ARYL HYDROCARBON RECEPTOR MEDIATED (DIOXIN-LIKE) RELATIVE POTENCY FACTORS FOR CHLORONAPHTHALENES

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Introduction

Chloronaphthalenes (CNs, PCNs) are environmental and food contaminants of multi-source origin that exert dioxin-like toxicity via binding to Ah receptor but affinity is weaker compared to 2,3,7,8-TCDD[1,2]. Recently a method for the complete separation and determination in one instrumental run and without any pre-separation or fractionation of analyte for all tetra-, penta- and hexaCNs as well as heptaCNs and octaCN (together 49 compounds) in a mixture by a set of two capillary columns of different polarity in two-dimensional GC and quadropole MS (GC×GC/QMS) has been reported[3]. This method provides a highly advanced analytical tool for the full resolution and improvements in risk assessment of all toxicologically relevant PCNs that contaminate food and environment. Rreviewed is information relating to the dioxin-like potency of PCNs and obtained *in vivo*, *in vitro* and *in silico*. This can help and improve the quality of data when assessing the contribution of CNs in the risk analysis of total exposure to dioxin-like contaminants from foods etc.

Materials and methods

There is cumulative toxicological evidence that both technical PCNs formulations and many individual chloro- and bromonaphthalenes resemble the highly toxic dioxin - 2,3,7,8-TCDD, in their biological action and effects on animals [2,4,5,6,7].

A key question regarding toxicological risk arising from the occurrence of PCNs in foods and through environmental diffusion, is the effects of AHH receptor binding, which is a common mechanism of action shown by dioxin-like compounds such as 2,3,7,8-TCDD and its planar analogues. Substituted with bromine or chlorine at 2,3,7,8- positions, dibenzo-*p*-dioxins and furans and dl-PCBs have assigned values of Toxic Equivalency Factor (TEF) or Relative Potency Factor (REF) that express compound toxicity relative to 2,3,7,8-TCDD, which as the most potent has an assigned TEF value of 1 [8]. Development of values of TEFs/RFPs for dioxins and other compounds of similar nature requires the meta-analysis of a significant number of high quality toxicokinetic and mechanistic data on each compound considered. Both, *in vitro* and *in vivo* data can be extremely useful, even though the volume of the latter are often limited.

Studies in vivo. A study reports on RPFs for CNs #66 and #67 (purity for both > 99.9%) determined using of dose response modeling [independent (ip) and common (cp) parameter models] of CYP1A1 and CYP1A2 (from *in vivo* data with female Harlan Sprague-Dawley rats) and of thymic atrophy[2]. An estimate of RPF for CN#66 (CYP1A1) is at 0.0017 (ip) and 0.0015 (cp), and (CYP1A2) at 0.0041 (ip) and 0.0022 (cp); and of REP for CN#67 is at 0.00029 (ip) and 0.00036 (cp) and at 0.00067 (ip) and 0.00032 (cp), and an estimate for thymic atrophy is 0.0072 for CN#66 and 0.00032 for CN#67 (cp) [2].

Studies in vitro. The RPFs and efficacies (% of TCDDmax) of several CN congeners were evaluated using *in vitro* cell-based bioassays [9,10,11,12], and results are collated in Table 2. Those bioassays are based on the aryl hydrocarbon (Ah)-receptor activity, using micro-EROD (ethoxy-resorufin-*O*-deethylase) that utilizes a wild type rat liver cell line (rat liver H4IIEC3/T cells), and DR-CALUX (Dioxin-Responsive-Chemical Activated Luciferase gene expression) that consists of a genetically modified rat hepatoma H4IIE cell line that incorporates the firefly luciferase gene coupled to dioxin-responsive elements (DREs) as a reporter gene.

Studies in silico. A few *in silico* studies [13,14], have predicted RPFs values of individual CNs, through quantitative structure-activity relationships (QSAR) computing, using the available EROD and luciferase activities of individual CNs from the *in vitro* tests referred to above [9,10,11,12]. Data from one study provided a complete set for all CN congeners [14]. Those data but with exception of a few congeners for which predicted values were marked as uncertain (including CN#75) are included in Table 1.

