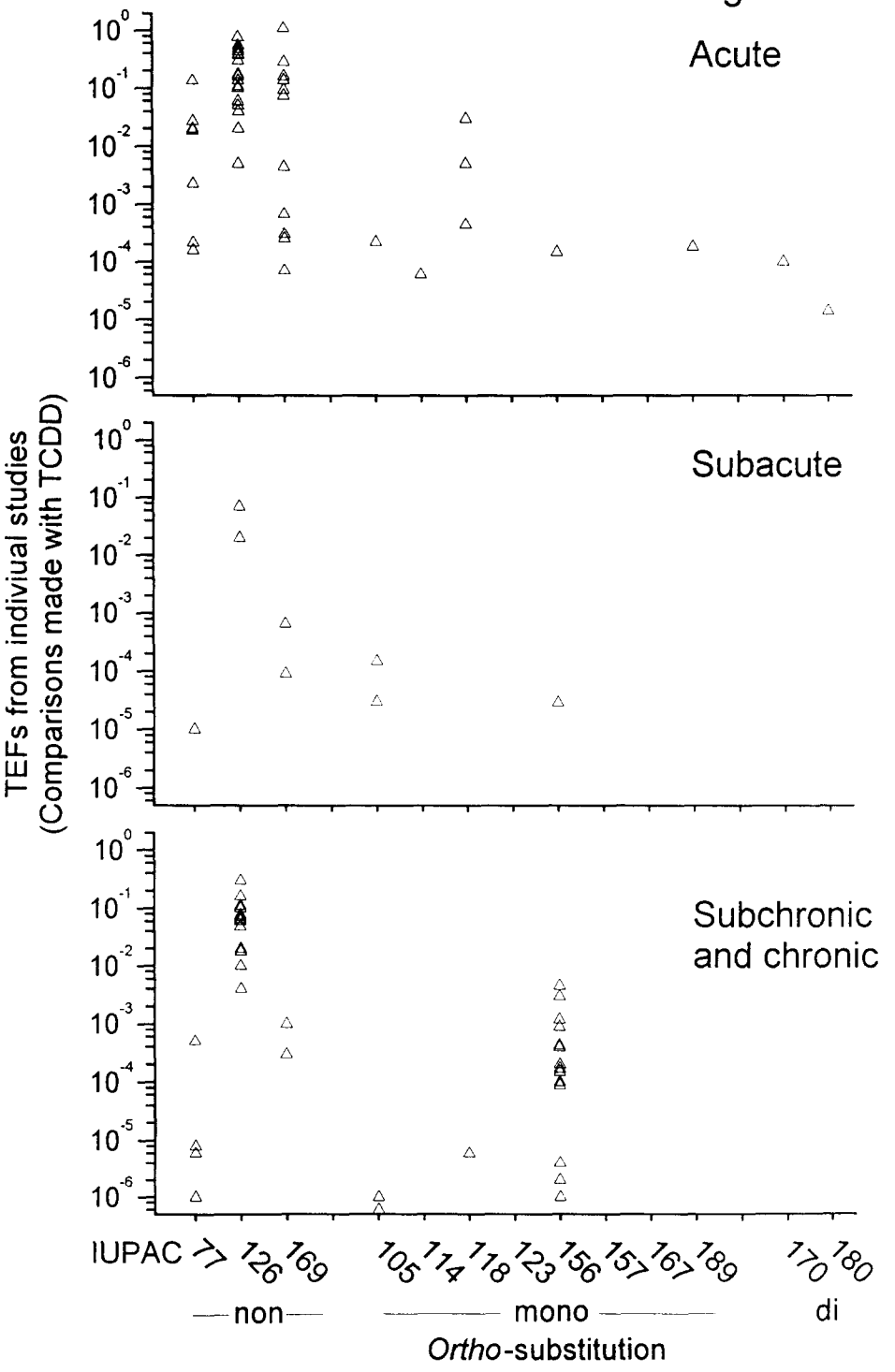
