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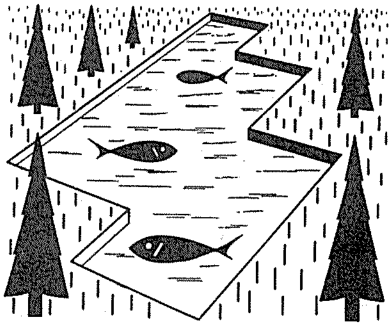

WATER QUALITY CRITERIA FOR EUROPEAN FRESHWATER FISH

REPORT ON AMMONIA AND INLAND FISHERIES

prepared by

EIFAC Working Party on Water Quality Criteria
for European Freshwater Fish

EUROPEAN INLAND FISHERIES ADVISORY COMMISSION
FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
Rome, 1970

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PREPARATION OF THIS DOCUMENT

The background of this paper is described in the Foreword to the report itself. The paper was prepared by the European Inland Fisheries Advisory Commission (EIFAC) Working Party on Water Quality Criteria for European Freshwater Fish.

The report is being issued in this series where the first four documents of the Working Party were published: "Report on finely divided solids and inland fisheries", EIFAC tech.Pap., (1):21 p., 1964;"Report on extreme pH values and inland fisheries", EIFAC tech.Pap., (4):24 p., 1968; "Report on water temperature and inland fisheries based mainly on Slavonic literature", EIFAC tech.Pap., (6):32 p., 1968; and "List of literature on the effect of water temperature on fish", EIFAC tech.Pap., (8):8 p., 1969.

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Water Quality Criteria for European Freshwater Fish. Report on ammonia and inland fisheries. EIFAC tech.Pap., (11):12 p., 1970.

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FOREWORD

This is the fifth technical paper on water quality criteria for European fresh-water fish prepared for the European Inland Fisheries Advisory Commission (EIFAC) - an intergovernmental organization with a membership of 23 countries. The Commission has concentrated its efforts on the establishment of water quality criteria for European freshwater fish since its Second Session, Paris, 1962 1/, when it took note of a recommendation of the United Nations Conference on Water Pollution Problems in Europe, 1961, that EIFAC take the initiative in this field.

As was stated in its first four reports 2/, the Commission "agreed that the proper management of a river system demands that water of suitable quality be provided for each use that is made or intended to be made of it and that the attainment and maintenance of such quality is normally to be sought through the control of pollution. It was necessary therefore to know the standards of quality required for each particular use in order to determine the degree of pollution control necessary and to forecast the probable effect of augmented or new discharges of effluents. It was pointed out that water quality standards for drinking water had been well defined by the World Health Organization (WHO) and that standards for certain agricultural and industrial uses are also well defined. However, water quality criteria for fish have not received the attention that they deserve. All too often, water has been considered quite adequate for fish as long as there has been no obvious mortality which can be ascribed to known pollutants. Degradation of the aquatic habitat through pollution and decrease in the annual production and subsequent harvest of fish have often passed unnoted.

With such reasoning in mind, it was agreed that the establishment of water quality criteria for European freshwater fish be undertaken by the Commission. This was to be accomplished by a critical examination of the literature, and very possibly experimentation to clear up contradictions and fill in gaps of knowledge, followed by recommendations as to desirable requirements for various aquatic organisms or groups of aquatic organisms with respect to the various qualities of water. The final criteria were to be published and given wide dissemination."

To accomplish this task, the Second Session of the Commission appointed a Working Party of experts selected on the basis of their knowledge of physical, chemical and biological requirements of European freshwater fish in relation to the topics to be studied. This Working Party prepared its first report on finely divided solids and inland fisheries, referred to above, which was submitted to the Commission at its Third Session, Scharfling am Mondsee, 1964, where it was unanimously approved. Its report on extreme pH values and inland fisheries (see footnote 2) was published early in 1968, in time for presentation at the Fifth Session of EIFAC (Rome, May 1968) where again it was unanimously approved.

In addition to the review of literature on water temperature and pH, the Commission had decided at its Fourth Session 3/, Belgrade, 1966, to study dissolved oxygen requirements and toxic substances including heavy metals, phenols and pesticides and herbicides. After the Fourth Session, FAO also

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1/ See, respectively: EIFAC Report, Second Session, 1962, p.21-2
UN (1961) Conference on Water Pollution Problems in Europe, held in Geneva from 22 February to 3 March 1961.
Documents submitted to the Conference. Vols I-III, United Nations, Geneva, 600 p.

2/ Report on Finely Divided Solids and Inland Fisheries, EIFAC tech.Pap., (1):21 p., 1964
Report on Extreme pH Values and Inland Fisheries, EIFAC tech.Pap., (4):24 p., 1968
Report on Water Temperature and Inland Fisheries based mainly on Slavonic Literature, EIFAC tech.Pap., (6):32 p., 1968.
List of Literature on the Effect of Water Temperature on Fish, EIFAC tech.Pap., (8):8 p., 1969

3/ EIFAC Report, Fourth Session, 1966, p.12

appointed a consultant to review the world literature on dissolved oxygen requirements for freshwater fish. At its Fifth Session 4/, Rome, 1968, the Commission, reviewing again the priorities for future studies, decided to undertake the critical reviews of the literature on the effects of ammonia and phenols. At its Sixth Session 5/, Krakow, 1970, the Commission decided to continue reviews on phenols, copper and zinc and to start work on mercury compounds and cyanides.