Results and discussion

Consolidated RPFs. This paper collates relevant information from toxicological studies on PCNs and including the relative potencies (RPFs) data produced for these compounds, to date. The RPFs derived *in vivo* that is available only for chloronaphthalene congeners #66 and #67 fits to most of data derived *in vitro* and *in silico* for those compounds (Table 2). And the RPF of 0.002 could be temporally assigned both to CN#66 and CN#67 as a reasonable compromise at the moment. And congener CN#70 appears as having adequately similar strength of the binding to the Ah receptor as CN#66, #67, and its assigned RPF of 0.003 seems adequate (Table 2). Hence, the chloronaphthalenes #66, #67 and #70 can be classified as having an order of magnitude greater dioxin-like potency compared to octachloro- and octabromo dibenzo-*p*-dioxin and -dibenzofuran [8]. They are an order of magnitude more potent than two dioxin-like non-*ortho* chloro- and bromobiphenyls that are substituted at positions 3,3',4,4'- and 3,4,4',5- (*i.e.* chlorobiphenyls and bromobiphenyls #77 and #81, respectively) or all mono-*ortho* chloro- and bromobiphenyls. Chloronaphthalene #73 could be considered as next the most potent among PCNs (Table 2) and its assigned RPF is 0.0006, and similarly for CN#68 is 0.0005, for CN#54 is 0.0002, for CN#69 is 0.0001, for CN#63 is 0.00002, for CNs #55, #64, #71 and #75 is 0.00001. For chloronaphthalene congener #56 and #62, the RPF is 0.000005 and for congeners: #57, #58, #59, #60 and #61 is 0.000001. For remaining chloronaphthalenes, the RPF values are less than 0.000001 (Table 1).

Conclusions. Chloronaphthalenes bind to the Ah receptor but affinity is lower compared to that of TCDD. The dioxin-like potency of 20% PCNs expressed by the RPFs are between 0.003 and 0.000001. Chloronaphthalenes #66, #67 and #70 with RPFs between 0.003 and 0.002 are likely to be an order of magnitude more potent compared to dl-tetraCB/tetraBBs and they are an order of magnitude more potent than octaCDD/F and octaBDD/F. Altogether 19 chloronaphthalenes can be attributed as showing dioxin-like activity, while extra and more rigorous data are necessary to confirm this conclusion. Some recent studies have estimated a small but relevant contribution to dioxin-like toxicity in foods, arising from these compounds. Given the additivity of response postulated for other dioxin-like compounds, it would be unwise to ignore this contribution. Additionally, although a greater emphasis in this review has been placed on dioxin-like toxicity, other toxicological effects have also been documented. Chloronaphthalenes will continue to be relevant environmental and food-chain trace contaminants because environmental exposure to PCNs (and probably less to PBNs and PXNs) by humans will continue in the future due to legacy and existing sources of pollution.

