

CRITERIA FOR THE
DERIVATION OF TOXIC
EQUIVALENCY FACTORS
FOR DIOXIN-LIKE PCBs



IPCS
GENEVA



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COPENHAGEN

TARGET 19

ENVIRONMENTAL HEALTH MANAGEMENT

By the year 2000, there should be effective management systems and resources in all Member States for putting policies on environment and health into practice.

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CRITERIA FOR THE DERIVATION OF TOXIC EQUIVALENCY FACTORS FOR DIOXIN-LIKE PCBs

Report on a WHO Consultation

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ABSTRACT

The WHO European Centre for Environment and Health and the International Programme on Chemical Safety initiated a project to create a database 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 analysed. A consultation was held at the WHO European Centre for Environment and Health, Bilthoven, 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 participants recommended that the programme should be continued to include PCDDs and PCDFs and other dioxin-like halogenated environmental pollutants. It was also recommended that the possibilities of separate TEFs for ecotoxicology should be explored.

Keywords

DIOXINS - toxicity
POLYCHLOROBIPHENYL COMPOUNDS
ENVIRONMENTAL POLLUTANTS
DATABASES, BIBLIOGRAPHIC
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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 are known to be present in fatty tissues of animals and humans, even if only at very low concentrations. 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 of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). These include skin effects, 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).

Since dioxin-like compounds normally exist in environmental and biological samples as complex mixtures of congeners, the concept of toxic equivalents 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 equivalents) are determined based on *in vitro* and *in vivo* studies. This approach is based on the fact 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 of which is that the toxic effects of the components of a given mixture would be additive, neglecting possible synergism or antagonism.

A number of different schemes of toxic equivalency factors (TEFs) have been developed for PCDDs and PCDFs, and some are being used for dioxin-like PCBs. Recognizing the necessity for a harmonized approach towards setting internationally agreed TEFs, the WHO European Centre for Environment and Health and the International Programme on Chemical Safety (IPCS) have initiated a project to create a database 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, data on the relative toxicities of dioxin-like PCBs with respect to a number of endpoints were collected and analysed by Professor Ulf G. Ahlborg and his collaborators at the Karolinska Institute in Stockholm, Sweden. Following this data collection exercise, a Consultation on Criteria for the Derivation of Toxic Equivalency Factors for Dioxin-like PCBs was held at the WHO European Centre for Environment and Health in Bilthoven, Netherlands, from 15 to 17 December 1993 to discuss the available data and possible means to analyse the database 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, one observer and WHO staff. Professor Ulf G. Ahlborg was elected Chairperson, and Dr Linda S. Birnbaum, Rapporteur. Financial support was provided by the US Environmental Protection Agency 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. The participants are listed in Annex 1.

DESCRIPTION OF THE DATABASE

From about 1200 articles on PCBs, 146 were selected which were considered useful for the PCB-TEF database. These articles were analysed, and the data to be included in the final database were selected using the following criteria:

- at least one PCB congener studied;
- 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;

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- endpoint affected by TCDD or the PCB-reference (PCB 77, 126 or 169).

The third criterion was meant to ascertain whether only dioxin-specific endpoints would be included in the database. It was recognized, however, that some endpoints, such as liver weight and tumour promotion, are affected by both dioxin-like and nondioxin-like compounds.

The selection resulted in 56 articles/manuscripts; 3 more were added at the Consultation. Of these articles, 12 were *in vitro* and 47 *in vivo* assays.

Several different methods were used to calculate TEFs from the reported data.

1. TEF taken as reported in the article. If experimental data were also reported, these were used in the first place, using one of the methods below.
2. TEF 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. Ratio of ED₅₀-, LD₅₀-, EC₅₀-, ED₂₅- or ED₁₂-values.
4. Ratio of NOEL-, LOEL- or minimum detectable concentration values.
5. Ratio of tumour promotion indexes.
6. Ratio of maximal induction levels.
7. Ratio of 80% effect levels.
8. Ratio of K_D-values.
9. Estimation from graph.

Methods 2 and 3 were used most frequently.

Several problems were encountered with the reported data when using them for calculating TEFs. For example:

- when different compounds have different maximal effect levels (especially when using ED₅₀-values for enzyme induction, this results in different effect levels being compared);
- when only one dose level has been studied (if this value is not in between the dose-response curve of the reference compound, this results in < or > values as TEFs;
- when data are presented only as graphs;
- when few, high doses have been studied (might have reached maximal effect);
- when there is dose unit confusion (e.g. 0.14 mmol and 140 nmol reported as the same dose!);
- when there is congener confusion (e.g. PCB 156 is reported to be 2,3,3',4,4',5'-hexachloro-biphenyl, which is IUPAC 157).

All evaluated data were compiled into a computerized spreadsheet format. The present database consists of more than 900 entries and the number of variables (congener, effect, doses used, estimated TEF, etc.) is about 50. The size of the present file is about 800 Kb. The database can easily be translated from Quattro Pro for Windows to other spreadsheet formats, to database formats or to DIF-format and different text formats.

DISCUSSION

The participants discussed the feasibility of setting TEFs and emphasized the importance of developing them through an

internationally agreed and harmonized procedure that has the support of international scientific bodies such as WHO and IPCS. They could then be used worldwide for regulatory and risk management purposes. The present database could be used to set interim TEFs for selected PCBs. It was noted that when international TEFs had been established for PCDDs and PCDFs, there were fewer data available than for the dioxin-like PCBs today. It was also recognized that there are some important limitations to the concept of TEFs and that values should be developed with strong caveats. Determination of the individual TEF values requires expert scientific judgement involving review of all the existing data.

