WATER QUALITY CRITERIA FOR EUROPEAN FRESHWATER FISH

Report on copper and freshwater fish

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

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REPORT ON COPPER AND FRESHWATER FISH

Prepared by

EIFAC Working Party on Water Quality Criteria

for European Freshwater Fish

with the cooperation of the United Nations Environment Programme (UNEP)

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
Rome, 1976

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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 with the cooperation of the United Nations Environment Programme (UNEP).

The report is being issued in this series where the first nine 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; "List of literature on the effect of water temperature on fish", EIFAC Tech.Pap., (8):8 p., 1969; "Report on ammonia and inland fisheries", EIFAC Tech. Pap., (11):12 p., 1970; "Report on monohydric phenols and inland fisheries", EIFAC Tech.Pap., (15):19 p., 1972; "Report on dissolved oxygen and inland fisheries", EIFAC Tech.Pap., (19): 10 p., 1973; "Report on chlorine and freshwater fish", (20):11 p., 1973; "Report on zinc and freshwater fish", EIFAC Tech.Pap., (21):22 p., 1973.

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FOREWORD

This is the tenth technical paper on water quality criteria for European freshwater fish for the European Inland Fisheries Advisory Commission (EIFAC) - an inter-governmental organization with a membership of 23 countries. The Commission has been active in its efforts to establish water quality criteria for European freshwater fish since its Second Session, Paris, 1962, 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 drawing up water quality requirements with respect to fisheries[1/].

As was stated in its first nine reports on water quality criteria[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 (see footnote[2/]) which was submitted to the Commission at its Third Session, Scharfling am Mondsee, 1964, where it was unanimously approved[3/].

The Third Session then suggested that the following studies be considered by the Working Party:

- water temperature (including a review of the effect of heated discharges);
- dissolved oxygen and carbon dioxide; pH; toxic substances including heavy metals, phenols, pesticides and herbicides.

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):18 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
Report on Ammonia and Inland Fisheries, EIFAC Tech.Pap., (11):12 p., 1970
Report on Monohydric Phenols and Inland Fisheries, EIFAC Tech.Pap., (15):18 p., 1972
Report on Dissolved Oxygen and Inland Fisheries, EIFAC Tech.Pap., (19):10 p., 1973
Report on Chlorine and Freshwater Fish, EIFAC Tech.Pap., (20):11 p., 1973
Report on Zinc and Freshwater Fish, EIFAC Tech.Pap., (21):22 p., 1973

3/ EIFAC Report, Third Session, 1964, p. 11

Elevated temperature was given first priority, and a draft on this subject was prepared by the Working Party during the following inter-sessional period. (At the Third Session the work of the Commission was re-organized into three Sub-Commissions, one of which, Sub-Commission III - Fish and Polluted Water - regrouped all the activities of EIFAC in the field of water pollution. The Working Party on Water Quality Criteria for European Freshwater Fish has since functioned under this Sub-Commission.)

The Fourth Session of the Commission, Belgrade, 1966, after having studied this first draft of review of literature on the effects of water temperature on aquatic life concluded that such a review required more effort than the resources of the Commission permitted at the time. Meanwhile, it suggested that a water quality report for extreme pH values be prepared for the next Session of EIFAC, and that a report on dissolved oxygen be prepared when funds become available for a full-time consultant[4/].

The report on extreme pH values and inland fisheries (see footnote[2/]) was published in 1968, in time for presentation at the Fifth Session of EIFAC, Rome, 1968, where it was unanimously approved[5/].

At its Fifth Session the Commission again reviewed priorities for future studies and decided to undertake critical reviews on the effects of ammonia and phenols on freshwater fishes.

It also recommended that guidance as to its future work in the field of water pollution control, including the development of water quality criteria, be taken from the FAO/EIFAC Symposium on the Nature and Extent of Water Pollution Problems affecting Inland Fisheries in Europe which was later held in Jablonna, Poland, 15-16 May 1970, just before the Sixth Session of EIFAC.

The Fifth Session also approved in draft a report on water temperature and inland fisheries based mainly on Slavonic literature. The report was published in November 1968 as the third in the EIFAC water quality criteria series, and was followed in 1969 by the fourth publication in the series, a list of literature on the effect of water temperature on fish. (See footnote[2/] for both papers.)

Following the Jablonna Symposium[6/], the Sixth Session of EIFAC, Krakow, 1970, again reviewed the Commission's programme with respect to water quality criteria[7/]. Noting that a report on ammonia was almost complete, it approved continuance of work on phenols, and the current work begun by the Working Party on copper, zinc and mercury, and recommended the addition of cyanides, detergents, chlorine and hydrocarbons as items for future reviews. It also recommended eventual resumption of work on water temperature and the preparation of a review based on a critical worldwide report on dissolved oxygen prepared for FAO[8/].

After the Sixth Session of EIFAC, the EIFAC Working Party has published reports on ammonia and monohydric phenols as the fifth and sixth reviews in this EIFAC series of water quality papers[2/] which were presented to the Seventh Session of EIFAC (Amsterdam, 1972[9/]) where they were unanimously approved.

After the Seventh Session, the EIFAC Working Party on Water Quality Criteria drafted reviews on dissolved oxygen, chlorine and zinc which were studied at its eleventh and twelfth meetings held in Rome (15-17 January 1973) and Karlsruhe (25 May 1973), respectively. The reports on dissolved oxygen, chlorine and zinc have been published as the seventh, eighth and ninth reviews of this series[2/] and were approved by the Eighth Session of EIFAC (Aviemore, Scotland, 1974)[10/]. The Eighth Session gave priority to cadmium as the subject of the next report. It recommended in addition (i) that all completed reports should be updated where necessary and offered to a publisher for printing in a single volume and (ii) that research in the field to provide information essential for the formulation of water quality criteria should be encouraged by EIFAC.

The tenth review, which follows, is the one on copper and freshwater fish. For the preparation of this report, the following experts were appointed to the EIFAC Working Party on Water Quality Criteria:

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

5/ EIFAC Report, Fifth Session, 1968, pp. 14-5

6/ Holden, A.V. and R. Lloyd (1972), Symposium on the Nature and Extent of Water Pollution Problems affecting Inland Fisheries in Europe. Synthesis of National Reports, EIFAC Tech.Pap., (16):20 p.

7/ EIFAC Report, Sixth Session, 1970, p. 13

8/ Doudoroff, P. and D.L. Shumway (1970), Dissolved Oxygen Requirements of Freshwater Fishes, FAO Tech.Pap., (86):291 p.

9/ EIFAC Report, Seventh Session, 1973, p. 18

10/ EIFAC Report, Eighth Session, 1975, p. 11

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The thanks of the Working Party are hereby conveyed to Messrs. V.M. Brown, J.F. de L.G. Solbé, Dr. J. Gardiner and Dr. J. Hall of the Water Research Centre, Stevenage (U.K.) who have provided constructive criticism of the draft copper report.

