Ammonia Toxicity in Teleost Fishes: A Review

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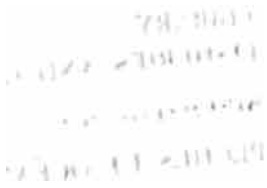

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ABSTRACT

Haywood, G. P. 1983. Ammonia toxicity in teleost fishes: A review. Can. Tech. Rep. Fish. Aquat. Sci. 1177: iv + 35 p.

Previous literature is reviewed, concerning ammonia toxicity to teleost fishes, and the manner is examined in which factors such as temperature, pH, carbon dioxide, dissolved oxygen and dissolved solute affect the un-ionized ammonia fraction. Some interpretations are suggested concerning the observable symptoms and possible physiological action of ammonia toxicity. A table of previous findings regarding ammonia toxicity to a number of teleost species is presented and units are standardized. From these findings the maximal permissible (safe) levels of un-ionized ammonia in polluted waters are suggested as 2 µg/L for salmonids, 10 µg/L for other freshwater teleosts, and 50 µg/L for other seawater teleosts. The corresponding maximum levels for total ammonia are suggested as 1, 2.5 and 2.5 mg/L respectively.

Key words: ammonia, ammonium, NH_3, NH_4^+, teleosts, toxicity.

RÉSUMÉ

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On passe en revue les travaux déjà publiés sur les effets toxiques de l'ammoniac chez les téléostéens et on étudie de quelle façon des facteurs comme la température, le pH, le gaz carbonique, l'oxygène et les produits dissous agissent sur la partie non ionisée de l'ammoniac. On émet certaines hypothèses sur les symptômes observables et les effets physiologiques possibles de la contamination par l'ammoniac. Un tableau des résultats antérieurs concernant les effets toxiques de l'ammoniac chez un certain nombre d'espèces de poissons téléostéens est présenté et les unités sont normalisées. À partir de ces résultats, on propose que les concentrations maximales acceptables (sécuritaires) d'ammoniac non ionisé dans les eaux polluées soient de 2 ug/L pour les salmonidés, de 10 ug/L pour les autres téléostéens d'eau douce et de 50 ug/L pour les autres téléostéens d'eau salée. On propose que les concentrations maximales équivalentes d'ammoniac total soient respectivement de 1, 2,5 et 2,5 mg/L.

Mots-clés: ammoniac, ammonium, NH3, NH4+, téléostéens, toxicité.

INTRODUCTION

The toxicity of ammonia to aquatic animals, and particularly fishes, has been fairly extensively investigated, and also reviewed (Alabaster and Lloyd 1980; EIFAC 1970). However, even after previous reviews, the literature remains somewhat confusing to many readers since different workers express ammonia in different terms and in different units. Furthermore, no concise guidelines have been established for defining the acute or chronic characteristics of ammonia toxicity. Previous work demonstrates that a wide variety of biological and environmental factors can influence the toxic action of ammonia. This paper assembles previous experimental findings for fishes, and attempts to make direct comparisons in common units, thereby simplifying the differences in "ammonia types" referred to by different workers.

Many previous workers have used different terminology and symbols to quantify toxicity measurements. Thus the reader will come across EC_{50}, LC_{50}, LD_{50}, TL_{50}, ET_{50} and terms such as "Median Effective Concentrations", "Median Lethal Concentrations", "Incipient Lethal Levels" and many others. Where appropriate, a brief explanation of terms used has been given in the text, but the reader is advised to consult the excellent explanation of toxicity terminology by Sprague (1969).

OCCURRENCE AND CHEMICAL FORM OF AMMONIA

Ammonia is both a substrate and an end-product of protein metabolism, its consumption or production depending on the diet and metabolic requirements of the fish. Normally protein nitrogen is in plentiful supply for teleosts on a normal diet; since it is not stored, there is a continual production of nitrogenous waste material. In teleost fishes the major nitrogenous wastes are ammonia or urea, since the majority of these fishes are ammoniotelic, excreted ammonia being produced from the catabolism of amino acids, purines and pyrimidines (Smith 1929; Wood 1958).

To avoid confusion, certain terminology will be used throughout this paper in connection with the various forms of ammonia. Thus "un-ionized" ammonia refers to the NH_3 moiety, which in solution becomes $NH_3.nH_2O$ (aq) - but at no time carries any ionic charge. Conversely "ionized" ammonia or the ammonium ion carries a positive change and is the ionic form of ammonia (NH_4^+). This ionized moiety exists most often in natural waters in association with hydroxide ions as part of the NH_4OH complex. Any general reference to ammonia in which both the above components are considered to be present, will be referred to as "total ammonia". In the past it has been common to report un-ionized or total ammonia concentrations (mg/L or µg/L) in terms of ammonia-nitrogen (NH_3-N or NH_4^+-N). However, none of the forms of ammonia exerts its effects through the nitrogen atom alone and it is considered more pertinent here to refer to un-ionized ammonia concentrations in terms of NH_3 and <u>not</u> NH_3-N. They are interconnected as indicated below:

$$0.8224 \text{ mg/L } NH_3\text{-N} = 1 \text{ mg/L } NH_3$$

FACTORS AFFECTING THE UN-IONIZED AMMONIA FRACTION

Ionization constants

In aqueous solution, ammonia exists in two forms, namely $NH_3.nH_2O$ (aq) and $NH_4^+ + OH^-$. Temperature affects the solubility of total ammonia in water, and together with pH, also affects the equilibrium between the un-ionized and ionized ammonia fractions present at any given temperature. The ionization constants of the ammonium ion were determined at 5°C temperature intervals over the range 0-50°C by Bates and Pinching (1949, 1950), using two different methods; and later the results were extended by Everett and Landsman (1954).

Several important tables giving the fractions of un-ionized ammonia per unit of total ammonia have been published since then. The first was by Burrows (1964) - although his table covered only a narrow range of pH (7.0 to 9.5) and temperature (10°, 15°, and 20°C) and contains some errors. Trussell (1972) published a larger table covering a wider range of these two variables. Both Burrows and Trussell used constants of Bates and Pinching (1950), with one exception: Trussell's value for the dissociation constant at 25°C differs from that given by Bates and Pinching and is based on figures given by Fleck (1966). Montgomery and Stiff (1971) prepared a nomogram for determining the un-ionized ammonia fraction at a given pH and temperature. Skarheim (1973) produced an extensive table giving the un-ionized fraction as a function of pH, temperature and total dissolved solids. Both the Montgomery and Stiff, and the Skarheim papers were criticized more recently (Emerson et al. 1975) for not being based on the most reliable determinations of the equilibrium constants, and for not describing how the calculations were done (Skarheim). According to Emerson et al., statistical analysis of Skarheim's reported equilibrium constants suggests that some questionable assumptions were made. Emerson's paper presents a table of calculated un-ionized fractions between 0° and 30°C and between pH values 6.0 and 10.0 - although the pH increments are only 0.5 - which we found too large for current research in this laboratory. A most comprehensive table of un-ionized ammonia concentrations, over a wide range of temperature and pH, was produced by Thurston et al. (1974), using computer techniques, which statistically provided an adequate fit to the Bates and Pinching data.

Temperature

A reduction in temperature reduces the fraction of un-ionized ammonia present, and hence the overall toxicity, as was demonstrated by Wuhrmann et al. (1947) and Wuhrmann and Woker (1948). Reduction in temperature has also been shown to affect the tolerance and susceptibility of fish to stress. Woker (1949) exposed chub (Squalius cephalus) to constant levels of un-ionized ammonia, and demonstrated that while their survival time decreased with rising temperature, the LC_{50} remained constant. Similarly Burrows (1964) exposed chinook salmon to un-ionized ammonia at 43°F (6.1°C) for 6 weeks and found that physiological changes in gill tissue occurred, including epithelial proliferation from which the fish did not recover when replaced in fresh water at the same temperature for a further 3 weeks.

However, the same experimental procedure carried out at 57°F (13.9°C)resulted in marked recovery from the gill hyperplasia when the fish were returned to fresh water suggesting a greater recovery capacity of the fish in warmer water.

