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Aluminium toxicity to rainbow trout at low pH

Daniel Dietrich and Christian Schlatter

Institute of Toxicology, Swiss Federal Institute of Technology and University of Zurich, Schwerzenbach, Switzerland

An acute toxicity study of aluminium at low pH, using one-year-old rainbow trout (*Salmo gairdneri* R.), was performed in a closed recirculating system at pH 5.2, 5.4, and 5.6 with nominal concentrations of 0, 100, 200, and 400 μg Al/l. Mortality (96 h) was dependent on the pH and aluminium concentration. Measurements of aluminium in the plasma of exposed fish, by electrothermal atomic absorption spectrometry (ETAAS), showed a dose-dependent uptake of aluminium, but no correlation of plasma aluminium concentration to the mortality observed. Three major mechanisms of pH-Al toxicity seemed to prevail:

i. Relatively low nominal aluminium concentrations (100 and 200 μg/l) at pH 5.2 as well as 200 μg Al/l at pH 5.4 led to electrolyte loss possibly due to an interaction of aluminium with enzymes and epithelial tight junctions in the gill of the exposed fish.

ii. Exposing fish to Al concentrations ≧100 μg/l and pH values ≧5.2 enhanced cell necrosis, proliferations, and fusions of the secondary lamellae in the gills resulting in the obstruction of the interlamellar space and thus most likely in the impairment of gas exchange. Aluminium fractionation suggested that inorganic monomeric Al was responsible for this tissue damage.

iii. High aluminium concentrations (≧200 μg/l) at moderately low pH (≧5.4) led to clogging of the gills with mucus and thus to an impairment of gas exchange. This mucification was thought to stem from the physical irritation of the gills by accumulating polymeric Al.

Key words: Plasma–Aluminium; Mucification; Cytotoxicity; Ionoregulation; Histopathology; Aluminium

INTRODUCTION

Acidified waters have been recognized as a problem for freshwater fisheries in Europe and North America. Intensive research resulted in the identification of aluminium (Al) as the major fish toxicant besides low pH. A review of the current literature on aluminium and pH–toxicity to fish (Wood and McDonald, 1987; Witters et al., 1987; Baker, 1982; Muniz and Leivestad, 1980a and b; Schofield and Trojnar, 1980, and Leivestad et al., 1987) reveals that the mechanism of aluminium toxicity is still a matter of controversy, especially in the pH range 5.0 to 6.0.

Correspondence and requests for reprints to: D. Dietrich, Institute of Toxicology, Swiss Federal Institute of Technology and University of Zurich, CH-8603, Schwerzenbach, Switzerland.

Many authors (Baker, 1982; Grahn, 1980; Dickson, 1983; Leivestad et al., 1980; Rosseland, 1980; and Muniz and Leivestad, 1980b) found that fish damaged by exposure to waters supersaturated with respect to $Al(OH)_3$, had gills clogged with mucus and showed symptoms of asphyxiation (reduced blood O_2 saturation, elevated blood lactate levels, and increased ventilation rates). This mucification and subsequent asphyxiation was attributed to the physical irritation of the gill epithelia by precipitating polymeric $Al(OH)_3$ onto the gill surface, an aluminium form which prevails even at low total aluminium concentrations at pH values between 5.0 and 6.0 (Burrows, 1977).

Contrary to the observations above, neither Skogheim et al. (1984), Fivelstad and Leivestad (1984), nor Hutchinson et al. (1987) could detect mucification on the gills of fish exposed under similar conditions in the laboratory as well as in the field. As only two of the numerous reported mucifications came from field experiments (Grahn, 1980, and Dickson, 1983), mucification and subsequent asphyxiation was considered to be an experimental artefact inherent to laboratory testing (Leivestad et al., 1987) and possibly due to the choice of $Al_2(SO_4)_3$ as aluminium source (Rosseland and Skogheim, 1984, Rosseland et al., 1988). Based on this controversy, we thought it important to conduct experiments with $AlCl_3$ as aluminium source with the aim to gain further information on the mechanisms of pH-Al toxicity.

In many cases without mucification, an important loss of body electrolytes (Na and Cl) was observed in fish exposed to aluminium at low pH. The electrolyte loss was thought to stem from the opening of the branchial tight junctions due to an interaction of the epithelia with accumulating aluminium on the gill (Wood and McDonald, 1987). This hypothesis though, is currently disputed by Lacroix and Townsend (1987), who could not find a correlation between aluminium accumulation on the gills and electrolyte loss. Kjartansson (1984) and Staurnes et al. (1984) were able to show that the Na-K-ATPase, responsible for electrolyte uptake, is inhibited by aluminium. Thus either accumulating aluminium on the gills of the fish and/or systemic aluminium must interact with the tight junctions of the gill epithelia and the enzymes therein. While the correlation of gill-aluminium with electrolyte loss is still being disputed, systemic aluminium has never been measured. It cannot be ruled out a priori that systemic aluminium may be responsible for the interactions mentioned above and thus for the toxicity, possibly explaining some of the controversial results obtained so far. A further objective of our investigation was therefore to measure the plasma aluminium concentration (PAC) of aluminium exposed fish and try to relate our findings to mortalities and electrolyte losses.