FAO Secretariat:

Mr. J.-L. Gaudet - Secretary to EIFAC

The Working Party used the same general basis for their work on which they had agreed for the preparation of their first report that:

"Water quality criteria for freshwater fish should ideally permit all stages in the life cycles to be successfully completed and, in addition, should not produce conditions in a river water which would either taint the flesh of the fish or cause them to avoid a stretch of river where they would otherwise be present, or give rise to accumulation of deleterious substances in fish to such a degree that they are potentially harmful when consumed. Indirect factors like those affecting fish-food organisms must also be considered should they prove to be important."

This report will be presented to the Ninth Session of EIFAC (Helsinki, Finland, 9-15 June 1976).

SUMMARY

Copper is a common pollutant in surface waters; its mode of action on aquatic organisms is not clear but toxicity is largely attributable to Cu^{2+}. The cupric form of copper (which is the species commonly found) is readily complexed by inorganic and organic substances and is adsorbed on to particulate matter. For this reason, the free ion rarely occurs except in pure acidic soft waters. The analytical techniques commonly used do not distinguish between toxic ionic copper and non-toxic soluble copper complexes and are not accurate at low concentrations, making the interpretation of field data difficult; where possible, copper concentrations are expressed in this report as "soluble copper", i.e., that which passes through a millipore filter of average porosity 0.45 µ.

Toxicity (LC50) is increased by reduction in water hardness, temperature, dissolved oxygen, chelating agents such as EDTA and NTA, humic acids, amino acids, and suspended solids but little is known of the effect of pH.

Acutely lethal concentrations (i.e., 48- or 96-h LC50) of copper to European species of fish in hard water range over 1½ orders of magnitude. No reliable comparative data are available for different species in soft water, for the young stages, or for sub-lethal effects.

Significant adverse effects on growth of some species, including rainbow trout, occur at about 0.1 of the 96-h LC50.

Aquatic plants and algae and invertebrates are generally more resistant than fish and there is no evidence that fisheries in waters containing copper have been adversely affected because of a reduction in food organisms.

The toxicity of copper in natural waters, except soft water free from organic matter and suspended solids, is less than that predicted from laboratory tests in clean water, probably because of the presence of non-toxic complexes and insoluble precipitates. Sewage effluents containing copper are also less toxic than would be predicted from laboratory data. The presence of non-toxic complexes may partly explain the existence of brown trout populations where the annual 50 and 95 percentile values of soluble copper were 0.17 and 0.38 of the 48-h LC50 to rainbow trout, and some non-salmonid species where the corresponding values were 0.17 and 0.66 respectively.

Only tentative water quality criteria can be formulated at present because there are virtually no field observations that indicate unequivocally the concentrations of copper that are not inimical to fish populations or fisheries. This is mainly because analytical methods for low concentrations are inadequate and the methods commonly used do not distinguish between toxic and non-toxic soluble forms. Also, quantitative data on the size and structure of the fish populations are not available and other poisons are frequently present with copper. Only meagre qualitative data are available for non-salmonid species.

In the absence of data on the precise effects of copper on natural fish populations, considerable reliance has to be placed on laboratory data; it is suggested that the maximum safe concentrations should be based on annual 50 and 95 percentile values of soluble copper of 0.05 and 0.2 respectively of the threshold LC50 to rainbow trout, taking into account the effect of water hardness as shown in Table I. Peak concentrations ($>$0.5 of the threshold LC50) may be more damaging in the winter than in the summer.

The presence of organic matter might allow the values in Table I to be increased up to 3-fold. The values should be decreased to allow for low temperature and for the presence of other poisons and also adjusted to allow for the different sensitivities of other species of fish, as illustrated in the appropriate sections of the report.

Table I Approximate maximum annual 50 and 95 percentile concentrations of soluble copper for rainbow trout. Adjustments can be made for the presence of organic matter, low temperature, harmful substances and other species (see main report)

Water hardness (mg/l as $CaCO_3$)	50 percentile (µg Cu/l)	95 percentile
10	1.0*	5.0*
50	6.0	22.0
100	10.0	40.0
300	28.0	112.0

* The presence of fish in waters containing higher concentrations may indicate the predominance of soluble, non-toxic, organo-copper complexes

1. INTRODUCTION

(1) Copper is found in natural waters as a trace metal, i.e., usually at concentrations of <5 µg/l, but can also be present at much higher concentrations (several mg/l), as a result of mining activities and other industrial processes, to the detriment of fish; it is often present with other heavy metals (especially zinc) at potentially harmful concentrations and with other poisons, making it difficult to distinguish the effect attributable to copper. It is also used as an algicide and molluscicide.

1.1 Chemistry of copper in fresh water

(2) In the aerobic conditions normally prevailing in natural surface fresh waters the only stable oxidation state of copper is the cupric form. This has a great tendency to form complexes (i.e., the stability constants of its complexes are large), and the chemistry of copper in water is dominated by this tendency. The proportion of the total dissolved copper present as the free ion has been found to be about 1 percent, but would be much smaller in waters with heavy organic loads and those of high pH (> 7.5). Only in water of unusually low pH, or very soft water, could a significant proportion be present as the free ion.

(3) Complexation in fresh water has been thoroughly investigated by Stiff (1971b). The complexes usually present are those with the carbonate and hydroxide ions and amino acids and, in waters receiving domestic and industrial wastes, cyanide and synthetic chelating agents such as the detergent builders polyphosphate and nitrilotriacetic acid (NTA). The stability constants of all these complexes are known (Sillen and Martell, 1964) and the extent of complexation can be calculated if the pH and concentration of the complexing agent are also known. Another important class of copper compounds found in natural waters is that containing humic substances, for which few accurate stability constants are known.

(4) Most copper complexes are labile and equilibria are rapidly established. Certain of the humic complexes, however, appear to be inert (Chau and Lum-Shue-Chan, 1974) and once formed, probably by slow rearrangement of a labile humic complex, their subsequent response to changes in the composition of the water which would lead toward dissociating the complex will be slow.

(5) The least soluble copper salt forming under normal aerobic conditions in natural fresh waters containing bicarbonate salts is the basic carbonate, malachite. At a bicarbonate concentration of 5×10^{-3} M the equilibrium solubility of free cupric ion is 8×10^{-8} M (or 5 µg/l) at pH 7, and 3×10^{-9} M (or 0.16 µg/l) at pH 8. Because of complexation, however, total dissolved copper concentrations in excess of this are thermodynamically stable and establishment of equilibrium is in any case a slow process.

(6) There has been discussion of the role of the chemical speciation of copper in accounting for differences in the toxicity of the metal in water of different chemical composition (Stiff, 1971a, 1971b; Calamari and Marchetti, 1974 and Pagenkopf et al., 1974). It has been suggested (para. 20) that the toxicity can be related to the total concentration of soluble copper, i.e., Cu^{2+} and $CuCO_3$ (Shaw and Brown, 1974), but recent work in the U.S.A. (R.W. Andrew, unpublished) appears to demonstrate adequately that it is the copper ion which is the most important form.