Brown (1968) also indicated, contrary to the general trend, that un-ionized ammonia is more toxic to trout at 3°C than at 13°C, as indicated by the 48-h LC_{50} values. Again such findings suggest a greater susceptibility of fish at lower temperatures. However, susceptibility to injected ammonium acetate is increased for goldfish and channel catfish by raising the temperature from 23.3°C to 32.2°C (Wilson et al. 1969). Maximum tolerance of most species to un-ionized ammonia is found between 10° and 20°C, most probably due to this being the optimal temperature range for most teleost fishes.

pH

Generally, a reduction in the pH of water results in a shift in the NH_3/NH_4^+ equilibrium toward the ammonium ion, resulting in a reduction in the un-ionized ammonia fraction. The result can be seen in the main tables for un-ionized ammonia previously mentioned, particularly in that of Thurston et al. (1974). Alabaster and Herbert (1954) found that carbon dioxide blown through water reduced the toxicity of ammonia solution by reducing the pH and hence the un-ionized ammonia fraction. The pH values determined during the experiments with ammonia solutions fell with increased concentrations of carbon dioxide from about 7.91, when none was added, to 7.4 with 30 ppm carbon dioxide added. Above 60 ppm, carbon dioxide itself was toxic and survival time of fish was greatly reduced, even in the absence of ammonia. Downing and Merkens (1955) reported that at pH 7.0 the concentration of ammonium chloride required to give the three concentrations of un-ionized ammonia used was about 10 times that required to produce the same concentrations of un-ionized ammonia at pH 8.0. They confirmed that the toxicity of the ammonia could be related directly to the un-ionized ammonia fraction, and this in turn was directly affected by pH.

By virture of the effect of pH upon the NH_3/NH_4^+ equilibrium, there will also be a direct effect of pH upon the compartmental distribution of ammonia within the body. Un-ionized ammonia, being lipid soluble and easily diffusible through lipid membranes, will equilibrate rapidly between body compartments, whereas movement of ammonium ions will be restricted and their concentration in any one compartment will be governed by the pH of that compartment. A reduction in pH will increase the concentration of ammonium ion present, shifting more of the total ammonia present into the non-diffusible state. Thus for two compartments separated by a lipid membrane, un-ionized ammonia concentrations will be in equilibrium across the membrane (since it can traverse the membrane) but the ammonium ions will be in greater concentration in the compartment with the lower pH, and hence the total ammonia concentration will also be greater in the compartment of lower pH. In mammals, compartmental pH has been shown to affect greatly the distribution of total ammonia within the body (Warren 1962; Moore et al. 1963), to the extent that the following equation, modified from the Henderson-Hasselbach equation, may be used to describe the distribution of total ammonia in a two-compartment system.

$$\frac{[\Sigma \text{ total ammonia}]_1}{[\Sigma \text{ total ammonia}]_2} = \frac{1 + 10(pKa-pH_1)}{1 + 10(pKa-pH_2)}$$

where 1 and 2 refer to the two compartments, total ammonia is the total concentration of ammonia (ionized + un-ionized) in each compartment, and pKa is the ionization constant for ammonium ions.

The general tendency is for un-ionized ammonia to be in equilibrium between intracellular and extracellular compartments. Total ammonia levels will tend to be higher in the intracellular compartment, since the majority of total ammonia is in the ionized form. The situation is far from simple, however, since increase in blood pH will tend to increase the un-ionized fraction in the blood, which itself will increase un-ionized ammonia diffusion into tissues. However, by the same increase in pH, the acceptable intracellular pool for total ammonia is reduced since intracellular pH is bound to rise although to a lesser degree. Slight acidosis of the blood may increase the acceptable intracellular ammonia pool, but by the same token acidosis will decrease the fraction of blood ammonia that may be easily diffused.

To indicate further the complexity of the situation, recent studies (Armstrong et al. 1978; Tabata 1962; Thurston 1980; Thurston et al. 1981; Tomasso et al. 1980) have indicated that there may be more to the pH effect upon ammonia toxicity, than merely altering the equilibrium between NH_3/NH_4^+. This is discussed in more detail under "Possible action of ammonia toxicity in fish".

Free carbon dioxide

Alabaster and Herbert (1954) have shown that increased CO_2 content in water can reduce the un-ionized ammonia fraction by reducing the pH, and hence reduce the overall toxicity of the ammonia. Lloyd and Herbert (1960) also showed that the toxicity of ammonia is not entirely dependent upon the pH value of the bulk of the solution. They reported the apparently paradoxical finding that a reduction in pH of aquarium water, from 8.2 to 7.0, increased the free CO_2 content from 3.2 to 48 ppm, and decreased the measured tolerance level for un-ionized ammonia. Thus the toxicity of un-ionized ammonia increased with decrease in pH. These findings have been explained (Lloyd and Herbert 1960; Lloyd 1961; Lloyd and Herbert 1962) in terms of pH changes at the branchial surface caused by respiratory CO_2 output. Lloyd (1961) indicated that the pH of the water in direct contact with the gill epithelium may be readily calculated from the bicarbonate alkalinity, temperature and free carbon dioxide concentration in the water, together with the free carbon dioxide excreted at the fish gills.

According to Lloyd (1961), the CO_2 liberated in respiration causes a drop in pH at the gill surface, which is independent of the pH of the rest of the medium. The reduction in pH is greater when the free CO_2 content of the water is low. Thus with little free CO_2 present (high pH),

respiratory CO_2 output at the gills gives rise to a greater lowering of pH in the immediate branchial vicinity, greater than if the free CO_2 level were higher (lower pH) in the rest of the medium. The greater pH lowering at the gill surface results in a greater conversion of NH_3 to NH_4^+ - which was once thought to represent the major branchial excretory mechanism for ammonia in teleosts (Maetz 1973; Maetz and Garcia Romeu 1964). The increased ammonia toxicity due to a raised free CO_2 level was therefore explained in terms of decreased detoxification of ammonia (by conversion from NH_3 to NH_4^+) at the branchial lamellae. However, in the light of recent findings (Hillaby and Randall 1979), which suggest that un-ionized NH_3 itself may well account for the major branchial ammonia loss, the above suggestion no longer seems appropriate.

Lloyd (1961), however, pointed out the additional effect of oxygen upon the above mechanism. He stated that "as oxygen concentration of the water is reduced, the concentration of excreted carbon dioxide at the gill surface is also reduced and the pH value of the water at this surface rises, resulting in an apparent increase in ammonia toxicity. This increase will be greater as the concentration of free CO_2 in the bulk of the water is reduced."This appears to be in conflict with the earlier statement regarding ammonia excretion at the gill surface, and EIFAC (1970) has pointed out that under conditions where dissolved oxygen concentrations are adequate, free carbon dioxide levels are very low and pH is high, the level of un-ionized ammonia toxic to fish may be about five times greater than that indicated for polluted waters where free CO_2 is high and pH is low. This somewhat puzzling situation has been suggested by Willingham (1976) to be due to an increased CO_2 pressure gradient across the respiratory epithelium (in the former situation), which may facilitate the rapid excretion of carbon dioxide.

In recent work (Haywood et al. 1980), involving recycled water, yearling coho salmon were found to tolerate up to 70% water re-use (30% replacement per cycle) without appreciable toxicity from waste ammonia. The very low levels of un-ionized ammonia (<2 ppb) were attributed to respiratory CO_2 and soft water maintaining a pH near 6.5 at all times. At no time, under the above conditions was there any evidence of ammonia toxicity.

Bicarbonate alkalinity

Bicarbonate alkalinity affects un-ionized ammonia levels where present, in terms of pH, the resultant rise in pH producing more of the un-ionized fraction (Lloyd 1961).

Water hardness

Wuhrmann and Woker (1953) showed that the toxicity of un-ionized ammonia to minnows (Phoxinus phoxinus) was not affected by water hardness, and Herbert (unpublished data referred to in Herbert 1961) showed similar results, using rainbow trout.

Dissolved oxygen

Several workers have demonstrated ameliorating effects of dissolved oxygen (DO) upon several toxic substances, in addition to ammonia. Southgate et al. (1933) found that when fish were placed in a fixed volume of potassium cyanide solution (0.11 ppm CN^-), toxicity decreased as the concentration of oxygen was increased, but the rate at which it decreased fell off as air saturation value was approached. Downing (1954) found that survival times of rainbow trout in concentrations of potassium cyanide (range 0.105-0.155 ppm) increased with increase in DO concentration between 10 and 100% of air saturation, the effect being most marked at the lower cyanide concentrations. Furthermore, no diminution in the rate of increase of survival times was apparent even as the 100% saturation value was approached.