MATERIALS AND METHODS

Experimental animals

All experiments were performed with approximately 1-year-old rainbow trout, *Salmo gairdneri* Richardson (21–142 g wet weight). The fish were purchased at a com-

TABLE I

COMPOSITION OF EPA-MEDIUM AS ANALYSED DURING ACCLIMATIZATION AND EXPERIMENTATION (WATER ANALYSIS, 1983)

NH_4-N	< 0.02 mg/l	Cl	1.6 ± 0.1 mg/l
NO_2-N	< 0.002 mg/l	SO_4	27.3 ± 1.4 mg/l
NO_3-N	< 0.1 mg/l	Ca-Hardness	14.8 ± 4.4 mg/l
PO_4-P	< 0.01 mg/l	Total-Hardness	52.5 ± 2.8 mg/l
Total-P	< 0.01 mg/l	O_2 (Winkler)	9.9 ± 0.4 mg/l
DOC	< 0.2 mg/l	Conductivity	162 ± 9 μS/cm

mercially run fish hatchery in Andelfingen, Switzerland. All fish were acclimatized to soft water conditions (Table I) for 48 h prior to the experiments. The water was exchanged every 24 h in order to reduce any accumulation of excretory products. The fish were not fed during the acclimatization and the experiments.

Experimental unit

All experiments were done by using a temperature-controlled (10 ± 1°C) recirculating system (Fig. 1). The flow rate was 1200 l/h and the total volume 436 l. The water

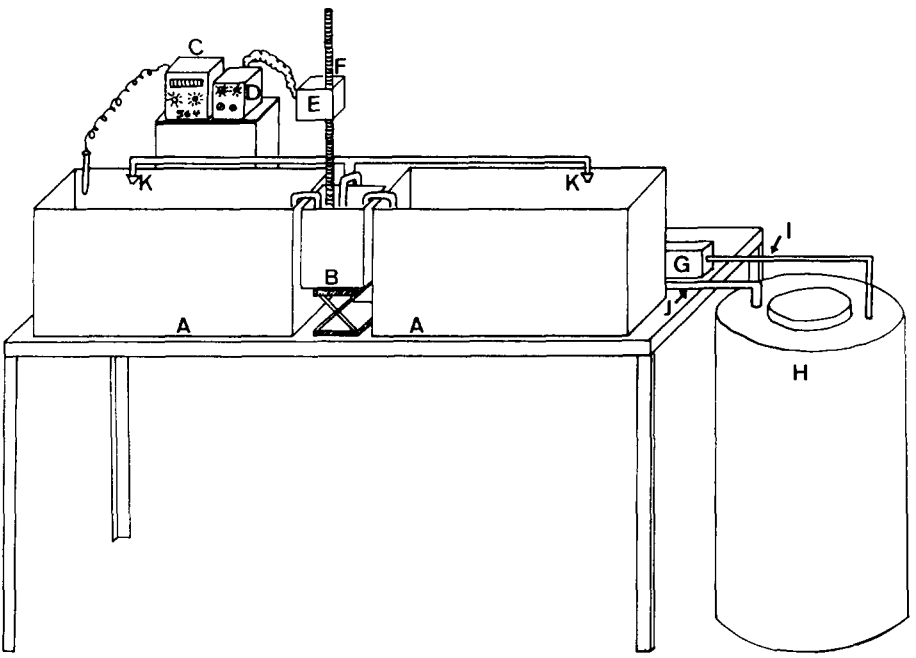

Fig. 1. Schematic diagram of the experimental set-up. A, 110 liter aquaria; B, mixing chamber; C, pH-meter; D, titration unit; E, solenoid valve; F, acid burette; G, water pump; H, exchangeable medium tank; I, medium outflow from tank; J, medium inflow into tank; K, injector.

(Table I) was prepared by adding inorganic salts to deionized water as described by the U.S. EPA guidelines (EPA-660/3-75.00). Aluminium was added as anhydrous $AlCl_3$. 50% of the test solutions or control water was replaced every 48 h to maintain the concentration of aluminium and the other electrolytes. pH was kept constant by an automatic titration unit (Metrohm 564 pH-meter) using 1 N HCl for titration. pH was adjusted whenever the value exceeded the nominal value by 0.05 pH units.

Experimental protocol

Ten one year old rainbow trout (*Salmo gairdneri*, R.) were put into each of the two experimental units, i.e. ten fish into the exposure unit and ten fish into the control unit, respectively. Six experiments, including the corresponding controls, were carried out (Table II) and lasted 96 hours or until 50% of the fish in the exposure unit had lost their equilibrium (Manifestation Time, MT_{50}, Mueller, 1980). Upon reaching the MT_{50} the remaining fish from the exposure unit and all fish from the control unit were removed from the tanks, anaesthetized with 100 mg MS-222/l (Sandoz AG, Basel, Switzerland), blood sampled, and dissected. Hematocrit was determined immediately thereafter and plasma samples were taken for Al, Na, and whenever sufficient plasma was available for Cl determinations. Gill, kidney, and liver samples were taken for histopathology. Samples for water analysis and aluminium fractionation were taken at the beginning, every 24 h, 2 h after the exchange of 50% of the medium, and at the end. The experiments were carried out in succession and within one year.