For the preparation of the report on water quality criteria for ammonia the following experts were appointed to the EIFAC Working Party on Water Quality Criteria:

Mr. J.S. Alabaster	(United Kingdom), Convener
Prof. T. Backiel	(Poland)
Dr. T.B. Hasselrot	(Sweden)
Mr. R. Lloyd	(United Kingdom)
Dr. V. Mitrovic	(Yugoslavia)

FAO Secretariat: Mr. William A. Dill - Secretary to EIFAC
Mr. A. Thorslund - Fishery Officer (Inland Water Pollution)

The preparation of the present report on Ammonia and Inland Fisheries was accomplished largely by Mr. R. Lloyd who prepared the basic manuscript to be reviewed by other members of the Working Party.

This report will be presented to the Seventh Session of EIFAC which is scheduled to be held in Amsterdam, 1972.

4/ EIFAC Report, Fifth Session, 1968, p. 13-5
5/ EIFAC Report, Sixth Session, 1970

SUMMARY

In establishing water quality criteria for European inland fisheries, the effect of ammonia is an important factor to be considered. Sewage effluent, effluents from certain industries and from agriculture are common sources of ammonia in water.

The harmful effects of ammonia on fish are related to the pH value and the temperature of the water due to the fact that only the un-ionized fraction of ammonia is poisonous. The un-ionized fraction increases with rising pH value, and with rising temperature.

Fish differ slightly in their tolerance to ammonia depending on species. The difference in tolerance being more significant for short periods of exposure. The difference in tolerance is, however, not great enough to justify different criteria for different species.

The lowest toxic concentration found for salmonids is 0.2 mg NH_3/l (un-ionized), but other adverse effects caused by prolonged exposure are only absent at concentrations lower than 0.025 mg NH_3/l (un-ionized). Concentrations of total ammonia which contain this amount of un-ionized ammonia vary from 19.6 mg/l (pH 7.0, $5^{\circ}C$) to 0.12 mg/l (pH 8.5, $30^{\circ}C$).

The criterion should not be applied to temperatures below $5^{\circ}C$ or to pH values above 8.5 when other factors have to be taken into consideration.

1. INTRODUCTION

(1) The purpose of this review is to summarise the state of present knowledge on the effect of ammonia on fish, to see whether firm criteria can be established for this poison, and to indicate areas where further research is necessary. Ammonia is present in most waters, as a normal biological degradation product of proteins although the concentrations may be very small and subsequent conversion to nitrate (nitrification) may take place. Probably the most common source of ammonia in water is sewage or sewage effluent, particularly if nitrification is inhibited at the sewage disposal works, although large quantities of ammonia can be produced by industries such as those producing coal gas, coke and fertilizers, and considerable amounts may be discharged to rivers or estuaries. Another frequent source is from silage and manure and it can also accumulate in fish ponds during winter as an excretory product of the fish. However, ammonia and ammonium salts have been used to fertilize fish rearing ponds, and also for aquatic weed control. Although ammonia is oxidised to nitrate in well oxygenated natural waters the reverse process may take place at low concentration of dissolved oxygen.

(2) As long ago as 1913, it was shown that the toxicity of ammonia to fish was considerably affected by the pH value of the water, but it was not until 1947 that Wuhrmann, Zehender and Woker, in a classic study, demonstrated that it was the un-ionised fraction of ammonia which was poisonous to fish, and that the ionised fraction had little or no toxicity. Subsequent research has shown that other environmental factors could affect the toxic concentration of ammonia to fish and these may account for some of the inconsistencies shown in the early data. As a result, the complexity of the problem appears to have precluded the setting of effective criteria in respect of this poison. Usually the criteria are based on the concentration of total ammonia in the water, and do not allow for the effect of pH value.

(3) Much of the literature issued prior to 1950 has been admirably and critically reviewed by Doudoroff and Katz (1950) and further reviews have been made by Marchetti (1961) and Jones (1964). This review will follow the same pattern as that for suspended solids (EIFAC, 1964) and extreme pH values (EIFAC, 1968), and will draw on data mainly from European sources, except where data on non-European species of fish serve to add collaborative evidence, or demonstrate the effect of a variable on which there is no European evidence. Although it is hoped that most of the literature has been surveyed, omissions will be inevitable; in some cases, papers may not have been quoted where it is felt they do not add a significant contribution to what is already known, or if the data given are incomplete. Too often, field observations on the mortality of fish caused by ammonia are unaccompanied by measurements of oxygen levels, pH values and temperature, without which the data cannot be compared with other field observations or results of laboratory experiments.

(4) There is some confusion in the terminology used to describe concentrations of ammonia. In this review, the terms "ionised ammonia" (NH_4^+) and "un-ionised ammonia" (NH_3) will be used to describe the two states of ammonia, and "ammonia" will refer to the combined concentrations of un-ionised and ionised ammonia ($NH_3 + NH_4^+$). Chemical methods of analysis give values for ammonia and the method used for calculating the amounts of un-ionised and ionised ammonia present are given below. Concentrations of ammonia will be expressed as $mgNH_3/l$.

