

# Ecological and biological factors controlling the concentrations of trace elements (As, Cd, Cu, Hg, Se, Zn) in delphinids *Globicephala melas* from the North Atlantic Ocean

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**ABSTRACT:** Trace elements (As, Cd, Cu, Hg, Se and Zn) were determined in liver and kidney of pilot whales *Globicephala melas* Traill, 1809 collected from 7 schools caught at different seasons around the Faroe Islands. These and other biological data at our disposal enabled us to confirm and to define more accurately the relations shown previously between age, sex and trace element concentrations in marine mammals. The most striking features were: (1) the elevated levels of Cd and Hg in pilot whales compared to other marine mammals and to minimum adverse-effect levels established for humans; (2) the biocumulative behaviour of Cd and Hg; (3) the high correlations between Hg and Se, predominantly influenced by age; and (4) the high correlations between Cd and Zn, the levels of which appear to be mainly dependent on the school to which the specimens belong. This last may be tentatively attributed to the fact that the schools sampled correspond to different sub-populations with different genetic characteristics. The apparent metal tolerance of pilot whales and the health consequences to consumers of flesh of marine mammals are discussed.

**KEY WORDS:** Marine mammals · Heavy metals · Interactions

## INTRODUCTION

The consumption of flesh of marine mammals has been recognized as a way of exposure to cadmium and mercury in the human Faroese population (Andersen et al. 1987, Jean-Caurant & Amiard-Triquet 1991). Metal concentrations in pilot whales *Globicephala melas* Traill, 1809 caught in the drive fishery of the Faroe Islands in 1977 and 1978 (Julshamn et al. 1987) were generally higher than those encountered in other species (Johansen et al. 1980, Helle 1981, Falconer et al. 1983, Wagemann et al. 1983, Ronald et al. 1984, André et al. 1990a, b, 1991). A significant fraction (about 75 %) of the population of pilot whales sampled off the Faroe Islands had blood Cd concentrations higher than minimum adverse-effect levels established for humans (Caurant & Amiard-Triquet unpubl.). It is difficult, however, to determine if anthropic activities

have a decisive influence on metal concentrations in animals living far from industrialized areas, such as pilot whales caught off the Faroe Islands or seabirds from the South Atlantic (Muirhead & Furness 1988).

Numerous trace elements are able to interact (Momcilovic 1988) inducing synergetic or antagonistic changes in metal accumulation or toxicity. Interactions can result from competition for binding sites on proteinic ligands, such as metallothioneins. The relations between zinc, copper and other metals with regard to metallothionein levels have been reviewed by Cosson et al. (1991). In some cases, direct interactions were demonstrated through the formation of metallic compounds such as HgSe crystals, the presence of which has been demonstrated in the hepatic cells of a marine mammal, *Ziphius cavirostris* (Martoja & Berry 1980). Consequently, in the present study the concentrations of essential and toxic elements were determined con-

comitantly in organs (liver, kidney) that play a key role in biotransformation and elimination.

As high-level predators with a long life span, delphinids can be regarded as ecotoxicological models for the study of cumulative and biomagnified pollutants. The present study was a part of a larger investigation on the biology of pilot whales *Globicephala melas* caught in the Faroe Islands drive fishery (Donovan in press). This year-round fishery presents an excellent opportunity to obtain numerous data on this species, because schools contain individuals of both sexes, and a wide range of ages and all stages in the sexual cycle are available for sampling. Ecotoxicological findings will be compared to classical factors mentioned above as well as food habits (Desportes & Mouritsen 1989) and genetic characteristics of schools (Andersen in press).

## MATERIALS AND METHODS

Liver and kidney samples for trace element analysis were collected from whales of 7 schools (Schools I to VII) caught at different seasons over a 1 yr period. Sampling characteristics are given in Table 2 (see 'Results').

Arsenic (As), cadmium (Cd), copper (Cu), mercury (Hg), selenium (Se) and zinc (Zn) were determined in fresh tissue. Tissue samples (about 2 g) were heated with supra-pure nitric acid at 65°C until the solution was clear.

Cd, Cu and Zn were determined by Flame Atomic Absorption Spectrophotometry (AAS) and As and Se by electrothermal AAS using the Zeeman effect (Amiard et al. 1987). Total mercury was analysed by Cold Vapour AAS (Boiteau & Pineau 1988).

As analytical quality control, standards of the NRC Canada (lobster hepatopancreas TORT1, dogfish liver DOLT1 and dogfish muscle DORM1) were analysed using the same procedure (Table 1). Measurements of As, Cu, Se and Zn were validated by International Intercalibration Exercises (IAEA 1987, 1988).

Trace element concentrations were analysed using a fixed model ANOVA (Sokal & Rohlf 1981) with a randomized block factorial design (RBF design). The randomized block procedure acknowledged the fact that concentrations of trace elements could vary substantially from block to block

i.e. school to school depending on various factors, genetical and environmental (Julshamn et al. 1987, Andersen in press). Two fixed factors were considered: sex with 2 classes (male and female), and age with 4 classes: 0+ (0 to 5 yr), 5+ (5 to 10 yr), 10+ (10 to 20 yr) and 20+ (older than 20 yr). Missing age data were estimated using a length/age relation (Fig. 1) from a previous study (Bloch & Lockyer 1989). According to Kirk (1968) this design is a RBF 2-4 design. It allowed elimination of school-to-school variation from the comparison of the respective influences of age and sex on trace metal concentrations.

Schools IV, V & VI with unsuitable numbers of male whales were eliminated from the ANOVA analysis. This arrangement of data allowed a complete blocks design with one mean concentration per treatment in spite of unequal school sizes and disproportionality in age frequency distribution within schools. However, in the case of kidney data, 2 missing values were encountered. They were estimated following the Yates procedure (Yates 1940). To satisfy the assumptions of normality and homoscedasticity, data were transformed ( $\log_{10} x$ ) prior to analysis, if necessary. The hypothesis of no interaction between schools (blocks) and treatments was tested using Tukey's test (Tukey 1949). Then, the Newman-Keuls test was used to perform a posteriori multiple comparisons among means (Kirk 1968).