TABLE II

pH VALUES AND NOMINAL ALUMINIUM ADDED TO THE EPA SOFTWATER MEDIUM (TABLE I) AND ALUMINIUM FRACTIONS ANALYSED (ARITHMETIC MEAN ± S.D.) DURING THE COURSE OF THE EXPERIMENTS

Exp. No.	pH	Aluminium added		Aluminium analysed					
		Control (μg Al/l)	Exposure	Total Al (μg Al/l)	(n)[a]	Labile Al (μg Al/l)	(n)	Suspended Al (μg Al/l)	(n)
1	5.2	0	100	137 ± 32	(5)	92 ± 10	(5)	43 ± 37	(5)
2	5.2	0	200	203 ± 12	(2)	175 ± 21	(2)	14 ± 11	(2)
3	5.4	0	200	142 ± 54	(6)	123 ± 41	(2)	39 ± 33	(2)
4	5.4	0	400	490 ± 140	(3)	193 ± 24	(3)	293 ± 97	(3)
5	5.6	0	200	173 ± 58	(6)	63 ± 46	(6)	102 ± 42	(6)
6	5.6	0	400	530 ± 514	(3)	341 ± 462	(3)	166 ± 164	(3)[b]

[a](n), the number of samples analysed. [b]In this experiment the first water sample for aluminium fractionation was taken from the mixing chamber before the aluminium was homogeneously dissolved in the total volume of the medium.

Blood samples

Blood was taken from the anaesthetized fish by puncturing the heart (Lehmann and Stuerenberg, 1980) with a heparinized hypodermic needle (Li-Heparin, Terumo-Luer 18G $\times 1^1/_2''$) and letting the blood run into a 2 ml heparinized polystyrol tube (Milian AG, Geneva, Switzerland).

Hematocrit

Microhematocrit capillaries (Capilet-C, American Dade, USA) were filled to the mark with wholeblood samples and sealed on both ends with tube sealer. The capillaries were then placed into a hematocrit-fuge (Readacrit, Clay Adams, USA) and centrifuged for 5 min. Hematocrit values were determined by laying the capillaries onto a microhematocrit scale (0–100% in 1% units, Heraeus Co., FRG).

Plasma Na and Cl analysis

Plasma Na was analysed on a Corning 435 flamephotometer (Corning Ltd, UK), having a detection limit of 1 mM Na/l. The flamephotometer was calibrated using Corning 140 mM Na/l standard.

All plasma Cl analyses were done at the Children's Hospital in Zurich using coulometric titration on an Analyzer 929 (Corning Ltd, UK), calibrated with aqueous chloride standards, and a detection range of 10–150 mM Cl/l.

Histopathology

Gill (2nd gill arch, left side, direction 'tail-head'), kidney (middle section), and liver samples were taken and put immediately into 4% buffered (pH 6.5–6.8, $CaCO_3$) formaldehyde solution. These samples were then dehydrated in an increasing series of ethanol i.e. 70%, 95%, and 100%, embedded in paraffin, and cut into 5 μm thin slices. After gently placing the tissue slices onto a microscope glass-slide, they were deparaffinized for 15 min in xylene, stained with hematoxylin-eosin, and inspected under the microscope.

Aluminium fractionation

Aluminium fractionation was carried out on 100 ml samples within 2 h after taking the samples. The fractionation technique used was basically the same as described by Barnes (1975) and later modified by LaZerte (1984) though no dialysis or cation exchange resin steps were carried out but an extra MIBK extraction step introduced in turn (Fig. 2).

Of the fractions A–G only the fractions A–D were analysed. Fractions E, F, and G were calculated by subtraction, e.g. A–B = E. The labile aluminium concentrations are identical with the inorganic monomeric fraction and thus can be calculated (C–D or G–F = labile aluminium). All fractions were acidified with 65% quartz-distilled HNO_3 to an approximate pH of 1 prior to analysis.

