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Toxicity and QSAR of Chlorobenzenes in Two Species of Benthic Flatfish, Flounder (*Platichthys flesus* L.) and Sole (*Solea solea* L.)

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Chlorinated benzenes were among the first large-scale produced aromatic compounds. They have found broad spectrum of uses in numerous domestic and industrial preparations, ranging from engine - block cleaners, solvents, pharmaceutical intermediates, synthesis of chlorophenols and in disinfectants.

Recent investigations have shown that they are present in the major environmental compartments and organisms, including fish tissues (Oliver and Nicol 1982), coastal waters (van de Meent et al. 1985) and estuaries (Harper et al. 1992). Comparatively little is known about the ecotoxicity of chlorinated benzenes particularly to economically important fish. A number of earlier investigations examined the toxicity of chlorobenzenes and they demonstrated differences in the toxicity values for the same species and isomers of the chemicals investigated (Konemann and van Leeuwen 1980; Calamari et al. 1983; Veith et al. 1983).
The present investigation has assessed the toxicity and QSARs of selected chlorobenzenes to two ecologically and commercially important flatfish, the flounder (*Platichthys flesus*) and sole (*Solea solea*). Both have a widespread geographic distribution in coastal and estuarine regions throughout Western Europe and are therefore highly representative test species.

MATERIALS AND METHODS.

Fish were collected from two locations on the Tidal River Thames. Flounder were collected at a brackish water site (5‰) by netting from the cooling - water reservoir of the London Underground Limited, Generating Station, Fulham, London, SW10 (0° 15'W, 51° 30'N). Sole were obtained from a more seaward site with a salinity of 22 ‰ at the National Power sub-station, West Thurrock, Essex (0° 20'E, 51° 30'N). The fish were taken immediately from filter screens into nylon mesh bags. In each case the fish were quickly transferred to an insulated fiberglass transit-tank equipped with a continuous source of aeration. Fish were then transported back to the laboratory and transferred to a 1000-L continuously recirculating aquarium system which contained diluted seawater with a salinity which matched that of the collecting site; 5 ‰ for flounder and 22 ‰ for sole. The system was housed in a constant temperature room maintained at 6 °C. Both species were acclimatised to laboratory conditions for 7 d prior to experimentation.

Chlorobenzenes were obtained as the highest purity grade available ('AnalaR' or reagent grade) (Fisons Scientific) and were certified as being between 97 - 99% (analysis by capillary GC). Stock solutions of the chlorobenzenes were prepared by dissolution using a solvent vehicle of glass - distilled acetone. Dissolution of the chlorobenzenes was optimized by subjecting the stock solutions to sonication.

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Chlorobenzene solutions were stored under conditions of darkness and refrigerated at 4 ^{o}C.

Toxicity (96-hr LC50) was determined using the standard protocol adopted by the OECD (1981). Tests were conducted using a semi - static regime. This consisted of six all - glass 30-L aquarium tanks which were filled to a volume of 20-L with the test media. Each tank was sealed with a tightly - fitting glass lid. Experimental vessels were gently aerated using a constant stream of filtered compressed air. Each exposure tank contained 12 of the respective test organisms. Fish were selected on a weight basis, flounder being within the range 56.2±2.5 g and sole being within the limit of 45.0±2.5 g. Tests were conducted under conditions of subdued illumination using a photoperiod of 12L:12D.

The exposure regime consisted of 1 control tank and 5 experimental exposures. These tests were conducted as combined duplicates. A control exposure with acetone at the same dilution used in preparing the toxicant exposures was used to ensure that the solvent vehicle caused no mortality to the test species. Regular monitoring of the exposure solutions by GC analysis showed that exposure concentrations could be maintained to within 80% of the nominal values by removing four-fifths (80%) of the exposure solution and replacing it with fresh exposure solution after 48 hr.