Polynomial regressions (Sokal & Rohlf 1981) were performed for all individual data to examine nonlinear

Table 1. Results of internal quality controls (concentrations in mg kg<sup>-1</sup> dry wt; mean and confidence interval at the 95% level) using standard reference materials from the National Research Council (NRC) Canada

	Lobster hepatopancreas TORT-1	Dogfish muscle DORM-1	Dogfish liver DOLT-1
<b>Arsenic</b>			
Certified value	–	–	10.1 ± 1.4
Our value	–	–	9.97 ± 1.48
<b>Cadmium</b>			
Certified value	26.3 ± 2.1	0.086 ± 0.012	4.18 ± 0.28
Our value	27.8 ± 1.5	0.087 ± 0.035	4.14 ± 0.18
<b>Copper</b>			
Certified value	439 ± 22	5.22 ± 0.33	–
Our value	434 ± 13	5.65 ± 0.40	–
<b>Selenium</b>			
Certified value	6.88 ± 0.47	1.62 ± 0.12	–
Our value	6.82 ± 0.33	1.26 ± 0.12	–
<b>Mercury</b>			
Certified value	0.33 ± 0.06	0.798 ± 0.074	0.225 ± 0.037
Our value	0.32 ± 0.02	0.788 ± 0.136	0.219 ± 0.010
<b>Zinc</b>			
Certified value	177 ± 10	21.3 ± 1.0	92.5 ± 2.3
Our value	173 ± 3	21.6 ± 0.8	92.3 ± 1.8

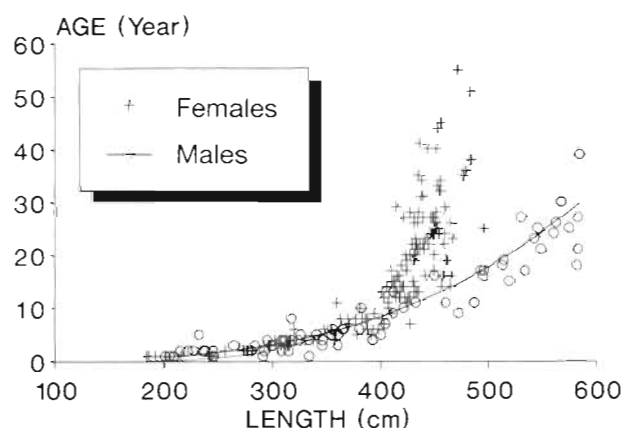


Fig. 1 *Globicephala melas*. Relationship between age and length of pilot whales

relations between trace metals, age, sex and schools when significant deviations around linear trend were found. Age was considered as a quantitative variable when sex and school were considered as qualitative variables. Thus, SEX = 1 if the individual is a female and SEX = 0 if not, and SCHOOL<sub>*i*</sub> = 1 if the individual belongs to school *i* and SCHOOL<sub>*i*</sub> = 0 if not. Selection of variables and power of variables depended on both ANOVA results and stepwise procedure in order to obtain an equation with the smallest set of significant variables. Coefficients were obtained by least squares nonlinear regression using the algorithm of Marquardt (1963). A posteriori multiple comparisons among coefficients were performed by Newman-Keuls test adapted for unequal number of data (Kramer 1956).

Linear correlations among trace metal concentrations were performed, but prior to analysis of correlation coefficients, a test of linearity was used (Dagnélie 1986). If the test of linearity was significant linear correlation coefficients were calculated.

## RESULTS

The mean concentrations of trace elements in the liver and the kidney are shown in Table 2. The variability of coefficients of variation (CV) reflected the individual variations typical of each element in one school: in both organs, the lowest % CVs were for the essential metals Cu and Zn, the individual variations of which are known to be limited as a consequence of homeostasis processes, whereas the highest ones were for Hg and Se.

Cu and Zn were higher in liver than in kidney, while the opposite was observed for Cd. However, the most striking feature is the higher concentrations of liver Hg and Se compared to kidney.

## Relations between trace element concentrations, age and sex of individuals

In both organs, Cd, Cu, Hg and Se concentrations increased with age ( $p < 0.01$ ; Tables 3 & 4, Figs. 2 & 3), however As and Zn in the liver were independent of age. However the significant interaction in the case of Zn in the liver (significant Tukey's test; Table 3) indicated that the influence of both factors, age and sex, cannot be described for all schools as a whole, but instead must be described individually for each school. Probably this interaction was a consequence of the presence of numerous pups in School II.

Except in the case of As and Zn in the liver, trace element levels were higher in females than in males (Table 3, Fig. 4). Trace elements did not differ between sexes in the kidney, except for Cu (Table 4, Fig. 5).

Table 2. *Globicephala melas*. Bioaccumulation of trace elements in the liver and in the kidney of pilot whales. For each school and each metal, the 3 values represent successively the mean concentration ( $\mu\text{g g}^{-1}$  wet wt), the standard deviation and the coefficient of variation (%). N: no. of individuals

School Sampling date	As	Cd	Cu	Hg	Se	Zn
<b>Liver</b>						
II	0.55	41	6.0	56	20	87
Sep 1986	$\pm 0.23$	$\pm 32$	$\pm 1.9$	$\pm 83$	$\pm 25$	$\pm 34$
N = 52	(42)	(77)	(31)	(148)	(127)	(39)
III	-	77	7.2	-	-	77
Nov 1986	-	$\pm 35$	$\pm 2.0$	-	-	$\pm 23$
N = 28	-	(45)	(28)	-	-	(29)
IV	0.20	80	5.6	52	13.6	76
Apr 1987	$\pm 0.12$	$\pm 29$	$\pm 0.9$	$\pm 38$	$\pm 8.9$	$\pm 16$
N = 11	(60)	(36)	(17)	(72)	(65)	(29)
V	0.46	57	4.9	62	16.5	78
Jul 1987	$\pm 0.19$	$\pm 29$	$\pm 1.2$	$\pm 57$	$\pm 14.6$	$\pm 15$
N = 22	(41)	(50)	(25)	(92)	(88)	(19)
VI	-	91	7.1	-	-	109
Jul 1987	-	$\pm 61$	$\pm 2.4$	-	-	$\pm 37$
N = 41	-	(66)	(34)	-	-	(34)
VII	0.43	33	6.0	84	23	54
Oct 1987	$\pm 0.13$	$\pm 19$	$\pm 1.7$	$\pm 92$	$\pm 24$	$\pm 9$
N = 40	(30)	(56)	(28)	(109)	(105)	(16)
<b>Kidney</b>						
I	-	86	4.4	-	-	43
Jul 1986	-	$\pm 49$	$\pm 0.9$	-	-	$\pm 9$
N = 43	-	(57)	(21)	-	-	(20)
II	0.37	93	4.8	5.7	4.5	37
Sep 1986	$\pm 0.19$	$\pm 45$	$\pm 0.9$	$\pm 3.8$	$\pm 1.6$	$\pm 5.3$
N = 23	(51)	(48)	(19)	(66)	(35)	(13)
VII	0.18	55	3.8	4.9	3.1	35
Oct 1987	$\pm 0.06$	$\pm 20$	$\pm 1.0$	$\pm 3.8$	$\pm 1.1$	$\pm 4.4$
N = 31	(33)	(36)	(27)	(78)	(35)	(13)

Table 3. Significance level, residual and Tukey's test table for RBF 2-4 ANOVA on liver trace elements. As, Hg and Se: Schools II and VII; Cd, Cu and Zn: Schools II, III and VII. Significant probabilities at the 5 % level are underlined