Wuhrmann (1952) demonstrated that the toxicity to minnows of solutions containing un-ionized ammonia (1-10 ppm) was modified by the DO concentration of the water. Downing and Merkens (1955) showed that survival times for rainbow trout exposed to un-ionized ammonia concentrations of 0.60-1.29 ppm nitrogen increased as the concentration of DO was raised from 1.5 to 8.5 ppm. As observed with earlier work (Downing 1954), the effect of oxygen in increasing the survival time was greater at the lower concentrations of the toxin. This general trend for better fish survival at higher DO levels was again found by Merkens and Downing (1957) and Lloyd (1961), and may be expected where the total ammonia level of the water is artificially maintained. Lloyd also felt that a reduction in DO concentration would be accompanied by an increased rate of ventilation by fish. This could increase the toxicity of ammonia in two ways; firstly, the increased rate of ventilation would effect a greater rate of exposure to the un-ionized ammonia moiety, and secondly, increased respiration would effectively reduce the concentration of carbon dioxide being excreted at the gill surface, thereby increasing the pH at that surface. The effects of varying concentrations of both DO and carbon dioxide are obviously related.

In a study of hatchery-type water reuse on rainbow trout (where the same water passes through seven or eight tanks in series) Larmoyeux and Piper (1973) found that fish from lower oxygen environments in 5th and 6th tanks (in series) demonstrated observable detrimental effects. When oxygen levels declined below 5 ppm and total ammonia -N exceeded 0.5 ppm (0.6 ppm total ammonia), a decline in fish quality was evident through reduction in growth rate, damage to gill tissue and occasional pathology of kidney and liver tissue. However, growth rate was not affected when trout were maintained for 6 weeks in an environment with an oxygen level in excess of 7 ppm and total ammonia-N at 0.8 to 1.0 ppm (0.9-1.2 ppm total ammonia). Smith (1972) also reported that as long as 5 ppm DO was maintained, trout growth was not significantly reduced until the average total ammonia concentration reached 1.6 ppm (0.033 ppm un-ionized ammonia) and then only after continuous exposure for at least 6 months. Alabaster, Shurben and Knowles (1979) reported a 24-h LC_{50} of un-ionized ammonia to Atlantic salmon smolts of 0.15 mg/L NH_3 at 12°C and DO close to the air saturation value (ASV). This is about half that reported by Herbert and Shurben (1965) under similar conditions. However, Alabaster et al. have suggested that the difference may be due to heavy mortalities in the stock of Herbert and Shurben resulting in increased

resistance in surviving fish. In addition rainbow trout shows a threefold rise in resistance to ammonia, associated with a rise in temperature from 5° to 18°C (Ministry of Technology, 1968).

SALINITY EFFECT -- TOTAL DISSOLVED SOLIDS

Herbert and Wakeford (1964) showed that low salinity could increase the resistance of yearling rainbow trout and Atlantic salmon smolts to certain toxins - with salinity up to about 30-40% seawater ($\geq 10‰$) Beyond this point, however, resistance was reduced, presumably by the increased energy requirements of osmotic and ionic regulation. Later, Herbert and Shurben (1965) demonstrated that the 24-h TL_M for rainbow trout kept at 13.6°C in a solution of pH 7.45 could be increased from 60 to 150 ppm NH_4Cl-N (182 ppm NH_4^+) by increasing the salinity to about 30% sea water. This effect was reversed upon further increase of salinity up to 100% seawater.

Acclimation to low ammonia concentrations

It has been established (Vamos 1963; Malacea 1968; Lloyd and Orr 1969) that exposure of fish to sub-lethal levels of un-ionized ammonia increases the subsequent resistance of the fish to lethal concentrations of un-ionized ammonia, although the effect has only been observed to last for 2-3 days and is lost after 3 days (Lloyd and Orr 1969).

Exertion

Herbert and Shurben (1963) have indicated a decrease in the resistance of rainbow trout to un-ionized ammonia toxicity at swimming speeds greater than two body lengths/sec. At three body lengths/sec the threshold LC_{50} (the level of environmental toxicity beyond which 50% of the population cannot live for an indefinite time) was 70% of that in still water.

Sex and size differences

Hemens (1966) reported greater resistance of female mosquito fish (Gambusia affinis) to total ammonia poisoning than shown by males of the species. Size differences had no apparent effect on susceptibility.

AMMONIA TOGETHER WITH OTHER TOXINS

Early experiments with solutions containing both ammonia and cyanide showed that the combination was more toxic than either substance alone (Wuhrmann and Woker 1948). Herbert (1962) showed that the LC_{50} threshold

of a mixture of phenol and ammonia was obtained when the sum of the individual concentrations, expressed as proportions of their separate LC_{50} values, reached unity. Later tests with zinc and ammonia (Herbert and Wakeford 1964) and copper and ammonia (Herbert and Shurben 1964; Herbert and Van Dyke 1964) again showed that the toxicity of the individual poisons could be summed in this manner. Hence, with more than one toxicant present in the same system, the combined effect may be such that the collective toxicity can be predicted from the responses to the separate individual toxins.

The empirical toxic unit method (reviewed by Sprague 1970) and additive index system (Marking 1977) have been used to evaluate the joint acute toxicity of combinations of toxins. However this strict summation method for toxic fractions, whilst apparently suitable for predicting the toxicity of certain combinations, is sometimes not successful in predicting median lethal levels and low response percentages (Sprague 1970; Calamair and Marchetti 1973; Eaton 1973; Marking 1977; Spehar et al. 1978). This lack of correlation may result from chemical and toxicological interactions between the toxins with a definite area of interaction or synergistic action between the two (Bliss 1952, 1956; Lloyd and Orr 1969) and the levels of one of the toxicants may not even be toxic, so that a summation of individual toxicities is not pertinent. Brown et al. (1969) showed, however, that mixtures of zinc, phenol and ammonia, in which the proportion of the total toxicity contributed by zinc was predominant, were significantly less toxic than the expected values. Vamos and Tasnadi (1967) reported antagonistic effects between copper and ammonia toxicity, as reported by Herbert and Van Dyke (1964); copper sulphate was shown to reduce the toxicity of ammonia to carp. It was suggested that on a short-term basis the copper-ammonia complex formed was not toxic to fish.

A thorough understanding of the chemical reaction of constituents in a toxicant mixture is necessary for prediction of joint effects. Where toxicants combine chemically, prediction of joint toxicity may be most difficult. Chromium and zinc can combine with cyanide to form coordination compounds. However, calculations from stability constants show that often the percentage of the combined ion complex is less than 1% in the case of cyanide, and even less for most metals.

The physiological basis for joint interaction of toxicants recently has received further attention (Marking 1977). Projected theories for the mechanisms of increased toxicity by mixtures include increase in uptake rate, formation of toxic metabolites, reduction of excretion rates, alteration of distribution and inhibition of detoxification. Lloyd and Swift (1976) concluded that for rainbow trout exposed to ammonia-phenol solutions, the rate of uptake and concentration of phenol in fish muscle was not influenced by elevation of ambient ammonia levels - nor was the urine flow rate increased. They found no physiological basis to account for the empirical additive toxic action of ammonia and phenol. The effects measured in the binary mixture were those corresponding to either ammonia or phenol alone, and the presence of one did not appear to enhance the effect of the other. Recently, Broderius and Smith (1979) determined mortality curves for rainbow trout and showed that Zn-HCN and ammonia-HCN mixtures were more acutely toxic, and Cr-HCN less toxic, than predicted by the strictly additive index approach. The concentration and response addition models could not be used to predict dosage-mortality curves for HCN mixtures. HCN, combined with ammonia, was the

only mixture that demonstrated some sublethal interactive effect on growth of rainbow trout, producing a greater effect than that determined for the corresponding toxicant, tested alone. Most other binary mixtures disrupted normal physiological processes in a manner essentially equal to that of each toxicant presented alone. Since it has not been established whether the adverse effects of toxicant mixtures on fish can be predicted from individual toxicant effects, a need still exists for development of a valid multiple toxicity approach to evaluate the toxicity of chemical combinations.

