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Effects of heavy metals on eels, *Anguilla* sp.

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Abstract

About 60 papers and reports dealing with heavy metal contents in different eel species were reviewed in order to document various patterns of uptake, elimination and accumulation of these metals and to underline their detrimental effects (lethality, histo-pathology). Two main toxicological topics were considered: observations on contaminant levels in eels taken from the wild, mainly from contamined waters, and experimental studies under laboratory conditions.

Keywords : *Anguilla*, heavy metal, toxicology.

Effets des métaux lourds sur les anguilles, Anguilla *sp.*

Résumé

Une synthèse présentée porte sur les modalités de contamination et d'accumulation des métaux lourds sur les diverses espèces d'anguilles, afin de souligner les effets toxiques de ces polluants (létalité, histocytopathologie). Deux thèmes principaux ont été considérés: études des anguilles des milieux naturels contaminés, et expériences de contamination en laboratoire.

Mots-clés : *Anguilla*, métaux lourds, toxicologie.

INTRODUCTION

Eels, *Anguilla* sp., can often be exposed to various pollutants in marine, brackish and freshwaters at each life cycle stage (glass-eel, elver, yellow eel, silver eel). Among the various pollutants, some heavy metals such as cadmium, chromium, lead and mercury are known to be toxic, even at low concentrations; some of them (copper, zinc) are essential heavy metals and are required metabolically at very low concentrations. The accident at Sandoz factories, which happened near Basel on 1 November 1986, also caused the death of about 200 tons of eels in the Rhine River (Anonymous, 1987*a*); it showed that eels are as susceptible to chemical pollution (mercury, pesticides) as any other fish species.

Knowing the effects of heavy metals may have on eels is of hight interest because:

– Eels may prove to be valuable test organisms in natural waters and may serve as target species for monitoring (Amiard-Triquet *et al.*, 1981, 1988).

– Alarming concentrations have been reported in eels from heavily polluted areas, for example: mercury poison in Japanese eels (Minamata Bay, Tsuchiya, 1969), in the Elbe River, northern Germany (Kruse, 1982), and from other areas such as 11 rivers in East Anglia (Barak and Mason, 1989). Metal accumulation in fish is, therefore, important for public health concerns because eels are used for human consumption in many areas. Eel is a long-lived fish and its relative resistance to toxic substances emphasizes the obvious danger of accumulation of heavy metals at sublethal

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doses which can lead to acute toxic effects at higher trophic levels, including man (Bouquegneau and Distèche, 1973).

– The potential vulnerability of these species to poisons despite their known resistance; ecological consequences of such vulnerability in eel stocks are possible when exploited in fisheries.

A comparative review of pertinent literature (60 publications) on the effects of heavy metals on eels was undertaken in our laboratory prior to experimental studies on cadmium effects in glass and yellow eels (Gony, 1987). The majority of these papers deal with the European eel *Anguilla anguilla*. The literature is discussed under two major headings: uptake, elimination and accumulation, and biological effects of heavy metals.

UPTAKE. ELIMINATION. ACCUMULATION OF HEAVY METALS

Uptake

Three pathways of contamination can be identified: directly through the water, through contact with sediment and by uptake with food.

Water

The uptake of Cd and Hg (as $CdCl_2$ and $HgCl_2$) was shown to be much higher from water (1.1 and 0.4 µmol/dm^3 respectively) than from food or the gills: the gill is the principal absorption site for these metals and seems to be less permeable to Cd than to Hg (Bouquegneau, 1972*a*, 1975; Noël-Lambot and Bouquegneau, 1977). During short term exposure to $HgCl_2$, inorganic mercury was accumulated chiefly in the gills (Bouquegneau and Distèche, 1973). Quantity of metal accumulated in the gill of eels, contaminated for 1 month in sea water containing 1 ng/g mercury as $HgCl_2$ or CH_3HgCl, was respectively 1 450 and 1 130 ng/g wet weight; these concentrations of mercury were much higher in the gills than in any other viscera as digestive tract, liver and kidney (Bouquegneau and Joiris, 1988).

Sediment

A relationship between mercury concentrations in sediments versus organisms was obtained from experiments by Cottiglia *et al.* (1984) and Hg concentrations were shown to be conspicuously higher in *Anguilla* than in other fish species (*Aphanius* sp.) and a crustacean (*Gammarus* sp.). On the other hand, because metal concentration in eels seems to depend on the availability of these metals in the bottom sediments, and because the length of their life in rivers (5-12 years) constitutes a favorable factor for including a chemical imprint, it was suggested to use the accumulation level and the ratio between these metals in the fishes to determine their geographical origin. Thus, Moreau and Barbeau (1982) identified eel samples from the St.-Lawrence River and its tributaries from different metal concentrations according to their geographic origin. High concentrations of Hg (22 µg/g dry weight) were found in sediments collected in the immediate vicinity of a chemical factory in Limfjord (Denmark) but eels caught in polluted areas showed lower mercury concentrations (319 µg/kg) than other bottom-dwelling fishes such as flounders *Platichthys flesus* (735-847 µg/kg) according to Kiorbe *et al.* (1983). Further studies are required to verify these results.