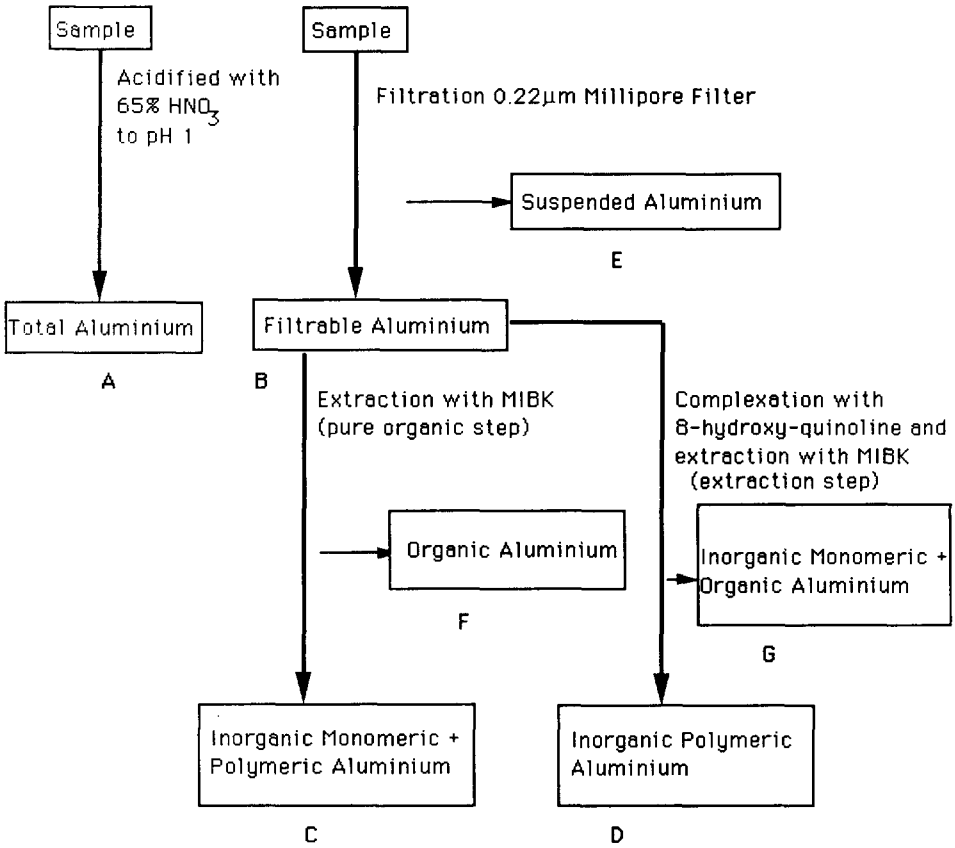

Fig. 2. Aluminium fractionation scheme. Bold letters denote different aluminium fractions.

Aluminium analysis

The analyses of the plasma samples and the different Al-fractions were done by electrothermal atomic absorption spectrometry (ETAAS) on a Perkin-Elmer model consisting of a HGA 500 graphite furnace, a model 5000 spectrophotometer, and an AS-1 autosampler. All samples were calibrated using the method of standard additions. More detailed information as to this method and the detection limits of ETAAS are given by Rickenbacher (1984) and Kaenzig et al. (1987). Each water sample was injected into the ETAAS four times. The standard deviation of these repetitive analyses was ± 5 µg Al/l. For all sample types one temperature program was used (Table III). The usual procedure was to mix 100 µl of sample diluent (0.25% Triton X-100 in doubly distilled H_2O), 50 µl of sample, and 50 µl of aluminium standard within the ETAAS sample cups. The standards 0, 50, and 100 ppb Al were made up from a 1000 µg Al/l solution (Fixanal, Riedel-de-Häen AG, Hannover, FRG) and acidified with quartz distilled HNO_3 to pH 3. The quality of the aluminium analysis

TABLE III

TEMPERATURE PROGRAM USED FOR Al ANALYSIS BY ETAAS

Steps	Temperature (°C)	Ramp (s)	Hold (s)
Drying	110	10	15
Ashing	1450	10	10
Cooling	20	1	2
Atomizing	2200	0	5
Cleaning	2500	1	3

by ETAAS was controlled by participating in a monthly round-robin 'Trace Element Assessment Scheme' (Trace Element Reference Center, Guildford, UK) analyzing aluminium in water, dialysis fluids, and sera.

Statistical analyses

The values in the tables are arithmetic mean $\pm$ SD. Significant differences between acid-exposed and acid-aluminium exposed fish were identified by the Wilcoxon, Mann–Whitney two-tailed *U*-test and are shown as ** for $P < 0.01$ and * for $P < 0.05$.

RESULTS

Aluminium fractionation

The total aluminium concentrations (labile and suspended) were not stable over the whole length of the experiments (Table II). Total aluminium concentrations declined with increasing time after initial addition and also after the initial concentration level was restored by exchange of 50% of the medium during the experiments (after 48 h). This decline, also registered as a decrease in labile-Al concentration (Fig. 3), was most pronounced in the experiments with high aluminium concentrations and moderately low pH, whereas this effect was almost negligible in the experiments with low pH and relatively low aluminium concentrations. Similar observations were already described by Witters et al. (1987) and Karlsson-Norrgren et al. (1986) who attributed this effect to an enhanced formation and precipitation of polymeric $Al(OH)_3$. The mean total-Al concentration in the waters of all control experiments was 10 ± 5 μg Al/l.