(7) Like other trace metals, copper is also readily adsorbed on to solid particles suspended in water and on to container surfaces. Substantial proportions of the total copper present in unfiltered natural water samples, therefore, can be associated with particulate material (Stiff, 1971b; Nachšina and Feldman, 1971).

(8) The more common methods for measuring copper in fresh water (atomic absorption spectrophotometry, activation analysis, mass spectrometry, and most colorimetric methods) measure the total concentration of copper in the sample after suitable pretreatment. The concentration of copper associated with particulate material can readily be obtained by filtration, but the differentiation and identification of even only some of the complexes present requires more elaborate procedures involving the copper specific ion electrode and colorimetric reagents (Stiff, 1971a) and possibly other methods. Anodic stripping voltammetry can also be used to a limited extent to measure complexation (Chau and Lum-Shue-Chan, 1974), and after the release of copper from inert complexes provides an alternative method of determining the total dissolved metal concentration. Complete analytical identification cannot, however, be made and because of the low concentrations at which the ionic form (the most important state) is present, this is very difficult to determine at levels of interest with regard to aquatic life.

(9) It will be evident from the foregoing paragraphs that the value of much of the published data on the toxicity of copper to aquatic life is severely limited by the inadequacy of analytical information

on water quality, especially in early publications, and the fact that conditions in the field are more complex than those produced in the laboratory. This is discussed again in para. 19 to 29, and 73 to 83.

2. DIRECT LETHAL ACTION ON FISH

2.1 Mode of action

(10) The toxicity of copper salts to fish was attributed by early workers, for example, Ellis (1937), to the precipitation of mucus on the gills thereby causing suffocation, and also to direct damage of the gill. Certainly, acutely lethal concentrations of copper cause a temporary reduction in the number of mucus cells in common carp (Cyprinus carpio) (Labat et al., 1974) and extensive breakdown of the gill of rainbow trout (Salmo gairdneri), the lamellae tending to collapse and overlie each other and showing hypertrophy and hyperplasia (Department of Scientific and Industrial Research, 1961). Effects on the gills of lower concentrations are less severe, the epithelial layer showing thickening of the apical cells, vacuolization, and containing myelin-like bodies and increased numbers of chloride cells (Baker, 1969).

(11) Bilinski and Jonas (1973) found that exposure of rainbow trout to rapidly lethal levels of copper reduced the capacity of excized gills to oxidize lactate, but with exposure to concentrations causing 33 percent mortality in 96 h there was no reduction in oxidation rate. Therefore the hypothesis that fish die from asphyxiation is probably an oversimplification, metabolic pathways involving other enzymes probably being implicated. For example, hepatic and renal disorders have been reported for the winter flounder (Pseudopleuronectes americanus) (Baker, 1969) and for killifish (or mummichog) (Fundulus heteroclitus) (Gardner and La Roche, 1973), and changes in the activity of some liver enzymes have been found in mummichog following exposure to the 96-h LC50 of copper (Jackim et al., 1970). In vitro measurements of the effect of copper on the plasma lactate dehydrogenase (PLDH) and plasma glutamic oxalacetic transaminase (PGOT) activity in white sucker (Catostomus commersoni) showed inhibition by 65 and 130 mg Cu/l respectively (Christensen, 1971), but these levels are certainly higher than those likely to be found in the plasma of fish exposed to copper solutions (see para. 56). However, with brook trout (Salvelinus fontinalis) PGOT activity was reduced at levels of copper that were detrimental to survival and growth (McKim and Benoit, 1971).

(12) Little is known of the significance of enhanced concentrations of copper in the blood, gill, liver and kidney of trout treated with sub-lethal concentrations (Calamari and Marchetti, 1973) and in whole fish exposed to lethal concentrations (Kariya et al., 1967). Increased levels occurred in the gill, liver and kidney but not in the opercula, red blood cells and blood plasma of brown bullhead (Ictalurus nebulosus) exposed to sub-lethal concentrations of copper (Brungs, Leonard and McKim, 1973); and somewhat similar results were found for bluegill (Lepomis macrochirus) (Benoit, 1975). Tissue levels in brown bullhead when killed by further exposure to lethal concentrations were higher than those of controls kept beforehand in clean water. Other relevant data are given in para. 56 to 62.

(13) It may therefore be concluded that little of the harmful mode of action of copper on fish is known with certainty.

2.2 Factors affecting acutely lethal levels

(14) The curve relating the logarithm of median period of survival to the logarithm of copper concentration can be used to estimate the median asymptotic or threshold LC50; the shape, and more importantly, the asymptotic values of the curves, are affected by environmental factors such as water hardness, dissolved oxygen concentration, pH value, and temperature, and are different for different species and stages in the life history. Since these concentration-response curves may cross each other, one curve may indicate a shorter time of survival at high concentrations than another and yet indicate a higher median lethal threshold concentration, i.e., a lower toxicity (para. 17).

a) Temperature

(15) Liepolt and Weber (1958) found that in hard water (250 mg/l as $CaCO_3$), the threshold concentration for rainbow trout was 0.5 mg Cu/l at both 15°C and 10°C although the survival time at concentrations higher than the threshold were shortest at the highest temperature.

(16) Similarly, the 8-day LC50 of 0.5 mg Cu/l for this species found by the Department of the Environment (1971) in water having a hardness of about 250 mg/l as $CaCO_3$ and a temperature of 11.5°C was close to that obtained by Calamari and Marchetti (1973) in water having a hardness of 290-310 mg/l as $CaCO_3$ and a temperature of 15.0-15.6°C.

(17) On the other hand, for juvenile rainbow trout (length 3 cm) acclimated to the test temperature for at least 14 days in hard water (250-260 mg/l as $CaCO_3$) at a dissolved-oxygen concentration of over

70 percent of the air-saturation value and at a pH value between 7.0 and 7.5, the 6-day LC50 at 6°C and 2°C was about 0.3 and 0.5 of the value at 15°C (Department of the Environment, 1973). Thus for rainbow trout at 6°C, as compared with 15°C, survival time at acutely lethal concentrations can be increased but the 6-day LC50 reduced by two thirds, providing an example of crossing over of the concentration-response curves. Reduction in the survival time of goldfish (Carassius auratus) acclimated to the test temperature and exposed to 1.5 mg Cu/l in very soft water has been found at 27°C as compared with 14°C (Marchetti, 1962). Similar results have been found for the minnow (Phoxinus phoxinus) though the 7-day LC50 was unchanged (Liepolt and Weber, 1958). Rehwoldt et al. (1972) also found no differences in 96-h LC50 values for six North American species tested at 17°C and 28°C.

b) Dissolved oxygen

(18) Low concentrations of dissolved oxygen increase the toxicity of poisons to fish (Lloyd, 1961a), and for copper the 48-h LC50 is reduced by about one third with a reduction in the dissolved-oxygen concentration from 100 percent to 40 percent of the air-saturation value.

c) pH value

(19) There are few data on the effects of pH on the toxicity of copper, but it is known that copper is precipitated in hard water at alkaline pH values. For example, for a nominal concentration of 5 mg Cu/l at pH 7.45, 2.94 mg Cu/l were in solution after 24 h whereas at pH 8 only 1 mg/l was in solution after 2 hours (Department of the Environment, 1971). It is not known whether the precipitate is toxic.