Food

^{203}Hg labelled methylmercury was accumulated in fish via feeding according to Jarvenpaa *et al.* (1970). Increased concentrations of ^{65}Zn in the natural aquatic food chain (*Gammarus* sp.) to eels were experimentally analyzed by Baudin (1981). Cd and Pb concentrations in adult eels from the Loire Estuary reflected those in their environment throughout the food chain (with higher values in stomach contents than in the flesh) and regarding the prey-predator relationships and trophic level of the eels (Amiard *et al.*, 1982). The results do not indicate any biomagnification of metal through the food chain (Amiard-Triquet *et al.*, 1983). However, high total mercury concentrations in flesh were associated with high trophic level predators and bottom feeders such as eels (Cottiglia *et al.*, 1984; Cosson, 1987) which were evaluated as indicators of pollution by Uysal (1980).

Elimination

Elimination rate of methylmercury was measured in *A. anguilla* by Jarvenpaa *et al.* (1970). Half-time of excretion was rather high: 910 to 1 030 days after peroral and intramuscular administration. Thus, the metal was efficiently retained in fish tissues when accumulated through food chains and extremely slowly excreted at a rate ranging from 0.5 to 1.1×10^{-3} µg/g day according to Zitko *et al.* (1971).

Zinc is excreted (unknown mechanism) into the digestive tract according to Baudin (1981) who showed that about 45% of the zinc was eliminated with the feces. In contrast, the rest of the fraction was eliminated very slowly (450 days for ^{65}Zn). Elimination of chromium salts occured according to two steps: a rapid one (about 20 days) followed by a slow one, but nothing is known about excretion mechanisms (Descamps *et al.*, 1973). Methylmercury, at first accumulated in the eel liver, was excreted via the bile while inorganic mercury, accumulated in the kidney, was excreted via the urine (Bouquegneau and Joiris, 1988). Localized pollution associated with an industrial complex was noted in the Severn River, near Bristol, and eels collected on the screens at Oldbury Power Station showed very high Cd concentrations: 25.6-123 µg/g dry weight tissue (liver) according to Romeril and Davis (1976). In eels from condenser

Table 1. – Eel and natural waters contaminated by metals.

Geographical area	Metals	Concentration values µg/g (dry weight)		Authors
		A. anguilla		
Dutch canals and lakes	Hg	0.01-1.62 (wet weight)	Muscle	Nuijt and van der Velden, 1973.
MMAE Lab. Burnham (UK)	Cd	0.3	*id.*	Portman, 1974 *in* Romeril and Davis, 1976.
Elbe Estuary	Hg	0.93	*id.*	Kruger *et al.*, 1975
Severn River and Bristol Channel (UK) (Oldbury Power Station)	Cd	67.3	muscle and liver	Romeril and Davis, 1976
	Cu	107.0		
	Fe	1850		
	Zn	2370	liver	
Lower Medway Estuary (UK)	Hg	0.16-0.87	muscle and liver	Wharfe and van den Broek, 1977
	Zn	13.9-36.4-40.4-95.8		
	Cu	0.3-10-50-36.9		
	Pb	0.25-1.82-0.84-5.29		
	Cd	0.08-0.50-0.15-0.59 (wet wt)		
Venezia lagoon (Italy)	Hg	0.15-0.57-0.17-0.67	*id.*	Campesan *et al.*, 1979
	Pb	1.0-1.20		
	Cd	0.15-0.18		
	Cr	0.15-0.15		
	Mn	0.33-1.70		
Izmir Bay, Bafa Lake, Egean coast (Turkey)	Cd	0.19-1.15 (wet wt)	whole fish	Uysal, 1980
	Fe	1.20-1.58		Uysal and Tuncer, 1982
	Pb	1.11-3.83		
	Mn	0.51-1.1		
	Zn	19.0-22.1		
	Hg	0.38-0.60		
	Cu	1.26-1.75		
	Cr	0.32-1.43		
	Co	0.67-1.43		
Elbe River (yellow eels + elvers)	Se	0.5-2.0	muscle	Kruger and Kruse, 1982
	Hg	0.2-1.5		
	Cd	0.05		
Loire Estuary (adult eels)	Cd	0.1	*id.*	Amiard *et al.*, 1982
	Pb	2.5		
	Zn	50.5		
Loire Estuary	Cd	0.02 (d.t.: 2.63)	whole fish, digestive tract (dt)	Amiard-Triquet *et al.*, 1983
	Pb	0.25 (d.t.: 9.70)		
	Cu	1.38 (d.t.: 24.04)		
	Zn	0.07 (d.t.: 0.12)		
Limfjord (Denmark)	Cd	0.31	whole fish	Kiorbe *et al.*, 1983
Gironde, Loire and Vilaine Estuaries (France) (glass eels)	Cd	0.41	*id.*	Andersen, 1984
S. Gilla lagoon (Sardegna)	Hg	0.04-5.32 (wet wt)	whole fish	Cottiglia *et al.*, 1984
id. (elvers + yellow eels)	Cd	0.02-0.61	*id.*	Metayer *et al.*, 1984
	Cu	0.11-20.98		
	Zn	2.59-989.38		
Camargue (France)	Cu	0.34 (l: 22.8)	muscle, liver (l)	Cosson, 1987
	Pb	0.09 (l: 3-4.6)		
	Hg	0.74-1.82 (l: 0.39-3.04)		
	Zn	37.9-76.9 (l: 95-380)		
Loire Estuary (elvers)	Cu	34.4	whole fish	Amiard *et al.*, 1987
11 rivers in East Anglia	Hg (+Cd and Pb)	0.3-1 (wet wt)	flesh and liver	Barak and Mason, 1989
		A. australis reinhardtii *		
Rivers and lakes of Victoria (Australia)	Hg	0.17-0.37 (wet wt)	muscle	Beumer and Bacher, 1982
		A. japonica *		
Agano River delta (Japan)	CH_3HgCl	41.0 (? wt)	whole fish	Tsuchia, 1969

