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Field Dose-Response Approach (DRAC-"Dose-Resposta para Avaliação da Contaminação") as a Tool for Environmental Mercury Contamination Assessment in Fish

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Methylmercury (MeHg) is a well known human neurotoxin (Clarkson, 1994). The general population is primarily exposed to MeHg through fish consumption (WHO, 1990; Clarkson, 1994; U.S. EPA, 2001). It has been demonstrated that Hg usually accumulates in fish tissues as MeHg, from inorganic Hg sources (Huckabee et al., 1979). Traditionally, the United States Environmental Protection Agency (U.S. EPA) has expressed, in its section 304(a), the water quality criteria guidance, to protect human health, as the pollutant concentration in ambient surface water. In January 2001, the U.S. EPA (2001) concluded that it is more appropriate, at this time, to derive a fish tissue (including shellfish) residue water quality criterion for MeHg rather than a water column-based water quality criterion. This criterion is based directly on the dominant human exposure route to MeHg.

Many factors have been considered as important in the bioaccumulation and/or biomagnification of Hg in fish. Among them, the Hg load-dependent factors in the aquatic environment, specially those related to Hg in sediments and environmental conditions, like bio-production (Häkanson, 1980; 1991); as well as local biota's physiological-dependent factors, like size, length, age and metabolic rate (Phillips, 1980; WHO, 1990); and, in addition, the food-chain characteristics (Cabana et al, 1994). In general, the Hg levels in fish muscles show large inter-individual variabilities, resulting in very high values for relative standard deviation (U.S. EPA, 1999). Hg levels in fish for spatial and/or temporal comparisons have been normalized by the average Hg content in 1 Kg of fish (as pike) (Johnels *et al*., 1967; Häkanson, 1991), by using only fish of one year old (Post et al, 1996), or a specific length (Scruton, et al., 1994), or a specific weight (Watras et al., 1998). Castilhos *et al* (2001) suggested a field dose-response approach as a tool for assessing the environmental Hg contamination, through the mercury analysis in fish. Dose-response approach is usually used for analysis of data from laboratory or epidemiologic studies. However, we have proposed its use for assessment of Hg contamination in fish sampled from field. This methodology was used to assess the Hg contamination, in the Tapajós River Region, caused by gold mining activities.

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The results showed different daily uptake doses by Tucunaré (*Cichla* spp.) between non-contaminated and contaminated areas (~4.0 times), and such differences could be attributed to different Hg loading rates between the studied areas.

We have tested the applicability of the proposed methodology (DRAC) by using the literature data. Seven species of fishes from 8 different aquatic ecosystems, including lentic and lotic freshwater, estuarine and marine ecosystems, that were chosen from scientific publications, in which Hg levels in muscles and estimated age and/or measured length for individual specimens, were available. The objectives of this work were: (i) to establish and compare the dose-response relationship for Hg accumulation by different fish species, from several ecosystem and collection time (ii) to estimate and compare the daily Hg uptake rate by those different fishes; (iii) to estimate and compare the potential exposure time necessary for Hg accumulation to reach 500μg.Kg^{-1}, the limit concentration for human consumption, adopted in many countries, and, (iv) to present this approach, in addition to that expressed by U.S. EPA, for water quality criteria for methyl-mercury.

MATERIALS AND METHODS

Seven species of fish, from 8 different aquatic ecosystems, including lentic and lotic freshwater, estuarine and marine ecosystems were chosen from scientific publications, in which Hg levels in muscles and estimated age or measured length for individual specimens were available. For the literature that shows the relationship between the Hg levels in tissues and the size and/or age of fish, in a graphical form, the program *"GIF Coordinates"* was used for the coordinate acquisition (Schneider, 2000).