2. LITERATURE SURVEY ON EFFECTS OF AMMONIA

Direct Lethal Action:

2.1 Laboratory Data

Variables Affecting the Lethal Levels

(a) pH Value

(5) Although there are several reports in the early literature that ammonia is more toxic in alkaline than in acid solutions, the chemical basis for this was first demonstrated by Wuhrmann and Woker (1948) who showed that only the unionized molecule was toxic, the ammonium having little or no toxicity. Further studies by Downing and Merkens (1955) confirmed that the toxicity of ammonia could be directly related to the concentration of un-ionised ammonia present.

(6) For practical purpose the formula for calculating the percentage of un-ionised ammonia present in an ammonia solution is as follows:-

$$\text{Per cent un-ionised ammonia} = \frac{100}{1+\text{antilog}\ (\text{pka}-\text{pH})}$$

where pka = the negative logarithm of the ionisation constant

Thus, for example, an increase in pH value of 0.3 units from 7.0 to 7.3 would double the concentration of un-ionised ammonia in an ammonia solution although the effect becomes less above pH 8.5. The value for pka depends on temperature and appropriate values from Bates and Pinching (1950) are given in Table 1, and shown graphically in Fig. 1.

Table 1. Values for pka of ammonia at temperatures between 5-30°C

Temperature °C	5	10	15	20	25	30
pka	9.90	9.73	9.56	9.40	9.25	9.09

It can be calculated that a rise in temperature of 10°C doubles the concentration of un-ionised ammonia present in an ammonia solution. It can also be shown that the proportion of undissociated ammonia increases with increase in ionic strength, the increase over that in distilled water is about 10 per cent in a water having a hardness of about 250 mg/l expressed as calcium carbonate and about 25 per cent in sea water.

(7) Recently, Tabata (1962) has claimed that the ionised fraction of ammonia has a demonstrable toxicity, although it was only one-fiftieth of the toxicity of un-ionised ammonia for the water flea (Daphnia pulex), and even less for some species of fish. These conclusions are at variance with the data given by Downing and Merkens (1955).

(b) Free Carbon Dioxide

(8) Alabaster and Herbert (1954) showed that the toxicity of a solution of ammonium chloride could be decreased by increasing the level of free carbon dioxide in the water, which reduced the pH value, until a concentration of free carbon dioxide was reached which was itself toxic to fish. More recently, Lloyd and Herbert (1960) proposed a second effect of free carbon dioxide. They suggested that it was not the pH value of the bulk of the solution which was important in determining the toxicity of ammonia, but the pH value of the water at the gill surface; this depends on the effect which the respiratory carbon dioxide produced by the fish has on the pH value of the water and its magnitude depends upon the concentration of free carbon dioxide already present in solution. If the concentration of free carbon dioxide in the water is very low, the amount excreted by the fish will considerably reduce the pH value at the gill surface, but the extent of this pH change will become less as the level of free carbon dioxide rises in the bulk of the water. Thus, in experiments in which the ambient free carbon dioxide levels are very low and the pH value high (following the addition of ammonium hydroxide, or sodium hydroxide for pH control) the levels of un-ionised ammonia found to be toxic may be about five times greater than those applicable to polluted waters where the level of free carbon dioxide is likely to be high and the pH value lower. So far, this effect has only been demonstrated for rainbow trout (Salmo gairdnerii) and its magnitude may be less for those species of fish with low respiratory efficiency and which would therefore excrete only relatively small amounts of carbon dioxide into the respiratory water flow.

(c) Dissolved Oxygen

(9) A reduction in the level of dissolved oxygen in the water increases the toxicity of several poisons to fish, and this has been found for ammonia by Wuhrmann (1952) and Downing and Merkens (1955), the latter showing that a reduction in the oxygen content of the water to 50 per cent of the air saturation value reduced the survival times of several species of fish in lethal solutions to one-third of the time in aerated water. Lloyd (1961a) showed that the effect of low oxygen concentrations on the threshold LC 50 for ammonia (the value where the curve relating median period of survival to concentration becomes parallel to the survival time axis) was greater than its effect on other poisons, and put forward a hypothesis to explain this difference and for variations caused by free carbon dioxide in the water. Using this hypothesis, it can be calculated that the effect of low oxygen levels on the toxicity of ammonia will become less as the level of free carbon dioxide in the water rises.

However, in field situations where a lowering of the dissolved oxygen levels is likely to be accompanied by an increase in the level of free carbon dioxide and a concomitant reduction in pH value, this latter factor is likely to reduce the toxicity of ammonia to a greater extent than the increased toxicity caused by low oxygen levels. In the experiments which have been reported here, fish have been transferred directly from clean aerated water to ammonia solutions of lower oxygen content; to our knowledge, similar experiments with fish already acclimated to the low dissolved oxygen level of the water have not been made.

(d) Water Hardness

(10) Both Wuhrmann and Woker (1955) using minnows (*Phoxinus phoxinus*), and Herbert (unpublished data referred to in Herbert, 1961) using rainbow trout, showed that variations in the hardness of the water had no effect on the toxicity of ammonia to these fish.

(e) Alkalinity

(11) So far as is known, alkalinity (bicarbonate concentration) only affects the toxicity of ammonia by its part in determining the pH value of the water in conjunction with the level of free carbon dioxide present. This effect has been described graphically by Lloyd (1961b).

(f) Temperature

(12) It has already been stated that an increase in temperature will increase the proportion of un-ionised ammonia present in an ammonia solution (para 6). It has been shown by Woker (1949) that although survival times of chub (*Squalius cephalus*) at constant levels of un-ionised ammonia decreased with a rise in temperature, the threshold LC 50 remained the same. Similar results for rainbow trout were found by Herbert (1962).