(20) Liepolt and Weber (1958) found that in hard water (250 mg/l as $CaCO_3$) the threshold concentration for rainbow trout was 0.5 mg Cu/l at pH values of both 5.6 and 6.4 although the survival time was reduced at the lower pH value. Also, Shaw and Brown (1974) found that the median period of survival for rainbow trout was similar for equal concentrations of copper (Cu^{2+} and $CuCO_3$) at pH values of 6.5 and 7.5 in hard water.

d) Hardness

(21) Copper has been shown to be more toxic in soft than in hard water (Department of Scientific Industrial Research, 1962); tests made with rainbow trout at three different levels of hardness (12, 42 and 320 mg/l as $CaCO_3$) to which the fish were acclimated beforehand showed that the 7-day LC50 was about 0.03, 0.8 and 0.5 mg Cu/l respectively.

(22) Tabata (1969) also found with both rainbow trout and carp that the 24-h LC50 increased almost 3-fold with an 8-fold increase in hardness effected by the addition of calcium chloride and magnesium sulphate. Lloyd and Herbert (1962) suggested that a linear relation exists between the logarithm of threshold LC50 of copper for rainbow trout and the logarithm of total water hardness.

e) Salinity

(23) With juvenile pompano (Trachinotus carolinus) acclimated to different salinities the toxicity of solutions containing copper sulphate decreased with increase in salinity (Birdsong and Avault, 1971), the 96-h LC50 being 1.42 mg Cu/l and 10 g/l and 1.97 mg Cu/l at 30 g/l.

f) Organic substances

(24) Some organic substances of low toxicity to fish such as ethylene-diaminetetraacetic acid (EDTA), NTA, citric acid, humic substances, and natural amino acids, can reduce the acute toxicity of copper (Sprague, 1968; Nishikawa and Tabata, 1969; Ministry of Technology, 1970).

(25) Grande (1966) found an increased survival of Atlantic salmon fingerlings (Salmo salar) in soft water containing 1 mg Cu/l after addition of an amount of EDTA sufficient for a complete chelation of the metal. Shaw and Brown (1974) observed a 50 percent mortality of rainbow trout in 8 days at 0.5 mg Cu/l in the absence of NTA, but no mortality with 4 mg Cu/l in the presence of 12 mg NTA/l under otherwise the same test conditions. In the presence of up to 2 mg Cu/l survival time of rainbow trout was progressively increased by increase in the concentration of humic acid (Brown et al., 1974).

(26) Zitko et al. (1973) also found that the presence of humic acid reduced the toxicity of copper; the 96-h LC50 for juvenile Atlantic salmon in water having a hardness of 14 mg/l as $CaCO_3$ and a temperature of 3.8 to 4.8°C was 0.09 and 0.165 mg Cu/l in the presence of 5 and 10 mg/l of humic acid respectively, and about 0.02 mg Cu/l in its absence, i.e., a reduction in toxicity of 0.22 and 0.12 respectively. Cook and Cote (1972) using a slightly harder water (24-96 mg/l as $CaCO_3$) found a

somewhat smaller reduction in toxicity, being about 0.3, 0.2 and 0.14 of control values in the presence of 10, 20 and 30 mg/l humic acid respectively.

(27) Increasing the concentration of glycine can also reduce the toxicity of copper sulphate solutions, and at a concentration of 10 mg glycine/l the 24-, 48- and 72-h LC50 values for rainbow trout were seven to ten times higher than in the absence of the amino acid. The survival time of this species in the presence of 2 mg Cu/l was increased from 8 h in solutions containing no sewage effluent to 80 h in those consisting of only sewage effluent (Brown et al., 1974) (see para. 74).

(28) The above observations (para. 24 to 27) suggest that these organo-copper complexes have little, if any, acutely toxic effect on fish but no information is available on long-term effects. However, some organic substances containing copper may be toxic (para. 89).

g) Suspended solids

(29) Investigations of Svenska Gruvföreningen (1960) and observations of the Norwegian Institute of Water Research (M. Grande, pers. comm.) indicate that the toxicity of copper and zinc is reduced when the metals are mixed with wastes from flotation processes. These wastes contain high levels of finely ground materials which may adsorb heavy metals. A 3-fold increase in the 48-h LC50 of total copper to rainbow trout was shown in the presence of organic or inorganic solids at a concentration of 50 mg/l (Brown et al., 1974). However, in terms of soluble copper, the toxicity of such solutions appears to increase with increase in the concentration of solids, the 48-h LC50 in the presence of 100 mg/l organic solids being about half of that observed when no solids are present.

h) Age and size of fish

(30) In a hard water (260-280 mg/l as $CaCO_3$) a copper concentration of 1 mg/l did not affect fertilization of rainbow trout eggs although it did increase the rate of development, all copper-exposed eggs hatching before any of the controls (Shaw and Brown, 1971).

(31) Experiments with eggs of rainbow trout at 10°C and in water having a hardness of 14 mg/l as $CaCO_3$ showed that at 0.02, 0.04 and 0.06 mg Cu/l there was 25, 70 and 100 percent mortality respectively compared with 10 percent in the controls (Grande, 1966). Mortality among eggs of Atlantic salmon under the same conditions was 82, 92 and 100 percent respectively compared with 15 percent in the controls. These concentrations had less effect on yolk-sac fry and fingerlings of Atlantic salmon for which the 21-d LC50 was 0.04 mg Cu/l. However, damage to the fish at 0.02 mg Cu/l was indicated by their unwillingness to eat food. Thus, there was relatively little difference in tolerance between eggs, yolk-sac fry and fingerling Atlantic salmon but salmon eggs were more sensitive than those of rainbow trout.

(32) Yearling and fingerling rainbow trout had a similar resistance to acutely lethal levels of copper in the range 0.8 to 1.0 mg/l in water having a hardness of 320 mg/l as $CaCO_3$ (Department of Scientific and Industrial Research, 1960).

(33) Hazel and Meith (1970) found that eggs of chinook salmon (Oncorhynchus tshawytscha) were more resistant to copper than fry; eyed eggs exposed to a concentration of 0.08 mg Cu/l at 13°C to 14°C and a hardness of 44 mg/l as $CaCO_3$ hatched successfully, but 0.04 mg/l was acutely toxic to fry and 0.02 mg/l caused a mortality of 33 and 12 percent among the swim-up stages and the sac fry respectively, compared with a mortality of 23 percent and 4 percent respectively in the controls. McKim and Benoit (1971) reported that 17 µg Cu/l did not adversely affect the survival of adult brook trout or the hatchability of the eggs but severely reduced the survival of the alevin/juveniles during 8-month experiments in water having a hardness of 45.4 mg/l as $CaCO_3$ and temperatures ranging from 6°C in March to 16°C in September; the 96-h LC50 for adults was 0.1 mg Cu/l. Thus it appears that the early stages of some salmonids are more sensitive to copper than other stages in the life cycle.