Mortality and observed symptoms

No mortality was recorded in any of the exposures without added Al (controls) with the exception of the experiment at pH 5.2 where one fish was lost due to a severe infestation with the protozoan ectoparasite *Costia necatrix*. No mucus accumulation or hyperventilatory response could be detected in any of the experiments without added Al.

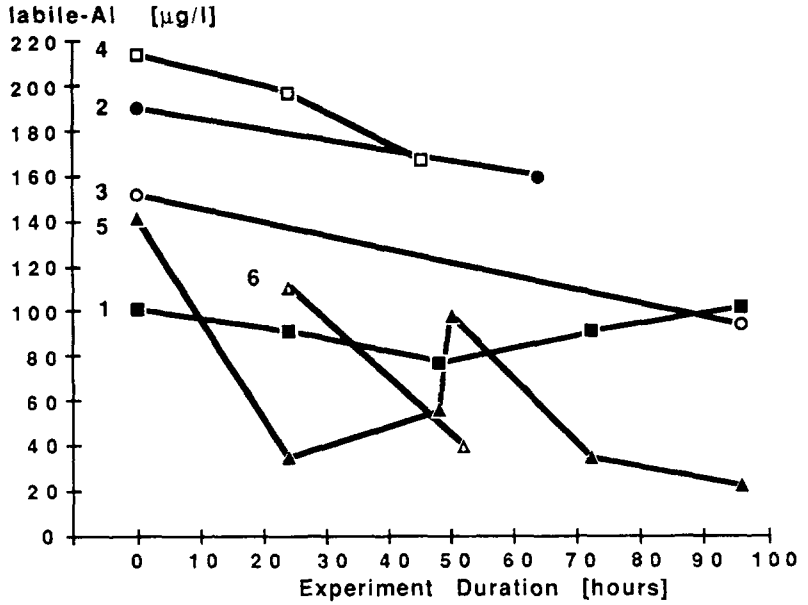

Fig. 3. Labile-Al concentrations in the six experiments. ●, pH 5.2 and 200 µg Al/l; ○, pH 5.4 and 200 µg Al/l; ■, pH 5.2 and 100 µg Al/l; □, pH 5.4 and 400 µg Al/l; ▲, pH 5.6 and 200 µg Al/l; △, pH 5.6 and 400 µg Al/l.

Coughing, increase of ventilation rate, slight darkening of the body coloration, frequent spasms interspersed with longer periods of immobility, and eventually loss of equilibrium before death could be observed in all of the Al-exposure experiments, although the intensity of the symptoms varied with pH and Al-concentration. No accumulation of mucus could be detected at pH 5.2. Slightly enhanced production of mucus was recorded at pH 5.4 and 200 µg Al/l, while moderate and extensive mucification resulted from exposing the fish to pH 5.4 and 400 µg Al/l, pH 5.6 and 200 µg Al/l, and pH 5.6 and 400 µg Al/l, respectively. Fish dying at pH 5.2 and 200 µg Al/l did not exhibit gaping opercula, although this was seen at pH 5.4 and 400 µg Al/l, pH 5.6 and 200 µg Al/l, or 400 µg Al/l. 200 µg Al/l was lethal to 50% of the exposed fish within 64 h at pH 5.2, no deaths occurred at pH 5.4, and three fish out of ten died within 96 h at pH 5.6.

Histopathology

Compared to fish kept at a pH 7.6 (Dietrich, 1988), the gills of the fish exposed to acid water alone revealed only minor alterations, regardless of the pH used. These alterations consisted mainly of lifting of the epithelial layer, some cell necrosis, and pyknosis of epithelial, chloride, and mucus cells.

The gills of the fish exposed to aluminium were all damaged. At pH 5.2, the damage increased with increasing aluminium concentration and consisted predominantly

of lamellar fusions, cell proliferation, and epithelial lifting leading to the loss of gill structure and congestion of the interlamellar space with cell debris and proliferating cells. At pH 5.4 and 200 μg Al/l the degree of tissue damage was moderate and in addition, enhanced mucification was observed. Almost complete obstruction of the interlamellar space with mucus was recorded at pH 5.4 and 5.6 with 400 μg Al/l. A similar observation was made in the experiment at pH 5.6 with 200 μg Al/l, though the mucification was not as extensive. The gill tissue damage in the latter three experiments was low in comparison to the damage reported for the experiment at pH 5.2 with 200 μg Al/l.

Liver or kidney tissue damage was not induced by exposing fish to aluminium. This observation stands in contrast to reports by Schofield and Trojnar (1980) and Tandjung et al. (1982) who found a correlation between increasing degrees of liver and kidney tissue damage in brook trout exposed to aluminium concentrations >200 μg Al/l.

