Chronic and Acute Aluminium Intoxication

Carlos Sandoval Hurtado[1], Enrique Paredes Herbach[2], J. Manuel Mejia Estrada[1], Manuel Ulloa[1].
[1]Research & Development Laboratorio Vehice. Ltda.
[2]Veterinary Doctor. Institute of animal pathology, Universidad Austral de Chile.

Aluminium (Al) is a metal that is not attributed to participation as a cofactor or functional element in cellular processes. It is a metal that occurs frequently in fresh water and its effect translates into mortality when it occurs in high concentrations, however, as for other metals, sub-lethal concentrations could alter production cycles (NIVA, 2011).

CHRONIC ALUMINIUM INTOXICATION

Chronic aluminium intoxication in freshwater fish is manifested by the alteration in respiratory function, associated with histopathological damage in gill filaments such as thinning of the lamellar epithelium, hyperplasia of the gill epithelium, which affects the exchange of gases (Youson and Neville, 1987, Evans *et al.*, 1988, Tietge *et al.*, 1988, Mueller *et al.*, 1991, Audet and Wood, 1993); however, morphometric changes can be observed in sub-lethal levels of Al with acid pH. There is also an alteration in swimming and in the process of muscular contractions (Brett, 1964). The alteration in muscle contractions is due to the ionic imbalance that occurs during acute periods of exposure in an acid medium with the presence of aluminium. When water is acidic (pH 5.2), it may cause alterations in appetite and growth (Wilson *et al.*, 1994).

Effects on reproduction

Alterations observed by Al exposure in acid media in the reproduction stage include an abnormal metabolism during vitellogenesis in mature females and indirectly affect the regulation of ions, delaying the maturation of oocytes and spawning, and there have even been cases where ovulation can be completely inhibited (Mount *et al.*, 1988).

Effects on hatching

High aluminium concentrations produce an early hatching and therefore is reflected in a high mortality, derived from early hatching. The mechanism of action is not established, but may be associated with dysfunction of the chorion structure, leading to an immaturity of vital organs and consequently poor adaptation to the environment. (Pressot & Kristiansen. 2011).

Toxic effects of Al in alkaline media

Toxic effects in alkaline media are lower than in acidic water, this because in alkaline media the predominantly aluminium charge is in the form of anion $[Al(OH)_4^-]$, which ameliorates the damage and even not considerable damage is observed at the gill level (Heming and Blumhagen, 1988).

Al Effects on salt water

There are few studies on the Al toxicity in estuaries or in the sea; however, acute mortalities have been reported during storms, in which large quantities of Al have been washed away from acidified rivers with high aluminium concentrations (Bjerkens *et al.*, 2000).

Effects on smoltification stage

Although there are not many studies in salt water, negative effects have been reported in the organisms in smoltification when they are exposed to aluminium in acidic media, affecting their osmoregulation and therefore their ability to adapt to seawater. These alterations are derived by the effect of Al to the gills, altering the activity of the NA^+/K^+ - ATPase (Saunders *et al.*, 1983, Staurnes *et al.*, 1993, Poleo and Muniz, 1993, Monette *et al.*, 2008); however, this is not the only process involved in ionic regulation during smoltification. Discussions are on-going on the possibility that there is a decrease in the mRNA expression of other ion transporters as is the case of CFTR-1 (Cl^- apical channel of Cl^- secretion) as well as the mitochondria of gill cells reduce in size and intensity of staining.