(34) Mount and Stephan (1969) found that no fathead minnow fry survived a 30-d exposure to water having a hardness of 31.4 mg/l as $CaCO_3$ at 19°C and containing 18 µg Cu/l whereas the 96-h LC50 for adults was 75 µg/l. Gardner and LaRoche (1973) found that the emergence of larval mummichog was greatly reduced in the presence of 0.25 mg Cu/l and that only 57 percent of the hatched individuals survived whereas the 48-h LC50 for the larvae was 1.2 mg/l. These studies suggest that for some non-salmonid fish also the early larval stages are less tolerant of copper than eggs or adults.

(i) Acclimation to copper

(35) There are no specific studies known to us on the effect of acclimation to copper on the resistance of fish, but in a 3-month experiment with brook trout alevins McKim and Benoit (1971) found that the mortality of those from parents not previously exposed to copper was similar to that of the progeny

from parents exposed for 8 months to copper. Results with fathead minnow (Mount and Stephan, 1969) in soft water were similar. On the other hand, Paul (1952) observed that fish were resident in the Sacramento River despite the presence of a concentration of copper high enough to prevent the survival of introduced hatchery-reared fish.

(36) Rainbow trout exposed to surfactant, after being kept for 1 week at 0.5 of the 14-d LC50 of copper, during which time they accumulated abnormally high concentrations of metal in some organs, survived about as long as those not previously exposed to copper (Calamari and Marchetti, 1973).

j) Acclimation to other poisons

(37) Oseid and Smith (1972) found that the time of survival of bluegill in solutions containing copper was two- to four-fold higher among fish acclimated to high concentrations of hydrogen sulphide (5 to 15 µg/l) than it was among those acclimated to low concentrations (1.5 µg/l).

k) *Joint effect of copper and other heavy metals*

(38) Doudoroff (1952) found that whereas fathead minnow (Pimephales promelas) usually survived an 8-h exposure to solutions containing either 8 mg Zn/l or 0.2 mg Cu/l in soft water, most succumbed within this period in a mixture of copper and zinc at one-eighth of these concentrations respectively. Lloyd (1961b) obtained somewhat similar results with rainbow trout in soft water (hardness 14 mg/l as $CaCO_3$) but found that at relatively low concentrations, corresponding to less than the 3-d and 7-d LC50 of copper and zinc respectively, the effect of the mixtures was simply additive in both the soft and a hard water (hardness 320 mg/l as $CaCO_3$). Sprague and Ramsay (1965), who used these metals in water having a hardness of 14 mg/l as $CaCO_3$, also found that the toxicity of mixtures to juvenile Atlantic salmon could be accounted for by the simple addition of the fractions of the corresponding LC50 of the separate metals.

(39) D. Calamari and R. Marchetti (pers. comm.) obtained similar data in terms of fractions of the 96-h LC50 values for rainbow trout with copper and mercury tested at 15°C in a water having a hardness of 320 mg/l as $CaCO_3$.

(40) The 48-h LC50 to rainbow trout exposed to mixtures of copper, zinc and nickel has also been found to be adequately predicted by summation of the fraction of the 48-h LC50 values of the separate poisons (Brown and Dalton, 1970).

(41) Eaton (1973) reported that the 96-h LC50 for fathead minnow was only 0.8 of that predicted for a mixture containing mainly copper and zinc in hard water (hardness 200 mg/l as $CaCO_3$) while, on the other hand, Eisler and Gardner (1973) found increased mortality of mummichog when non-lethal concentrations of cadmium were present with lethal concentrations of copper and zinc.

(42) Thus it can be concluded that the acute lethal toxicity of mixtures of copper and other heavy metals can be largely accounted for by summation of the individual toxicity of the metals expressed as fractions of the threshold LC50.

l) Joint action of copper and other poisons

(43) Herbert and Van Dyke (1964) tested rainbow trout in solutions containing ammonium chloride and copper sulphate in hard water and found that the estimated value for the threshold concentration of the mixture was close to the value predicted from the sum of the fractions of the threshold concentrations of the individual poisons. Similar results have been found for mixtures of copper and phenol, and for copper, zinc and phenol (Brown and Dalton, 1970). Cairns and Scheier (1968) also report an additive effect on bluegill of a mixture of acetic acid, acetaldehyde, acetone and copper in soft water. Calamari and Marchetti (1973) tested rainbow trout in mixtures of copper and surfactants and found that with the non-ionic nonyl phenol ethoxylate the survival time was longer than that expected, but with the anionic ABS or LAS the survival time was greatly reduced; the 96-h LC50, expressed as a sum of the fractions of the individual LC50 values, was about 0.8 of that predicted assuming a simply additive effect, i.e., the mixture was slightly more toxic than expected.

(44) Thus the acute lethal toxicity of a number of common poisons tested in mixtures with copper demonstrate a simple additive effect which could be largely predicted on the basis of the sum of the fractions of the 48-h LC50 values of the individual poisons.

m) Uptake in food

(45) An oral dose of 400 mg Cu/kg was reported as lethal to common carp after 3 days (Nehring, 1964) but no information is apparently available on chronic effects.

2.3 Summary of toxicity data

a) Acutely lethal values

(46) Concentrations from 0.02 to over 10 mg/l of copper are reported to be lethal to fish, the difference being attributed mainly to different water hardness, species of fish, duration of test and stage in the life cycle. However, as is clear from para. 2 to 8 and 19 to 29 there is considerable uncertainty as to the amount and speciation of soluble copper especially at low concentrations, and often the data relate to the total concentration of copper apparently in solution.

i. Salmonid eggs

(47) Concentrations of 0.02 to 0.04 mg Cu/l impaired the hatch of rainbow trout and Atlantic salmon in very soft water (14 mg/l as $CaCO_3$), while in slightly harder water (44 mg/l as $CaCO_3$) 0.08 mg Cu/l did not affect the hatching success of chinook salmon (para. 33). In harder water (260-280 mg/l as $CaCO_3$), 1 mg Cu/l increased the rate of development of rainbow trout eggs (para. 30).

ii. Salmonid fry, juveniles and adults

(48) The early stages of the life cycle of salmonids are more sensitive to copper than the adult (para. 31 and 33). Brook trout appear to be less sensitive and Atlantic salmon more susceptible than rainbow trout (McKim and Benoit, 1971; Sprague, 1964a; Zitko et al., 1973; Lloyd and Herbert, 1962).

(49) For adult rainbow trout in soft water (14 to 45 mg/l as $CaCO_3$) the 96-h LC50 is between 0.02 to 0.1 mg Cu/l whereas in hard water (200-300 mg/l as $CaCO_3$) the values are from 0.5 to 1 mg Cu/l.

iii. Coarse fish

(50) No work has been reported for the young stages of European coarse fish but studies on other non-salmonid species (para. 33 and 34) show that these stages are more sensitive than adults.