Source	Trace element					
	As	Cd	Cu	Hg	Se	Zn
Sex (2)	0.34	<u>0.02</u>	<u>&lt;0.01</u>	<u>&lt;0.01</u>	<u>&lt;0.01</u>	0.22
Age (4)	0.08	<u>&lt;0.01</u>	<u>&lt;0.01</u>	<u>&lt;0.01</u>	<u>&lt;0.01</u>	0.40
Age x Sex	0.46	<u>0.44</u>	0.40	<u>0.02</u>	0.15	0.91
School (Block)	<u>&lt;0.01</u>	<u>&lt;0.01</u>	<u>0.01</u>	0.24	0.24	<u>&lt;0.01</u>
Residual	13 %	26 %	12 %	30 %	30 %	17 %
Tukey's test	0.58	0.71	0.45	0.69	0.23	<u>&lt;0.01</u>

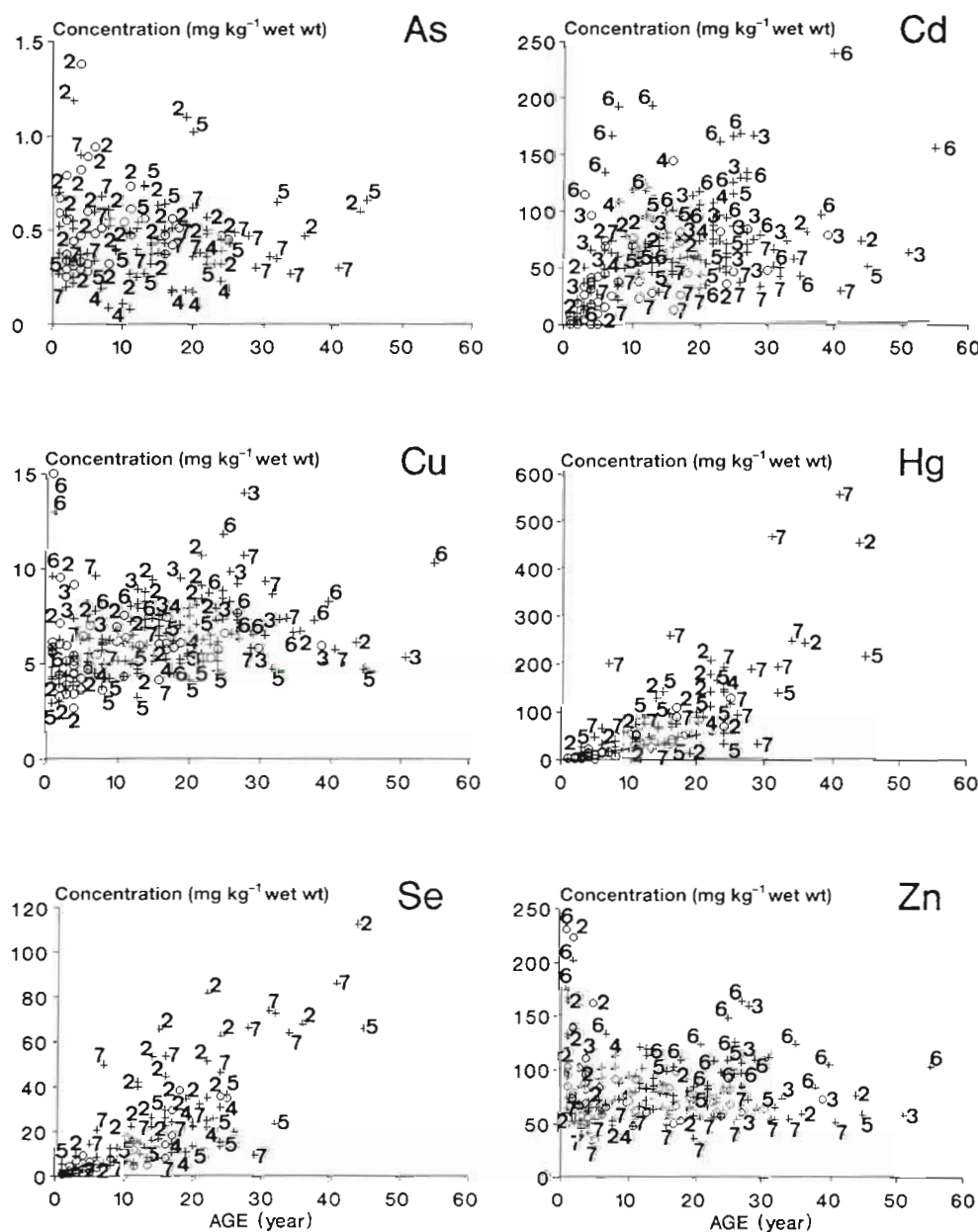


Fig. 2. *Globicephala melas*. Coded scatterplot of liver trace metal concentrations (As, Cd, Cu, Hg, Se and Zn) versus age of pilot whales. Each point code provides the school number (1 to 7) and the sex of individuals (○: male; +: female)