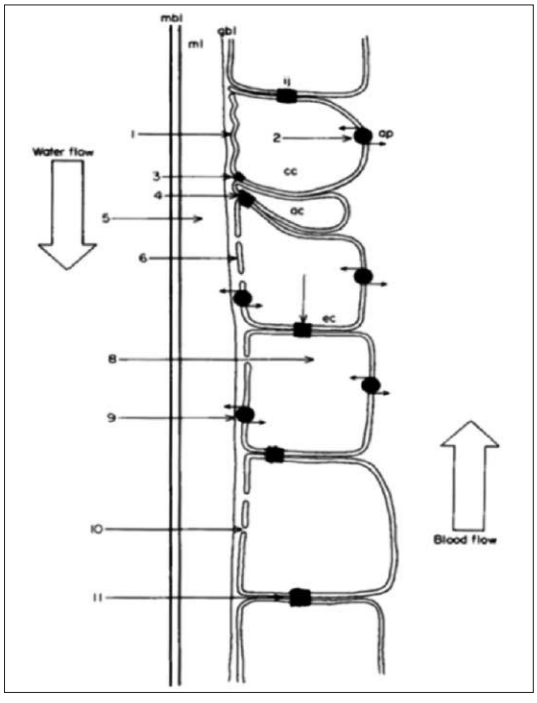
ACUTE ALUMINIUM INTOXICATION

Fish that have been acutely exposed to aluminium are typically characterized by macroscopic and/or microscopic damage to the gill tissue. Symptoms may include respiratory dysfunction characterized by plasma acidosis, hypoxia, and hypercapnia with osmoregulation loss. Other observations related to the acute aluminium toxicity include the excessive production of mucoid cells (Muniz & Leivestad, 1980), inhibition of the activities of the carbonic anhydrase

enzymes and Na-K-ATPase (Staurnes *et al.*, 1984) and the apical and intracellular accumulation of aluminium in the gill epithelium (Youson and Neville, 1987, Exley, 1989). Other findings include necrosis and detachment of epithelial cells (Exley, 1989). The alterations observed depend on the chemistry of the water. The acute aluminium toxicity in fish is clearly the result of the decomposition of the properties of the gill epithelium.

There are two theories about how acute aluminium poisoning affects fish. The first describes the alteration of the gill epithelial cells caused by the precipitation of aluminium hydroxide on the gill surface, a condition that is exacerbated by the excessive production of mucoid cells on the gill surface. The second theory is about surface bonding. The aluminium present in the gill surface joins functional groups in the gill epithelium. Initially, it was postulated that aluminium hydrolysis products, mainly AI (H_20) 3+, AI (H_20) 5 (OH) 2+, AI (H_20) 4 (OH) AI (H_2O) 2 (OH) competed to join groups that were part of the structure and function of the membrane (Sadler & Lynam, 1987). It was observed that the toxicity was more acute when it was calculated that AI (H_20) 5 (OH) 2+ was the dominant species in solution and later this species was the suggested toxic molecule.

In acute aluminium toxicity, this metal is attached to groups located apically in the gill laminar epithelium. Specific binding sites, for example, the aluminium-phospholipid phosphatidylserine complex (Shi & Haug, 1988) neutralizes the charge of one or more carboxylates and/or phosphate, subsequently reducing the membrane fluidity (Viersta & Haug, 1978). Similarly, it is expected that the substitution of aluminium by the metal cofactors of the transport proteins will alter the membrane permeability.



Schematic representation of the potential interaction sites of aluminium in the lamellar epithelium of the gill. mbl (mucosal borderline layer); ml (mucous layer); gbl (gill boundary layer); ij (intercellular junction); ap (ATPase bomb); cc (chloride cell); ac (accessory cell); ec (epithelial cell). The numbers indicate the interaction sites. (1) Apical surface of the chloride cell. (2) Active transport system basically located. (3) Narrow apical junction joining chloride and accessory cells. (4) Broad apical junction joining accessory and epithelial cells. (5) Mucoid polyanionic layer that includes the carbonic anhydrase enzyme. (6) Apical surface of the epithelial cell. (7) Intracellular effects on intercellular junctions. (8) Aluminium intracellular accumulation. (9) Active transport system apically located. (10) Apical membrane channels. (11) Extracellular effect on the intercellular junctions.

It is known that aluminium inhibits the active absorption of NaCl (Dalziel *et al.*, 1987) and the inhibitory mechanism may involve the aluminium substitution, either by a metal cofactor in the functional domain of an active transport protein or by the transport species itself.