Nitrite and nitrate toxicities

The previous section on joint toxicities has been included because un-ionized ammonia may sometimes exert its toxic effects in the presence of, and added to, those of other chemical toxins. In the case of nitrite (NO_2^-) and nitrate (NO_3^-), while both these ionic species may be present in the presence of ammonia, their appearance is usually due to the bacterial action in organic or sand filters, often used in water recirculation (hatchery) systems. The condition which promotes the abundance of NO_2^- and NO_3^- is usually a generally high bacterial activity and organic content in the water with a resultant softening of the water, (pH decrease) and reduction of DO level. Under such conditions, total ammonia does not become a problem since the un-ionized ammonia fraction is very small. The conditions which increase the toxicity of total ammonia, (by increasing the un-ionized fraction) decrease the toxicity of nitrite and nitrate. Wedemeyer and Yasutake (1978) reported that "Increasing the pH from 6.0 to 8.0 decreased (nitrite) toxicity by a factor of about eight for the smaller and three for the larger fish". Thus it is unlikely for ammonia toxicity to be a problem at the same time as toxicity due to NO_2^- and NO_3^- and for this reason, while the toxicity of these two ionic species to fishes is appreciated, it is felt that they represent a separate subject altogether, and will not be dealt with here.

Exposure of fish to ammonia

Exposure of fish to ammonia solutions generally raises the blood ammonia levels (Fromm and Gilette 1968), either due to ammonia retention in the fish or by entry of ammonia from the external medium resulting in a reduction in overall ammonia excretion. Exposure of rainbow trout to total ammonia levels greater than 12% of the threshold LC_{50} value also increased their absorption of water (Lloyd and Orr 1969). This may or may not play a role in facilitating increased un-ionized ammonia uptake by fish when exposed to raised ambient total ammonia levels, as observed in trout and goldfish (Fromm 1970). Where rise in the environmental level (external level) ammonia is not great or where the species is able to effect some form of internal regulation, blood ammonia may be stabilized. Thus Buckley et al. (1979) found no change in blood total ammonia in coho salmon in fresh water when the environmental ammonia level was raised. They also observed a rise in plasma sodium levels and ammonia regulation might have been effected by an increase in branchial NH_4^+/Na^+ exchange.

In the majority of cases, however, blood ammonia is raised, following a rise in external ammonia, and there is evidence that elevated

ammonia levels produce metabolic changes within the fish. Fromm and Gillette (1968) have observed increased pyruvate and lactate levels in rainbow trout exposed to raised ammonia levels. Fish do respond to elevated ammonia levels since they are able to acclimate to these raised levels (Malacea 1968; Schulze-Wiehenbrauck 1976), although the nature of the acclimation process is still obscure. One method of reducing ammonia toxicity is by increasing the conversion of ammonia to urea, a non-toxic excretory product. Whilst the majority of teleosts are ammoniotelic, there is evidence of low levels of the ornithine-urea cycle enzymes in many teleosts (Huggins et al. 1969; Read 1971) indicating the possibility of ammonia conversion to urea.

Converting ammonia to urea is not the only way of reducing ammonia toxicity. Levi et al. (1974) have recorded raised levels of glutamine in goldfish brain, following exposure to raised ambient ammonia levels. Glutamine is less toxic than ammonia and can be used to "buffer" sudden rises in nitrogenous waste production. The high glutamine synthetase activity in fishes, and their ability to detoxify ammonia by producing glutamine in the brain, may account for reduced susceptibility to raised ammonia levels. Under conditions where the ambient ammonia level rises too far or too quickly, or the fish is unable to regulate blood ammonia levels for some reason, ammonia toxicity becomes apparent.

The above findings apply to conditions where fish are exposed to a uniform raised total ammonia environment. However, under conditions where natural waters may be polluted with ammonia from point source discharges, thus producing dilution zones and ammonia concentration gradients, fish avoidance reactions might be expected. Stickleback (Jones 1948) and green sunfish (Summerfelt and Lewis 1967) have been reported to show avoidance to lethal solutions of total ammonia in a gradient tank. However green sunfish were not repelled by concentrations producing sublethal distress and sticklebacks were attracted to sublethal concentrations of total ammonia. Hepher (1959) observed that common carp showed avoidance to ammonia fertilizer added to ponds but there is no evidence to suggest that fish actively avoid sublethal levels of total ammonia.

Fish responses to ammonia toxicity

Ammonia toxicity manifests itself chiefly as a neurological disorder, with resultant secondary distress symptoms similar to those observed in the chick and mouse, regardless of the method of exposure. Carp (Flis 1968a) and rainbow trout (Smart 1975), responding acutely to ammonia in water, show hyperexcitability, hyperventilation, violent erratic movements, coma and convulsion, rapidly leading to death. If returned to ammonia-free water in time, these symptoms may cease (Smart 1975). Similar symptoms appeared in goldfish, rainbow trout and channel catfish 10-15 min after intraperitoneal injection (Wilson et al. 1969). Hillaby (1978) noted similar effects with rainbow trout and reported reactions to NH_4Cl injection (Hillaby and Randall 1979) consisting of gulping at the surface, coughing, increased ventilation followed by sporadic ventilation, loss of equilibrium and muscle spasms - again terminating in violent convulsions and death. Blood pH was lowered in this latter case, and blood appeared dark red (methaemoglobin). Similar symptoms were produced after injection of NH_4HCO_3, although blood pH was raised slightly; the fish also changed colour, turning from light to dark, to light, and back to normal again. The blood appeared bright red.

Ammonia has also been found to produce histopathological changes in fish gill structure. The usual effects appear as a general thickening of the epithelial membrane with associated hyperplasia; sometimes there is a breakdown of the pillar cell structure of the secondary lamellae. This general disruption of branchial structure effectively reduces the surface area of the gill membrane, with resultant reduction in oxygen-diffusing capacity (Burrows 1964). Impaired physiological function, due to ammonia effects, implies reduced resistance to disease. Burrows (1964) observed that the susceptibility of salmonids to bacterial fish disease was closely linked with extensive gill hyperplasia caused by ammonia. More recently, Smart (1975) qualitatively analysed specific effects of acute ammonia toxicity upon gill structure in rainbow trout. For fish with mean survival times less than 2 h in solutions of 0.50-0.65 µg/mL (ppm) un-ionized ammonia, macroscopic appearance of gill tissue was generally normal; with occasional swelling and mucus production, but no hemorrhaging. Microscopic examination of this tissue showed the beginning of epithelial lifting with a marked dilation of blood spaces in the secondary lamellae. Smart concluded that histological changes in gill tissue are not serious after very short-term exposure to high ammonia levels.

Exposure to ammonia at lower concentrations but over a longer period (chronic toxicity) may result in the diminution or total absence of neurological disorders, while paradoxically often producing serious disruption of gill structure. By contrast with Smart's findings, Burrows (1964) showed that un-ionized ammonia at concentrations as low as 0.006 ppm, in continuous exposure for 6 wk, can produce extensive hyperplasia of the gill epithelium. Larmoyeux and Piper (1973) observed similar damage to gill structure in rainbow trout exposed to 4 and 8 mo of water re-use, and further concluded that exposure to low levels of ammonia may well predispose fish to bacterial gill infections.

Smith and Piper (1975) exposed rainbow trout to serial re-use water in which a maximum un-ionized ammonia level of 0.0166 ppm was recorded. Although exposure of fish to this level of un-ionized ammonia for 9.5 mo showed no significant increase in percentage mortality between control and experimental fish, these latter fish became emaciated and lethargic. Microscopic examination of prepared tissue sections from lethargic fish showed extensive proliferation of gill epithelial tissue and severe fusion of gill lamellae, preventing normal respiration. Live tissue showed reduced glycogen storage and areas of dead and dying liver cells, which became more extensive as exposure time increased. During the twelfth month, fish exposed to this level of un-ionized ammonia, incurred bacterial gill disease infection, resulting in severe mortalities among the population.

Marchetti (1960) observed severe caudal damage in Crucian carp (Carassius carassius) after prolonged exposure to sublethal ammonia levels. Reichenbach-Klinke (1967) also observed reductions in erythrocyte numbers in the blood of rainbow trout exposed to un-ionized ammonia. Flis (1968b) showed that severe tissue damage after 35 days exposure to 0.11 mg/L un-ionized ammonia killed 8% of fish in one test and caused damage to the liver and kidneys that was attributed to disruption of blood vessels. Rudd have shown histopathological changes in the epidermis after 95 days exposure to sub-lethal un-ionized ammonia levels of 0.10 mg/L (Department of the Environment 1972).

