DOSE-RESPONSE ASSESSMENT USING THE BENCHMARK DOSE APPROACH OF CHANGES IN HEPATIC EROD ACTIVITY FOR INDIVIDUAL POLYCHLORINATED BIPHENYL CONGENERS

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Introduction

The benchmark dose (BMD) approach was proposed as an alternative to the no-observed-adverse-effect-level (NOAEL) or the lowest-observed-adverse-effect-level (LOAEL) as point of departure (POD) for extrapolation of data from animal studies to the low dose human exposure situation[1,2]. In the risk assessment process using the NOAEL/LOAEL parameter, the reference dose (RfD) or the admissible daily intake (ADI) is obtained by dividing the NOAEL/LOAEL value by uncertainty factors. The uncertainty factors are incorporated in order to take into account variability in the sensitivity of different species, inter-individual differences in sensitivity within the human population, and variability in experimental data. In the BMD approach a dose-response curve is fitted to experimental data (Figure 1) and the BMD is calculated from the equation of the curve as the dose corresponding to a predetermined change in the response defined as the benchmark response (BMR). The 95% lower confidence bound of the BMD, usually referred to as BMDL, can be used as the POD in the extrapolation process to get a RfD or an ADI. The advantages of using the BMD approach are many. First, all the experimental data are utilized to construct the dose-response curve; second, the variability and uncertainty are taken into account by incorporating standard deviations of means; and third, it represents a single methodology for cancer and non-cancer endpoints [3]. In this study the BMD methodology was applied to evaluate dose-response data of seven chlorinated biphenyl (CB) congeners (Table 1), some of which are *dioxin-like* while others are not. The data were obtained from subchronic dietary exposure studies in male and female Sprague Dawley rats [4-10]. Elevation in ethoxyresorufin-*O*-deethylase (EROD) activity was selected as biological response because it is known to be an endpoint sensitive to the exposure of dioxin-like PCBs. Since this response is not an adverse effect *per se*, in this paper we will refer to the no-observed-effect-level (NOEL) or to lowest-observed-effect-level (LOEL), instead of to the NOAEL or LOAEL. The objectives of the study were to evaluate the applicability of the BMD approach,

and to compare the values derived from BMD and BMDL with those obtained from the conventional NOEL and LOEL approach.

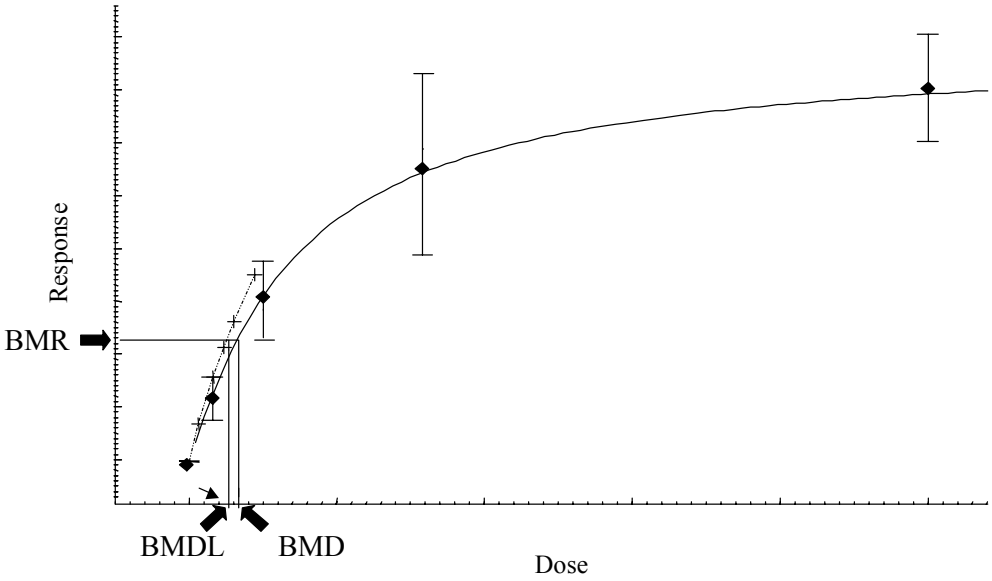

Figure 1. Dose-response curve showing the benchmark dose approach.