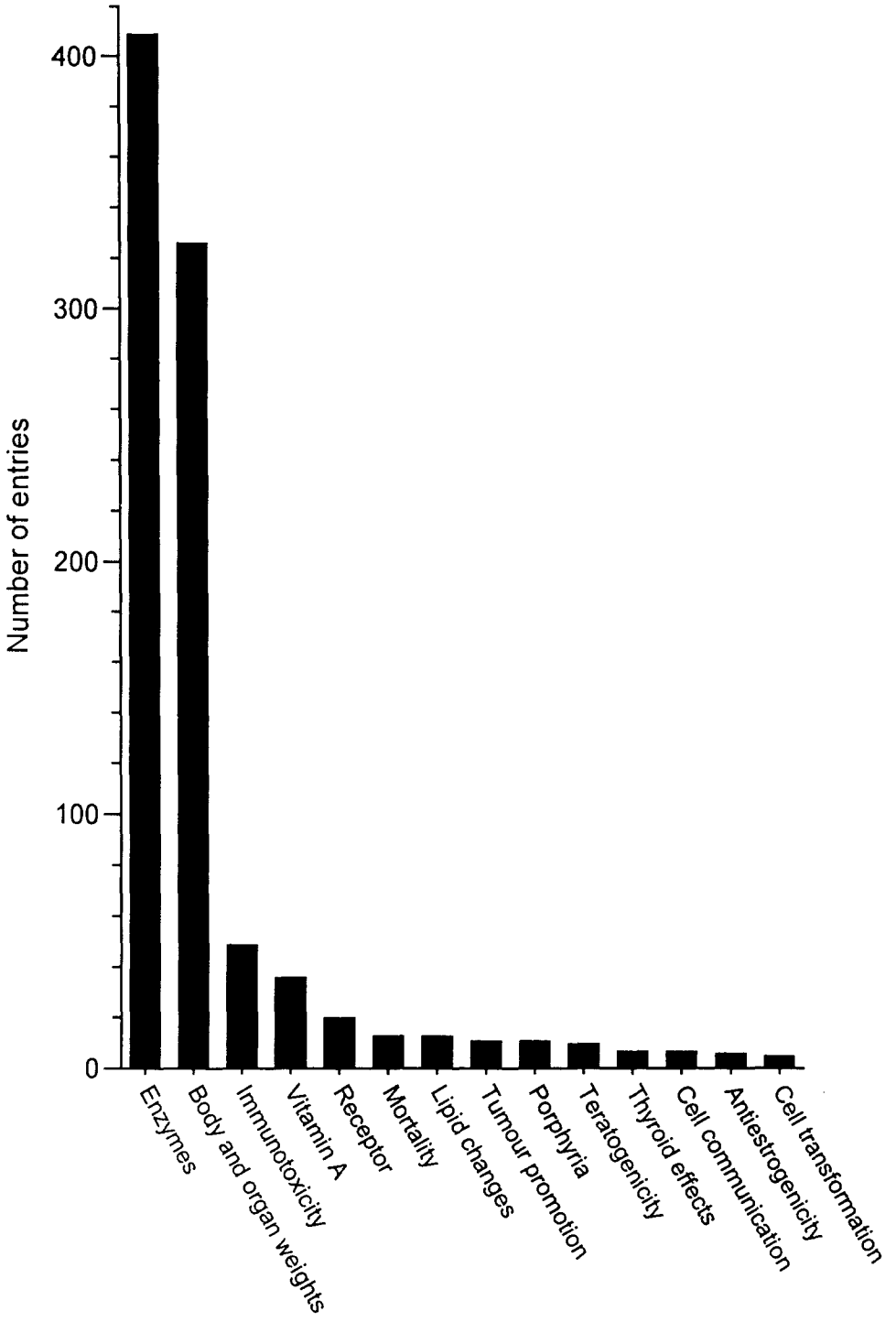