References

1. Falandysz J. (2003) *Food Addit Contam*. 20: 995-1014
2. Hooth MJ, Nyska A, Fomby LM, Vasconcelos DY, Vallant M, DeVito MJ, Walker NJ. (2012); *Toxicology* 301: 85-93
3. Hanari N, Falandysz J, Petrick, Nakano T, Yamashita N. (2013); *J Chromatogr A*. submitted
4. Campbell MA, Bandiera S, Robertson L, Parkinson A, Safe S. (1981); *Toxicology* 22: 123-32
5. Campbell AM, Bandiera S, Robertson L, Parkinson A, Safe S. (1983); *Toxicology* 26: 193-205
6. Birnbaum LS, Darcey DJ, McKinney JD. (1983); *J Toxicol Environ Health*. 12: 555-73
7. Robertson LW, Thompson K, Parkinson A. (1984); *Arch Toxicol*. 55: 127-31
8. Van den Berg M, Denison MS, Brinbaum LS, DeVito M, Fiedler H, Falandysz J, Rose M, Schrenk D, Safe S, Tohyama C, Tritscher A, Tysklind M, Peterson RE. (2013); *Toxicol Sci*. 132: in press doi:10.1093/toxsci/kft070
9. Hanberg A, Waren F, Asplund L, Haglund E, Safe S. (1990); *Chemosphere* 20: 1161-4
10. Blankenship AL, Kannan K, Villalobos SA, Villeneuve DL, Falandysz J, Imagawa T, Jakobsson E, Giesy JP. (2000); *Environ Sci Technol*. 34: 3153-8
11. Villeneuve DL, Kannan K, Khim JS, Falandysz J, Nikiforov VA, Blankenship AL, Giesy JP. (2000); *Arch Environ Contam Toxicol*. 39: 273-81
12. Behnisch PA, Hosoe K, Sakai S. (2003); *Environ Int*. 29: 861-77
13. Falandysz J, Puzyn T. (2004); *J Environ Sci Health A – Tox/Hazard Sub Environ Eng* 39:1505-23
14. Puzyn T, Falandysz J, Jones PD, Giesy JP. (2007); *J Environ Sci Health A* 42: 573-90

Table 1. Reported ranges of combined *in vivo*, *in vitro* and *in silico* relative potencies (REPs) for selected individual CNs