Table 1. – Continued.

Geographical area	Metals	Concentration values µg/g (dry weight)		Authors
		A. rostrata *		
New Brunswick, Bay of Fundi, Nova Scotia Banks (Canada)	CH_3HgCl	0.07-2.8 (wet wt)	whole fish	Zitko *et al.*, 1971
Chesapeake Bay (US)	Hg	0.10 (? wt)	*id.*	Bender *et al.*, 1972
Medway River (New Scotland, Canada)	Hg, CH_3HgCl	0.40-0.72 (wet wt)	muscle	Freeman and Horne, W973
St.-Lawrence River and tributaries	Hg	l: 5.53 (? wt) k: 2.40 (? wt) s: 0.11 (? wt)	liver kidney skin	Turgeon *et al.*, 1972
id.	Hg	0.05-2.46 (? wt)	liver	Turgeon and Beaulieu, 1973
Inshore waters along the coasts of Georgia and Florida (US)	Cu Hg Cd As Zn	0.8 2.7 <0.1 <1.0 25	whole fish	Windom *et al.*, 1973
St.-Lawrence River and tributaries	Hg	>0.9 (? wt)	*id.*	Moreau and Barbeau, 1982
id.	Hg Cd Cu Pb Zn	0.34 (wet wt) 0.03 0.77 0.33 34-78	*id.*	Dutil *et al.*, 1987

* Yellow eel.

waters, the total iron and copper contents of the liver was nearly, respectively, thirty and ten times greater than in eels from the river water. However, these authors thought that eels have developed, at Hinkley Power Station, a high capacity to detoxify the metals in the liver, especially cadmium. Similary, wild eels caught in the Elbe River, contamined with Hg, kept for 6 months or more in clean water, showed a dilution of their mercury content during their growth (Koops *et al.*, 1982). In both cases, nothing is known about the mechanisms involved.

Accumulation

Concentrations of metals in different species of eels were measured in many natural water bodies and contaminated areas by waste water effluents (*table* 1).

Since eels are long lived, they can accumulate high concentrations of pollutants, so that less polluted waters can also be potentially harmful. In addition, the oldest eels (silver eels) may be considered the most dangerous ones for human consumption. Thus, in a sample of 405 American eels *Anguilla rostrata* from Canadian rivers, 307 fish (75.8%) in which mercury concentrations were higher than the safety level (0.5 µg/g); hence, the commercial eel fishery was stopped and forbidden in Canada in 1970 (Turgeon *et al.*, 1972; Turgeon and Beaulieu, 1973) owing to the warning given by Minamata's disease (methyl mercury poisoning) in Japan. However, other eels from Nova Scotia were thought acceptable for consumption, their total mercury and methylmercury contents being 0.72 and 0.40 µg/g, respectively (Freeman and Horne, 1973). Similary, most of the Australian eels collected in rivers and lakes of Victoria had mercury concentrations well below the allowable statutory level (Beumel and Bacher, 1982). In European eels, whereas the cadmium level was generally low and would not appear to constitute a human health risk (Romeril and Davis, 1976), 42% of the eels from the Elbe River exceeded the recommended limit of 1 µg/g Hg in edible parts (Kruger and Kruse, 1982). Yellow and especially silver eels were found in the Elbe River with high mercury levels (Koops *et al.*, 1982). Recently, commercial sale of Rhine River eels was forbidden in F.R.G. (Anonyme, 1987*b*), and levels of mercury in eels from a dutch polder lake (Tjeukemeer) call for concern in view of their human consumption (Badsha and Goldspink, 1988). Commercial eel fishing in the River Brain (East Anglia) should not be allowed because of mercury levels which exceeded the standard concentration in most eels (Barak and Mason, 1989).