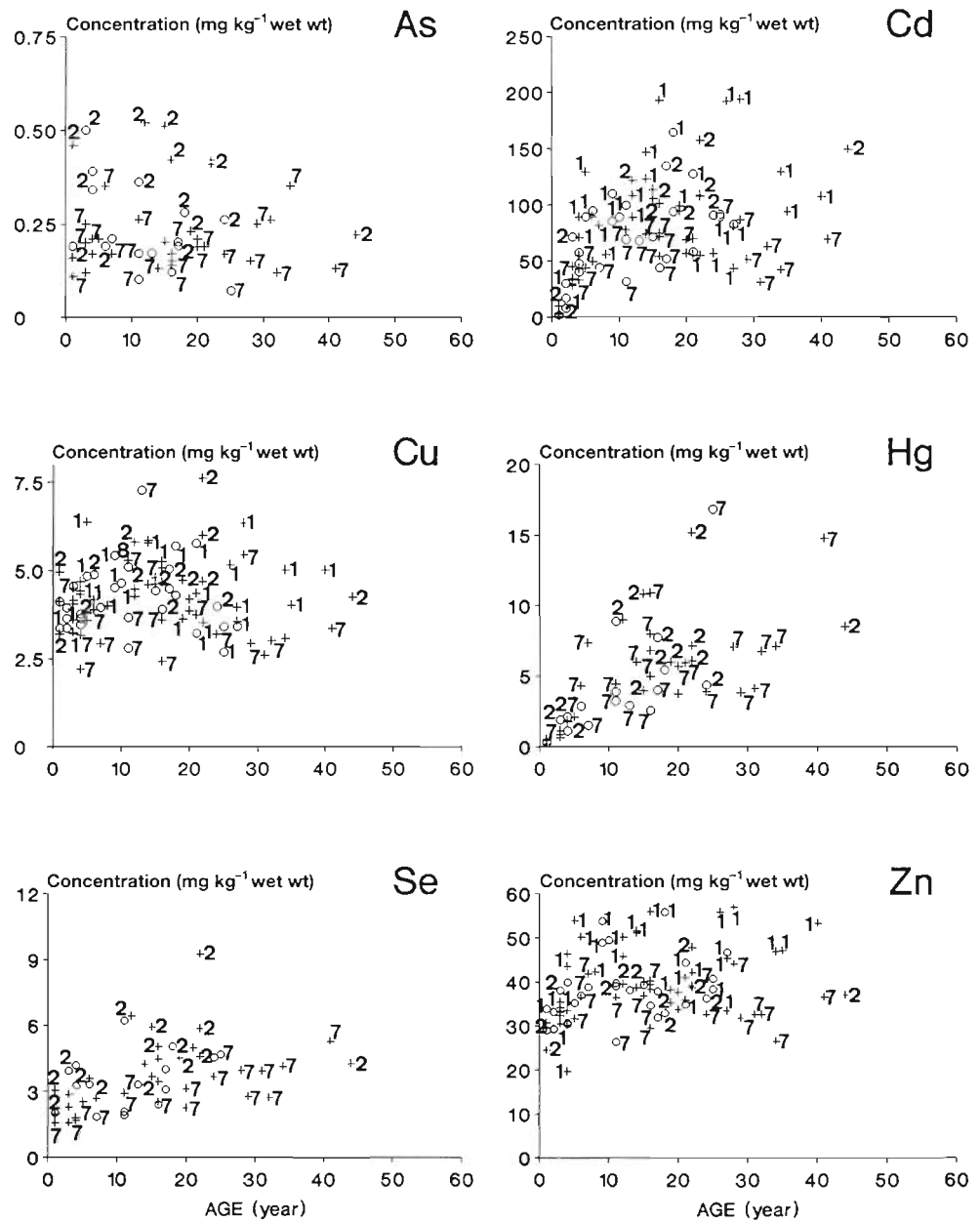


Fig. 3. *Globicephala melas*. Coded scatterplot of kidney trace metal concentrations (As, Cd, Cu, Hg, Se and Zn) versus age of pilot whales. Each point code provides the school number (1 to 7) and the sex of individuals (o: male; +: female)

Table 4. Significance level, residual and Tukey's test table for RBF 2-4 ANOVA on kidney trace elements. As, Hg and Se: Schools II and VII; Cd, Cu and Zn: Schools I, II and VII. Significant probabilities at the 5 % level are underlined

Source	Trace element					
	As	Cd	Log Cu	Log Hg	Log Se	Zn
Sex (2)	0.06	0.30	0.31	0.19	0.15	0.28
Age (4)	0.62	<u>&lt;0.01</u>	<u>&lt;0.01</u>	<u>0.03</u>	<u>&lt;0.02</u>	<u>&lt;0.01</u>
Age × Sex	0.35	0.80	<u>&lt;0.01</u>	0.52	0.24	0.80
School (Block)	0.06	<u>&lt;0.01</u>	<u>&lt;0.01</u>	0.54	<u>&lt;0.01</u>	<u>&lt;0.01</u>
Residual	23 %	23 %	49 %	41 %	13 %	8 %
Tukey's test	0.58	0.38	0.76	0.38	0.35	0.11



The interaction between sex and age (Tables 3 & 4) was only significant in the case of Hg in liver and Cu in kidney. The accumulation rate of Hg in liver was higher for females than males (Fig. 4) and can be modeled as a function of age and a second order term, i.e. interaction between sex and age<sup>2</sup>, following the equation:

$$\text{Hg} = A_1 \cdot \text{AGE} + A_2 \cdot \text{AGE}^2 \cdot \text{SEX} \quad (1)$$

with  $A_1 = 3.20$  (SD = 0.57)  
and  $A_2 = 0.12$  (SD = 0.02) (N = 127) (Fig. 6)

This model explained 66 % of the data variability.

In the case of Cu in the kidney, the oldest females (over 20 yr old) showed significant higher levels than males (Fig. 5).

### Comparison of trace element levels in different schools (blocks)

Besides age and sex, which appeared as major factors controlling the levels of trace elements, a significant variability existed among schools (Tables 3 & 4), except for Hg and Se in liver, and As and Hg in kidney. However a separate analysis performed on data from females also showed a significant variability among Schools II, IV, V & VII for Hg and Se.

Multiple regression analysis performed on the Cd data tested for differences among schools, taking into account the influence of sex and age revealed by the ANOVA. Cadmium concentrations for both liver and