PCN congener	ID	*In vitro*						*In vivo***		*In silico*	
		*Sp	H4II-EROD	H4II-EROD	H4II-luc		DR-CALUX- (pM)	Micro-EROD (pM)	CYP1A1	CYP1A2		
2-CN	2		< 2.2 x 10^{-7}				1.8 x 10^{-5}	*< 1.5 x 10^{-6}*			1.0 x 10^{-8}	1.5 x 10^{-7}
1,2-DiCN	3						*< 2.9 x 10^{-7}*				2.3 x 10^{-7}	2.2 x 10^{-7}
1,4-DiCN	5		5.1 x 10^{-9*}				3.5 x 10^{-5}	*< 1.6 x 10^{-6}*			4.3 x 10^{-9}	1.5 x 10^{-7}
1,5-DiCN	6						*< 1.2 x 10^{-6}*	*< 6.6 x 10^{-7}*			6.5 x 10^{-9}	2.6 x 10^{-7}
1,8-DiCN	9						*1.5 x 10^{-5a}*	*< 1.7 x 10^{-6}*			1.4 x 10^{-7}	1.6 x 10^{-6}
2,3-DiCN	10						2.7 x 10^{-5}	*< 5.9 x 10^{-6}*			2.2 x 10^{-8}	3.5 x 10^{-7}
2,7-DiCN	12		< 4.2 x 10^{-7}								3.5 x 10^{-7}	4.9 x 10^{-7}
1,2,3-TrCN	13						*< 4.4 x 10^{-6}*	*< 2.0 x 10^{-6}*			4.2 x 10^{-8}	9.1 x 10^{-7}
1,2,7-TrCN	17		< 8.4 x 10^{-7}								6.6 x 10^{-7}	1.7 x 10^{-7}
1,2,3,4-TeCN	27						*< 2.3 x 10^{-6}*	*< 1.6 x 10^{-6}*			9.1 x 10^{-7}	2.3 x 10^{-6}
1,2,4,7-TeCN	34	DVC	< 4.2 x 10^{-7}			< 6.9 x 10^{-7}					4.7 x 10^{-7}	1.3 x 10^{-6}
1,2,5,6-TeCN	36	DDV					*< 4.1 x 10^{-7}*				1.1 x 10^{-6}	2.3 x 10^{-6}
1,2,6,8-TeCN	40	TVC				1.6 x 10^{-5*}					1.3 x 10^{-7}	1.4 x 10^{-5}
1,3,5,7-TeCN	42	NVC	< 4.2 x 10^{-6}			< 6.9 x 10^{-6}	7.5 x 10^{-6}	*< 1.9 x 10^{-6}*			1.2 x 10^{-6}	3.2 x 10^{-6}
2,3,6,7-TeCN	48	DDV					4.1 x 10^{-5}				2.3 x 10^{-4}	1.0 x 10^{-5}
1,2,3,4,6-PeCN	50	DVC; αβ					6.8 x 10^{-5}	4.3 x 10^{-5}			4.2 x 10^{-5}	3.0 x 10^{-5}
1,2,3,5,7-PeCN	52	NVC	4.2 x 10^{-6}				*< 3.4 x 10^{-6}*	*< 1.8 x 10^{-6}*			8.5 x 10^{-6}	3.8 x 10^{-5}
1,2,3,5,8-PeCN	53	DVC; ββ					*< 1.8 x 10^{-6}*	*< 1.2 x 10^{-6}*			1.3 x 10^{-8}	5.2 x 10^{-6}
1,2,3,6,7-PeCN	54	DVC; αα	9.2 x 10^{-5}		< 0.00069	0.00017	0.00058				2.8 x 10^{-5}	5.5 x 10^{-5}
1,2,3,6,8-PeCN	55	DVC; αα									7.1 x 10^{-6}	6.8 x 10^{-5}
1,2,3,7,8-PeCN	56	TVC	2.4 x 10^{-5}		0.00049						2.3 x 10^{-5}	5.6 x 10^{-5}
1,2,4,5,6-PeCN	57	DVC; αβ	1.7 x 10^{-6}		3.7 x 10^{-6}						1.5 x 10^{-6}	1.5 x 10^{-6}
1,2,4,6,7-PeCN	60	NVC	< 4.2 x 10^{-7}			< 2.8 x 10^{-5}					1.3 x 10^{-6}	2.8 x 10^{-5}
1,2,4,6,8-PeCN	61	NVC	< 4.2 x 10^{-7}								2.9 x 10^{-7}	1.3 x 10^{-5}
1,2,3,4,5,6-HxCN	63	DVC; αβ		0.002							2.2 x 10^{-5}	2.2 x 10^{-5}
1,2,3,4,5,7-HxCN	64	NVC		2 x 10^{-5}							1.1 x 10^{-4}	1.0 x 10^{-5}
1,2,3,4,6,7-HxCN	66	NVC	0.00061		0.0024	0.0039	0.0012	0.00054	0.0015-0.0017	0.0022--0.0041	0.00069	0.0029
1,2,3,5,6,7-HxCN	67	NVC	0.00028	0.002		0.001	0.00048		0.00029-0.00036	0.00032-0.00067	0.001	0.0017
1,2,3,5,6,8-HxCN	68	NVC		0.002		0.00015	0.00049				0.00027	0.00011
1,2,3,5,7,8-HxCN	69	NVC		0.002			1.1 x 10^{-4}	6.4 x 10^{-6}			8.3 x 10^{-7}	1.5 x 10^{-4}
1,2,3,6,7,8-HxCN	70	DVC; αα	0.0021		0.0095	0.00059	0.0028				0.0028	0.00071
1,2,4,5,6,8-HxCN	71	NVC					*< 1.1 x 10^{-6}*				4.3 x 10^{-5}	1.6 x 10^{-7}
1,2,4,5,7,8-HxCN	72	NVC					6.0 x 10^{-5}	*7.1 x 10^{-6}*			1.0 x 10^{-4}	8.9 x 10^{-8}
1,2,3,4,5,6,7-HpCN	73	NVC	0.00040	0.003	0.0006	0.001	0.00052				0.00038	0.0018
1,2,3,4,5,6,8-HpCN	74	NVC					*4.1 x 10^{-6}*					1.0 x 10^{-7}
1,2,3,4,5,6,7,8-OCN	75	NVC					1.0 x 10^{-5}	*< 4.3 x 10^{-6}*				
References		1	11	9	10		12		2		14	

Notes: *Sp (Substitution pattern; NVC, DVC, TVC, and DDVC refers to CNs that have no any or have two, three, and two pairs of adjacent (vicinal) carbon atoms unsubstituted with chlorine); **An estimated RPFs from dose response modeling for thymic atrophy was 0.0072 for CN #66 and 0.00032 for CN #67).