Acute
Subacute
Subchronic and chronic
TEFs from indiviual studies (Comparisons made with TCDD)
10^{0}
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
10^{-6}
IUPAC 77 126 169 105 114 118 123 156 157 167 189 170 180
non
mono
di
Ortho-substitution

Figure 4


Number of entries
400
300
200
100
0
Enzymes
Body and organ weights
Immunotoxicity
Vitamin A
Receptor
Mortality
Lipid changes
Tumour promotion
Porphyria
Teratogenicity
Thyroid effects
Cell communication
Antiestrogenicity
Cell transformation

CONCLUSIONS

On the feasibility of setting TEFs

The consultation recognized the importance of developing TEFs by an international harmonized procedure with the support of international scientific bodies such as WHO and IPCS. These TEFs could then be used worldwide for regulatory and risk management purposes. It was concluded that the present data base could be used to set interim TEFs for selected PCBs. It was noted that when international TEFs (I-TEFs) were established for PCDDs and PCDFs, there was less data than currently available for the dioxin-like PCBs. It was also recognized that there are some important limitations to the concept of TEFs and that the TEF-values should be developed and used with care. Determination of individual TEF-values requires expert scientific judgement involving the review of all the existing data.

The concept of TEFs assumes strict additivity. There is no evidence for nonadditivity for Ah-receptor agonists. There appears to be a potential for nonadditivity only in very weak agonists or non-Ah-receptor agonists.

On criteria for inclusion

The criteria for including a compound in a TEF-scheme has previously been discussed (Safe 1990, Barnes *et al.* 1991, Ahlborg *et al.* 1992b) and the following criteria should be met for a compound to be considered:

It should show structural relationship to the PCDDs and PCDFs
It should bind to the Ah-receptor
It should elicit dioxin-specific biochemical and toxic responses
It should be persistent and accumulate in the food chain

On the basis of these criteria, the following PCBs were included:

non-*ortho*-substituted	IUPAC 77, 126 and 169
mono-*ortho*-substituted	IUPAC 105, 114, 118, 123, 156, 157, 167 and 189
di-*ortho*-substituted	IUPAC 170 and 180

IUPAC 170 and 180 were included because they are active as inducers of EROD activity and are present in significant amounts in environmental samples. However, this decision was taken based on limited experimental data. Other di-*ortho* substituted congeners were not included because of their weaker activity as Ah-receptor agonists and their lack of biological significance in the context of TEFs for dioxin-like chemicals. However, it is possible that certain congeners have not been included due to lack of data.

TEF-values for selected PCBs

Based on the available data base, and recognizing that the setting of interim TEFs dictates the choice of values which are more, rather than less conservative in order to be protective of public health, the following TEF-values were recommended (Table 1). TEFs were based on studies with repeated dosing *in vivo* when available. When such data were lacking, TEFs were chosen based on single exposure studies, structure-activity considerations and data from *in vitro* studies.

Table 1. WHO/IPCS interim TEFs for human intake.

Type	Congener		TEF
	IUPAC No.	Structure	
Non-*ortho*	77	3,3',4,4'-TCB	0.0005
	126	3,3',4,4',5-PeCB	0.1
	169	3,3',4,4',5,5'-HxCB	0.01
Mono-*ortho*	105	2,3,3',4,4'-PeCB	0.0001
	114	2,3,4,4',5-PeCB	0.0005[1, 2]
	118	2,3',4,4',5-PeCB	0.0001
	123	2',3,4,4',5-PeCB	0.0001
	156	2,3,3',4,4',5-HxCB	0.0005[2]
	157	2,3,3',4,4',5'-HxCB	0.0005[2]
	167	2,3',4,4',5,5'-HxCB	0.00001[1]
	189	2,3,3',4,4',5,5'-HpCB	0.0001[1]
Di-*ortho*	170	2,2',3,3',4,4',5-HpCB	0.0001[1]
	180	2,2',3,4,4',5,5'-HpCB	0.00001[1]

[1] Based on very limited data.

[2] IUPAC 114, 156, and 157 are expected to have similar TEF-values based on similar responses. Although the data is limited, the determination of TEFs for these congeners is supported by their structural similarity.

The interim TEFs proposed here are based on molar comparisons, but are applicable on a weight basis for this class of compounds. In future, it may be necessary to make comparisons on a weight basis (i.e., for the more fully chlorinated compounds or for brominated congeners).

Consequences of the new TEFs

To illustrate the consequences of the recommended WHO/IPCS TEFs, a comparison is made between the application of earlier TEFs (Safe 1990, Ahlborg *et al.* 1992a) and the present recommendations (Table 2). For further comparison, the TEQ-values based on the I-TEFs for PCDD/Fs (NATO/CCMS 1988) are also included.