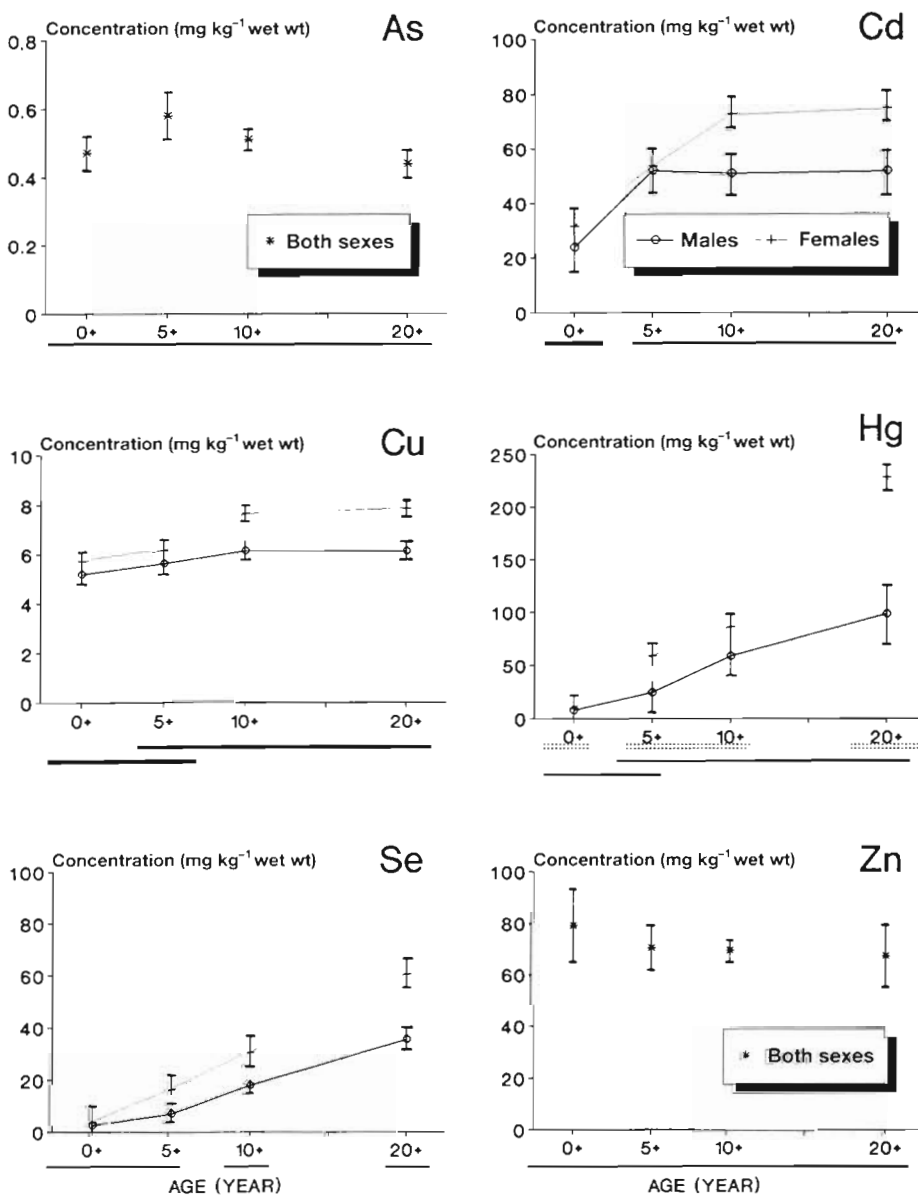


Fig. 4. *Globicephala melas*. Influence of age and sex on liver trace metal concentrations according to RBF 2-4 ANOVA. Verticals bars indicate 95% confidence intervals. Lines under the horizontal axis indicate homogeneous groups of means that are not significantly different ( $p > 0.05$ ). See Table 3 for details on significant level of factors and interactions

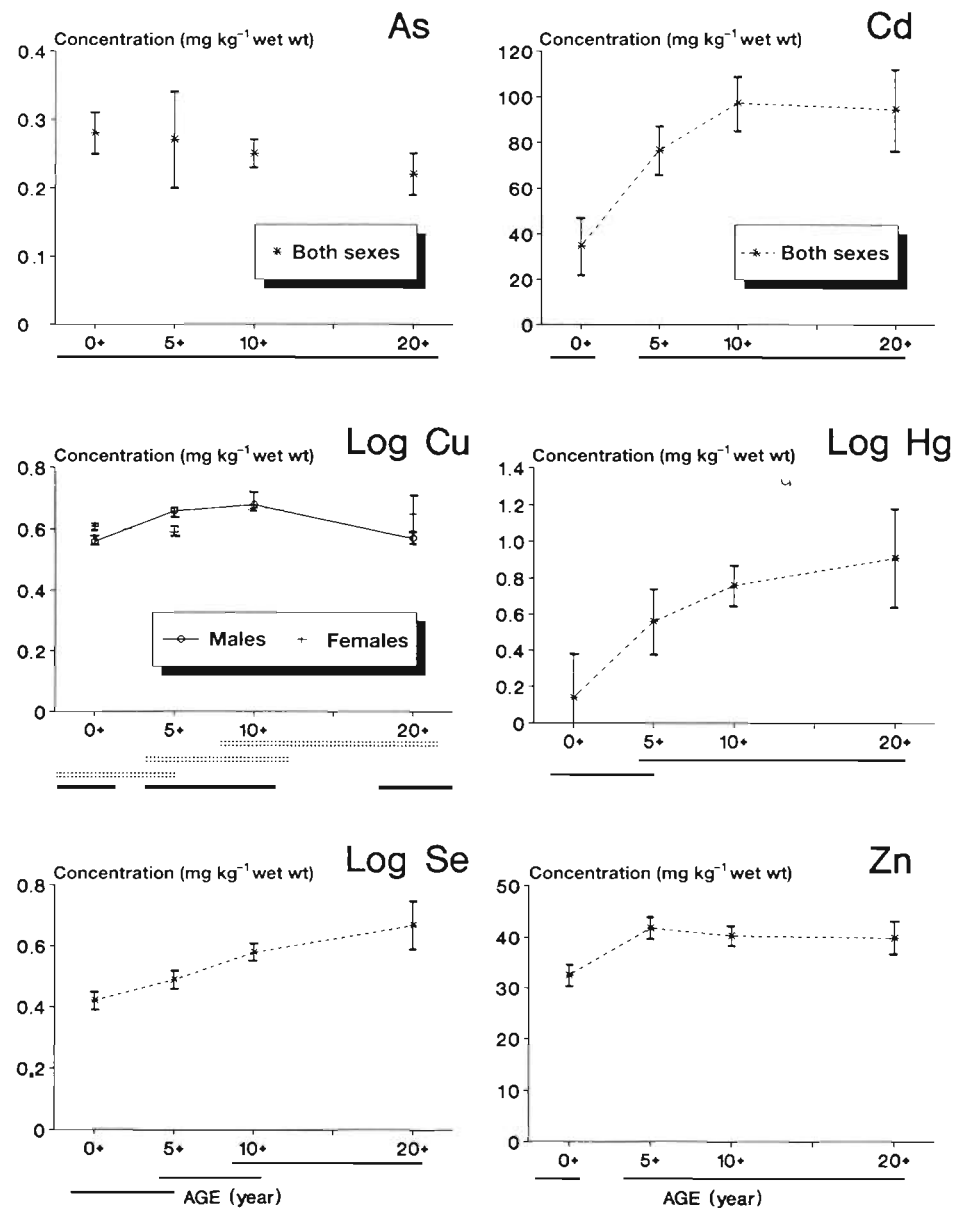


Fig. 5. *Globicephala melas*. Influence of age and sex on kidney trace metal concentrations according to RBF 2-4 ANOVA. Verticals bars indicate 95% confidence intervals. Lines under the horizontal axis indicate homogeneous groups of means that are not significantly different ( $p > 0.05$ ). See Table 4 for details on significant level of factors and interactions

kidney were modeled as a function of age, sex and school following the equation:

$$\text{Cd} = (A_0 \cdot \text{SEX} + \sum_{i=1}^7 A_i \cdot \text{SCHOOL}_i) \cdot \text{AGE} / (\text{AGE} + A_8) \quad (2)$$

This model explained 55% of the data variability.

Coefficients  $A_0$  and  $A_8$  depended on the sex and the age of whales, respectively. Each  $A_i$  coefficient was the asymptote of a response curve depending on each school<sub>*i*</sub>.

Multiple comparisons among school asymptotic coefficients for Cd liver could be summarised as School VII < School V = School II < School IV = School III < School VI (Table 5). In the case of kidney Cd data, School VII was significantly different from Schools I and II.

Hg liver concentrations appeared to be independent of schools (cf. Eq. 1).

The presence of a school × treatment interaction indicated that the effects of a treatment cannot be described for all schools as a whole but must be described individually for each school.