Copper and Zinc concentrations appear to be independent of levels of these metals in natural (or experimental) environments because of regulation mechanisms versus non-essential metals, such as cadmium and lead, concentrations of which depends in the organism mainly on their environmental levels. In the latter case, the equation $y = ax^b$ describes the relation between the level of metal in eels (y) and water (x), except for cadmium in the elver; *A. anguilla* is able to control its entire body levels of essential trace metals (Cu, Zn) from its early stages (elver) of life

cycle, but metal regulation may impose a metabolic cost (Amiard *et al.*, 1987).

A correlation between the quantity of metals in the eel with respect to its weight was shown for Pb, Cd, Cu and Zn, as early as the pigmented stage of elvers, with an evolution throughout growth of the eel: from 26 ng of Cd in an elver (stage VI A3) to 602 ng in an eel 12 g dry weight (Metayer *et al.*, 1984). Thus, mercury values in American eels from Nova Scotia did not indicate any correlation between metal levels and weights and sex of the eel (Freeman and Horne, 1973). In contrast, a positive correlation between mercury level and size and weight of eels was shown in Québec by Turgeon and Beaulieu (1973), in the Netherlands by Nuijt and van der Velden (1973) and in Cadiz, Spain, by Establier (1975).

However, the problem of metal accumulation rates in organisms is complex, because the specific chemical form of the metal (as an ion or as a molecule, organic or inorganic) must be taken into account (Amiard-Triquet *et al.*, 1987), and also a possible synergistic-antagonistic effect, as that of Cd on the bio-accumulation of Cu as shown by Andersen (1984) and that of Zn on the accumulation of Cd (Pally and Foulquier, 1976). The most striking example of the importance of the speciation of a metal with regard to its rate of accumulation was the effect of mercury, whether it was present in the environment in an inorganic ($HgCl_2$) or an organic form (CH_3HgCl_2). It appeared (Bouquegneau and Joiris, 1988) that the lipophilic methylmercury compound was accumulated 2.5 times more rapidly than in the inorganic one, the main route of intake of both toxicants being through the gills. Methylmercury was not retained in the gill tissue and readily passed into the other organs via the blood. On the other hand, methylmercury from food contamination passed much more efficiently through the intestinal wall than inorganic mercury according to these authors.

The highest concentration of heavy metals were generally found in liver and kidney tissue (Turgeon *et al.*, 1972, Bouquegneau, 1973*a*; Descamps *et al.*, 1973; Romeril and Davis, 1976; Wharfe and van den Broeck, 1977; Noël-Lambot and Bouquegneau, 1977; Bouquegneau and Joiris, 1988), organs which function as the main reservoirs for a number of substances. Metals are bound to metallothionein, a protein rich in metal chelating SH groups. Next to these organs, the digestive tract and spleen accumulated metals to higher concentrations than in muscle tissues. When accumulation of a heavy metal pollutant takes place through mainly gill tissue (high surface/volume ratio) the uptake was correlated with damage of gill tissue, following alterations induced by heavy metals at the cell membrane level (Bouquegneau and Distèche, 1973).

However, there was considerable variation in metal concentrations among eels collected from the same polluted area, *i.e.* the Bristol Channel (Romeril and Davis, 1976). A high variation of individual accumulation levels also occured in eels contaminated by radioactive chromium, showing heavy accumulation in the digestive tract and higher levels in the liver and kidney than in the gills, skin and muscles (Descamps *et al.*, 1973). Similary, mercury concentrations in American eels from a Canadian river were highly variable, ranging from 0.015 to 2.46 µg/g (Turgeon and Beaulieu, 1973).

BIOLOGICAL EFFECTS OF HEAVY METALS

Acute and chronic poisoning of eels by heavy metals were studied by different physiological and ecotoxicological parameters (*table* 2).

Lethal effects

Acute intoxication of $HgCl_2$ (4 µmol/dm^3) and $CdCl_2$ (445 µmol/dm^3) caused mortality after 5-10 hours of exposure in European eels (Noël-Lambot and Bouquegneau, 1977). It was shown that accumulation of Hg is greater than that of Cd in contamined eels and this occurs in all organs. Ells are much more susceptible to $HgCl_2$ exposure than $CdCl_2$ exposure. Thus, eels were alive for more than 6 months in seawater containing 110 µmol/dm^3 Cd. LC_{50} and LC_{100}, 96 hours, of Cd, Cu, Pb and Zn in juvenile eels was evaluated by Denuit *et al.* (1981); LC_{50}, 96 hours, of Cd, Cu and Zn was studied in elvers by Andersen (1984) and in juvenile eels by Amiard-Triquet *et al.* (1981). Denuit *et al.* (1981) showed that toxicity was higher at low salinity (10 $^0/_{00}$) than at 25 $^0/_{00}$ salinity.