The "DRAC" methodology was described in a previous work (Castilhos et al., 2001), and a brief description is presented as follows. The dose-response relationship has the competence to absorb inter-individual variabilities. The responses are of two kinds: Quantal and quantitative. In a quantitative test, an organism either or not shows the response under study. Thus, a certain percentage of test organisms will show the response within some stated conditions. In a quantitative or graded test, each organism responds to a distinct degree. The Quantal test are designed to estimate the concentration of a test material that affects 50% of the test organisms, the mean effective dose (ED 50% or ED50). One must choose the effect to be observed. Thus, this is a quantal rather than a graded response, since the specific effect is either present or absent. The ED50, for accumulation of Hg by fish, indicate the exposure time necessary to attain those tissue concentration levels by half of the exposed individuals. Some methods are used to calculate ED50, and, among them, there is the "probit" method (American Public Health Association, 1985; Ross and Gilman, 1985). The potential exposure times were inferred from estimated age and were transformed in their logarithms. The frequency of responses were transformed in "probit" units. The D_{50} for accumulation of Hg by fish (accumulation dose 50 or AD_{50}) indicates the exposure time necessary to attain those tissue concentration levels by half of the exposed individuals. This resulting time can be related to response, as follows: $t_{exposure} * C$ = constant (adapted from Dämgen & Grünhage, 1998); in which a certain response (K, constant) can be achieved from a exposure time ($t_{exposure}$) and the concentration in the aquatic environment C; such concentration will result as a potential dose or daily uptake rate (DUR), expressed in $\mu g.Kg^{-1}.d^{-1}$. From these results one could estimate the exposition time to reach either 300 $\mu g.Kg^{-1}$ or 500 $\mu g.Kg^{-1}$ and compare the contamination magnitude (or bioavailability) among different aquatic ecosystems.

RESULTS AND DISCUSSION

In environmental hazard analysis, the ecological effect of a specific substance on an organism or group of organisms in an aquatic system can be described as a function of the dose during a defined period of time. The direct bioaccumulation factor (BAF) or bioconcentration factor (BCF) of Hg is defined as the ratio of Hg concentration in fish tissue to the Hg concentration in water. The indirect bioaccumulation or biomagnification is the accumulation of a chemical in a given species according to its trophic levels in the food chain (Bruggeman, 1982). Bioaccumulation and/or biomagnification are the most direct study of mercury reaction at the environmental interface of the aquatic organisms. In this particular context, the ecological effect is defined as Hg-content in fish.

Although the bioaccumulation/biomagnification processes have not been traditionally interpreted as a pharmacological/toxicological "effect", one could suggest that as higher the internal dose (bioaccumulation), the higher is the potential aquatic risk. For instances, MeHg has been classified as clastogen substance and toxic effects such as icthyomutagenesis have been associated to MeHg exposure and dose intensity (probabilistic risk) (Schoeny, 1997). Moreover, several effects show a positive correlation with exposure but a causal-effect relationship is a difficult task to access, but bioaccumulation/biomagnification processes, which have to have, at least, exposure conditions for a determinant chemical agent.

The U.S. EPA (2001) concluded that it is more appropriate to derive a fish tissue (including shellfish) residue water quality criterion for MeHg rather than a water column-based water quality criterion, by using the following equation.

$$TRC = \frac{BW \times (RfD - RSC)}{\Sigma^4_{i=2} FI_i}$$

Where:

TRC = Fish tissue residue criterion (mg methyl-mercury/ kg fish) for freshwater and estuarine fish

RfD = Reference dose (based on non-cancer human health effects) of 0.0001mg / kg body weight-day

RSC = Relative source contribution (subtracted from RfD to account for marine fish consumption) estimated to be 2.7×10^{-5} mg methyl-mercury / kg body weight-day

(RfD – RSC) = (1x 10^{-4} – 0.27x10^{-4}) = 0.73x10^{-4} mg methyl-mercury / kg body weight-day or 0.073 μg methyl-mercury / kg body weight-day

BW = Human body weight (default value of 70 kg) (for adults)

FI = Fish intake at trophic level (TL) i (i = 2, 3, 4); total intake is 0.0175 kg fish / day for general adult population.

The resulting Fish Residue Criterion is 0.3 mg methylmercury/kg fish. This is the concentration in fish tissue that should not be exceed based on a total fish and shellfish consumption-weighted rate of 0.0175 kg fish.d^{-1}.