### Correlations within pairs of trace elements

Linear correlations were established between metals using individual liver concentrations from all schools. The linearity was tested (Dagnélie 1986). Deviations from a linear trend were partly due to school-to-school variability. Liver As concentrations were independent

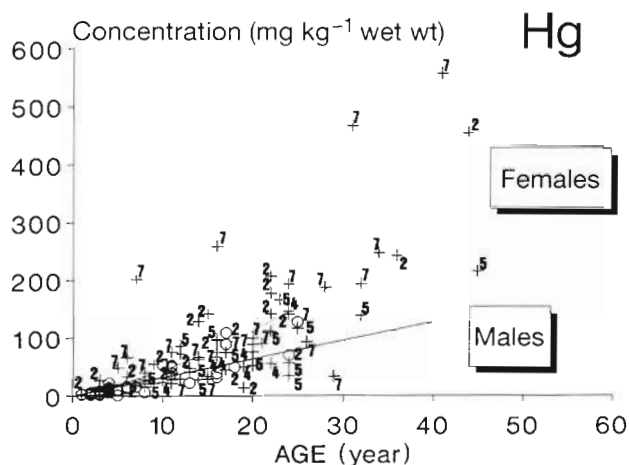


Fig. 6. *Globicephala melas*. Hg liver concentrations as a non-linear function of age and sex. Numbers 2, 4, 5 and 7 correspond to Schools II, IV, V and VII for males (o) and females (+) of pilot whales

Table 5. Coefficient values for nonlinear regression model established for Cd concentrations in liver and kidney. Organ, coefficient, mean, standard deviation (SD), number of data (n) and group (homogenous school coefficients are indicated by block capitals)

Organ	Coefficient	Mean	SD	n	Group
Liver	Sex	22.93	7.32	–	–
	Age	3.26	0.96	–	–
	School				
	II	58.08	9.37	53	C
	III	87.85	9.97	28	B
	IV	77.23	14.17	11	B
	V	55.44	11.52	23	C
	VI	102.79	11.15	40	A
	VII	31.14	8.83	40	D
Kidney	Sex	11.03	9.24	–	–
	Age	4.16	1.16	–	–
	School				
	I	122.79	12.55	39	A
	II	132.79	14.26	21	A
	VII	68.84	11.19	31	B

of all other elements (Table 6). On the other hand, the highest correlation coefficient was reached for the pair Hg/Se. The y-intercept of the linear regression was not significantly different from zero ( $p < 0.05$ ) for both organs. In liver, regression slopes were significantly different for males and females.

For males:  $\text{Hg} = 1.085 (\pm 0.076) \text{Se}$  ( $n = 39$ ,  $r^2 = 0.74$ )  
 For females:  $\text{Hg} = 1.447 (\pm 0.061) \text{Se}$  ( $n = 87$ ,  $r^2 = 0.78$ ) (Fig. 8A)

The difference between sexes was consistent with our previous ANOVA results (Table 3).

In kidney, both sexes had a common regression slope:

$\text{Hg} = 0.582 (\pm 0.039) \text{Se}$  ( $n = 52$ ,  $r^2 = 0.47$ ) (Fig. 8B)

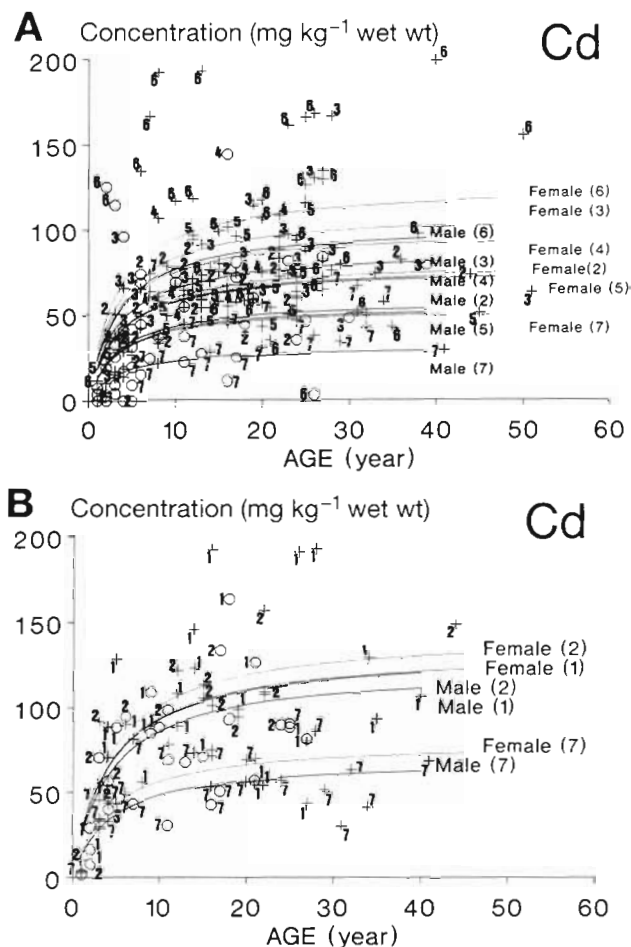


Fig. 7. *Globicephala melas*. Cd concentrations as a nonlinear function of age, sex and school. Nonlinear regression lines are shown for (A) liver and (B) kidney. Numbers 2 to 7 correspond to Schools II to VII for males (o) and females (+)

Concerning intermetal correlations in the kidney the most striking difference was that As levels were significantly related to Cd, Cu and Se levels (Table 7).

## DISCUSSION AND CONCLUSIONS

Most metal concentrations in *Globicephala melas* liver are higher in females than in males. Likewise in spotted dolphin *Stenella attenuata*, clear sexual differences have been registered for Hg in numerous organs (André et al. 1990b), for Se in liver and heart (André 1988), and Cd in the intestine (André et al. 1990a). Differences in metal concentrations of striped dolphin *Stenella coeruleoalba* (Honda et al. 1983) and narwhal *Monodon monoceros* (Wagemann et al. 1983) as a function of sex were not statistically significant.

Male pilot whales grow more rapidly than females and attain a greater length at the same age (Bloch &



Table 6. *Globicephala melas*. Correlation matrix between trace element levels in the liver of pilot whales. Values represent data for 105 individuals, except those marked a = 164 individuals. Significant correlation coefficients are underlined ( $p < 0.05$ ). \* Deviation from linear trend

	As	Cd	Cu	Hg	Se	Zn
As						
Cd	-0.07					
Cu	-0.11	<u>0.58<sup>a</sup></u>				
Hg	-0.09	<u>0.24*</u>	<u>0.52</u>			
Se	-0.06	<u>0.37*</u>	<u>0.69</u>	<u>0.86</u>		
Zn	0.03	<u>0.65<sup>a</sup></u>	<u>0.43<sup>a</sup></u>	-0.04	0.05	

Table 7. *Globicephala melas*. Correlation matrix between trace element levels in the kidney of pilot whales. Values represent data for 54 individuals, except those marked a = 93 individuals. Significant correlation coefficients are underlined ( $p < 0.05$ ). No deviation from linear trend

	As	Cd	Cu	Hg	Se	Zn
As						
Cd	<u>0.33</u>					
Cu	<u>0.28</u>	<u>0.64<sup>a</sup></u>				
Hg	<u>0.09</u>	<u>0.68</u>	0.20			
Se	<u>0.43</u>	<u>0.72</u>	<u>0.53</u>	<u>0.70</u>		
Zn	<u>0.25</u>	<u>0.76<sup>a</sup></u>	<u>0.56<sup>a</sup></u>	<u>0.57</u>	<u>0.58</u>	

Lockyer 1989). This phenomenon may be related to sexual differences in feeding rate or metabolism. Moreover, in males the weight continues growing to a greater age than in females (Bloch & Lockyer 1989).