The MTL (median tolerance limit) for 24, 48 and 96 hours of Cu, Ni and Zn exposure was determined in the American eel by Rehwoldt *et al.* (1971), and for Cu (Mushiake *et al.*, 1984) and Zn (Cruz and Muroga, 1985) in the Japanese eel.

In field studies, for example the River Rhine which is heavily polluted below Basel by chemical plants as well as sewage and chloride mines, synergistic effects of various micropollutants, among them heavy metals especially mercury, may be responsable for fish kills including eels (Anonymous, 1987*a*). Additionally, it seems that heavy metals appear to be more toxic to estuarine species, which have to withstand osmoregulatory stress, than to marine species which have no osmoregulatory problems. This is especially true for stressful activities correlated with anadromous and catadromous migrations of eels. Thus, heavy annual mortalities (100 metric tons in 1972 and 1973) of migrating silver eels, *A. rostrata*, were reported in the freshwater section of the St.-Lawrence River. It is likely that diseases are linked to osmoregulatory failure and are associated with ion loss in freshwater, perhaps as a result of increased permeability of the gill membrane due to damage caused by pollution of heavy metals in the St.-Lawrence (Dutil *et al.*, 1987).

Table 2. – Eel and experimental contamination by metals.

Metals	Concentration (µmol/dm³)	Experimental conditions		Authors
		Anguilla anguilla *		
CH_3HgCl (proteinate or ionic, oral and intramuscular)	$^{203}Hg_2$ (2-5 µCi)	6‰, 2-16°C, 9 months	Whole fish, rate of elimination	Jarvenpaa *et al.*, 1970
$HgCl_2$ (water)	0.04	sea water, ?, 5 hours, 16 days	brain, gill, kidney, liver and spleen	Bouquegneau, 1973*a*
id.	*id.*	*id.*	gill ATPases, osmoregulation disturbances	*id.* 1973*b*
id.	4	sea water, ?, 30 hours	*id.*	*id.*, 1977
id.	*id.*	*id.*, 10 hours	metallothioneins synthesis	*id.*, 1979
id.	1.6	*id.*, 2 weeks	*id.*	Bouquegneau *et al.*, 1975
id.	0.4 4	*id.*, 4 hours	gill ATPases, osmoregulation disturbances	Bouquegneau and Gilles, 1979
$HgCl_2$ CH_3-HgCl	0.04 40 0.05-50	sea water, 1-42 days	mortality rate, osmoregulation disturbances	Bouquegneau and Distèche, 1973; Bouquegneau and Joiris, 1988
^{51}Cr (Na_2CrO_7)	5 µCi	freshwater, 15°C, 1 day-45 days	digestive tract and elimination	Descamps *et al.*, 1973
$CdCl_2$	0.09 0.445	freshwater, 16°C, >44 days, starvation	air bladder, gill, heart, kidney, liver and spleen	Pally and Foulquier, 1975
$ZnCl_2$	0.76 76 0.4	freshwater, 16°C, 76 days, starvation	*id.*	*id.*, 1976
$ZnCl_2 + CdCl_2$ (mixture)	*id.*	*id.*	*id.*	*id.*
^{65}Zn	5 µCi			
$CdCl_2$	1.1 0.4	seawater, 18°C, 1 day-6 months	Concentration factor, gill histopathology	Noël-Lambo and Bouquegneau, 1977
id. (elvers)	0.1	25-35‰, 15°C, 1-16 days	whole fish	Amiard *et al.*, 1981
$CdCl_2$	0.9 nmol-0.9 mmol/	10.20, 25‰	LC_{50} 96 hours, LC_{100} 96 hours	Amiard-Triquet *et al.*, 1981, 1986 and 1988
$CuSO_4$ (elvers + adults)	1.6 nmol-1.6 mmol/dm³			
$ZnSO_4$	1.5 nmol-	4 days	*id.*	*id.*
Pb $(NO_3)_2$	1.5 mmol/dm₃			
Zn (^{65}Zn) water and food chain (elvers + adults)	63 nmol/dm³ (0.11-0.81 µCi)	12.8‰, 20°C, 76 days 25-35‰, 15°C, 1-16 days	muscle, viscera accumulation, elimination	Baudin, 1981 Denuit *et al.*, 1981
$CdCl_2$ water (glass eels)	0.1-100		LC_{50} 96 hours	
$CdCl_2$	27 nmol/dm³	sea water, 18°C, starvation	intestinal corpuscules	Noël-Lambot, 1981
$CuCl_2$	0.26			
$ZnCl_2$ water (yellow eels)	0.22			
$CdCl_2$ water i.m. injection	7.12	*id.*	viscera	*id.*
$CuCl_2$	1.6-3.9 nmol/dm³	freshwater, 8-21°C.	riboflavin (chemical mediator) perturbation	Fontaine *et al.*, 1982
$NiSO_4$	1.7-4.3 nmol/dm³	LD 12:12, LD 18:6, LD		
$ZnCl_2$	1.5-3.8 nmol/dm³	6:1. L: light, D: dark		
$ZnSO_4$ water (glass eels)				
$CdCl_2$ (yellow eels)	15	sea water	kidney histo-and MEB	Martoja *et al.*, 1982
$CdCl_2$	0.53-22.2	2‰, 15°C	whole fish histo-immunopathology of digestive tract	Andersen, 1984
$CuCl_2$	0.79-4.7			
$ZnSO_4$ water, glass eels	1.5.76.5			
$Cd(NO_3)_2$ glass eels elvers yellow eels	4.5-45 nmol/dm³ ?	?	gill, kidney, liver, skin and spleen histology	Biagianti *et al.*, 1986; 1987
		Anguilla japonica		
$CuCl_2$	1.6-15.7	freshwater, 25°C, 24 hours-14 days	TLm (median tolerance limit) 24-48-96 hours and susceptibility to bacteriosis	Mushiake *et al.*, 1984
id.	3.9	*id.*, 0-96 hours	*id.*	*id.*, 1985
$ZnSO_4$	15.3-458.9	freshwater, 25°C, starvation	*id.*	Cruz and Muroga, 1985