The Reference Dose (RfD) is an estimate (with total uncertainties spanning up to five orders of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious health effects during a lifetime. In the risk assessment discussion U.S. EPA uses the National Research Council (NRC)-recommended Benchmark Dose Lower Limit (BMDL) of 58 ppb mercury in cord blood as an example of RfD calculation. By using an one-compartment model, the methylmercury ingestion dose that corresponds to a cord blood level of 58ppb, is 1.081 μg.Kg^{-1} bw.d^{-1}. Considering the uncertainties factors, the RfD for methylmercury resulted in 0.1 μg.kg^{-1} bw.d^{-1} or 1x10^{-4} mg.kg^{-1} bw.d^{-1}. Based on available data, human exposures to methylmercury from all media sources except freshwater/estuarine and marine fish are negligible, both in comparison with exposures from fish and compared with RfD. However, ingestion of marine fish is a significant contributor to the total methylmercury exposure. For the methylmercury criterion, the RSC is the estimated exposure from marine fish intake. This is substracted from the RfD when calculating the water quality criterion, and represents almost 30% of the RfD.

It has been repeatedly shown that mercury in fish accumulates throughout the lifetime of the individual, and BAF values for given species vary as a function of ages of the animals examined. Most of the water body-specific BAFs and resulting level distributions, are based on "opportunity" (whatever you catch, you include), composite samples and do not report age or size of individuals sampled (U.S EPA, 2001). Then, one only can sample very small fish specimens to show adequacy to the TRC value.

The results are presented in Table 1, which displays the popular name of the fish, the local and time of sampling, food habit, number of collected specimens, observed effect (OE), daily uptake rate estimate (DUR), estimate of time of exposition to reach 500 μg.Kg^{-1}, and references. Comparing the OE values (mean Hg concentration in μg.kg^{-1}) in the population sampled, for piscivorous and zooplanktivorous fishes, one could suggest the following crescent order of contamination or crescent contamination magnitude/bioavailability: Sepetiba Bay (corvina) < Ilha Grande Bay (corvina) = Guanabara Bay (corvina) = lagoons-Santarém (tucunaré) < Conceição Lagoon (corvina) < Tongue River- reservoir (white crapie) < Tongue River-reservoir (walleye) = Tongue River-reservoir (pike) = Tapajós River (tucunaré) < Tongue

River- reservior (sauger) < Gulf of Trieste (conger-conger) < Tucuruí River-reservoir (tucunaré). However, considering the daily uptake rate estimates and/or the time necessary for half of the specimens to reach $500\mu g.Kg^{-1}$, the order of contamination is altered to: Tongue River-reservoir (white crapie) < Conceição Lagoon (corvina) < Ilha Grande Bay < Guanabara Bay < Sepetiba Bay < Tongue River-reservoir (pike = sauguer= walleye) = lagoons-Santarém (tucunaré) < Tapajós River (tucunaré) < Gulf of Trieste (conger-conger) < Tucuruí River-reservoir (tucunaré).

Considering TRC, sauger (Tongue River), tucunaré (Tucuruí Reservoir) and conger-conger (Gulf of Trieste) showed Hg levels above the limit. However, looking at the estimated daily uptake rates, northern pike, sauger and walleye (Tongue River) and tucunaré (lagoons=Santarém) showed the same value: $0.2\mu g.kg^{-1}d^{-1}$. The estimated daily uptake rates for conger-conger (Gulf of Trieste), tucunaré from Tapajós River and tucunaré from Tucuruí Reservoir resulted in 2 times, 4 times and 8 times higher than $0.2\mu g.kg^{-1}d^{-1}$, respectively.

Considering data from the work relative to *Micropogonias furnieri*, when the average of Hg levels are normalized with respect to the average weight of the fish (not presented here), no alteration in the order of contamination is achieved, as compared to the order obtained by using DRAC. On the other hand, the zooplanktivorous fish from Tongue River reservoir became, as expected, the less contaminated specie compared to carnivorous and piscivorous fish. For the Tucuruí system the possible differences in the tucunaré growth rate should be investigated, since the literature data indicate the existence of dwarf species in reservoirs (Doyon et al., 1998).