The conclusion is that aluminium is not merely irritating to fish in acidic waters, but that it exerts a major effect. It induces an increase in the permeability of the gill epithelium with consequent alterations in the transmembrane ion fluxes. This allows aluminium to accumulate intracellularly in epithelial cells and reach a toxic threshold altering homeostasis, accelerating cell death, exacerbating the breakdown of the gill barrier and resulting in the death of fish.

Impacts of aluminium on the success of the migration to brackish water and sea

Observation of osmoregulation is of particular concern and environmental relevance, since it can be interrupted by Al exposure in fresh water at very low concentrations (e.g., 6 µg/L to pH 5.8) (Kroglund *et al.*, 2003) for a short period (h) (Stauernes *et al.*, 1996) and that cannot really cause a significant deterioration of the osmoregulatory capacity in freshwater of these fish.

The most obvious objective of the Al effect is the activity of Na+/K+-ATPase in the gills, which is known to increase during smoltification in preparation for migration to the sea (Stauners *et al.*, 1993, 1995, 1996, Magee *et al.*, 2003).

Monette *et al.* (2010) also found a decrease in the mRNA expression of other transporters in seawater caused by Al exposure, such as CFTR-1 (the Cl-apical channel involved in Cl secretion), as well as the decrease in the size and intensity of the mitochondria in the gill cells.

Accumulation of aluminium in the body

The accumulation of Al is associated with mucus, but it can also be observed in intracellular deposits. Accumulation has been reported in organs such as brain, kidney, liver, gonads, heart, white muscle and scales in chronic exposures. Sandoval *et al.*, (2016) have observed in experimental aluminium poisonings (unpublished study) an accumulation of metal in bone/cartilaginous tissue of fish, as well as an accumulation in these tissues in poisonings in the field, evidenced by special histochemical techniques for aluminium.

This correlates to what Malcolm observed. *et al* (1981), who evidenced by histochemical techniques the accumulation of aluminium in bone tissue in people intoxicated with aluminium.

Potential for bioconcentration and/or biomagnification of aluminium

The adsorption on the gill surface before Al exposure in water is fast, while the cellular uptake of water is slow, but gradual accumulation in the internal organs (muscle, liver, and kidney) can occur through the diet. (10 g Al/kg of dry mass of the diet) (Handy, 1993).

Characterization of uptake routes

Gill The mucus accumulates Al very quickly (minutes to hours) (Goossenaerts *et al.*, 1988). Cellular accumulations through the gills are slow, but gradual accumulation in internal organs occurs over time. Al toxicity arises from the actions of bound and precipitated Al or a polymerization on the gill surface (Exley *et al.*, 1991). Almost all of the gill Al is found in the gill surface (Goossenaerts *et al.*, 1988) and in particular in areas rich in mucus between the gill lamellae (Norrgren *et al.*, 1991), however, after prolonged exposure (from 1 week to 1 year), the Al deposit can be found within the cells of the same gills.

Other routes

It has not been documented

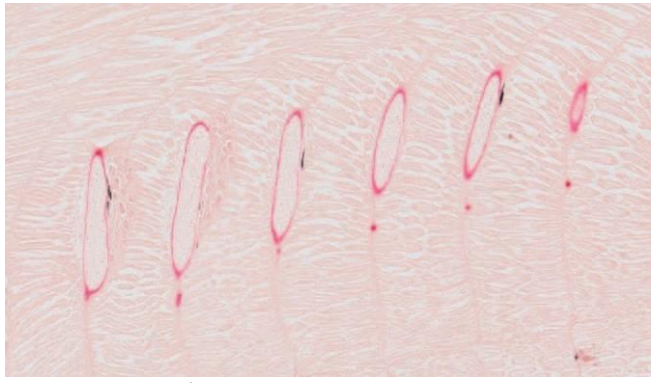


Figure 1. Cartilage/bone. Aluminol. Moderate positive reaction to aluminium (red colour) in cartilage/bone.