(51) The 96-h LC50 for adult goldfish in a hard water (hardness about 220 mg/l as $CaCO_3$) was found to be 0.46 mg Cu/l by Calamari and Marchetti (1970). In soft water (53 mg/l as $CaCO_3$) the values were less than 1 mg Cu/l for this species and common carp while for other species including the eel (*Anguilla rostrata*) they were 0.81 and 6.4 mg Cu/l under static conditions (Rehwoldt et al., 1971). Values were about 3 mg/l for pike (*Esox lucius*) and 4 mg/l for eel (*Anguilla anguilla*) at 10°C in recent tests in a hard water (250 mg/l as $CaCO_3$) and continuous flow conditions, and for rudd (*Scardinus erythrophthalmus*), common carp, and perch (*Perca fluviatilis*) tested concurrently they were about 0.6, 0.6 and 0.3 mg Cu/l respectively, compared with 0.9 mg Cu/l for rainbow trout (Department of the Environment, 1971). For stone loach (*Nemacheilus barbatulus*) in water of the same hardness at 12°C the 4-d LC50 was 0.76 mg Cu/l (J.F. de L.G. Solbé and Cooper, in press). In a slightly softer water (about 100 mg/l as $CaCO_3$) the lethal threshold for tench (*Tinca tinca*) at 16°C and pH 7.7 was 0.08 to 0.15 mg Cu/l (Haider, 1966).

b) Long-term lethal values

i. Salmonids

(52) A mortality of 24 percent was found among rainbow trout kept for 17 weeks in a hard water (250-260 mg/l as $CaCO_3$) containing 0.28 mg Cu/l (about 0.6 of the 96-h LC50) but little or no mortality was found at lower concentrations (Department of the Environment, 1973). McKim and Benoit (1971) kept brook trout for 8 months in soft water (hardness 45 mg $CaCO_3$/l) containing copper; with adults there was a 57 percent mortality at 32 μg Cu/l (0.32 of the 96-h LC50) and no mortality at 17 μg Cu/l but with the alevins there was a complete mortality at the lower concentration. With the same species in water of the same hardness McKim et al. (1970) found a mortality of 10 percent at 69 μg Cu/l in 21 days and 60 percent mortality at 32 μg Cu/l in 337 days.

ii. Coarse fish

(53) With fathead minnow in hard water (198 mg/l as $CaCO_3$) (Mount, 1968; Mount and Stephan, 1969), there was in one test a low mortality and in another a complete survival after 11 months at 33 μg Cu/l and a 30 percent kill at 95 μg Cu/l (0.2 of the 96-h LC50). On the other hand, in soft water (30 mg/l as $CaCO_3$) there was a low mortality or complete survival at 11 μg Cu/l and 50 percent kill at 18 μg Cu/l (0.24 of the 96-h LC50). Fry from exposed and unexposed parents survived equally well when kept for 1 month at 10 μg Cu/l but all died at 18 μg Cu/l.

(54) In replicate tests Calamari and Marchetti (1970) found a 50 percent mortality among goldfish at 20 and 30 days respectively at a concentration of 0.12 mg Cu/l (0.26 of the 96-h LC50) in water having a hardness of about 220 mg/l as $CaCO_3$ and a temperature of 15.5°C. Recently the 50-d LC50 for the stone loach was found to be 0.29 mg Cu/l in water having a hardness equivalent to 250 mg/l as $CaCO_3$ (J.F. de L.G. Solbé and Cooper, pers. comm.).

3. SUB-LETHAL EFFECTS ON FISH

3.1 Enzymes

(55) The few enzymes that have been studied (para. 11) have been inhibited only at concentrations of copper that are either rapidly or potentially lethal to fish. With brown bullhead (Christensen *et al.*, 1972) as with brook trout (McKim and Benoit, 1971) the only measured long-term effect was a reduced level of PGOT at above 27 µg Cu/l (0.15 of the 96-h LC50).

3.2 Tissue residue analyses

(56) No accumulation of copper occurred in the opercula, red blood cells, or blood plasma of brown bullhead exposed for up to 20 months to copper levels up to 104 µg/l in water of total hardness 202 mg/l as $CaCO_3$ (96-h LC50, 170-190 µg Cu/l) (Brungs *et al.*, 1973). However, gill and liver levels increased with exposure to concentrations greater than 27-53 µg Cu/l (0.16 to 0.31 of the 96-h LC50) and kidney levels increased at 104 µg/l within 30 days, but not at 27 µg/l within 20 months. Also, brown bullhead exposed to sub-lethal concentrations of copper had higher levels of copper in their tissues when killed by copper solutions than those so killed after having previously been held in clean water. After 24 months exposure in the laboratory to copper solutions in Lake Superior water (total hardness 45 mg/l as $CaCO_3$), bluegill showed increased copper levels in the gills at concentrations above 40 µg Cu/l, and higher liver and kidney levels were measured at 162 µg Cu/l and above (Benoit, 1975). However, survival was reduced at levels higher than 40 µg/l for fry and 77 µg/l for adults. KcKim and Benoit (1974) found that progeny of brook trout which had been exposed to 4.5 and 9.4 µg Cu/l (total hardness 44 mg/l as $CaCO_3$) were not more susceptible, nor did they accumulate copper in their tissues, when exposed to these copper levels.

(57) Increased copper in fish tissues, especially the gill, has also been observed in 1-year-old common carp fed on a diet enriched with copper (Iozepson, 1971). Prolonged feeding of this species with a copper ammonium compound caused elevated levels of copper in the tissues, especially the liver, disturbed protein synthesis and reduced serum globulins (Semčuk and Avdošev, 1972).

(58) Elevated levels of copper in the tissues (gill and liver) of some species have therefore been associated with adverse physiological effects.

3.3 Blood analyses

(59) When channel catfish (*Ictalurus punctatus*) were exposed to 2.5 mg Cu/l for 4 days (total hardness 206-236 mg/l as $CaCO_3$), a non-lethal level, the serum osmolarity decreased during the first 2 days, followed by a recovery to normal levels (Lewis and Lewis, 1971). However, exposure to 5 mg Cu/l was lethal in 2 days. Similar results were obtained with golden shiner (*Notemigonus crysoleucas*) and in both species the effects were eliminated by the addition of 235 mOsm NaCl to the solution. At these copper concentrations both species were coated with precipitated mucus.

(60) Measurements of red blood cells, haematocrit and haemoglobin levels of brook trout exposed to 24, 39 and 67 µg Cu/l (total hardness 46 mg/l as $CaCO_3$) showed a transient increase, and chloride a decrease, during the first 21 days, but after 337 days there was no measurable difference between fish exposed to up to 32.5 µg Cu/l and the controls (McKim *et al.*, 1970). Higher levels were detrimental to survival and growth of this species (McKim and Benoit, 1971). Similar experiments with brown bullhead exposed to 3.4 to 104 µg Cu/l for up to 20 months showed that glucose and haematocrit increased within 6 days at 104 µg/l, the highest concentration, and that at levels above 49 µg Cu/l (0.26 of the 96-h LC50), chloride, haematocrit, haemoglobin and glucose increased at 30 days (Christensen *et al.*, 1972).