In addition, chronic ammonia toxicity may produce abnormal behavioural responses. Woltering, Hedtke and Weber (1978) reported that the normal predator-prey relationsip between largemouth bass and mosquitofish can be altered by un-ionized ammonia build-up in the water. At levels in excess of 0.63 mg/L (ppm) NH_3, predatory activity of the bass often ceased and the mosquitofish began chasing and nipping the bass, especially when the "prey" were at high densities. In six tanks with high un-ionized ammonia levels they reported that bass died within 10 d with continuous harassment by mosquitofish, which appeared otherwise unaffected by the ammonia.

POSSIBLE ACTION OF AMMONIA TOXICITY IN FISH

In the past it has generally been considered that the un-ionized ammonia (NH_3) in polluted water is the toxic moiety. Rapid movement of this uncharged non-polar molecule across the gills of fish was postulated long ago by Wuhrman and Woker (1948). They suggested that because of its solubility in lipids, NH_3 rather than NH_4^+ was the form of ammonia most likely to traverse lipoprotein membranes. Such movement would be facilitated by simple diffusion not requiring any carrier mechanism, and hence would only be limited by diffusional vapour pressures and concentrations as indicated by Fick's principle. Whilst there is much published work indicating a definite branchial carrier mechanism in teleosts whereby NH_4^+ ions are exchanged for either Na^+ or H^+ ions as part of the ioniuptake mechanism (Maetz and Garcia Romeu 1964; Maetz 1972, 1973; Payan and Maetz 1973; Payan 1978), it has also been shown that un-ionized ammonia does traverse fish gills and may well be much less restricted in so doing than its ionic counterpart. Smart (1975) showed a more rapid increase in blood ammonia levels in trout maintained in ammonia solutions at high water pH values, as compared with fish under identical ammonia conditions, but at lower pH levels. Very recent research however has further complicated the overall understanding of ammonia toxicity by indication that un-ionized ammonia may not be the sole toxic fraction, and that the ionized NH_4^+ moiety and/or the total ammonia level exerts a toxic effect also. Based on the findings for ammonia toxicity to larvae of the freshwater prawns Macrobrachium rosenbergii (Armstrong et al. 1978) where un-ionized ammonia was not found to be the exclusive toxic agent, a model was proposed to explain observed effects. The modal proposed that prawn larvae exposed to pH values greater or equal to about 8.4 would be mainly affected by un-ionized ammonia (NH_3), which is nonpolar and readily diffusible through biological hypoprotein membranes (Warren 1962). At this high pH, about 10% of the total ammonia would exist as un-ionized ammonia, and would exceed postulated blood levels (for un-ionized ammonia) resulting in NH_3 diffusion into the animal. Once entering the blood, the NH_3 would be protonated to NH_4^+ thereby maintaining the NH_3 diffusion gradient into the animal. Thus, unless some means of excreting the ammonia (both NH_3 and NH_4^+) were activated, ammonia build-up with resultant toxicity would follow. Armstrong et al. (1978) found that toxicity due to inward diffusion of un-ionized ammonia was rapid for prawn larvae at a pH of 8.34, causing mortalities within 2-18 hr.

With respect to ammonia toxicity at low pH values (e.g. 6.83), Armstrong et al. (1978) have suggested that the effect of the low pH upon alternate routes for ammonia removal, may cause the observed toxicity. Thus,

inhibition of sodium influx may be a significantly contributing factor. At this lower pH value nearly all the external ammonia present exists in the NH_4^+ form. By successfully competing with sodium ions, the NH_4^+ will reduce the influx of Na^+ ions into the animal, and since Na^+ is lost by passive diffusion in freshwater, a general depletion in body sodium will result. In addition, body levels of ammonia will rise, since NH_4^+ ions will use the active transport to be taken into the animal (in place of sodium). Prawn larvae showd more resistance to low pH ammonia toxicity which has been postulated as more of an osmoregulatory imbalance (as just mentioned) than the toxicity due to copious un-ionized ammonia diffusion.

Hillaby and Randall (1979) have demonstrated acute toxic reactions in rainbow trout after intra-arterial injection of NH_4Cl and NH_4HCO_3. Blood hydrogen ion and total ammonia concentrations were measured in the dorsal aorta before and after injection. Ammonium chloride is a slightly acidic salt, whereas the bicarbonate salt is slightly alkaline, and injection of the former therfore resulted in a decrease in blood pH whilst the converse was true with the bicarbonate.

The same quantity of each salt was required to produce acute toxic symptoms but the effects were more rapid following the NH_4Cl injection. Final blood total ammonia and ionized ammonia levels were also higher following NH_4Cl injection; conversely NH_3 concentration in the blood washigher after injection of NH_4HCO_3. Their findings also showed that for two groups of fish injected with NH_4Cl, one of which died, the un-ionized ammonia levels were the same. On the strength of this they theorised that the un-ionized ammonia in the blood was not the toxic factor. The delay in toxicity following injecton of the bicarbonate salt was explained in terms of increased ammonia excretion and loss after the injecton. Since the bicarbonate salt increased the pH of the blood, more of the ammonia was in the un-ionized form and they observed a greater ammonia loss across the gill epithelium. In addition, as mentioned before, a change in the blood pH will also affect the compartmental distribution of ammonia between the blood and tissues. Raising the pH tends to shunt more ammonia into the tissues and out of the blood, even though the ammonia pool is somewhat reduced by the rise in pH. Since there was no significant difference in the injected doses of the two salts required to produce the toxic symptoms, but there was significantly more un-ionized ammonia in fish injected with ammonium bicarbonate, they concluded that, unlike mammals (Warren 1958; Warren and Schenker 1962) there is no increased toxicity as a result of increased un-ionized ammonia in the blood. Therefore, in trout, according to Hillaby and Randall (1979) it appears that either the ionized ammonia or the total ammonia is the toxic factor inside the fish, and not the un-ionized ammonia.

If the un-ionized form of ammonia were solely responsible for the observed toxicity of ammonia, it might be expected that un-ionized ammonia LC_{50} values would be reasonably similar for a given fish species, regardless of water pH and total ammonia present. Not only do the findings of Hillaby and Randall (1979) suggest that this is not the case, but recent work by Thurston et al. (1981) has shown that for both rainbow trout and fathead minnows, un-ionized ammonia LC_{50} values are markedly less at pH 6.5 than at pH 9.0, and conversely total ammonia LC_{50} values are much higher at pH 6.5 than at pH 9.0. It was not clear whether the un-ionized ammonia LC_{50} value reached a peak at pH 9.0 with subsequent decrease, or whether they remained at

a peak at pH 9.0 with subsequent decrease, or whether they remained at a plateau. Thurston et al. (1981) suggested, as explanation for their observations, that the toxicity of un-ionized ammonia is not constant over the pH range 6.5 to 9.0, so that increased H^+ concentration may increase the toxicity of NH_3, or perhaps the NH_4^+ ion exerts a toxic effect, which is more pronounced at low pH.

Ammonia appears to affect neuronal activity, as indicated by the convulsions observed in many experiments. Hillaby and Randall (1979) observed hyperventilation, hyperexcitability, disorientation, and convulsions in rainbow trout after the above injections, and concluded that ammonia toxicity is due to the action of ammonia on the central nervous system since hyperexcitability, convulsions and coma in fish are symptomatic of neurological disorders. Smart (1975) measured ATP and PC (phospocreatine) in fish medulla, after ammonia exposure, and found both to be significantly decreased, this depletion possibly explaining the neurotoxicity. Since the lipid-soluble NH_3 moiety is known to pass through lipoprotein membranes with ease, it would be most suspect as a neurotoxin, particularly at synaptic and lipoprotein membrane junctions. Neurotoxicity implies a traversing of the blood-brain barrier separating the brain and cerebrospinal fluid (CSF) from the blood. Although there is evidence that this barrier is not altogether complete in fishes (Rapoport, 1976) there also is evidence that only un-ionized ammonia (NH_3) can cross the blood-brain barrier in mammals (Stabenau et al. 1958). Bromberg et al. (1960) found no increase in the CSF/blood total ammonium concentration ratio, after sublethal injection of ammonium acetate into dogs, indicating no transition of ammonium ions across the blood-brain barrier.