Figure 3. Cartilage/bone. Aluminol. Moderate positive reaction to aluminium (red colour) in cartilage/bone.

Characterization of excretion pathways

Depuration is initially fast from the gills after returning to the Al-free water after acute exposure in the water. This rapid clearance is probably related to mucosal detachment largely bound to Al (Playle and Wood, 1991); however, the depuration of Al that has accumulated in the gills of brown trout took longer (40 days) in returning to the levels found in the control fish that was close to 15 days.

The information on the Aluminium removal rate from the internal organs seems to be slower than for the gills (>15 days for the head, the kidneys and the liver), or it may not occur at all as is the case with the white muscle (25 days). An aluminium enriched diet may cause Al to be detected in the Rainbow trout mucus, and Al concentrations were relatively higher in the gills than in the liver or kidney after 42 days of Al dietary intake, which suggests that excretion occurs through the gills (mucus) and is the detoxification pathway of internal stores (Handy, 1996).

Al interaction with other metals

Although it is known that Al causes alterations in the biota of water, the interaction it may have with other metals is not completely known. Some studies reveal the interaction of Al with Zinc (Zn), Copper (Cu) and Hydrogen (H+), observing similar mechanisms of inhibition of ion regulation in gills (Hutchinson and Sprague, 1986).

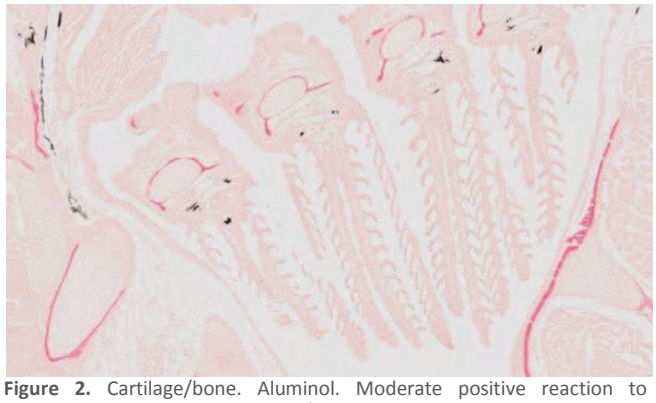


Figure 2. Cartilage/bone. Aluminol. Moderate positive reaction to aluminium (red colour) in cartilage/bone.