The applicability of the recommended TEFs

It was recognized that the recommended TEFs have been developed for use in exposure scenarios, i.e. they are intake TEFs. These values may, or may not, be appropriate for body burden assessments. They may also need to be reexamined for ecotoxicity purposes. There is some data suggesting that TEFs for mammalian systems may not be applicable for fish and birds. The selection of a TEF-value should be driven by the question being addressed. Thus there may be different classes of TEF-values depending upon whether the considerations relate to intake, body burden, or ecological concerns. The ecological concerns may be further subdivided into categories for fish, birds, or other species of wildlife.

On nondioxin-like PCBs

There is some evidence that there may be nonadditive (in particular antagonistic) interactions between nondioxin-like PCBs and dioxin-like compounds. Such interactions could make the strict assumption of TEF additivity for complex mixtures highly conservative. Likewise, nondioxin-like PCBs also have their own independent toxicities, which, in certain cases, may be as important as those associated with dioxin-like compounds (e.g., cancer, neurotoxicity). For example, nondioxin-like PCBs appear to be responsible for most of the tumour promotion associated with higher chlorinated mixtures, e.g., Aroclor 1260 or Clophen A60. Effects due to PCB metabolites, e.g. estrogenicity of hydroxylated metabolites, pulmonary toxicity of sulphonated metabolites, may also be critical confounders. The toxicity of nondioxin-like PCB congeners and metabolites should be assessed in future studies.

Questions of nonadditivity of complex mixtures must be further investigated since these are environmentally relevant. Additivity, synergism, and antagonism may be effect and species specific (Safe 1994). Furthermore, great care will have to be exercised when evaluating effects that can be caused by multiple mechanisms (e.g. increased liver weight, tumour promotion).

Table 2. Toxic equivalents (TEQs) calculated for fish, cow's milk and human milk samples using the present interim WHO/IPCS TEFs are compared to the previously used TEFs by Safe 1990, Safe 1994 and Ahlborg et al 1992a. Chemical data for the human milk are from Norén & Lundén 1991, data on cow's milk are from WHO/EURO 1994 and data on salmon are from Great Lakes Health Effects Division, Health and Welfare Canada (unpublished data).

IUPAC	TEF-system				Human milk					Cow's milk					Salmon				
					Conc. pg/g fat	TEQ				Conc. pg/g fat	TEQ				Conc. pg/g wet weight	TEQ			
	WHO/ IPCS	Ahlborg	Safe 1990	Safe 1994		WHO/ IPCS	Ahlborg	Safe 1990	Safe 1994		WHO/ IPCS	Ahlborg	Safe 1990	Safe 1994		WHO/ IPCS	Ahlborg	Safe 1990	Safe 1994
77	0.0005	0.0005	0.01	0.01	27	0.01	0.01	0.3	0.3	3.4	0.002	0.002	0.03	0.03	930	0.5	0.5	9.3	9.3
126	0.1	0.1	0.1	0.1	98	9.8	9.8	9.8	9.8	23.3	2.3	2.3	2.3	2.3	660	66.0	66.0	66.0	66.0
169	0.01	0.01	0.05	0.05	47	0.5	0.5	2.4	2.4	9.9	0.1	0.1	0.5	0.5	120	1.2	1.2	6.0	6.0
105	0.0001	0.0001	0.001	0.001	6000	0.6	0.6	6.0	6.0	590	0.06	0.06	0.6	0.6	120000	12.0	12.0	120.0	120.0
114	0.0005	0.0005	0.001	0.0002	NA[1]					NA					ND[2]				
118	0.0001	0.0001	0.001	0.0001	25000	2.5	2.5	25.0	2.5	3000	0.3	0.3	3.0	0.3	240000	24.0	24.0	240.0	24.0
123	0.0001	0.0001	0.001	0.00005	NA					NA					ND				
156	0.0005	0.001	0.001	0.0004	14000	7.0	14.0	14.0	5.6	NA					20000	10.0	20.0	20.0	8.0
157	0.0005	0.001	0.001	0.0003	NA					NA					ND				
167	0.00001		0.001		NA					NA					17000	0.2		17.0	
189	0.0001		0.001		NA					NA					6500	0.6		6.5	
170	0.0001		0.00002		NA					NA					62000	6.2		1.2	
180	0.00001		0.00002		64000	0.6		1.3		3600	0.04		0.07		210000	2.1		4.2	
Sum of TEQ for non-*ortho* PCBs						10.3	10.3	12.5	12.5		2.4	2.4	2.8	2.8		67.7	67.7	81.3	81.3
Sum of TEQ for mono-*ortho* PCB						10.1	17.1	45.0	14.1		0.4	0.4	3.6	0.9		46.8	56.0	403.5	152.0
Sum of TEQ for di-*ortho* PCBs						0.6		1.3			0.04		0.07			8.3		5.4	
Total TEQ for all PCBs						21.0	27.4	58.8	26.6		2.8	2.8	6.5	3.7		122.8	123.7	490.2	233.3
Sum of TEQ for PCDDs and PCDFs						20.6[3]	20.6[3]	20.6[3]	20.6[3]		5.6	5.6	5.6	5.6		56.0	56.0	56.0	56.0