Methods and Materials

Chemicals: CB 126 (3,3',4,4',5-pentachlorobiphenyl) was purchased from Accustandard (New Haven, CT); CBs 28 (2',4,4'-trichlorobiphenyl), 77 (3,3',4,4'-tetrachlorobiphenyl), 105 (2,3,3',4,4'-pentachlorobiphenyl), 118 (2,3',4,4',5-pentachlorobiphenyl), 128 (2,2',3,3',4,4'-hexachlorobiphenyl), 153 (2,2',4,4'5,5'-hexachlorobiphenyl), and 156 (2,3,3',4,4',5-hexachlorobiphenyl) were synthesized at the Department of Environmental Chemistry at Stockholm University. The identity and the purity (> 99%) of the chemicals were confirmed using gas chromatography-mass spectrometry.

Animals and experimental design: Detailed descriptions of the experimental designs, dietary preparation, animal treatment, and laboratory assays have been published previously [4-10]. Briefly, Sprague Dawley rats of both sexes were fed *ad libitum* water and diet mixed with different concentrations of the CB congener under investigation (Table 1) for 90 days. Control animals received diet containing an equivalent amount of corn oil only.

Data analysis and BMD computation: Data were first analyzed by one way ANOVA and Dunnett's test for multiple comparisons. When at least one dose group showed a response significantly different from the control ($p<0.05$), they were modeled using the USEPA's Benchmark Dose Software 3.1.1[11]. A variation of 100% of hepatic EROD activity, in comparison to the control animals, was selected as the BMR in the computation.

Table 1. Daily consumption of chlorinated biphenyl (CB) congeners in rats administered through the diet for 13 weeks.

Congener	Treatment (µg/kg diet)	Ingested dose (µg/kg bw/day)		Animals/ group
		male rats	female rats	
CB 28	0, 50, 500, 5000, 50000	0, 2.8, 36, 359, 3783	0, 2.9, 37, 365, 3956	10
CB 77	0, 10, 100, 1000, 10000	0, 0.73, 7.1, 75, 768	0, 0.92, 8.7, 89, 892	10
CB 105	0, 50, 500, 5000, 50000	0, 3.9, 39, 404, 4327	0, 4.2, 44, 449, 3960	10
CB 118	0, 10, 100, 1000, 10000[a]	0, 0.66, 6.9, 70, 683	0, 0.17, 1.8, 17, 170	10
CB 126	0, 0.1, 1, 10,100	0, 0.01[b], 0.08, 0.74, 0.71[b], 7.4	0, 0.01, 0.09, 0.84, 0.83; 8.7[b]	10
CB 128	0, 50, 500, 5000, 50000	0, 4.2, 42, 425, 4210	0, 4.5, 45, 441, 4397	10
CB 153	0, 50, 500, 5000, 50000	0, 3.6, 34, 346, 3534	0, 4.2, 42, 428, 4125	10
CB 156	0, 10, 100, 1000, 10000	0, 0.7, 6.87, 67.7, 697	0, 0.8, 8.1, 81.2, 809	10

[a] In female rats the doses were: 0, 2, 20, 200, 2000 µg/kg diet.
[b] Nine rats in these groups.

The Hill model was selected among the available continuous models because it provided the best description of the data.

$$\mu(d_i) = A + B \times \frac{d_i^n}{k^n + d_i^n}$$

Where :

- µ is the mean value of the response (e.g. EROD activity) for a given dose d_i ;
- A is the intercept of the curve, corresponding to the background of the response (e.g. average EROD activity in the control group);
- B is the range of the response and its sign could be negative or positive depending on whether treatment caused a decrease or increase of the responses
- d_i is the dose that in this investigation is expressed as average daily intake of CB congener ;
- *n* and *k* are the power and the slope of the curve, respectively.

Results and Discussion

Data were found suitable for BMD dose-response modelling because, for all of the congeners in both male and female rats, there was at least one group with a significant increase of the EROD activity (data not shown)[4-10]. All the congeners were modelled with an acceptable goodness-of-fit (P>0.05) even if data for CB 118 did not show any dose-related trend until the highest dose. This implies that there was very limited information about the shape of the dose-response curve. As a result there was more uncertainty in BMDL calculation for this congener.