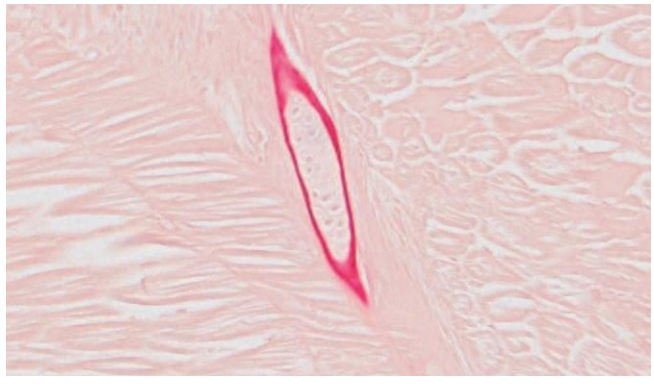
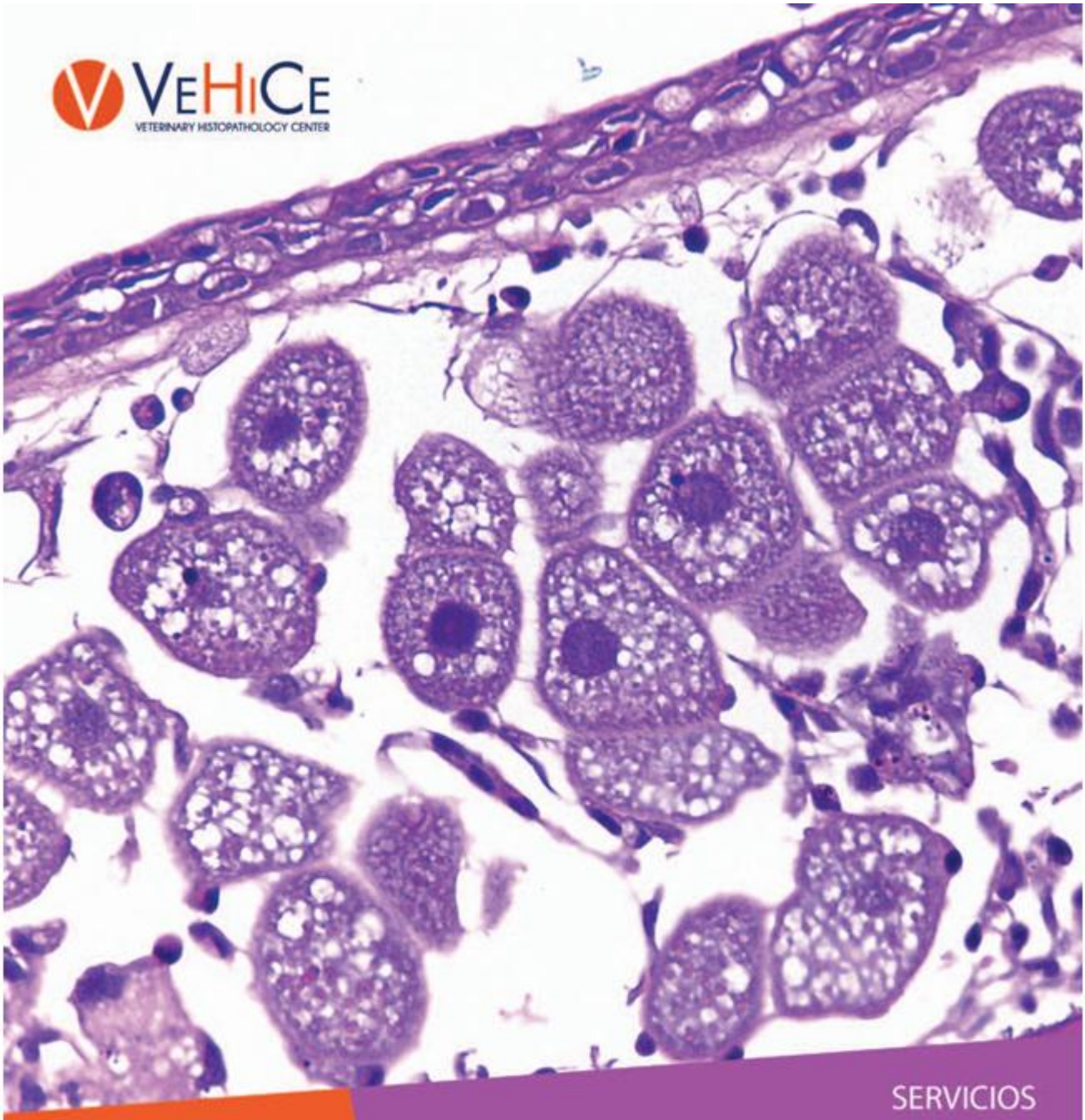


Figure 4. Cartilage/bone. Aluminol. Moderate positive reaction to aluminium (red colour) in cartilage/bone.


VeHiCe
VETERINARY HISTOPATHOLOGY CENTER
SERVICIOS
Determination of toxicity of aquaculture products
Evaluation of gonadal development
Detection of parasites and fungi
Detection of heavy metals
Detection of viruses and bacteria
Health status evaluation
FAN detection
Diagnosis of diseases
Metabolic diseases
Smoltification status
Exotic diseases
Evaluation of diets
Aquatic toxicology
Histological score
PatologíaVeterinaria
PATOLOGIA-VETERINARIA.COM
Libertad 590, Puerto Montt X Región de Los Lagos, Chile
Teléfono: +56 9 7575 4923 / +56 9 8414 0421. E-mail: info@vehice.cl
PATOLOGÍA DÍGITAL
WWW.VEHICE.CL