Mortality was taken to be when there was a cessation of branchial irrigation characterized by a swollen head, widely opened buccal and opercular cavities and when the fish had turned onto their dorsal side. Exposure vessels were inspected routinely and dead fish were immediately removed. The number of dead fish was recorded at the end of every 24 hr. The 96-hr LC50s were determined by probit analysis.

The octanol:water partition coefficients (logKow), zero order (O-χ-V)and second order (2-χ-V) molecular connectivity values, which were used to formulate QSAR by regression analysis with the LC50 values, are given in Table 1.

RESULTS AND DISCUSSION

The order of toxicity was HCB>1,3,5-TCB>1,2,4-TCB>1,2-DCB>MCB>Benzene (Table 2). Both species exhibited similar sensitivity to the chlorobenzenes although the LC50 values for sole were consistently lower than those of flounder. The LC50 values for 1,2,4- and 1,3,5- trichlorobenzene were respectively 20.1 µM for flounder and 16.5 µM for sole and 12.4 µM for flounder and 10.8 µM for sole, which indicates that 1,3,5 TCP was more toxic than 1,2,4-TCP.

Significant QSARs were established for the chlorobenzenes in both species of flatfish (Table 3). The correlation coefficients (r values) were all significant at $p<0.01$ and the F-ratio of the regression constants were also highly significant. Consistently superior relationships were obtained when the molecular connectivity indices were regressed as opposed to logKow. Although it was found that when two molecular descriptors were regressed in combination it gave rise to improved r values the F - ratios were less significant. Toxicity has been predicted for thirteen members of the chlorobenzene series based on the most significant QSAR regression equations.

Table 1. Octanol:water partition coefficient (logKow), zero (0-χ-V) and second (2-χ-V) order molecular connectivity values for benzene and chlorobenzenes.

Compound	logKow[a]	0-χ-V[b]	2-χ-V[b]
Benzene	2.14	3.46	1.98
MCB	2.86	5.11	2.74
1,2-DCB	3.55	5.98	3.24
1,3-DCB	3.60	5.98	3.38
1,4-DCB	3.62	5.98	3.37
1,2,3-TCB	4.11	6.80	3.74
1,2,4-TCB	3.93	6.80	3.87
1,3,5-TCB	4.14	6.80	4.02
1,2,3,4-TeCB	4.45	7.70	4.25
1,2,3,5-TeCB	4.50	7.70	4.38
1,2,4,5-TeCB	4.52	7.70	4.39
Pentachlorobenzene	4.88	8.59	4.77
Hexachlorobenzene	5.10	9.46	5.16

[a]Konemann et al (1979); [b]Sabljic (1983)

The QSAR predictions of toxicity using LogKow or 2-χ-V produced values which corresponded reasonably well with observed values (Tables 4 and 5). The two variables discriminated between the molecular and physical properties of each of the isomers and this highlighted isomer - specific differences in toxicity. Predicted toxicities based on zero order molecular connectivity values were more limited as this descriptor has the same value for the isomers of each member of the series.

Toxicity of chlorinated benzenes has been determined for several organisms in earlier investigations (e.g., Calamari et al. 1983; Veith et al. 1983; Carlson and Kosian 1987; Van Hoogen and Opperhuizen 1988). Reported toxicity values for trichlorobenzenes, from other experimental investigations involving fish, include, toxicity of 1,2,4 - TCB to rainbow trout (*Salmo gairdneri*) [1.95 mgL^{-1}; 10.7 μM] (Calamari et al. 1983), to zebrafish (*Brachydanio rerio*) [6.3mgL^{-1}; 34.7 μM], (Calamari et al. 1983), and to fathead minnow, (*Pimphales promelas*) [2.9 mgL^{-1}; 16.0 μM]; 1,2,3 -TCB toxicity to guppy (*Poecilia reticulata*) [2.3 mgL^{-1}; 12.9 μM] (Konemann and van Leeuwen 1980), fathead minnow (*Pimphales promelas*) [1.09 mgL^{-1}; 6.0 μM] (Carlson and Kosian 1987) and guppy (*Poecilia reticulata*) [0.35 mgL^{-1};1.9 μM] (Van Hoogen and Opperhuizen 1988).