Not surprising, some data may be lost during their transference from the plots of original articles to the "GIF coordinates", and the results might be improved by using the original data. However, the present objective was to test the applicability of the dose-response approach as a tool for environmental assessment mercury contamination by using the literature data. One of the ways to try to establish the weakest, most sensitive and uncertain part of a model is to conduct sensitivity tests. The selection of dose-response models (in this work, probit) and the effect of uncertainties in these models should be evaluated. Indeed, the results might not represent, actually, the magnitude of mercury contamination/availability among sites, because there are significant differences in temporal sampling as well as in analytical procedures.

We suggest that, in addition to TRC (0.3 mg methylmercury / kg fish) for specific fish specie, one could estimate the daily uptake rate, which may express the bioavailability of mercury in a defined aquatic ecosystem, and compare the time necessary to attain the TRC value. For using the dose-response approach, one should collect about 30 individuals (within intervals related with percentage of the specie-specific maximum length resulting three points in the dose-response relationship). Such approach might permit an integrate comparison among different aquatic ecosystems using the same or different fish species. The DRAC is a simple and fast

Table 1. Popular and scientific names, food habit, locality, date and number of fish collected (N), observed effect (OE;$\mu g.Kg^{-1}$), estimated daily uptake rate (DUR; $\mu g.Kg^{-1}.d^{-1}$), estimated time of exposure to attain either 300$\mu g.Kg^{-1}$ (T300, years) or 500$\mu g.Kg^{-1}$ (T500, years) and references.

Popular name	Scientific name	FH*	Locality	Date	N	OE ($\mu g.Kg^{-1}$)	DUR ($\mu g.Kg^{-1}.d^{-1}$)	T300 (years)	T500 (years)
Northern Pike	*Esox lucius*	P	Tongue River- Reservoir (EUA)[1]	1978	56	300	0.2	4.1	6.8 [5]
Sauger	*Stizostedion canadense*	P	Tongue River- Reservoir (EUA)[1]	1978	31	350	0.2	4.1	6.8 [5]
Walleye	*Stizostedion vitreum*	P	Tongue River- Reservoir (EUA)[1]	1978	26	300	0.2	4.1	6.8 [5]
White crapie	*Pomoxis annularis*	Z	Tongue River- Reservoir (EUA)[1]	1978	36	200	0.05	16.4	27.0 [5]
Corvina	*Micropogonias furnieri*	C	Guanabara Bay (RJ, BR)[2]	1992	56	100	0.15	5.5	9.1 [6]
Corvina	*Micropogonias furnieri*	C	Ilha Grande Bay (RJ, BR)[2]	1990-1991	57	100	0.13	6.3	10.5 [6]
Corvina	*Micropogonias furnieri*	C	Sepetiba Bay (RJ, BR)[2]	1990-1991	60	80	0.10	8.2	13.7 [6]
Corvina	*Micropogonias furnieri*	C	Conceição Lagoon (SC, BR)[2]	1990-1991	42	130	0.08	10.3	17.0[6]
Tucunaré	*Cichla spp*	P	Lagoons –Santarém (PA, BR)[3]	1992	28	100	0.2	4.1	6.8
Tucunaré	*Cichla spp*	P	Tapajós River (PA, BR)[3]	1992	41	300	0.8	1.0	2.0
Tucunaré	*Cichla spp*	P	Tucuruí River- Reservoir (PA, BR)[4]	1995	61	1000	1.65	0.5	0.8 [5]
	Conger conger	P	Gulf of Trieste (Slovenia) [7]	1995-1996	25	610	0.4	2.1	3.4

* P = piscivorous; C= carnivorous benthic; Z = zooplanktivorous

[1]-Phillips et al., 1980; [2]-Kherig, 1992 (data non-normalized); [3]-Castilhos et al., 2001; [4]-Porvari, 1995; [5]-Castilhos & Lima, 2001; [6]-Castilhos, 1999; [7]-Horvat et al.,1999.

methodology and can be applied to any data bank including spatial and temporal contamination assessment for environmental contaminants that show bioaccumulation.

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