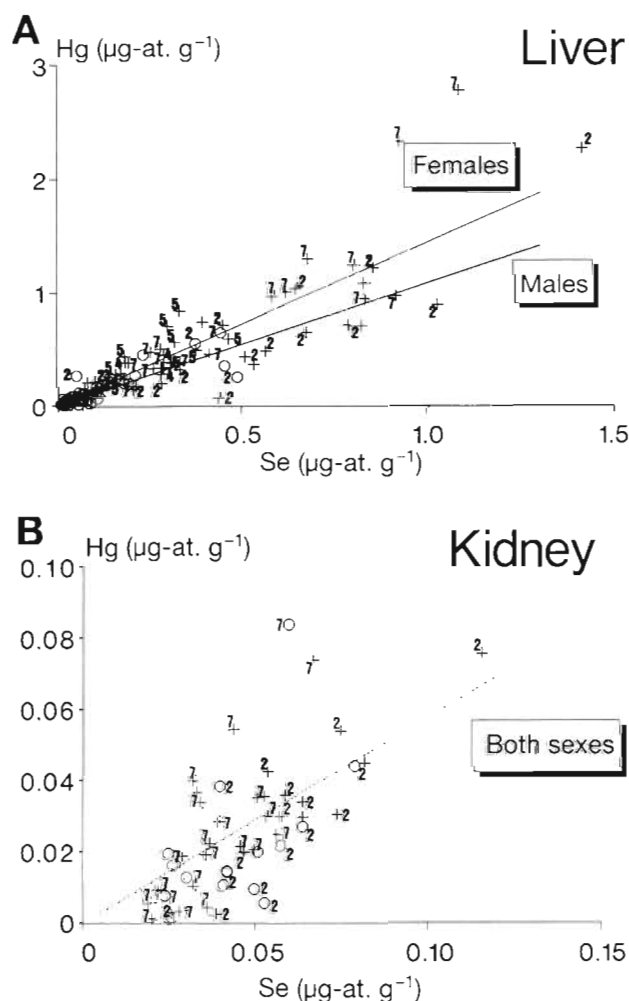


Fig. 8. *Globicephala melas*. Coded scatterplot of Hg concentrations versus Se concentrations in (A) liver and (B) kidney. Each point code as in Figs. 2 & 3

This could imply a greater effect of dilution and so could induce the lower metal concentrations in males. In humans, numerous studies have shown that plas-matic Cu was higher in women than in men, and was attributed to differences in hormone metabolism (Chappuis 1991). This second explanation could also be valid for the whales. The accumulation of trace elements in tissues depend on the food intake but retention from identical intake may vary: the Se balance compared to the Se intake with food is more positive in women than in men (Levander & Morris 1985).

Cd and Hg concentrations are low in the body at birth and accumulate with age. This corresponds to their long biological half-life: about 10 yr for Hg, 10 to 30 yr for Cd (as quoted in Wagemann et al. 1990). However, in pilot whales the rate of accumulation of these 2 metals differed. In liver, Hg was incorporated throughout life at a rate which did not decrease on maturity (Fig. 2), whereas the increase in Cd was particularly rapid during the first years, stabilizing at about 15 yr old (Fig. 2), which is also the age at which the pilot whales ceased growing (Bloch & Lockyer 1989). This supposes a higher absorption rate and/or a lower elimination rate of Hg than Cd in adult whales. Hg has been shown to be cumulative in numerous marine mammals from different regions (Gaskin et al. 1979, Falconer et al. 1983, Honda et al. 1983, André et al. 1990b, Wagemann et al. 1990), with the same pattern of accumulation in each study. The higher accumulation rate of Hg for females has not been previously reported and could be simply a sampling problem. Out of 30 individuals over 20 yr old, only 2 were males. Honda & Tatsukawa (1983) reported a similar relation between Cd concentration and age in young striped dolphin and attributed this to a higher absorption efficiency and accumulation of Cd through the digestive tract of the calf compared to adults.

Se concentrations in the tissues reflect the level of dietary Se over a wide range (Underwood 1977) and Se balance is linearly related to Se intake (Levander & Morris 1985). It is assumed that the increase of Se in

kidney and liver in pilot whales with increasing age is related to Hg accumulation since a reciprocal influence of these metals has been well documented (Cuvin-Aralar & Furness 1991).

Zinc is an essential element in all species, including humans. Zinc concentrations in liver are higher in newborns than in adults. Various studies on terrestrial mammals suggest that Zn absorption and retention decrease with increasing age (Chappuis 1991), the change occurring around weaning (Kirchgeßner & Weigand 1983). This is also the case for whales in School II, since 61.5% of the individuals of age <1 yr, which had milk or mixed milk and cephalopod beaks in their stomachs, typical of the weaning stage, showed the highest levels of Zn. Levels of Zn were much lower in the older whales. Considering the data for all the schools the influence of age is concealed due to the fact that newborns are included in the first age class (0 to 5 yr). A similar trend in the relation between Zn concentration and age has been shown in porpoises (Falconer et al. 1983) and striped dolphins (Honda & Tatsuoka 1983). The presence of very young individuals in School II and not in School VII probably explained the interaction between school and other factors revealed in the ANOVA analysis (Table 3).

In human liver, Cu concentrations gradually decrease from birth to maturity, and decrease again after the age of 60, whereas they remain unchanged in the kidney (Yunice & Hsu 1984). The decrease in Cu concentrations in the kidney of male whales of the oldest age class probably has no biological significance, since this class included only 3 males compared to 15 females (Fig. 3). Variations in plasmatic Cu also reveal metabolic changes associated with age (In Chappuis 1991). However the status of Cu in relation to age varies greatly from one species to another (Underwood 1977).

Contamination levels also appear to be dependent on the school to which the individuals belong. Julshamn et al. (1987) tentatively attributed the differences between Cd and Hg concentrations in organ tissues from catches in 1977 and 1978 to the possibility that the pilot whales belonged to different sub-populations. The analysis of genetic differences between schools using isozyme electrophoresis also illustrated variability between schools (Andersen in press). Three schools have been shown to diverge significantly from the others: Schools III, VI & VII. Multiple regression analysis performed with Cd concentrations is in agreement with these results (Table 5).