[1] NA = Not analyzed [2] ND = Not detected [3] Not all 2,3,7,8-penta, hexa and hepta substituted congeners were measured.

RECOMMENDATIONS

On expansion of the data base

The consultation recommended that the data base should be expanded to include not only the PCDDs and PCDFs, but other dioxin-like compounds which meet the criteria of Ah-receptor binding, identity of effects, structural similarity, and persistence (brominated analogs of the biphenyls, dioxins, and furans, halogenated naphthalenes and diphenyl ethers, and other related compounds). This expansion of the data base should occur preferably within a year.

WHO and IPCS should ensure that this interim data base for TEFs for dioxin-like compounds will be updated every 2 years.

On the need for further studies

There are obvious deficits in the data base which require additional experimental information. WHO and IPCS should encourage the design and conduct of experiments specifically to address the issue of TEFs. *In vivo* studies should receive greater weight than *in vitro* studies. Likewise, studies conducted by environmentally relevant routes of exposure are more useful in TEF determinations than studies conducted ip or sc. Multiple doses are essential for accurate TEF determinations since analysis of the dose-response curves provides more accurate estimates of relative potency than do NOEL- and LOEL-values which are driven by the experimental design. ED_{50} determinations may be model specific or are often based on graphic evaluations rather than statistical considerations. Repeated dosing studies, especially long term studies, more clearly mimic environmental exposure situations and thus should be preferred. However, for effects such as teratogenicity, short term exposures are clearly relevant. Whenever possible, and the group recognized that there are practical and safety issues, TCDD should be included as a positive control. In addition, more analytical data is needed regarding the occurrence of IUPAC 114 and 170.

On TEFs for body burdens and for use in ecotoxicology

The consultation recommended that the feasibility for developing separate TEFs for body burden and ecotoxicology should be explored.

More measurements should be made of body burdens (e.g., blood, liver, fat, target organ dosimetry, etc.) in order to allow for development of TEFs based on body burdens. Tissue distribution may be species, chemical, and dose dependent.

Studies need to be conducted in order to develop TEFs for various forms of fish and wildlife. There is data suggesting differential sensitivity to compounds such as IUPAC 77, particularly for avian species.

Other related issues

WHO and IPCS should explore the feasibility of developing endpoint specific relative potency values (i.e. not TEFs). An example of an area where this might be plausible is tumour promotion which is caused both by dioxin-like and nondioxin-like PCBs.

WHO and IPCS should encourage the development of panels of bioassays as measures of TEQs for mixtures, i.e., an integrated measure of response to be used as complimentary techniques to chemical analyses and for prescreening environmental samples. However, this is not intended as a substitute for the TEF-concept.

REQUESTS

The work on the data base and its expansion will continue. Comments on the work as well as reprints of articles that contain data suitable for calculating TEFs will be appreciated. Please address communications to U. G. Ahlborg.

ACKNOWLEDGEMENTS

Financial support was kindly provided by the US-EPA for data collection and analysis, and by the Netherlands Ministry of Welfare, Health and Cultural Affairs and the Netherlands National Institute of Public Health and Environmental Protection (RIVM) to organize the consultation. This paper does not necessarily reflect US-EPA policy.

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