However, there appears to be an absence in the literature on the toxicity of chlorobenzenes to commercially significant species, in this instance, bottom dwelling flatfish. The values for the toxicity of 1,2,4 - TCB to flounder (*Platichthys flesus*) [3.65 mgL^{-1}; 20.10 μM] and sole (*Solea solea*) [2.99 mgL^{-1}; 16.50 μM] compare favourably with the previously cited literature.

Table 2. Toxicity of benzene and five selected chlorobenzenes to flounder and sole.

Compound	Flounder LC50 (μM)	Flounder 95% C. I.	Sole LC50 (μM)	Sole 95% C.I.
Benzene	136.8	74.8 - 197.2	115.6	104.5 - 126.9
MCB	58.7	42.9 - 75.9	51.7	38.8 - 63.8
1,2-DCB	31.4	17.4 - 44.5	28.5	19.6 - 37.6
1,2,4-TCB	20.1	16.5 - 26.6	16.5	10.5 - 23.3
1,3,5-TCB	12.4	6.9 - 16.5	10.8	2.9 - 18.4
HCB	0.7	0.07 - 1.1	0.5	0.06 - 1.1

Table 3. Quantitative Structure - Activity Relationships (QSAR) for chlorobenzenes to flounder (*Platichthys flesus*) and sole (*Solea solea*).

Parameter	log(1/LC50)=	r - value	F - ratio	p - value
Flounder:				
logKow (x)	-3.90+0.724 x	0.949	36.17	<0.004
0-χ-V (x')	-3.68+0.382 x'	0.973	70.19	<0.001
2-χ-V (x")	-3.68+0.686 x"	0.962	49.99	<0.002
logKow+0-χ-V	-3.47-0.482x+0.628 x'	0.977	31.50	<0.01
logKow+2-χ-V	-3.36-0.860x+1.490x"	0.967	22.11	<0.016
0-χ-V+2-χ-V	-3.66+0.515x'-0.243 x"	0.974	27.09	<0.012
Sole:				
logKow (x)	-3.90+0.747 x	0.943	31.89	<0.005
0-χ-V (x')	-3.68+0.395 x'	0.969	61.23	<0.001
2-χ-V (x")	-3.68+0.709 x"	0.958	44.64	<0.003
logKow+0-χ-V	-3.43-0.594 x+0.698 x'	0.975	28.87	<0.011
logKow+2-χ-V	-3.28-1.09 x+1.73 x"	0.966	21.05	<0.017
0-χ-V+2-χ-V	-3.66+0.548 x'-0.279 x"	0.970	23.69	<0.015

Molecular connectivity indices have been developed for describing the topology of molecules and the significance of substituent position and molecular structure in relationship to toxicity, toxicokinetic parameters and bioaccumulation.

Octanol:water partition coefficients have been used for predicting the toxicity and and fate of organic compounds. However, it has been suggested that this dependence on logKow alone is of a questionable nature in assessing toxic mode of action (Opperhuizen et al. 1988; Sangster 1989).

Table 4. Predicted toxicity of chlorobenzenes to flounder from QSARs with logKow and 2-χ-V (Δobs-pred is the difference between observed and predicted values).

Compound	Log Kow		2-χ-V	
	LC50(μM)	Δobs-pred	LC50(μM)	Δobs-pred
Benzene	200.2	-63.4	188.9	-52.1
MCB	58.2	+0.7	54.7	+4.1
1,2-DCB	17.7	+13.7	24.1	+7.3
1,3-DCB	16.2		19.2	
1.4-DCB	15.7		19.5	
1,2,3-TCB	6.8		10.7	
1,2,4-TCB	9.2	+10.9	8.6	+11.5
1,3,5-TCB	6.4	+6.0	6.8	+5.6
1,2,3,4-TeCB	3.8		4.6	
1,2,3,5-TeCB	3.5		3.8	
1,2,4,5-TeCB	3.4		3.7	
PeCB	1.8		2.0	
HCB	1.2	-0.5	1.0	-0.4

Table 5. Predicted toxicity of chlorobenzenes to sole from QSARs with logKow, and 2-χ-V (Δobs-pred is the difference between observed and predicted values).