Plasma aluminium concentration (PAC)

A slight increase of PAC with increasing exposure time can be detected in the experiments without added Al (Fig. 4): The mean PAC in the experiments of a shorter duration (45.5 to 64 h) was 33 ± 6 ng Al/ml whereas the corresponding value in the experiments lasting 96 h was 50 ± 12 ng Al/l. The stress of being exposed to low pH could have caused a redistribution of the body Al which would explain the higher PAC levels and the considerable variation of the PAC levels in the control experiments lasting 96 h. The mean PAC levels of fish exposed to acid water alone are not different from the mean PAC levels measured in rainbow trout kept in Lake Zurich water at a pH 7.6 for four weeks (Dietrich, 1988). The PAC values in the Al experiments were only slightly higher than in the corresponding experiments without added Al (Table IV and Fig. 4).

Plasma electrolytes and hematocrit

Na The plasma Na concentrations in the acid-experiments varied in the range between 142–167 mEq/l whereas the lowest values can be found in the pH 5.2 experiments (Table IV). Compared to published values (Neville, 1985; Wheatley et al., 1984; and McDonald et al., 1980) they still are in the normal range. With the exception of the experiment at pH 5.6 and 200 μg Al/l, all Na values of the acid-Al experiments are lower than the Na values found in their corresponding experiments without added aluminium. The most pronounced effects were induced at pH 5.2 and 5.4 and 200 μg Al/l.

Cl Plasma chloride concentration measurements revealed that the mean plasma chloride concentrations are generally equal to or slightly lower than values found in literature (Neville, 1985, Wheatley et al., 1984, Thomas and Hughes, 1982, and McDonald et al., 1980). High aluminium concentrations (400 μg Al/l) seemed to induce a loss of plasma chloride. On the whole, the effects of pH and Al on plasma chloride concentration were almost negligible (Table IV).

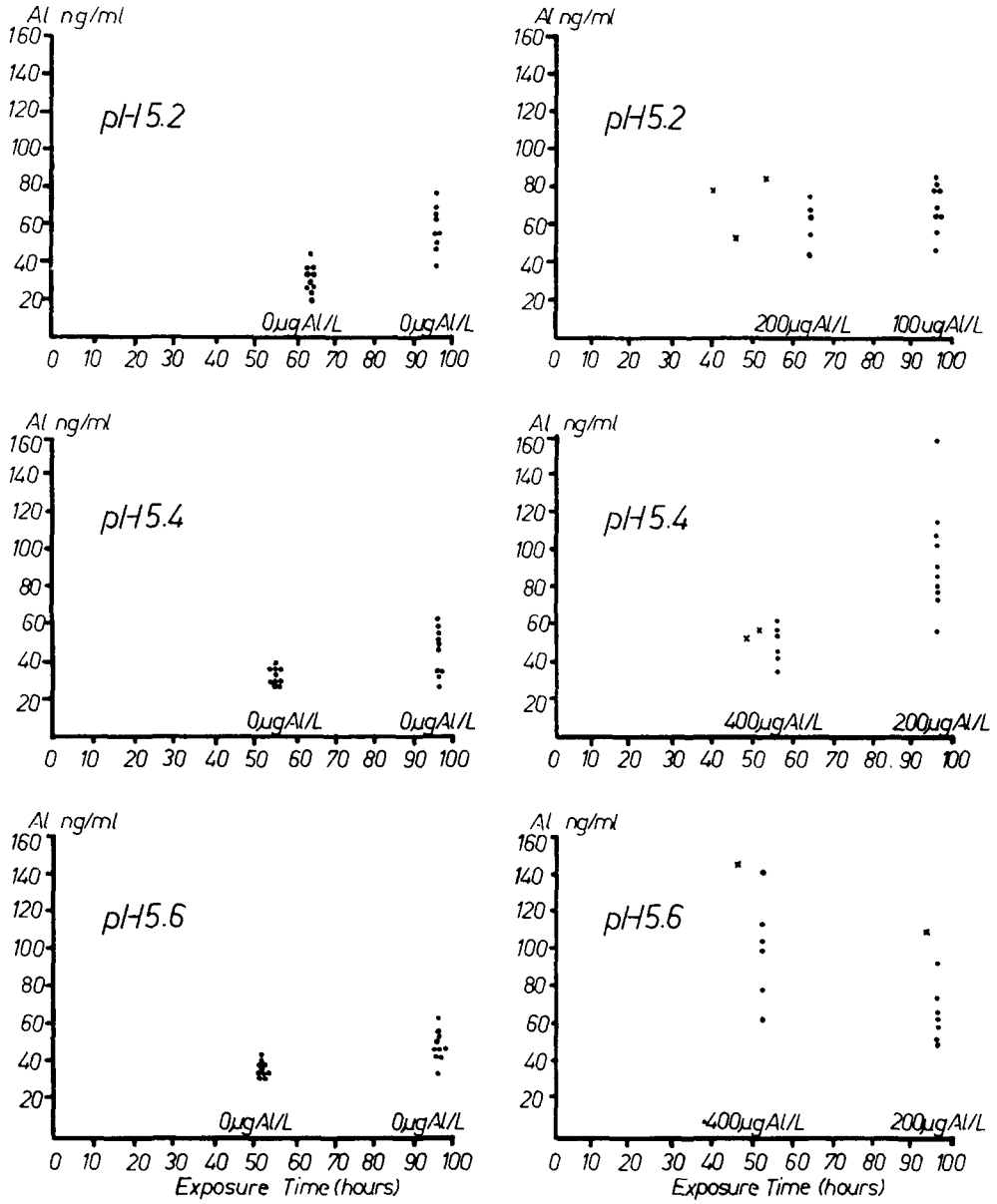

Fig. 4. Plasma aluminium concentrations (PAC). + denote fish sampled just before death and · fish sampled after termination of the experiment. The figure does not show fish that had died without being sampled. The nominal aluminium concentration added is indicated below the symbols.