The high correlation between Hg and Se found in pilot whales was noticed in tissues in the earliest studies (Koeman et al. 1973, 1975, Smith & Armstrong 1975) and in more recent studies on dolphins (Itano et al. 1984, Julshamn et al. 1987, André 1988) as well as

on seals (Reijnders 1980, Ronald et al. 1984, Wagemann & Muir 1984, Perttilä et al. 1986, Nielsen & Dietz 1990). The protective effect of Se against Hg toxicity has been observed in a number of different organisms (Haguenoer & Furon 1982) and different mechanisms have been proposed for it (Cuvin-Aralar & Furness 1991). Experiments on the protective effect of different chemical forms of Se against the renotoxicity of Hg in rats support the role of Hg-Se complex formation (Magos et al. 1987). Concerning marine mammals, tiemannite granules were identified in the liver of *Ziphius cavirostris* and *Tursiops truncatus* (Martoja & Berry 1980). This Hg-Se complex could be the last step of the detoxification process leading to the fossilization of Hg and Se. In the present study, the mean molar ratio of total mercury to selenium was 1.5 in liver and <1 in kidney of pilot whales. Hg is distributed among insoluble compounds (including Hg-Se granules) and cytosol including methylmercury (MeHg) and Hg-binding proteins. In the liver of pilot whales, the MeHg fraction represents 30% of the total Hg as a mean, but the proportion of organic Hg is negatively correlated with age (Schintu et al. 1992).

In the present study, significant correlations between levels of As and other elements were shown only in the kidney. Evidence of As-Cd and As-Se interactions has been shown when toxic effects were considered (Haguenoer & Furon 1982). Moreover, metabolic interferences have been evoked as a consequence of the similarities between metabolism of As and of Se (Chappuis 1991).

In both tissues of pilot whales examined here, significant and positive correlations were shown between Cd and Cu and all the other elements analysed (except Cu and Hg in the kidney). Molecular interactions between the essential metals Cu and Zn and the toxic metals Cd and Hg may be envisaged as a consequence of their different affinities for metallothionein (Webb 1987). The competition hypothesis proposed by Wagemann et al. (1990) to interpret the negative correlation between Cd and Hg in the kidney of beluga whales cannot be considered in the present study since the intermetal correlations were positive. Se has been recognized as a modifier of the toxicity of several trace elements probably through the formation of inactive selenides (Cd, Pb, Pt, Ag, Hg). Another process would be a Se-promoted binding of some metals (Cd, Hg) with less critical proteins after the formation of S-trisulphides groups with a high affinity for these toxic elements (Chappuis 1991). Irrespective of the above-mentioned potential molecular interactions, it is clear that some correlations may be due at least partly to the similar behaviour of some metals. These correlations include cumulative effects of Cd and Hg with increasing age, similar behaviour of Cd and Zn according to

the school to which pilot whales belong, and the concomitant fate of Zn and Cd in the environment due to their association in the earth's crust.

The assessment of ecotoxicological hazards associated with the presence of trace elements in pilot whale tissues may be based upon the relation between biological indicators and health effects, which have been documented by occupational toxicology and agricultural studies. However it is necessary to keep in mind that such instruments for risk assessment are imperfect since bioaccumulation and toxicological effects may be species-related (Underwood 1977, Chappuis 1991).

Liver copper overloads as high as  $411 \mu\text{g Cu g}^{-1}$  dry wt have been observed in humans affected by hepatic disease, but even normal concentrations in humans ( $31$  to  $58 \mu\text{g g}^{-1}$ ; Chappuis 1991) are high compared to those determined in pilot whales (about  $19 \mu\text{g g}^{-1}$  dry wt using a dry weight/wet weight ratio of 0.3). Thus pilot whales do not seem to incur a toxic risk due to Cu.

Health disturbances affecting liver, kidney and heart have been observed in cattle as a consequence of food poisoning through seleniferous plants or through Se supplementation. Liver and kidney Se concentrations were  $10$  to  $25 \text{ mg Se kg}^{-1}$  and  $1$  to  $5 \text{ mg Se kg}^{-1}$  respectively (Chappuis 1991). These levels are of the same order of magnitude as those determined in pilot whales. However, a reciprocal protective effect of Hg and Se may be envisaged (Cuvin-Aralar & Furness 1991).

Renal Hg concentrations of  $10$  to  $70 \mu\text{g g}^{-1}$  wet wt have been reported in workers with Hg-induced proteinuria (Tsalev & Zaprianov 1983). These are considerably higher than mean concentrations determined in the kidneys of pilot whales: 5 specimens among 54 exhibited renal concentrations higher than  $10 \mu\text{g g}^{-1}$  ( $11$  to  $17 \mu\text{g g}^{-1}$ ).

In humans, a Cd renal cortex concentration of  $200 \mu\text{g g}^{-1}$  wet wt has been established as a minimum adverse-effect level (Tsalev & Zaprianov 1983, Lauwerys 1990). Considering the present study and additional data obtained from 21 pregnant females (unpubl.), 31.6% of individual whales exhibit Cd levels in the kidney higher than  $100 \mu\text{g g}^{-1}$ . In humans, Cd is stored mainly in the cortex, the metal level of which is approximately twice as high as in the medulla (Pesch et al. 1989). Thus it may be considered that a significant fraction of the pilot whales sampled in the vicinity of the Faroe Islands had Cd levels approaching those considered as critical levels for humans. The same is true when other indicators (blood and urine Cd concentrations) are taken into account (Caurant & Amiard-Triquet unpubl.).

Data are lacking on the long-term health effects of environmental exposure to Cd. A 15 yr follow-up study on renal dysfunction among humans living in a

Cd-polluted area of Japan suggest that a threshold of Cd exposure could exist. It is hypothesized that under this threshold renal tubular dysfunction is reversible (Teranishi et al. 1992).

It is particularly difficult to determine sublethal effects of heavy metals on feral marine mammals. The frequency-at-age data has not shown anomalies likely to reveal a major toxic problem in pilot whales of the northeastern Atlantic (Perrin 1990) but gastric erosion and ulcers have been observed in numerous individuals (Caurant unpubl.), a type of disturbance which has been described in dead or stranded beluga whales in the polluted St. Lawrence estuary, Canada (Martineau et al. 1988).

The apparent metal tolerance of pilot whales is now under investigation. Once established, the risk to human consumers may be quantified in terms of dietary total mercury, methyl mercury, cadmium and selenium in the Faroe Islands population. In the 1980's, it was calculated that toxic metal intakes in the Faroe Islands population exceeded the Provisional Tolerable Weekly Intakes recommended by WHO (Andersen et al. 1987) and the population was encouraged to avoid consumption of the most highly-loaded organs (kidney, liver). In 1986, Cd and Hg levels in hair were significantly higher in consumers of muscle of marine mammals than non-consumers (Jean-Caurant & Amiard-Triquet 1991).

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