* Yellow eel.

Indeed, the lethal effect of $HgCl_2$ could be attributed to a disruption of the NaCl balance in the eels: mercury in the gills acts on active ionic movement, NaCl transport and on the osmoregulatory process (Bouquegneau, 1973 and 1977). The breakdown of osmoregulatory mechanisms seems to result from the specific action of the pollutant on the Na pump (rapid loss of K and water, an increase of Na content – through an inhibition of Na^+K^+ATPase activity, with 3×10^{-6}M $HgCl_2$ or 8×10^{-6}M CH^3 $HgCl_2$, according to Bouquegneau, 1977).

This failure may be correlated with alterations of the membrane permeability and disruptions of enzymatic systems (Bouquegneau and Distèche, 1973). Thus, Dutil mentioned that metal pollutants indirectly caused the death of silver eels since their injured gills could not maintain the internal balance of salts (Eel Working Party, Bristol, April 1987).

Sublethal effects

Detoxifying effects of metallothionein

Eels react to sublethal doses of heavy metals by producing in the liver a low molecular-weight protein which belongs to the metallothionein-like family. It is characterized by a high content of cysteine and functions as a protective agent against injuries caused by metal uptake (Bouquegneau, 1979; Bouquegneau *et al.*, 1975). Metallothioneins were shown to be present in the liver of normal eels, but in lower amounts than in chronically Cd intoxicated animals (ten times higher for 180 days = 110 µmol/dm^3 $CdCl_2$ more than the normal amount) (Noël-Lambot *et al.*, 1978). $CdCl_2$ accumulates to the greatest extent in the liver (331 µg/g weight) and especially in the soluble fraction of this organ (247 µg/g), where it binds to metallothionein (it competes with Zn and Cu for SH binding sites: Cd binding more firmly to metallothionein than Zn or Cu). Metallothioneins play a role in regulating the metabolism of some essential metals and are essential to the process of detoxification. Thus, it is an adaptive tolerance for chronic heavy metal intoxication. In the field studies, levels of metallothionein in Cd and Hg contamined eels from Camargue (southern France) were shown to be two to four times higher (758 µg/g and 1 436 µg/g respectively) than the levels in uncontaminated eels (350 µg/g) according to Cosson (1987). Opposed to liver metallothionein, gill metallothonein binds only a very small amount of Zn and Cu, and no Cd (Noël-Lambot *et al.*, 1978). The protective role of metallothioneins has been established in eel gills and its synthesis reduces the toxicity of $HgCl_2$, and effectively protect the fish against mercuric injuries (Bouquegneau, 1979). However, the threshold of synthesis of metallothioneins is quickly reached in the eels and the rate of mercury accumulation was quite linear for the 32 days of the experiment (Bouquegneau and Joiris, 1988).

Other mechanisms of elimination

An interesting protective mechanism against heavy metal intoxication was described by Noël-Lambot (1981) wherein corpuscules are present in the intestinal lumen of *A. anguilla* before elimination with feces. These contain high concentrations of $CdCl_2$, $ZnCl_2$ and $CuCl_2$ and most of the cadmium burden (99% of 100 µg/g Cd after 6 hours) accumulated in these corpuscules from water ingested by the eel. This therefore reduces the Cd content in water inside the intestine and probably limits the amount of metal absorbed by the level of intestinal wall.