Tabata (1962) showed interesting effects of pH upon ammonia toxicity to Daphnia spp. He observed that at low pH values ammonia could be quite toxic, under conditions where there was virtually no un-ionized ammonia present. He attributed a definite toxicity to the ammonium ion and stated "...it is clear that the ammonium ion provides a large proportion of the total ammonia toxicity, contributing up to 80% of the total toxicity at pH 7.0 and 30% at pH 8.0". His work with guppies indicated a more pronounced effect of pH upon ammonia toxicity in teleosts, but there remained the definite possibility of toxicity due to ammonium ions. Neurotoxicity by NH_4^+ ions implies that these ions can traverse the blood-brain barrier. It is true that the structure of the teleost brain is different from that of mammals and it is possible, owing to the incompete barrier, that localized areas of the brain/CSF system might be exposed to small ionic infiltration. However, there is no direct evidence of this as yet. Binstock and Lecar (1969) have demonstrated that NH_4^+ can substitute for the passive movement of both Na^+ and K^+ in a nervous impulse passing down giant squid axons. Once inside the axon, NH_4 tends to block any inflow of Na^+, possibly producing NH_4^+ excitability of the nerve cell. Evidence of direct competition of NH_4^+ ions for Na^+ sites of inward movement across human erythrocyte membranes is provided by Post and Jolly (1957).

The findings by Thurston et al. (1981) are again clearly indicative that toxicity is not simply due to the un-ionized ammonia fraction. The lower LC_{50} values for un-ionized ammonia at lower pH (6.5) clearly indicate an increased toxicity under conditions where the un-ionized ammonia fraction is

itself decreased due to the reduced pH. The overall decrease in total ammonia LC_{50} value with increase in pH is understandable, and conforms with the concept of increased ammonia toxicity at higher pH, formerly attributed to the increasing fractions of un-ionized ammonia under these conditions. However, if this were the case, one would expect the un-ionized ammonia LC_{50} slope to parallel that of the total ammonia, or at least to show a similar directional slope.

The issue is made more confusing by consideration of the following points. Tabata (1962) has indicated a definite toxicity due to the ammonium ion itself, with a possible ratio of NH_4:NH_3^+ of 1:40-200 required to produce similar toxic symptoms. In addition, since fish have been shown to possess circulating ammonia levels of 1-4 ppm (Smith 1929; Goldstein et al. 1964) and since the majority of this ammonia is in the ionized NH_4^+ form, the tissues are accustomed to appreciable circulating amounts of NH_4^+ ions. Presumbably some active mechanism prevents entry of these ions into the CSF/brain system, except perhaps under conditions of gross overloading. On the other hand, the build up of NH_3 in the blood, which would affect neuronal synaptic transmission, is somewhat self-regulating, since passive branchial loss of NH_3 will also increase as blood levels rise.

It seems therefore that toxicity may not be limited solely to the NH_3^+ moiety, and that as the external pH changes, the different ammonia forms show varying degrees of toxicity. As shown by recent workers (Armstrong et al. 1978; Thurston et al. 1981) sufficiently high concentrations of the ionized NH_4^+ moiety in water of low pH, produces marked toxicity effects, even though the un-ionized ammonia concentration present may be sublethal. It may be an over-simplification to attribute ammonia toxicity solely to the un-ionized ammonia at high pH, and solely to the ammonium ion, at low pH, since there is probably always a contribution from both forms whenever the total ammonia concentration is high enough to produce toxicity. However, the model proposed by Armstrong et al. (1978) suggests that the bulk of toxicity is attributable to these two forms, and the change in pH that alters the ratio between them.

Thus perhaps ammonia toxicity is brought about by a combination of factors. Doubtless the actual concentration of total ammonia present will affect the observed toxicity, but in addition, the pH may produce effects through altering the equilibrium between NH_3 and NH_4^+. Thurston et al. (1981) concluded that where total ammonia levels in the water are low (below 18-45 m/L) the NH_4^+ ions do not exert any pronounced toxicity, due to the more toxic un-ionized NH_3 effects, particularly noticeable where the pH of the water is above 7. However, the effects of NH_4^+ toxicity become more noticeable as the concentration of total ammonia is increased and pH is decreased. They showed that un-ionized ammonia (NH_3) is some 300-400 times more toxic than the NH_4^+ ion on a molecular comparison basis.

Within the internal physiology of the fish, whether caused by influx of NH_3 or by reduced excretion of ammonia as either NH_3 or NH_4^+ ions, it may well be the total ammonia or indeed the NH_4^+ ions that exert an effect upon the CSF/brain. With respect to histopathological gill damage, it is still unclear which moeity is responsible, although the lypid-soluble NH_3 would appear high suspect since the hyperplasia and epithelial lifting so often observed in fish gills is largely due to the degradation of

lipoprotein membranes. In any event, the field remains open for further research to determine more precisely the ionic or molecular configuration of the neurotoxic ammonia moiety.

RECOMMENDATIONS BASED ON FINDINGS

Table 2 consolidates toxicity findings under differing conditions and for a wide range of species. Findings have been divided arbitrarily into three groups - the first group being formed with the west coast Pacific salmon and trout hatcheries in mind. The three groups are (i) salmonids, (ii) other freshwater fishes, and (iii) other marine fishes. In all cases toxicity refers to un-ionized ammonia (NH_3) levels.

Salmonids

Most ammonia toxicity work on salmonids has been carried out on rainbow trout (Salmo gairdneri); these findings are assumed pertinent to both Pacific and Atlantic salmonid species.

Acute toxicity. Twenty-four to 96-h LC_{50}'s show considerable variation, even for the same species, but the lowest figure appears to be in the order of 0.2-0.3 mg/L (ppm) NH_3. Results suggest that levels above 0.2 mg/L NH_3 will produce toxic symptoms in most salmonids, including histological and pathological changes in gill structure, within 24-48 hours. The Environmental Protection Agency (EPA) (1976) similarly found a lowest lethal level, for salmonids, of 0.2 mg/L NH_3 and applied a safety factor of 0.1, resulting in a suggested maximal level of NH_3 of 20 µg/L (ppb) to avoid acute toxicity.

Chronic toxicity. Burrows (1964) reported that levels as low a 6-8 µg/L NH_3 can still produce proliferation of gill epithelium in chinook salmon over a 6-wk period, and obviously results in sub-lethal (chronic) toxicity. Exposure time appears as important as the ammonia concentration, since Burrows also reported that fish can tolerate exposure to levels as high as 15 µg/L NH_3 with impunity, provided they are only exposed for 1 h per day. In a water re-use system, Risa and Skjervold (1975) reported a level of 5 µg/L un-ionized ammonia as being harmless to Atlantic salmon fingerlings and Hampson (1976) suggests a safe level of 12 µg/L NH_3. Using Thurston's (1974) equation, however, a calculation of toxicity based on Burrows' observed gill damage, suggests that progressive gill damage in fingerling chinook salmon may occur near 3 µg/L NH_3. In a water re-use system with yearling coho salmon, Haywood et al. (1980) observed distress partly attributable to ammonia when the un-ionized level reached 3.5 µg/L. Recent findings (J. E. Bailey, Fisheries Research Biologist, N. W. Alaska Fisheries Centre, Auke Bay, Alaska, personal communication to Sigma Resource Consultants) indicates that a level of only 2 µg/L NH_3 may cause reduced growth rates in pink salmon alevins.

In view of these findings, but with consideration to the more recent implications that un-ionized ammonia may not be the only toxic moeity, it

seems pertinent that maximal permissible levels for both un-ionized ammonmia and total ammonia should be suggested for the three groups of fishes in question.

It is therefore suggested that THE MAXIMUM PERMISSIBLE (HARMLESS) LEVEL FOR BOTH REARING AND INCUBATION OF SALMONIDS BE RESTRICTED TO 2 µg/L (ppb) UN-IONIZED AMMONIA OR 1 mg/L (ppm) TOTAL AMMONIA.

Other freshwater fish

Results show a much lower sensitivity to ammonia in non-salmonid freshwater fish.