(13) However, it is possible that these findings only apply to temperatures above 10°C. Burrows (1964) showed that at lower temperatures, un-ionised ammonia became markedly more toxic to chinook salmon (*Oncorhynchus tshawytscha*) and recent work by Brown (1968) suggests that at 3°C the threshold LC 50 of un-ionised ammonia for rainbow trout is about half that at 10°C, which would cancel the effect of temperature on the dissociation of ammonia. This is of some importance if ammonia concentrations rise in rivers or carp ponds during the winter.

(g) Salinity

(14) The toxicity of ammonia to rainbow trout decreases with a rise in salinity up to 30 per cent seawater (a concentration approximately isotonic with the blood) but increases again up to 100 per cent seawater (Herbert and Shurben, 1965) under laboratory conditions of constant pH value. Examination of the data shows that the curve relating threshold LC 50 of ammonia to log concentration of seawater is symmetrical about the concentration isotonic with fish blood, the threshold in isotonic solutions being just over twice that found in freshwater. The dissociation constants for ammonia used in this study were those for fresh water.

(h) Acclimation to low ammonia concentrations

(15) It is well established that exposure of fish to sub-lethal levels of ammonia increases their subsequent resistance to lethal concentrations. (Vamos, 1963; Malacea, 1968; Lloyd and Orr, 1969). The resistance obtained by rainbow trout is held for at least one day, but is lost after three days (Lloyd and Orr, 1969). None of the data available allow an estimate to be made of the maximum concentration to which fish can be acclimatised.

(i) Other Factors

(16) It has been shown by Herbert and Shurben (1963) that the resistance of rainbow trout to ammonia poisoning was unchanged at swimming speeds up to 2 body lengths/sec but decreased thereafter, and at 3 body lengths/sec the threshold LC 50 was 70 per cent of that in still water.

(17) Hemens (1966) found that female mosquito fish (*Gambusia affinis*) were slightly more resistant than males to ammonia poisoning, but that size differences had no effect on their susceptibility.

(18) There is some circumstantial evidence (Lloyd and Orr, 1969) that the physical handling of fish immediately before exposing them to ammonia increases their resistance to this poison (para 20).

Summary of Toxicity Data

(a) Salmonids

(19) Data has been presented by Penaz (1965) which show that the ova of brown trout (Salmo trutta v. fario) are very resistant to short exposures (120 min) to concentrations as high as 50 mg NH3/l of un-ionised ammonia at 10°C, although there was some indication that hatching success was reduced if the eggs were exposed to this high concentration during the later stages of development. Wuhrmann and Woker (1948) found that the threshold for trout spawn was 0.3 - 0.4 mg NH3/l un-ionised ammonia.

(20) Further experiments by Penaz (1965) on the fry of brown trout gave a 10 hr LC 50 of 3.60 mg NH3/l although the data presented show that there was a 60 per cent mortality in 0.4 mg NH3/l at the end of this period, and this concentration was suggested as the threshold level. Lloyd and Herbert (1960) showed that the threshold LC 50 at the gill surface of rainbow trout was 0.49 mg NH3/l, although higher concentrations were required in the bulk of the solution to produce these levels. This accounted for the high threshold LC 50 values (1.8 mg NH3/l) found by Merkens and Downing (1957) who used water of very low free carbon dioxide content. In order to take into account the many different variables which affect the toxicity of ammonia solutions, Lloyd (1961b) published a series of graphs from which the threshold LC 50 of this poison for rainbow trout could be calculated. From the data then available, there was a close correlation between predicted and observed threshold LC 50 values. However, recent experiments by Ball (1967) gave a one-day LC 50 value for rainbow trout of 0.50 mg NH3/l, similar to that found by Herbert and Shurben (1963) of 0.50 mg NH3/l and by Herbert and Shurben (1965) of 0.49 mg NH3/l, although a second test gave a one-day LC 50 of 0.70 mg NH3/l. Lloyd and Orr (1969) found a one-day LC 50 of 0.47 mg NH3/l for rainbow trout fitted with a urinary catheter and kept in aerated water. These values are lower than those predicted from the graphs of Lloyd (1961b) for the experimental conditions and it has been suggested (Lloyd and Orr, 1969) that differences in the experimental techniques used, such as the handling of the fish immediately before the start of the experiment, may cause variations in the results. Even lower threshold LC 50 values of 0.2 mg NH3/l have been given for rainbow trout fry by Liebmann (1960) and for rainbow trout fingerlings by Danecker (1964) but no suggestion can be put forward to account for the increased susceptibility of these fish, except that Danecker used diluted liquid manure to produce the required ammonia concentration.

(21) Most data on the toxicity of ammonia to fish referred to in para 20 have been obtained from tests which continued for a few days only. However, tests over a three-month period with batches of 200 rainbow trout have shown that a small proportion of the fish population are killed by concentrations lower than the two-day LC 50; at 0.22 mg NH3/l, fifteen per cent died, and at 0.11 and 0.06 mg NH3/l, five per cent died (Water Pollution Research, 1967). On the other hand, there was no mortality of chinook salmon fingerlings exposed to 0.018 mg NH3/l or less for a six-week period, (Burrows, 1964), though some hyperplasia of the gill lamella epithelium was observed (para 35).