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

The participants also considered the criteria that should be met for a compound to be included:

- it should show structural relationships to the dioxins and dibenzofurans;
- 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, it was agreed that the following PCBs should be included: non-*ortho*-substituted IUPAC 77, 126 and 169; mono-*ortho*-substituted IUPAC 105, 114, 118, 123, 156, 157, 167 and 189; and di-*ortho*-substituted IUPAC 170 and 180. IUPAC 170 and 180 were included because they are quite active as EROD inducers and are present in significant amounts in environmental samples. This decision was taken, however, based on very limited information. Other di-*ortho*-substituted congeners were not included because of their extremely weak agonist activity, and their lack of biological significance in the context of TEFs for dioxin-like chemicals. The possibility remains, however, that certain congeners have been excluded due to lack of data.

Recognizing that the setting of interim TEFs has involved the choice of values which are more, rather than less conservative in order to be protective of public health, the participants recommended the TEF values for selected PCBs listed in Table 1.

Table 1. WHO interim TEFs for human intake

Type	Congener		TEF
	IUPAC No.	Structure	
Non-ortho	77	3,3',4,4'-TCB	.0005
	126	3,3',4,4',5-PeCB	.1
	169	3,3',4,4',5,5'- HxCB	.01
Mono-ortho	105	2,3,3',4,4'-PeCB	.0001
	114	2,3,4,4',5-PeCB	.0005 ^{a,b}
	118	2,3',4,4',5-PeCB	.0001
	123	2',3,4,4',5-PeCB	.0001
	156	2,3,3',4,4',5- HxCB	.0005 ^b
	157	2,3,3',4,4',5'- HxCB	.0005 ^b
	167	2,3',4,4',5,5'- HxCB	.00001 ^a
	189	2,3,3',4,4',5,5'- HpCB	.0001 ^a
Di-ortho	170	2,2',3,3',4,4',5- HpCB	.0001 ^a
	180	2,2',3,4,4',5,5'- HpCB	.00001 ^a

^a Based on very limited data.

^b IUPAC 114, 156 and 157 are expected to have similar TEF values based on similar responses. Although the data are 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

compound. In future, it may be necessary to make comparisons also on a weight basis (i.e. for the more fully chlorinated compounds or for brominated congeners).

The participants 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 closely examined for ecotoxicity purposes. There are some data suggesting that TEFs for mammalian systems may not be applicable for fish and birds. The selection of a TEF value should be determined by the question being addressed. Thus there may be different classes of TEF values depending on whether the considerations relate to intake, body burden or ecological concerns. The ecological concerns may be further subdivided into issues of fish, birds or other species of wildlife.

There is growing evidence that there may be non-additive (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 have been demonstrated to have their own independent toxicities, which, in certain cases, may be more important than the effects of the dioxin-like compounds (for example, cancer, neurotoxicity). For instance, nondioxin-like PCBs appear to be responsible for most of the tumour promotion associated with higher chlorinated mixtures such as Aroclor 1260 or Clophen A60. Effects due to PCB metabolites, for example estrogenicity of hydroxylated metabolites, pulmonary toxicity of sulfonated metabolites, may also be critical confounders. Additional evaluation is thus required to examine the effects of nondioxin-like PCBs and PCB metabolites.

Questions of non-additivity must be examined for complex mixtures, as they reflect the real world situation. Additivity, synergism and antagonism may be effect- and species-specific. Furthermore, great care will have to be exercised when evaluating effects that can be caused by multiple mechanisms such as increased liver weight and tumour promotion.

CONCLUSIONS AND RECOMMENDATIONS

1. The database should be expanded, preferably within one year, to include not only PCDDs and PCDFs but other dioxin-like compounds that 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).
2. WHO and IPCS should ensure that this interim database for TEFs for dioxin-like compounds be updated at regular intervals. A two-year interval was suggested.
3. There are obvious deficits in the database that 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 intraperitoneally or subcutaneously. 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 determined by the experimental design. ED₅₀ determinations may be model-specific but are often based on graphic evaluations rather than statistical considerations. Repeated dosing studies, especially long-term studies, approximate environmental exposure situations better and thus should be preferred. For effects such as teratogenicity, however, short-term exposures are clearly relevant. The group recognized that there are practical and safety issues, but whenever possible TCDD should be included as a positive control.
4. More analytical data are needed regarding the occurrence of IUPAC 114 and 170.

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5. The feasibility of developing separate TEFs for body burden and ecotoxicology should be explored.
 6. More measurements should be made of body burdens (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.
 7. Studies need to be conducted in order to develop TEFs for various forms of fish and wildlife. Data exist suggesting exquisite sensitivity to these compounds, for example, IUPAC 77, for some avian species.
 8. WHO and IPCS should explore the feasibility of developing endpoint-specific relative potency values (that is, not TEFs). An example of an area where this might be possible is tumour promotion caused both by dioxin-like and nondioxin-like PCBs.
 9. WHO and IPCS should encourage the development of panels of bioassays as a measure of the toxic equivalent of mixtures, that is, an integrated measure of response to be used as complementary techniques to chemical analyses and for prescreening of environmental samples. This is not, however, intended as a substitute for the TEF concept.

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