Acute toxicity. Twenty-four hour LC_{100}'s (concentration lethal to 100% fish in 24 hr) by McKee and Wolf (1963) indicate toxicities for coarse fish such as chub and perch to be as high as 14.5 mg/L (ppm) un-ionized ammonia (NH_3) - although LC_{100} values have questionable value, in view of the large error involved with this type of measurement. Twenty-four and 48-h LC_{50}'s show considerable variation with values for guppies and channel catfish varying between 1.5 and 3.0 mg/L (ppm) NH_3, but fathead minnows and bluegill sunfish show 48-h LC_{50}'s as high as 8 or 9 mg/L (ppm) NH_3. Most 96-h LC_{50}'s for coarse fish such as perch, roach, rudd and bream are below 1 mg/L NH_3 and a minimal figure for 96-h LC_{50} may be drawn around 0.3 mg/L (300 ppb) NH_3. Applying a safety factor of 0.1 would result in a suggested maximal level of 30 µg/L NH_3, to avoid acute toxicity effects.

Chronic toxicity. Long-term toxicity studies with the common carp (Ball 1968; Flis 1968) suggest that sublethal effects of un-ionized ammonia may still be demonstrable at levels above 100 µg/L (ppb) NH_3. Chronic toxic effects of ammonia on non-salmonid freshwater fish are poorly documented to date and in the light of what information is available it is suggested that little toxic effect would be expected below 50 µg/L (ppb) un-ionized ammonia. However, since a level of 30 µg/L NH_3 has been suggested to avoid acute toxic effects, it is suggested that a level below 30 µg/L be recommended for complete safety.

Based on the data available at present, THE MAXIMAL PERMISSIBLE (HARMLESS) LEVEL FOR NON-SALMONID FISHES IS SUGGESTED AS 10 µg/L (ppb) UN-IONIZED AMMONIA OR 2.5 mg/L (ppm) TOTAL AMMONIA.

Other seafish

Very little information is available regarding ammonia toxicity to marine fishes. Measurements on feeding habits and growth in two species of flatfish, the Dover sole (Solea solea) and the turbot (Scophthalmus maximus) (Alderson 1979) suggest that marine fish are far less susceptible to ammonia toxicity than are their freshwater relatives.

Chronic toxicity. Little information exists for acute toxicity of un-ionized ammonia on marine fishes, but Alderson (1979) has reported definite sublethal effects of ammonia upon growth rates. Depending upon pH, growth ceased in Dover soles when un-ionized ammonia was in the range of 0.46-0.94 mg/L NH_3, and for turbot in the range of 0.37-1.09 mg/L NH_3. Paradoxically, the level of un-ionized ammonia required to fully arrest growth appeared higher at the higher pH values--a fact Alderson suggested may be attributable to pCO_2 levels. In an earlier report (personal communication to B. Hampson, 1976), Alderson showed no observable effect of un-ionized ammonia upon growth of Dover soles at 51 µg/L (ppb) NH_3, and for turbot at almost twice this level. Alderson (1979) has confirmed threshold levels of un-ionized ammonia below which little or no effect upon growth is evident. These levels are 80 µg/L NH_3 for soles, and 134 µg/L NH_3 for turbot, at 16°C and 34 salinity.

In order to leave a safe margin below the lowest level quoted above, A MAXIMAL PERMISSIBLE (HARMLESS) LEVEL FOR MARINE FISHES IS SUGGESTED AS 50 µg/L (ppb) UN-IONIZED AMMONIA OR 2.5 mg/L (ppm) TOTAL AMMONIA.

It appears that salmonids are by far the most susceptible fish to ammonia toxicity. This has also been demonstrated in the case of intraperitoneal injections of ammonium salts (Wilson et al. 1969), where trout were found to be more sensitive than channel catfish, which in turn were more sensitive than goldfish. Hampson (1976) also pointed out that chinook salmon are more susceptible than rainbow trout and of the fish mentioned, theorder of sensitivity appears to be:

salmon > trout > channel catfish > goldfish > sole > turbot

The above figures suggest a fivefold difference in the maximum permissible recommended un-ionized ammonia level between salmonids and other freshwater fishes, and a fivefold difference between freshwater fishes and marine fishes. With respect to total ammonia, the difference between salmonids and other freshwater fish is only two and a half fold, and the same value is suggested between freshwater and saltwater fishes.

Summarized, this may be expressed as follows:

Maximum Acceptable Ammonia Levels	Salmonids	FW Fish	SW Fish
Un-ionized ammonia (µg/L or ppb)	2.0 (x 5 =)	10.0 (x 5 =)	50.0
Total ammonia (mg/L or ppm)	1.0 (x 2.5 =)	2.5 (x 1 =)	2.5

These total ammonia values have been based on average water parameters (with respect to temperature and pH) under the majority of conditions. Permissible total ammonia figures have thus been arrived at, by calculating the % un-ionized ammonia present under such conditions (from Thurston et al. 1974). It is recognized that these figures are calculated still assuming the un-ionized ammonia to be the toxic moeity, since the

total ammonia levels given should result in un-ionized ammonia levels not exceeding the recommended respective values. There is still insufficient evidence to calculate acceptable total ammonia levels based strictly on effects due to total ammonia itself or the ammonium ion (NH_4^+). It is hoped however, that further research may render this possible soon. Obviously, there will be cases where the total ammonia level and the un-ionized ammonia level do not correspond in the proportions indicated. In such cases it is recommended that THE LOWER VALUE OF THE TWO be taken as maximum permissible level, in order to avoid any health risk to the fish. It should be remembered that seawater has an average pH of around 8, which is over one whole unit above the pH of many freshwater sources. Consequently, this will increase the un-ionized ammonia fraction by a factor of 10, over that present in freshwater at lower pH and consequently similar values of total ammonia will represent higher proportions of un-ionized ammonia under seawater conditions. For this reason, the acceptable value of total ammonia for seawater fishes has not been raised above that for freshwater fishes.

In conclusion therefore, it is clear that although much evidence exists concerning the various effects of ammonia upon teleost fishes, it is still not clear exactly how ammonia exerts its toxic effects. Much evidence in the past has suggested that un-ionized ammonia is the moeity responsible, but more recent findings suggest that this may not always be the case. It is possible that ammonia toxicity is brought about by a complex interplay between un-ionized, ionized and total ammonia, the various fractions becoming more or less significant as the environmental conditions are altered. There is certainly scope for further experimentation that will hopefully highlight this problem and perhaps clarify the overall enigma concerning the mechanisms of ammonia toxicity in teleost fishes.

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Table 1. Sources and types of ammonia found in natural waters (Hillaby 1978, after EPA, 1971).

Source	Type of ammonia
Agricultural	
Fertilizers	Ammonium sulphate
Insecticides	Ammonium flouride
Industrial	
Chemical	Ammonium nitrate Ammonium sulphide Ammonium thiocyanate
Dye and pigment	Aluminum ammonium sulphate Ammonium chloride
Dye and tanning	Ammonium chloride
General industry	Ammonium sulphate
Ore processing	Ammonium chloride Ammonium molybdate
Petroleum	Ammonium carbonate Ammonium sulphide Ammonium thiocyanate Ammonium ferrocyanide
Photography	Ammonium dichromate Ammonium molybdate Ammonium sulphite
Pottery and porcelain	Ammonium dichromate
Pyrotechnics	Ammonium nitrate Ammonium thiocyanate
Textile plants	Ammonium fluoride Ammonium thiocyanate
Wood preservation	Ammonium fluoride

Table 2. List of toxic levels of un-ionized ammonia as reported in the literature.