(22) Atlantic salmon smolts (Salmo salar) in fresh water are more sensitive to ammonia poisoning than rainbow trout of the same size, having a one day LC 50 of 0.28 mg NH3/l (Herbert and Shurben, 1965) but the difference in sensitivity is lost at increased salinities.

(23) Since the pH value of natural waters may not remain constant, but show a diurnal fluctuation, the level of un-ionised ammonia present will certainly vary. Results of experiements by Brown, Jordan and Tiller (1969) on the toxicity of fluctuating ammonia levels, are difficult to evaluate, in that fluctuating concentrations between 1½ and ½ times the two-day LC 50 on a two-hour cycle caused a greater mortality of rainbow trout than would have been expected from the two-day LC 50 alone (the average concentration) with the survival time being about twice as long as that found for 1½ times the two-day LC 50. On this evidence therefore, one could assume that successive exposures to lethal concentrations of ammonia were cumulative. However, fluctuations of a similar magnitude on a one-hour cycle produced mortalities similar to that at a constant two-day LC 50, and in this case the fish could be assumed to be reacting to the average value of the ammonia concentrations to which they were exposed. One explanation for these different results might be that it takes one to two hours for ammonia to have a physiological effect on the fish (Lloyd and Orr, 1969) and that this might affect the reactions of fish to exposure to ammonia under these test conditions. Further experiments are needed to determine the effect of diurnal variations of un-ionised ammonia on the survival of fish, with associated changes in pH value and temperature, which can occur in both natural and polluted waters.

(b) Other Species

(24) A recent study by Ball (1967) on the toxicity of ammonia to roach (Rutilus rutilus), rudd (Scardinius erythrophthalmus) bream (Abramis brama) and perch (Perca fluviatilis), showed that the threshold LC 50 values for these species at British summer temperatures were 0.42, 0.44, 0.50, and 0.35 mg NH_3/l (un-ionised ammonia) respectively, and that the experiments had to be continued for between 2½ to 4 days before an estimate of the threshold LC 50 could be made. These values were comparable with those obtained within 24 hours for rainbow trout under the same test conditions. Therefore, although these species have a greater resistance than trout to ammonia during tests lasting two days, their survival time in 0.73 mg NH_3/l being eight times longer, the threshold LC 50 for all five species were similar. These results make a comparison between data from other experiments difficult where tests were short-term only, since survival times in lethal solutions can be affected by many factors.

(25) Flis (1968a) working with common carp (Cyprinus carpio) in a water temperature of 11°C, found that there was a 16 per cent mortality in un-ionised ammonia concentrations of 1.3 mg NH_3/l in one test, and an 18 per cent mortality in 0.9 mg NH_3/l in another, during a 10-day test period. In these experiments, ammonia was added as ammonium hydroxide, and the high pH values of 8.3 to 8.7 would indicate a low concentration of free carbon dioxide, so that the toxic concentrations may be slightly higher than normal. In further tests at an average pH value of 8.05, Flis (1968b) found an 8 per cent mortality of common carp in 35 days at 0.11 mg NH_3/l, although in a second series at this concentration there were no mortalities. Although Danecker (1964) estimated that an un-ionised ammonia concentration of 1.5 mg NH_3/l to be the lethal level for common carp at about 16°C, the tests were of less than two days duration, and a replotting of the data indicates that a threshold concentration might not have been reached within this period. Vamos (1963) found that the concentration of un-ionised ammonia required to overturn common carp was 0.5 mg NH_3/l in tests of only a few hours duration.

(26) Since Ball (1967) showed that tests of several days duration were required to obtain a measure of threshold LC 50 of ammonia for coarse fish, tests of shorter duration are unlikely to give threshold values, and therefore it is difficult to make a comparison between the results obtained for other species. In general, however, the toxic levels of un-ionised ammonia for short-term tests for several species of fish lie between 0.6 mg NH_3/l for perch and 2.0 mg NH_3/l for carp and tench (Tinca tinca), with chub and minnow being intermidiate in sensitivity. (Woker and Wuhrmann, 1950; Liebmann, 1960; Nehring, 1962; Danecker, 1964; Malacea, 1966).

(27) Therefore, it is likely that the various species of coarse fish have similar sensitivities to ammonia poisoning after prolonged exposure, but over a short period some are more resistant, especially carp and tench. This may be important in situations where the level of un-ionised ammonia fluctuates, either by variations in concentration of total ammonia or by changes in the pH value or temperature of the water, and some species may be able to survive high concentrations for short periods which would be lethal to more sensitive fish.

(c) Toxicity of Ammonia in the presence of other Poisons

(28) Several tests have been made with rainbow trout on the toxicity of mixtures of ammonia with other poisons. Experiments with solutions containing both ammonia and cyanide showed that the combination was more toxic than either substances alone (Wuhrmann and Woker, 1948). Herbert (1962) showed that the threshold LC 50 of a mixture of ammonia and phenol was obtained when the sum of the individual concentrations, expressed as the proportion of their separate threshold LC 50 values, equalled unity. Further tests with zinc and ammonia (Herbert and Shurben, 1964), and copper and ammonia (Herbert and Van Dyke 1964) gave similar results, in that the toxicity of the individual poisons could be added together in this manner. However, Brown, Jordan, and Tiller (1969) showed that mixtures of zinc, phenol, and ammonia, in which the proportion of the total toxicity contributed by phenol and ammonia was small, were less toxic than the predicted values and it is possible that this method of summing toxicities is not valid for low concentrations of poisons (para 34). Recently, Vamos and Tasnadi (1967) have used copper sulphate to reduce the toxicity of ammonia in carp ponds, and it is suggested that the cupro-ammonium compounds formed are not toxic, a finding opposite to that of Herbert and Van Dyke (1964) in laboratory experiments.