TABLE IV

MEANS AND STANDARD DEVIATIONS (IN BRACKETS) OF BLOOD PARAMETERS AND MANIFESTATION TIMES (MT_{50}).

pH	Water aluminium nominal (μg/l)	Al (ng/l)	Plasma Na (mEq/l)	Cl (mEq/l)	Hematocrit (%)	MT_{50} (h)
	0	56 (12)	142 (2)	124 (5)	38 (5)	–
5.2	100	67 (13)	132**(5)	124 (8)	45* (5)	–
	0	31 (8)	144 (10)	n.m.	39 (7)	–
5.2	200	63**(14)	113**(8)	n.m.	54**(3)	64
	0	45 (13)	167 (17)	n.m.	38 (7)	–
5.4	200	93**(29)	117**(15)	n.m.	41 (9)	–
	0	32 (4)	152 (4)	122 (6)	50 (8)	
5.4	400	50**(9)	139**(3)	108**(9)	61* (9)	45.5
	0	47 (8)	149 (3)	119 (2)	51 (5)	–
5.6	200	68**(21)	145 (5)	118 (8)	56 (7)	–
	0	35 (4)	150 (4)	128 (4)	46 (6)	–
5.6	400	105**(30)	142**(4)	117**(9)	61**(3)	52

Asterisks denote values significantly different from the corresponding control value (**$P<0.01$ and *$P<0.05$; n.m. = not measured).

Hematocrit The mean hematocrit values of the acid only exposed fish were all in the range considered normal (Lehman et al., 1976). In the experiments with added Al the hematocrits were increased (Table IV). The mean hematocrit values were always considerably elevated in groups where deaths occurred.

DISCUSSION

Mortality and symptoms

The main symptoms at pH 5.4 and 5.6 combined with 200 or 400 μg Al/l were high coughing rates and extensive mucification of the gills. Similar observations were reported earlier by Neville (1985), who exposed rainbow trout (*Salmo gairdneri* R.) to 75 μg Al/l and pH 5.5, using $Al_2(SO_4)_3$ as an aluminium source. In view of the fact that mucification of the gills occurred irrespective of the aluminium compound used for experimentation, the suggestion of Rosseland and Skogheim (1984) and Rosseland et al. (1988), that the use of aluminium as sulphate salt may be the cause for the reported mucifications, can be disproved. Clogging of the gills with mucus will raise the diffusional resistance to O_2 and reduce the water flow through the sec-

ondary lamellae (Ultsch and Gros, 1979) leading to asphyxia. As no substantial sodium loss was registered, it seems that the gills' function in regulating electrolytes was at least not heavily impaired.

Mucification is always, with the exception of Neville's observation (1985), reported in connection with the use of high (> 200 μg Al/l) aluminium concentrations (Muniz and Leivestad, 1980b, Rosseland and Skogheim, 1984). Especially in the pH range 5.4 to 5.8, where aluminium solubility is lowest (Burrows, 1977), aluminium will tend to polymerize and precipitate. Precipitating polymeric-Al may accumulate on the gill surface as a result of the negatively charged mucus (McDonald, 1983). As the gill serves as an excretion organ for ammonia, the slight increase in pH directly on the gill surface may further enhance the precipitation and accumulation of aluminium (Wood and McDonald, 1987). The reported mucifications in our experiments coincided with polymeric-Al concentrations that were higher than 100 μg Al/l in the medium (Table II). The precipitating polymeric-Al probably exerts a moderate cytotoxic effect on the gills leading to mucus secretion. Precipitation of polymeric-Al onto the gills thus represents a mechanism of aluminium toxicity inherent to conditions with high aluminium concentrations and moderately low pH.