(61) Transient increases in cortisol and cortisone during a 24-h period were observed in sockeye salmon (*Oncorhynchus nerka*) exposed to 6 µg Cu/l in water having a hardness of 12 mg/l as $CaCO_3$; levels of 0.6 mg Cu/l were lethal within 24 h (Donaldson and Dye, 1975).

(62) Thus transient changes only have been observed in blood analyses of some species exposed to concentrations of copper lower than between 0.5 and 0.26 of the 96-h LC50.

3.4 Life cycle studies

(63) Several long-term studies have been made on the effects of chronic exposure to copper solutions on the survival, growth and spawning success on species of North American fish. McKim and Benoit (1971) found that, in the laboratory in Lake Superior water (hardness 45 mg/l as $CaCO_3$, pH 6.9 to 8.0 and seasonal temperature 4°C to 21°C) the growth and survival of yearling brook trout was affected at a copper concentration of 32.5 µg/l over an 8-month exposure period, although spawning was successful at this level. However, this concentration affected the hatching success of the eggs, and copper levels above 17.4 µg/l affected the growth and survival of alevins, both from exposed and non-exposed parents, over a 3-month period. A transient effect of copper on growth rate was noted at copper concentrations of 3.4 to 32.5 µg/l, but after 23 weeks the growth rate of brook trout was equal to that of the controls at concentrations up to 9.5 µg/l. It was concluded that the maximum concentration of copper which did not significantly affect brook trout under these experimental conditions was between 0.1 and 0.17 of the 96-h LC50 value. More recently, McKim and Benoit (1974) have shown that the progeny from brook trout exposed as above to 4.5 and 9.5 µg Cu/l were not more susceptible to these concentrations and conclude that a single life cycle experiment gave results which were probably applicable to exposure for many generations.

(64) Tests with bluegill over a 24-month exposure period showed that survival was reduced at copper levels greater than 40 µg/l and that growth was retarded at 77-162 µg/l (Benoit, 1975). Growth of larvae was slightly reduced at 77 µg/l and survival was less at 40 and 70 µg/l (water hardness 45 mg/l as $CaCO_3$ and a seasonal range of temperature of 13-28°C). The level of 40 µg Cu/l was 0.04 of the 96-h LC50 value for this species under these conditions.

(65) Similar tests with fathead minnow exposed for 11 months to copper solutions in a soft water (hardness 31.4 mg/l as $CaCO_3$; temperature 19-25°C) showed that spawning potential was reduced at 18.4 µg/l Cu but that at levels up to 10.6 µg/l growth and reproduction were normal (Mount and Stephan, 1969); these levels were 0.22 and 0.13 of the 96-h LC50 respectively. However, tests in a hard water (198 mg/l as $CaCO_3$) showed that growth and fecundity were unaffected below the range 14.5 to 33.0 µg Cu/l which was 0.03 to 0.07 of the 96-h LC50 value of 450 µg/l (Mount, 1968).

(66) Comparable data are not available for European species but Grande (1966) found that salmon fry were unwilling to feed when held in solutions containing 20 µg Cu/l in soft water which was equivalent to 0.4 of the 21-d LC50 (para. 31). Rainbow trout kept for 17 weeks at concentrations of 0.16 to 0.05 mg Cu/l (about 0.32 to 0.1 of the 96-h LC50) grew less rapidly in the higher than in the lower concentrations and controls (Department of the Environment, 1973).

(67) Thus long-term tests have shown that the highest levels of copper that failed to produce adverse effects on fish were (expressed as fractions of the 96-h LC50) between 0.1 and 0.17 for the growth of brook trout and rainbow trout and 0.13 and 0.07 for the growth and reproduction of fathead minnow in soft and hard water respectively; growth and survival of bluegill however were affected at 0.04. No comparable data are available for European species.

3.5 Behaviour

(68) Experiments on the effects of heavy metals on the palatal chemo-receptors of common carp showed that a concentration of 6.4 mg Cu/l (10^{-4}M) depressed the response of the sugar and salt receptors (Hidaka, 1970). Also olfactory responses of sockeye salmon, coho salmon (Oncohrynchus kisutch) and rainbow trout to food extracts, amino acids and hand rinses were extinguished after more than 12 h exposure of the fish to a concentration of 40 µg Cu/l (Hara, 1972). However, the nature of the dilution water was not given in either of these studies. The "cough frequency" of brook trout was found to increase within the range 6 to 15 µg Cu/l (water hardness 45 mg/l as $CaCO_3$, pH 7.5 to 7.7 and temperature 8.5°C), reaching a peak 5 to 20 h after exposure and decreasing thereafter (Drummond et al., 1973). A similar, though more prolonged, transitory response was found by Morgan and Kuhn (1974) who found that large-mouth bass (Micropterus salmoides) exposed to 100 µg Cu/l (water hardness 54 mg/l as $CaCO_3$, temperature 25°C) increased their respiratory frequency during the first 3 days of exposure with a gradual return to normal in the following 4 days.

(69) Increase in activity of fish exposed to sub-lethal copper solutions is well known; for example, brook trout exposed to copper solutions of 6 to 115 µg/l (Drummond et al., 1973) increased their activity to 4-6 times that of the controls during the first 8 h of exposure; activity patterns returned to normal after 3 days in those fish exposed to up to 12 µg Cu/l. Similarly, brook trout exposed to copper levels greater than 17 µg/l stopped feeding and at 12 µg/l an initial reduced feeding rate returned to normal (albeit sluggishly) after 4 days; these levels were similar to those at which the survival and growth of brook trout began to be affected (McKim and Benoit, 1971) (para. 63).

(70) Sprague (1964b) showed that Atlantic salmon parr could detect levels of copper in a soft water (20 mg/l as $CaCO_3$) as low as 2.4 µg/l, which was 0.05 of the lethal threshold concentration; in these laboratory experiments the fish were given a choice of clean or polluted water in a short tube with a sharp interface between the two solutions, and in the absence of other stimuli. However, field observations at a counting fence (Saunders and Sprague, 1967) showed that adult salmon migrating upstream were turned back only by much higher concentrations, equivalent to 0.35 to 0.43 of the joint lethal threshold concentration for the mixture of zinc and copper present in the river. Kleerekoper et al., (1973) found that goldfish, which avoided water with a copper concentration of 10 µg/l (total hardness 5.4 mg/l as $CaCO_3$), were attracted to this water when the temperature was increased by 1.0 to 1.4°C above the acclimation temperature and this interaction between temperature and copper provided a stronger attractant than temperature alone. In other experiments, Timms et al. (1972) found that the characteristics of the copper gradient were more important in behaviour studies than the actual copper levels present and that goldfish, channel catfish and large-mouth bass only displayed slight changes of behaviour in such solutions; a concentration of 50 µg Cu/l was a slight attractant in water of total hardness 5 mg/l as $CaCO_3$ for goldfish and channel catfish.