2.2 Field Observations

(29). Although there are many recorded cases of fish kills following the discharge of ammonia to streams or rivers and from the accumulation of ammonia in carp ponds, the chemical data available are insufficient to enable a correlation to be made with the laboratory evidence. Furthermore, it is uncommon for ammonia to be the only poison present and it is difficult to make an estimate of the part played by other toxic substances.

(30) Recently, however, Vamos and Tasnadi (1967) have made some interesting observations on the mortality of carp in fish ponds. Mortality occurred when the concentration of un-ionised ammonia reached 0.5 mg NH3/l with a level of dissolved oxygen of 6 mg/l, but when the oxygen concentration was 2 mg/l, the lethal level of un-ionised ammonia was 0.2 mgNH3/l. These observations also support the laboratory data on the effect of low oxygen levels on the toxicity of ammonia to fish. There are several other instances recorded of common carp mortality in ponds where a combination of high temperatures, high pH value and high ammonia concentration were present in varying degrees. Usually the lethal conditions persist for a few hours only, and insufficient measurements are made of the water quality before and during this period to make a correlation with laboratory data possible. Kempinska (1968) recommends that ammonia should not be used as a fertilizer for fish ponds if the pH value of the water exceeds 8.5.

(31) Recent examination of data on water quality in the River Trent system in England (Garland and Hart, personal communication) shows that species of coarse fish are present where the median concentration of un-ionised ammonia over a period of a year is between 0.01 and 0.04 mg NH3/l (with median dissolved oxygen concentrations between 6.8 and 8.5 mg/l).The upper value is about 10 per cent of the threshold LC 50 found for coarse fish found by Ball (1967).

2.3 Mode of Toxic Action

(32) Statements are sometimes made that ammonia acts on the nervous system of fish but this is based mainly on mammalian data; several authors have demonstrated that exposure of fish to toxic ammonia solutions results in damage to the gill epithelium (Kuhn and Koecke, 1956; Burrows, 1964; Reichenbach-Klinke, 1967; Flis, 1968a) and Marchetti (1960) found that prolonged exposure to sublethal levels of ammonia caused severe caudal damage to Crucian carp (Carassius carassius). Reichenbach-Klinke (1967) also described some effects on the blood of rainbow trout in which the number of erythrocytes falls as the ammonia level reaches a toxic concentration, and he considers that trout fry are seriously affected by a concentration of 0.27 mg NH3/l. In a more extensive study on the effects of lethal and sub-lethal concentrations of ammonia on various organs of common carp, Flis (1968b) demonstrated severe tissue damage after 35 days exposure to an un-ionised ammonia concentration of 0.11 mg NH3/l which although not lethal in one test, killed 8 per cent in a second experiment. Damage to the liver and kidneys appeared to be associated with disruption of the blood vessels. Danecker (1964) reports both gill damage and haemolysis occuring in carp.

(33) Lloyd and Orr (1969) demonstrated that exposure to ammonia concentrations greater than 12 per cent of the threshold LC 50 increased the absorption of water by rainbow trout. By measuring changes in the rate of urine production, they showed that the threshold LC 50 was associated with a urine flow rate of 12 ml/kg/h, compared with a normal rate of 2 ml/kg/h at 10.5°C, showing a six-fold increase in absorption. These findings may account for the greater resistance of rainbow trout to un-ionised ammonia in isotonic saline solutions (para 14); also, the stress imposed by this water intake on the kidneys, and the water balance of the fish generally, may account for the effects found by other authors on the blood system and tissues. Lloyd and Orr (1969) further suggest that any factor which affects the water balance in fish will also influence their susceptibility to ammonia poisoning, and this may account for the increased susceptibility of ulcerated fish (Vamos, 1963) and of salmon smolts (Herbert and Shurben, 1965).

(34) The reasons for the increase in the permeability of fish to water is not known. Fromm and Gillette (1968) found evidence of some accumulation of ammonia in the blood of rainbow trout exposed to ammonia solutions, although the amount was small and is considered to have been derived endogenously. It is possible that the increase in permeability occurs only when the normal excretory mechanism becomes over-loaded and Fromm and Gillette (1968) showed that the proportion of ammonia to total nitrogen excreted decreased with increasing ambient ammonia levels. In those solutions where the amount of ammonia entering the fish can be readily excreted, or de-toxified, there would be no harmful effect, and this may occur in ammonia concentrations below 12 per cent of the leathal level. If fish change from ammonia to urea excretion at temperatures below 11°C, as shown for the chinook salmon by Burrows (1964) their resistance to ammonia might be expected to decrease as shown by Brown (1968).

(35) There is conflicting evidence on the permanence of damage caused by short-term exposure to ammonia; Grindley (1946) reported that few overturned rainbow trout survived on transfer to clean water, whereas Vamos (1963) found the reverse with carp, and that overturned fish had a greater resistance to subsequent exposure to ammonia solutions. Burrows (1964) exposed chinook salmon to low concentrations of ammonia for six weeks, and found progressive hyperplasia of the gill epithelium during the first four weeks of exposure; subsequent exposure to clean water led to recovery of the gill epithelium where the water temperature was 14°C, but not at 6°C.