Organism	Toxic effects	Un-ionized Ammonia Conc. NH_3-N (ppm)	Un-ionized Ammonia Conc. NH_3 (ppm)	Source
Brown trout spawn	LC_{50}	0.25-0.33	0.30-0.40	Wuhrmann & Woker (1948)
Brown trout fry	10-hr LC_{60}	0.33	0.40	Penaz (1965)
Brook trout	24-hr LC_{100}	2.5	3.0	McKee & Wolf (1963)
Cutthroat trout fry	96-hr LC_{50}	0.4-0.7	0.5-0.8	Thurson et al. (1978)
	36-day LC_{50}	0.2-0.5	0.3-0.6	Thurson et al. (1978)
Rainbow trout eggs	Could still be fertilized	1.47	1.79	Rice & Stokes (1975
Rainbow trout fertilized eggs and alevins	24-hr TLM up to 50 days	2.94	3.58	Rice & Stokes (1975)
85-day old fry	24-hr TLM	0.056	0.068	Rice & Stokes (1975)
Adult rainbow trout	24-hr TLM	0.080	0.097	Rice & Stokes (1975)
Rainbow trout fertilized eggs	42-days exposure no effect mortality	0.05-0.37	.0006-0.45	Burkhalter & Kaya (1977)
Rainbow trout sac fry	21-day incipient LC_{50}	0.25	0.30	Burkhalter & Kaya (1977)
Rainbow trout	Toxicity reduced by CO_2 - (low pH) 12-hr LC_{50}	0.10	0.12	Alabaster & Herbert (1954)
Rainbow trout fingerlings	500-min LC_{50}	0.60-1.29	0.73-1.57	Downing & Merkens (1955)
Rainbow trout	1-hr LC50	1.5	1.82	Merkens & Downing (1957)

Table 2 (cont'd).

Organism	Toxicity effects	Un-ionized Ammonia Conc. NH_3-N (ppm)	Un-ionized Ammonia Conc. NH_3 (ppm)	Source
Rainbow trout	24-hr LC_{50}	0.16	0.19	Liebmann (1960)
Rainbow trout	Acute 500-min LC_{50}	0.30	0.36	Lloyd & Herbert (1960)
Rainbow trout	Incipient 500-min LC_{50}	0.41	0.50	Lloyd & Herbert (1960)
Rainbow trout	24-hr LC_{50}	0.50	0.61	Herbert & Shurben (1963)
Rainbow trout	24-hr LC_{50} (no salinity)	0.49	0.61	Herbert & Shurben (1965)
Rainbow trout	48-hr LC_{15}	0.18	0.22	Ball (1967)
Rainbow trout	48-hr LC_{5}	0.09	0.11	Ball (1967)
Rainbow trout	48-hr LC_{5}	0.55	0.67	Ball (1967)
Rainbow trout	48-hr LC_{50}	0.2-0.6	0.2-0.7	Brown (1968)
Rainbow trout	Affects haemoglobin's combining ability	-	>10	Fromm & Gilette (1968)
Rainbow trout	500-min L_{50}	0.39	0.47	Lloyd & Orr (1969)
Rainbow trout	Harmless	0.046	0.059	Lloyd & Orr (1969)
Rainbow trout	Upper tollerance limit (UTL)	0.05	0.06	Scott & Gillepsie (1972)
Rainbow trout	Highest level (6th reuse water. 6 weeks - gill damage	0.014	0.017	Larmoyeux & Piper (1973)

Table 2 (cont'd).

Organism	Toxicity effects	Un-ionized Ammonia Conc. NH_3-N (ppm)	Un-ionized Ammonia Conc. NH_3 (ppm)	Source
Rainbow trout	500-min LC_{50}	0.45	0.55	Schulz-Weihenbrauck (1974)
Rainbow trout	No effect on growth	0.13	0.16	Schulz-Weihenbrauck (1974)
Rainbown trout fingerlings	Toxicity threshold	0.3	0.4	Hampson (1976)
	No growth restricting below	0.01	0.01	Hampson (1976)
Rainbown trout	2-hr LC_{50} Acute toxicity	0.65	0.79	Smart (1976)
Rainbown trout	55-hr LC_{50} Acute toxicity	0.50	0.61	Smart (1976)
Rainbown trout	24-day LC_{50} Chronic Toxicity	0.25-0.30	0.30-0.36	Smart (1976)
Rainbown trout	96-hr LC_{50}	0.573	0.697	Broderius & Smith (1976)
Chinook salmon fingerlings	Proliferation of gill epithelium after 6 weeks	0.005-0.007	0.006-0.008	Burrows (1964)
	Extensive gill damage exposed 24 hr/day for 6 weeks	0.008-0.10	0.010-0.012	Burrows (1964)
	Lamellar fusion in 4 weeks (24 hr/day exposure)	0.011-0.015	0.014-0.018	Burrows (1964)
	Fish can tolerate with impurity for 1 hr/day	0.015	0.018	Burrows (1964)

Table 2 (cont'd).

Organism	Toxicity effects	Un-ionized Ammonia Conc. NH_3-N (ppm)	Un-ionized Ammonia Conc. NH_3 (ppm)	Source
Sockeye salmon eggs	LC_{50} from fertilization	0.08	0.10	Rankin (1979)
Sockeye salmon eggs	Developing eggs cannot survive (fertilization to hatching)	0.403	0.490	Rankin (1979)
Atlantic salmon (fingerling)	Harmless mean water value	0.004	0.005	Risa & Skjervold (1975)
Atlantic salmon (fingerling)	Lethal to fry	0.013	0.016	Risa & Skjervold (1975)
Atlantic salmon (fingerling)	24-hr LC_{50} in fresh water with oxygen level at air sturation value (ASV)	0.12	0.15	Alabaster et al. (1979)
Atlantic salmon (smolts)	24-hr LC_{50} in fresh water at 3.5 ppm oxygen	0.07	0.09	Alabaster et al. (1979)
Atlantic salmon (smolts)	24-hr LC_{50} 30% sea water - oxygen at ASV	0.2	0.3	Alabaster et al. (1979)
Atlantic salmon (smolts)	24-hr LC_{50} in 30% sea water oxygen a 3.1 ppm	0.10	0.12	Alabaster et al. (1979)
Small fish	24-hr LC_{100}	12.0	14.5	McKee & Wolf (1963)

Table 2 (cont'd).

Organism	Toxicity effects	Un-ionized Ammonia Conc. NH_3-N (ppm)	Un-ionized Ammonia Conc. NH_3 (ppm)	Source
Creek chub	24-hr LC_{100}	12.0	14.5	McKee & Wolf (1963)
Perch	24-hr LC_{100}	12.0	14.5	McKee & Wolf (1963)
Perch	96-hr LC_{50}	0.29	0.35	Ball (1968)
Roach	96-hr LC_{50}	0.23	0.28	Ball (1968)
Rudd	96-hr LC_{50}	0.36	0.44	Ball (1968)
Bream	96-hr LC_{50}	0.4	0.5	Ball (1968)
Common carp	10-day LC_{17}	0.74-1.1	0.90-1.3	Ball (1968)
Common carp	35-day LC_8	0.09	0.11	Flis (1968)
Goldfish	24-hr LC_{100}	1.6-2.0	1.9-2.4	McKee & Wolf (1963)
Carp, shiner	24-hr LC_{100}	4.0	4.9	McKee & Wolf (1963)
Golden shiner	96-hr LC_{50}	0.99	1.20	Baird et al. (1979)
Fathead minnows	48-hr LC_{50}	7.0	8.5	McKee & Wolf (1963)
Suckers, trout	24-h LC_{100}	4.0	4.9	McKee & Wolf (1963)
Suckers, carp	24-hr LC_{100}	5.2	6.3	McKee & Wolf (1963)
Bluegill, sunfish	48-hr LC_{50}	7.4	9.0	McKee & Wolf (1963)
Guppies	24-hr LC_{50}	1.47	1.79	Rubin & Elmaraghy (1973)

Table 2 (cont'd).

Organism	Toxicity effects	Un-ionized Ammonia Conc. NH_3-N (ppm)	Un-ionized Ammonia Conc. NH_3 (ppm)	Source
Guppies	48-hr LC_{50}	1.27	1.54	Rubin & Elmaraghy (1977)
Guppies	72-hr LC_{50}	1.26	1.53	Rubin & Elmaraghy (1977)
Dover sole	Cessation of feeding. Zero growth	0.75	0.91	Alderson (1976)*
	Gowth at 13% rate when NH_3 conc is zero (ph8)	0.41	0.50	Alderson (1976)*
	No effect on growth	0.042	0.051	Alderson (1976)*
	Growth ceased	0.38-0.77	0.46-0.94	Alderson (1979)
	Lowest threshold causing any effect	0.066	0.080	Alderson (1979)
Turbot	No effect on growth	0.09	0.11	Alderson (1976)*
Turbot	Growth ceased	0.3-0.9	0.4-1.1	Alderson (1979)
	Lowest threshold causing any effect	0.11	0.13	Alderson (1979)

*Personal communication to B. L. Hampson (1976).