Precipitation of aluminium in experimental waters and accumulation of aluminium on the gills is not only reported for recirculating test systems (our experiments, Karlsson-Norrgren et al., 1986), but also for flowthrough systems (Muniz and Leivestad, 1980b, Schofield and Trojnar, 1980, Neville, 1985), and for field experiments (Grahn, 1980, Dickson, 1983). It may thus be concluded further that the observed mucifications do not represent a laboratory artefact as has been suspected by Rosseland et al. (1988). The fact that Skogheim et al. (1984) did not detect mucification in their studies with atlantic salmon (*Salmo salar*) in the pH range 4.7 to 6.0 with aluminium concentrations ranging between 47 and 394 μg Al/l remains unexplained. In the low pH/low aluminium experiments a loss of sodium and the lack of mucification were the most outstanding observations made. As the electrolyte loss was quite comparable at pH 5.2 and pH 5.4 with 200 μg Al/l, another factor, not seen at the other conditions, had to be responsible for the mortality at pH 5.2 and 200 μg Al/l: histopathological preparations of the gills of the fish exposed in experiments 1, 2 and 3 (Table II) revealed that heavy damage resulted from exposure to pH 5.2 and 200 μg Al/l, whereas the gills of the fish exposed to 100 μg Al/l at pH 5.2 and to 200 μg Al/l at pH 5.4 were only moderately damaged. The gill damage consisted of fusions of the secondary lamellae, hyperplasia, and desquamation of the gill epithelia, leading to an obstruction of the interlamellar space and thus to a reduction of water flow and gas exchange within the gills. The obvious question arises why a concentration of 200 μg Al/l is markedly more cytotoxic at pH 5.2 than at pH 5.4. Our Al-fractionation shows that a greater amount of aluminium exists in the labile (inorganic monomeric) form at pH 5.2 than at pH 5.4 (Fig. 3), thus suggesting that the labile-Al exerts a marked cytotoxic effect on the gills. It must be added though that our method of Al-fractionation is far from being perfect and may overestimate the labile fraction,

due to the fact that some polymeric-Al might pass the 0.22 μm filter.

These findings corroborate the observations of Jagoe et al. (1987) who reported similar gill damage in *Salmo salar* exposed to pH's between 4.5 and 5.5 and aluminium concentrations greater than 75 μg Al/l, though these damages were not explicitly attributed to monomeric-Al species. Rosseland and Skogheim (1984), on the other hand, found no gill damage in brown trout (*Salmo trutta*, L.) exposed to pH 5.0 and 130–462 μg Al/l, dying of electrolyte loss. This discrepancy may be explained by strain differences in the susceptibility towards the damaging effects of Al and low pH, shown previously to be of importance in the effects of Al and low pH on electrolyte loss (Rosseland and Skogheim, 1987).

Plasma aluminium

Though various previous efforts to measure aluminium in blood of fish exposed to aluminium at low pH by the pyrocatechol method (Dougan and Wilson, 1974) were undertaken previously (Wood and McDonald, 1987, Neville, 1985, and Dalziel et al., 1986 and 1987), no aluminium was detected. Whether the lack of aluminium detection was due to the detection limits of the pyrocatechol method or to the inadequacy of the method for aluminium detection in biological materials cannot be determined, especially as no detailed information about the detection limits, procedures of analysis, and results were given. Our plasma analyses, on the other hand, revealed higher PAC levels in fish exposed to aluminium than in fish of the experiments without added Al (Table IV). It is surprising that the plasma aluminium values of the control fish are approximately a factor ten higher than the normal values found in man. This might be due to the Al content in the water and in the trout feed, which was high (135 ± 24 μg Al/g wet weight, Dietrich, 1988) compared to the human diet (daily human intake of 4 mg Al, Knutti and Zimmerli, 1985). It seems however that the uptake of aluminium from the water is small since the increase of plasma concentration in the groups with 200–400 μg Al/l was only slight, going from 31–56 ng Al/ml of the controls to 50–105 ng Al/ml of the exposed fish (Table IV). It is not likely that this slight increase of aluminium in the plasma of aluminium exposed fish would have any pronounced effects on either mortality, hematocrit, or electrolyte loss; indeed no correlation was found between plasma Al and these parameters. Our findings thus suggest that plasma aluminium is not a factor in the acute toxicity of aluminium to fish.

Hematocrit

Leivestad (1982) and Rosseland and Skogheim (1984) found that an increase in hematocrit was always associated with an extensive electrolyte loss in fish exposed to aluminium at low pH. We could not confirm these findings, as we recorded high hematocrit values in our experiments whether the fish exposed faced an increased electrolyte loss or not. On the other hand, high hematocrit values were always associated with dying fish. Lehmann and Stuerenberg (1981) found that any kind of stress

can induce an increase in hematocrit in fish. Thus our opinion is that the high hematocrits reported in many studies may reflect an unspecific response of the fish to an extremely stressing situation, closely associated with a preterminal condition, rather than an indication of extreme electrolyte loss.

In conclusion, at pH values lower than 5.4 and Al concentrations higher than 100–200 μg Al/l, two major mechanisms may lead to the death of trout: one being electrolyte loss possibly being induced by an interaction of aluminium with enzymes and epithelial tight junctions; and the second being the cytotoxicity of labile-Al to the gill epithelia leading to necrosis, proliferations, and fusions of the secondary lamellae, finally resulting in the impairment of gas exchange.

At higher pH levels these two effects decrease in intensity whereas mucification increases, an effect which seems to be induced mainly by polymeric Al. The predominance of one of the three mechanisms may depend on the susceptibility of the fish strain, the pH, and the aluminium concentration.

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