2.4 Avoidance Reactions

(36) Both Jones (1948), using sticklebacks (*Gasterosteus aculeatus*) and Summerfelt and Lewis (1967), using green sunfish (*Lapomis cyanellus*), found that these fish were repelled by lethal solutions of ammonia in a gradient tank. Green sunfish were not repelled by concentrations in which the fish showed obvious signs of distress, and sticklebacks were attracted to sub-lethal levels. Hepher (1959) observed that common carp avoided local high concentrations of ammonia after it had been applied as a fertilizer in pond culture. There is no evidence to suggest that fish avoid sub-lethal levels of this poison.

2.5 Effect on Aquatic Food Organisms

(37) In an extensive study of invertebrate organisms typical of different zones of pollution, Stammers (1953) found that species inhabiting the most polluted zone were the most resistant to ammonia poisoning. In general, all the organisms tested were more resistant than trout. Malacea (1966) gives the two day LC 50 for *Daphnia magna* as 0.66 mg $NH3/l$ which is close to values obtained from trout.

(38) It is unlikely, therefore, that the presence of ammonia in a river at concentrations lower than those toxic to fish would affect adversely the food supply of fish; indeed, it may have a significant beneficial effect on productivity, and therefore the total biomass present, as can also occur when carp ponds are fertilized with ammonia.

3. CONCLUSIONS

(39) It is clear that the major factor controlling the toxicity of ammonia is the pH value of the water which, together with temperature, governs the concentration of un-ionised ammonia present in ammonia solutions. Many laboratory experiments of relatively short duration have demonstrated that the lethal concentration of ammonia for a variety of fish species lie in the range 0.2 to 2.0 mg $NH3/l$, with trout being the most sensitive and common carp being the most resistent. Discrepancies between results for any one species may reflect differences in other environmental variables, such as the level of free carbon dioxide in the water, which have a small, but significant, effect on the toxicity of ammonia, or they may be caused by differences in experimental technique, such as in the handling of the fish. Although it is clear that the more sluggish of coarse fish species survive much longer in toxic solutions than do salmonids, the difference in sensitivity between various fish species to prolonged exposure is probably less than the ten-fold range given above. Therefore, it seems that a water quality criterion for ammonia based on a trout standard would not be too harsh for waters containing only resistant species of coarse fish.

(40) From the laboratory evidence which is available, the lowest toxic concentration found for salmonids is 0.2 mg $NH3/l$ for rainbow trout fry (Liebmann, 1960), with values for Atlantic salmon smolts (Herbert and Shurben, 1965) and for rainbow trout at 3°C (Brown, 1968) being only slightly higher. It is possible, therefore, that an effective criterion could be based on this concentration, although lack of experimental data would prevent its confident adoption for temperatures below 10°C.

(41) Although concentrations of un-ionised ammonia below 0.2 mg $NH3/l$ may not kill a significant proportion of a fish population, they may still exert an adverse physiological or histopathological effect (Flis, 1968b; Lloyd and Orr, 1969). The only evidence from laboratory tests on which a level of no adverse effect can be based is that of Lloyd and Orr (1969) who showed that concentrations lower than 12 per cent of a lethal threshold concentration did not increase the permeability of rainbow trout to water. This evidence relates to sudden exposure of the fish to ammonia solutions, and there is some evidence that acclimation to sub-lethal concentrations can take place. It is possible, therefore, that a criterion based on 12 per cent of a threshold LC 50 would be unduly low. However, acclimation to sub-lethal levels has only been shown to give an increased resistance to toxic concentra-

tions and a reduced physiological response, and it is still possible that the fish migh undergo deleterious histophathological changes during a prolonged exposure (paras 21 and 32). It is very unlikely that a constant concentration of un-ionised ammonia could be maintained in any natural water system and fish which have been adversely affected might recover when the level of un-ionised ammonia fell, but such recovery may not occur in cold water (para 35). There is some field data (para 31) to suggest that coarse fisheries can be maintained in the presence of a median concentration of ammonia which is about 10 per cent of the threshold LC 50 with higher levels being present at times. On balance, therefore, a concentration of 0.025 mg NH_3/l (12 per cent of the threshold LC 50 of 0.2 mg NH_3/l un-ionised ammonia) may be the maximum which can be tolerated by fish for a long period. Concentrations of ammonia which contain this amount of un-ionised ammonia are given in Table 2 and expressed graphically in Figure 2.

Table 2. Concentrations of ammonia $(NH_3+NH_4^+)$ which contain an un-ionised ammonia concentration of 0.025 mg NH_3/l.

Temperature °C	pH value					
	7.0	7.5	8.0	8.5	9.0*	9.5*
5	19.6	6.3	2.0	0.65	0.22	0.088
10	13.4	4.3	1.37	0.45	0.16	0.068
15	9.1	2.9	0.93	0.31	0.12	0.054
20	6.3	2.0	0.65	0.22	0.088	0.045
25	4.4	1.43	0.47	0.17	0.069	0.039
30	3.1	1.00	0.33	0.12	0.056	0.035

* Criteria under these headings may be unduly low if there is little free carbon dioxide in the water.