Table 2. Comparison between BMDL values (μg/kg bw-day) derived for 100% increase of hepatic EROD activity and NOEL and LOEL values (μg/kg bw-day), following 13 weeks of dietary exposure to different CB congeners in male and female rats.

Male rats	**BMDL** (μg/kg bw-day)	**NOEL** (μg/kg bw-day)	**LOEL** (μg/kg bw-day)
CB 28	634	359	3783
CB 77	39	75	768
CB 105	54	404	4327
CB 118	70	70	683
CB 126	0.005	<0.01	0.01
CB 128	179	425	4210
CB 153	2	3.6	34
CB 156	14	68	697
Female rats			
CB 28	2795	365	3956
CB 77	29	8.7	89
CB 105	32	449	3960
CB 118	56	17	170
CB 126	0.005	<0.01	0.01
CB 128	834	441	4397
CB 153	0.5	<4.2	4.2
CB 156	7	81	809

As expected, CB 126 was the most potent congener with respect to hepatic EROD induction (Table 2). The di-*ortho* substituted CB 153 was the second most potent EROD-inducing congener in both male and female rats based on the data modelling (Table 2). However, for CB 153, the increase in EROD activity in female rats was not clearly dose related [6] and the shape of the dose-response curve was different from that of the other congeners, since it was steeper at low dose and flatter to the high doses. The mono-*ortho* substituted congener CB 156 was the third most potent EROD-inducing congener followed by CB 77 and CB 105 which both showed similar BMDL values (Table 2).

In general, BMDL values were quite similar between male and female rats. Some differences can be observed for CBs 28 and 128 which showed the lowest EROD-induction, and for CB 153. Comparison between the BMDL and the NOEL, calculated as the higher dose not inducing any significant statistical change in the response in comparison to the control, is shown in Table 2 and Figure 2. In most cases the BMDL and NOEL values are in the same order of magnitude. In some cases the BMDL values are lower while in others higher than the corresponding NOELs. In all the cases BMDLs are lower than the corresponding LOELs (Table 2). The BMDL and NOEL values indicate that the two approaches provide similar results in grading the potency of the congeners. Figure 2 shows that several congeners have the same value of Log NOEL (*y* axis) but different values of Log BMDL (*x* axis). Thus, a distinct advantage of the BMD approach is that it discriminates the potencies of these congeners with a greater sensitivity than that of the NOEL method.

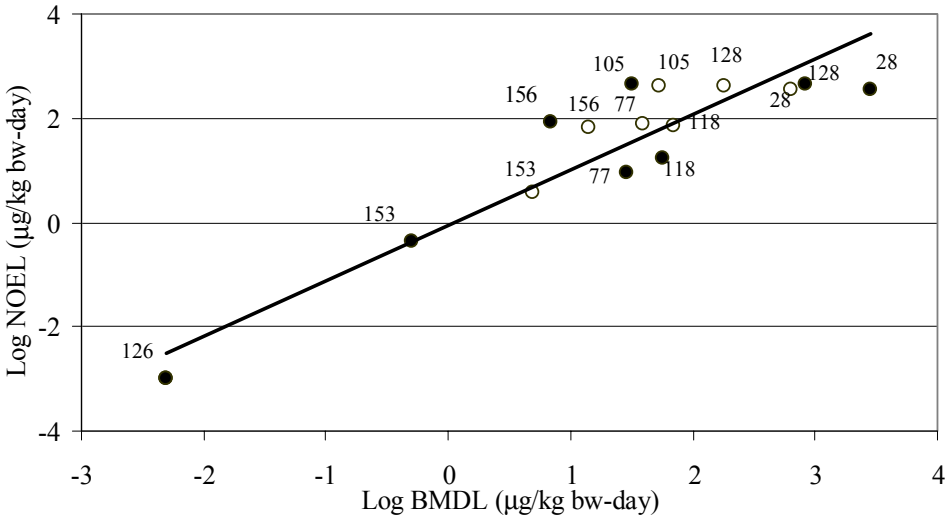

Figure 2. Graph in logarithmic scale showing the BMDL *vs* NOEL values, for hepatic EROD increase, for the individual congeners (IUPAC numbers on graph) in male (●) and female (○) rats.

Acknowledgements
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