Histopathology

Heavy metals concentrated in some target-organs generally produce harmful effects. Anatomical, histological and cytological changes can be observed in eels but it is sometimes difficult to decide whether these modifications are adaptive or pathological. However, some cases of pathology induced by metals have been described. Gill tissue was one of the first tissues which would suffer from effects of various pollutants, especially heavy metals. Incubation of isolated gills from *A. anguilla* (in $HgCl_2$ 50 µmol/dm^3) induced a necrosis characterized by the formation of "bag-like" structures (Bouquegneau, 1975). Because Hg is taken up and accumulated mainly by the gills during short term exposure to $HgCl_2$, they are shown altered (through permeability modifications, enzyme disturbance and cellular injuries) in both of their essential functions: respiration and especially osmoregulation (Bouquegneau and Distèche, 1973). Physiological effects of heavy metals are mainly on the active transport processes which implies a change in enzymatic systems: heavy metals are known to be potent inhibitors of ATPases (Reid and McDonald, 1988).

Exposure to cadmium (15 µmol/dm^3 as $CdCl_2$, 30 days) induces severe injuries in the kidney of teleosts since this organ is known to be involved in regulation of Ca^{++} and Mg^{++} levels in the blood. Histopathology of the kidney by SEM showed (Martoja *et al.*, 1982) the following $CdCl_2$ contamination: nephropathic casts grew in the lumen of nephrons and collecting tubules.

The effects of low cadmium concentrations (<45 µmol/dm^3) on the structure of various target organs in glass-eels and young yellow eels were histologically and cytologically (TEM) analyzed by Biagianti, *et al.* (1986 and 1987); Gony *et al.* (1988). Exposure to cadmium-nitrate for short terms (1-3 days) induced severe injuries. Extensive gill (œdema, lamellar fusion, epithelial bulbing, rupture and necrosis), liver (fibrosis, hepatocytic disturbances and acute cirrhosis) and skin (erosions and ulcerations) lesions were observed. During short chronic exposures, degenerated cirrhotic nodules, which correspond to acute cirrhosis of the liver, were observed. After a purging phase (28 days), no recovery was observed in the gills and only a partial recovery of the liver and skin. Moreover, structural lesions were dependent on the salinity level and were more serious in freshwater than in brackish water; thus, salinity seemed to act as an effective buffer against cadmium toxicity (Gony *et al.*, 1988).

Silver eels entering into their reproductive phase can have the quality of their gametes suffer heavily from these toxic effects. Thus, a decreased rate of maturation was observed in Canadian *A. rostrata* by Dutil *et al.* (1987).

Hematology

Exposure of European eels to $CdCl_2$ for 15 days (45 µmol/dm^3) produced a blood anemia as well as disturbances in carbohydrate metabolism (hyperglycemia, glucosuria) and plasma electrolytes (decrease in K^+ and Ca^{++} and increase in Mg^{++} Larsson, 1975).

Secondary effects

Stress factors, resulting from pollutant response, affect physiological and biochemical homeostasis of fishes and make eels more vulnerable to pathogens. Pollutants, and especially heavy metals, have been shown to be related with outbreaks of infectious diseases. Increased susceptibility of eels exposed to sublethal concentrations of heavy metals to pathogenic organisms were demonstrated experimentally. Copper exposure ($CuCl_2$ at a sublethal concentration) resulted in an increase in the susceptibility of Japanese eels to bacteriosis (*Edwardsiella tarda* and *Pseudomonas anguilliseptica*), which has been suggested to be a result of stress response to copper (Mushiake *et al.*, 1984). Numbers of lymphocytes and granulocytes decreased while the phagocytic level of leucocytes against *E. tarda* in the stressed eel blood decreased (Mushiake *et al.*, 1985). In contrast, susceptibility to *Edwardsiella* was not demonstrated in eels exposed to zinc (Cruz and Muroga, 1985); possibly because it did not induce a significant degree of stress response in the eels.

In the field studies, the critical pollution levels will tend to increase the susceptibility to a number of diseases as stated by Kinne (1984). In the lower Elbe, Moller (1985) noted that the eel population was affected by water pollution resulting in papillomatosis and granulomata (prevalence: 9.2%).

Ethology

Fontaine *et al.* (1982) raised the question of a possible chelation phenomenon which can occur between certain metals and substances capable of biological activity, such as riboflavins produced by glass eels and consequently looseing their capability as pheromones. In addition to speculative modifications and disturbances in the structure of chemoreceptive papillae in eels, these metals can impair, through inhibition of these chemical mediators, the ethology of the eels and especially their migratory behavior. Such mechanism, although not demonstrated, can potentially stop the anadromous migration of glass eels and elvers. A possible impediment of migration in the Po Delta was speculated by Ambrogi (1986).

Some trace metals such as copper, cobalt and nickel were found in Sargasso Sea and Gulf Stream waters at elevated levels (Hanson *et al.*, 1988; Jickells and Burton, 1988). These metals can have harmful effects on the eggs and leptocephala larvae of eels spawning in the Atlantic and may induce abnormal development and reduce survival while also affecting migratory activity.