The values given in this Table are related to the pH value and temperature of the water; for example, where the pH value of the water is 8.0, and the temperature 20°C, the total ammonia concentration should not exceed 0.65 mg NH_3/l. It is of interest to note that the recent report of the National Technical Advisory Committee on Water Quality Criteria (US Federal Water Pollution Control Administration) concluded that above a pH value of 8.0, the total ammonia concentration should not exceed 1.8 mg NH_3/l, a criterion not at variance with those given in Table 2. It must be stressed, however, that these concentrations may add to the toxicity of other poisons present in the water, although more research is required to demonstrate whether this actually occurs.

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FIG. 1. Relation between temperature and pK_a values for ammonia.

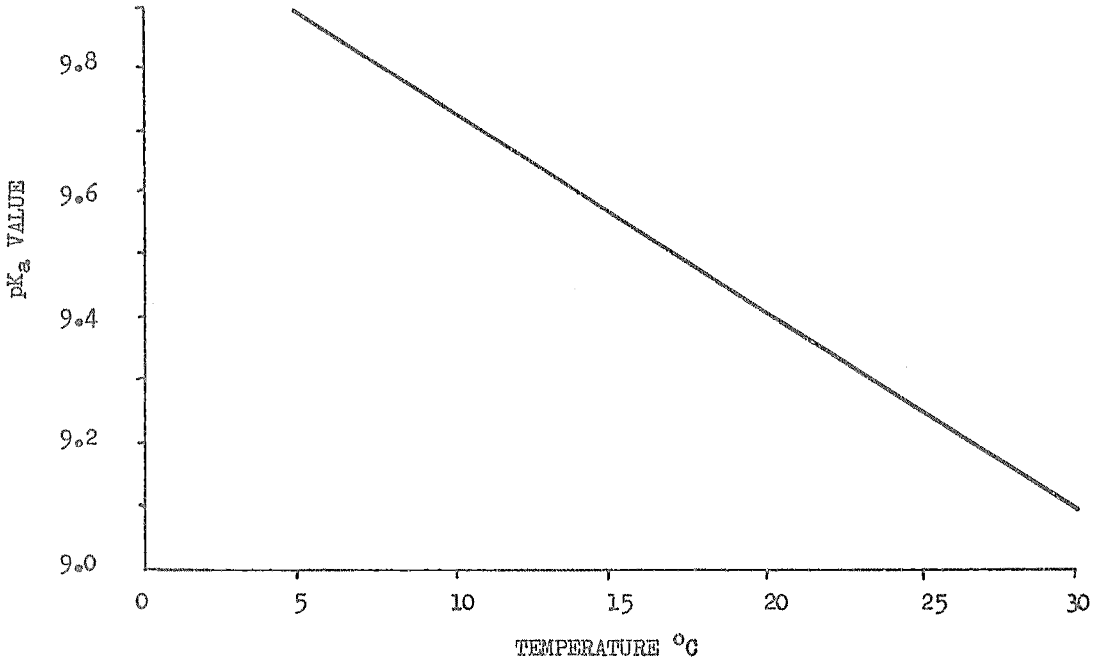
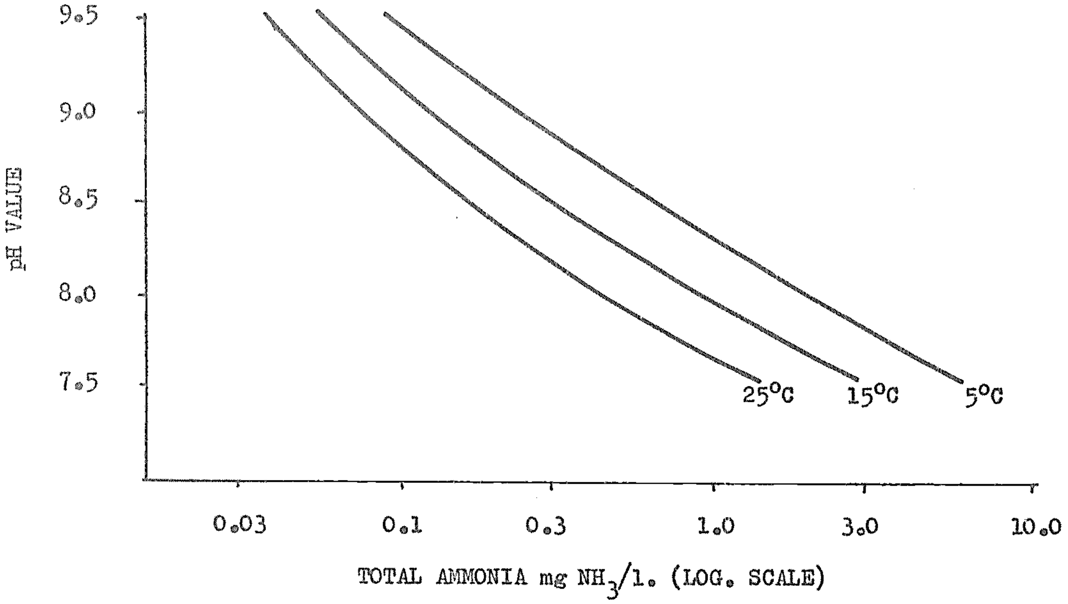


FIG. 2. Concentrations of total ammonia which contain 0.025 mg NH_3/l. un-ionised ammonia for waters of different pH and temperature.



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