Compound	logKow		2-χ-V	
	LC50(μM)	Δobs-pred	LC50(μM)	Δobs-pred
Benzene	224.2	-108.6	209.8	-94.2
MCB	69.8	-18.1	63.1	-11.5
1,2-DCB	21.4	+7.13	28.7	-0.2
1,3-DCB	19.7		23.0	
1,4-DCB	19.0		23.2	
1,2,3-TCB	8.4		13.0	
1,2,4-TCB	11.3	+5.2	10.6	+5.9
1,3,5-TCB	8.0	+2.8	8.4	+2.4
1,2,3,4-TeCB	4.8		5.8	
1,2,3,5-TeCB	4.4		4.7	
1,2,4,5-TeCB	4.2		4.7	
PeCB	2.3		2.6	
HCB	1.6	-1.1	1.4	-0.9

There has been much effort to consolidate the use of molecular connectivity indices in aquatic toxicology (Sabljic and Piver 1992). Chlorobenzenes are highly lipophilic and therefore become rapidly incorporated into biotic lipid. However, molecular connectivity can add an additional facet to understanding of toxic mode of action. The molecular connectivity is related to molecular size and it can be observed that concomitantly,with increasing molecular dimensions, the toxicity increases. Furthermore, this variable can be useful in determining partitioning into biotic lipid in this sense and can be used as a means to confirm observations based on octanol:water partition coefficient - dependent QSARs (Protic and Sabljic 1989; Sabljic and Piver 1992).

The pattern that emerged in this investigation indicated that toxicity was correlated well with both the 0-χ-V and 2-χ-V indices and logKow giving consistently high significance values for r and the F-ratio. The second order (2-χ-V) molecular connectivity with specific values for isomers for each member in the series was found to have greater predictive value. The QSARs based on logKow yielded relationships which indicated that the toxic mode of action of the chlorobenzenes in the flatfish was non - specific and could therefore be attributed to a non - polar narcotic effect. This observation concurs with that of other investigations involving fish (Buccafusco et al. 1981; Calamari et al. 1983; Veith et al. 1983; Carlson and Kosian 1987; Ikemoto et al. 1992).

The non - polar narcotic effect for the chlorobenzenes in flatfish is in contrast to that found for chlorophenols using the same two species (Smith et al. 1993). This study has reported that the mode of action of the chlorophenols is more specific and can be accounted for in terms of a Type II polar - narcotic effect (Verhaar et al. 1992). The difference in mode of action of chlorobenzenes and chlorophenols in both species of flatfish is evident from the differences in the logKow QSARs that were obtained, particularly, the value of the gradient (0.4 for chlorophenols as compared to 0.7 - 0.8 for the chlorobenzenes).

There does, however, require to be further consideration made of the *in vivo* biological significance of molecular connectivity in elicitation of toxic effects other than the purely chemical considerations based on molecular structure. The mode of action of lipophilic organics remains obscure and the concept of narcosis remains a loosely defined concept. The relationship between molecular connectivity and toxicity, because of the structural differences between isomers, could very well indicate there maybe more specific effects between isomers, which may suggest interference with more specific cellular components. Earlier work of Franks and Lieb (1978) gives credence to the theory that narcosis is more than a non - specific retardation of cytoplasmic activity. Certain structural attributes of organic compounds may have interactive effects with functionally important cellular entities or even with intrinsic or extrinsic membrane bound proteins which may induce a more specific action which leads to the toxic response or mode of action.

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