DISCUSSION

Despite the relatively large amount of data available on contamination of various eel species by heavy metals, our knowledge on their effects is limited and no clear-cut opinion exists on their biological pathways and significance in these species. Many questions still remain to be solved and there is an increasing need for current information on metal concentration in eels, as underlined by Barak and Mason (1989) but researchers have to pay attention to a standardization of the protocols, taking into account the metal species and the controlled conditions of the experiments or all the etiological factors in field studies.

Comparison of fish species versus pollutants is quite difficult. Extensive variability occurs among non-standardized toxicological parameters as well as experimental conditions, with many intrinsic and extrinsic factors, as stated by Amiard-Triquet *et al.* (1981). All these biological and ecological factors differ from one experiment to another and from one author to another, so it is not too surprising to be faced with a pathwork of data of low value and a great deal of non-conclusive findings. Data from eel investigations confirm the complexity of problems and difficulties that arise with chooseing a particular methodology.

In addition to species specific responses (e.g. acute doses of Hg, first disturb respiration in freshwater fishes, whereas osmoregulation first in seawater species...) or differences in environmental conditions (water quality, temperature, oxygen concentration, nitrogen metabolites, light...) to heavy metal exposure, toxicological effects depend on: the *nature of the chemical compound* and the type of salt(s) used (*i.e.* chemical speciation as ionic or non-ionic, organic or non-organic). These are very important parameters that unfortunately have scarce data in the literature; the *length of intoxication;* the *vector of transport* (water, sediment or food); *physiological condition* (well fed or starved, age, sex...) and the *synergistic effects* of other stress factors.

In laboratory experiments, the heavy metals are added to sea water containing little or no suspended matter, whereas, in nature, a high amount of suspended matter probably absorbs a large part of the pollutants. Thus, metals modified by inorganic and organic ligands present in marine waters would be altered in their reactivity. The absorption-desorption behavior of metals, the exchanges between sediment and sea water, and the bio-availability of trace metals all depend upon metal speciation (Bouquegneau and Gilles, 1979). Consequently, it is not possible to predict any biological effect on early stages of eel development in the Sargasso Sea (no eggs have been sampled and few leptocephali have been caught).

The greatest caution must be recommended in the choice of test-organisms and target-species. Some biological characteristics of eels seem to favor such a choice.

- Their extensive distribution which, coupled with the fact that their internal concentrations of heavy metals, seems to reflect the environmental levels, allows an extensive comparison of contamination

levels of marine and estuarine areas as well as river basins according to their level of contamination by chemicals.

- They are resident fishes over a long time (about 8-12 years), which separates anadromous and catadromous migrations. Oweing to home range and sedentary habits, concentrations largely reflect the local conditions. For example, in the lower Medway Estuary, mercury residues found in several species of fishes (whiting, flounder, sand goby, sprat) were comparable with reported values from the literature on other regions, while eel livers from the larger size class (>30 cm TL) had mean concentrations (1.55-2.06 µg/g wet wt) higher than other fishes (0.08-0.33 µg/g wet) according to Wharfe and Van Den Broek (1977).
- They are bottom-dwelling fishes in close contact with sediments.
- They are carnivorous species with a unique position in the benthic food chain.
- They are also migratory fishes, juvenile stages of which (glass eels, elvers) pass through estuaries which frequently are highly contamined waters, during their anadromous movement towards continental waterbodies; silver eels also swim across the same areas during their catadromous migration to the Sargasso Sea and can consequently die, as observed in the St.-Lawrence River (Dutil *et al.*, 1987).

Finally, they are rather easy to rear in aquaria and under experimental conditions and are very available, both in numbers and sizes, for eco-toxicological tests. Glass eels and juvenile yellow eels were adopted as a particulary suitable material for tests of metal pollution by Uysal (1980) and Beumer and Bacher (1982). They are also recommended for hydrocarbon tests (Anonymous, 1977). Larval stages, such as glass eels, are useful to establish long term toxicity tests on water contamination, as recommended by McKim (1977). Natural fasting of the juvenile stages does not interfere with nutritional problems of metal intake with food. However, wild samples used in experiments are not always free of contaminants and some of them, used as control fish, even showed a concentration higher than the contaminated and experimental eels (Pally and Foulquier, 1976).

Eels may be used as a useful bio-indicator of metal pollution in field studies, but with factors limiting its use, as recently shown by Amiard-Triquet *et al.* (1987 and 1988) and Mason (1987). Mercury levels in muscles of Australian eels accurately reflect those of the sampling locality, due to the sedentary habits of the fishes: these eels were considered by Beumer and Bacher (1982) as suitable indicators of mercury pollution in Australian waters. Further investigations at cytological (ultrastructural), biochemical (enzymology) and physiological levels are needed in order to increase our understanding of the number of metal pollution experiments which need to be conducted for assessing long-term toxicity, using low doses, similar to doses actually observed in contaminated waterbodies.

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