3.6 Mixtures of poisons

(71) Although mixtures of copper and other heavy metals are approximately additive in their acute lethal toxicity at combined lethal levels (para. 38 to 42), Eaton (1973) found that the specific sub-lethal effects of copper on fathead minnow - reduced growth and inhibition of secondary sexual characteristics - were only slightly enhanced in the presence of sub-lethal levels of cadmium and zinc which produced other specific sub-lethal effects.

3.7 Summary

(72) Much of the work summarized in this section is fragmentary, and only very general conclusions can be made. Several authors indicate that the sub-lethal effects measured in copper solutions are transitory and persist for a few days only, which could imply that some acclimation takes place. Except for work on the long-term effects of copper solutions on the fathead minnow (Mount, 1968) and bluegill (Benoit, 1975) in hard waters, concentrations below 0.1 of the threshold or 96-h LC50 appear to exert no measurable effect.

4. FIELD OBSERVATIONS ON FISH

4.1 Toxicity in natural waters and sewage effluents

(73) The majority of the laboratory experiments reported so far in this review have determined the toxicity of soluble copper to fish in the absence of organic matter. But, as shown earlier (para. 24 to 29), the toxicity of copper can be reduced if it is precipitated or complexed with organic compounds, both of which can occur in field situations, so that values of total copper usually measured may or may not be greater than the actual toxic amount present. For example, Brungs et al. (1976) found that for fathead minnow the 96-h LC50 for total copper in a natural stream water containing sewage effluent varied between 1.6 and 21.0 mg/l (using static tests), whereas the corresponding values for "dissolved" copper (after filtration to remove particles >0.45 µ) were 0.60 and 0.98 mg/l. The water hardness varied between 88 and 352 mg/l as $CaCO_3$ and the pH value between 7.5 and 8.5; considerable precipitation occurred at the higher hardness and pH values. Chronic tests showed that the maximum level of no observed effect was between 0.07 and 0.13 of the 96-h LC50 value for "dissolved" copper, and these values were higher than those given by Mount (1968) for this species, as were the 96-h LC50 values themselves (cf. para. 53).

(74) Laboratory tests on sewage effluents containing trade waste residues (Lloyd and Jordan, 1964) showed that when copper was present in relatively high concentrations the observed toxicity was less than that predicted from the poisons present. Further experiments with sewage effluents showed that the toxicity of copper present was not reduced by a constant proportion but varied with the total amount of copper present (R. Lloyd, pers. comm.). These "sliding values" were used in the prediction of copper in river waters containing copper-bearing sewage effluents, but the general validity of such an approach is doubtful. Zitko et al. (1973) showed that the presence of 10 mg/l humic acid increased four-fold the 96-h LC50 of copper in soft water (14 mg/l as $CaCO_3$) for Atlantic salmon parr; these authors as well as Montgomery and Stiff (1971) recommend the use of a specific ion electrode for the calculation of copper toxicity. Stiff (1971a) has added amino acids and polypeptides to the list of soluble organic compounds capable of complexing copper. Similar complexing effects of humic acid have also been reported by Grande (1966)(para. 24 to 28).

(75) Wilson (1972) found that the 96-h LC50 for copper to Atlantic salmon parr in water taken from the Exploits River, Newfoundland (total hardness 8-10 mg/l as $CaCO_3$) was 125 µg/l, compared with about 30 µg/l as predicted from laboratory experiments of Sprague and Ramsay (1965). The humic acid content of this water was 4.5 to 5.0 mg/l, and an addition of spent sulphite liquor (SSL) increased the 96-h LC50 still further, so that, in the presence of 450 mg/l SSL, there was only a 10 percent mortality at 250 µg/l copper. Wilson (1972) concludes that it is impossible to predict the toxicity of copper in natural waters; even with a specific ion electrode there is some interference with other ions at low copper concentrations. This difficulty has been demonstrated by Zitko et al. (1973).

(76) Recently, Pagenkopf et al. (1974) have presented theoretical calculations to support the conclusion that, in the absence of organic matter, only ionic copper (Cu^{2+}) is toxic to fish, and that increasing alkalinity reduces the proportion of copper ions present. However, in experimental studies Shaw and Brown (1974) observed that the concentrations of both copper carbonate and copper ions were related to the lethal toxicity of copper to rainbow trout. Calamari and Marchetti (1974) kept rainbow trout in cages in Lake Orta, Italy, in the presence of copper and found that the 48-h LC50 derived from data relating to 5-12°C, pH 5.5 to 6.4 and hardness of 21-26 mg/l as $CaCO_3$ was 70 µg Cu/l (95 percent confidence limits of 60-82 µg/l) which is in reasonable agreement with laboratory data (para. 21 and 22).

4.2 Comparison of field observations with laboratory data

(77) Because of the factors discussed above, it is unrealistic to expect to find a close correlation between field observations of the relation between toxicity or fisheries and total copper concentration except in very pure, soft water, free from organic compounds; the position is complicated further by the fact that rivers and lakes which contain copper also frequently contain zinc, and sometimes other metals such as cadmium, and it is very difficult to separate their individual contributions when present in low concentrations.

(78) Grande (1966) found that salmonids were present in some Norwegian lakes when the copper levels may have been as high as 60 µg/l (total hardness 2-15 mg/l as $CaCO_3$), even in the presence of considerable concentrations of zinc; this upper copper level is at the upper limit of the 21-d LC50 of 40 to 60 µg/l obtained by this author for a soft water (14 mg/l as $CaCO_3$). In streams in North Wales containing copper and zinc and studied intensively for a year from August 1973 (Cremer and Warner, pers. comm.), moderate populations of brown trout (Salmo trutta) were present when the 50 and 95 percentile values for the concentrations of copper were 0.17 and 0.38 of the 48-h LC50 for rainbow trout (in the presence of zinc at concentrations equivalent to 0.16 and 0.28 for the corresponding percentiles); fish were virtually absent where the corresponding percentiles for copper were 0.34 and 0.78 (in the presence of zinc equivalent to 0.01 and 0.02 for the corresponding percentiles).

(79) Observations on the River Churnet, England, showed that brown trout, bullhead (Cottus gobio), three-spined stickleback (Gasterosteus aculeatus) and minnow were present where the annual 50 and 95 percentile values for the concentration of copper were 34 and 83 µg/l (equivalent to 0.12 and 0.30 respectively of the predicted 48-h median lethal concentration to rainbow trout). The median and 95 percentile for the sum of all pollutants were 0.16 and 0.33 of the LC50, the difference being largely attributable to zinc. The average concentration of organic carbon was 11.3 mg/l and a single sample of soluble copper at this site had the following percentage composition: labile copper complexes 61; $CuCO_3$, 24; strong copper complexes 10; inert humic complexes 3; and free cupric ion 3 (J.F. de L.G Solbé, pers. comm.). In the River Ouse in Yorkshire, England, which contains sewage and industrial wastes, data supplied by the Yorkshire Ouse River Authority from 1966 to 1973 indicated that minnow, stickleback, roach (Rutilus rutilus) and chub (Leuciscus cephalus) were present where the 50 and 95 percentile concentrations of copper were 50 and 130 µg Cu/l respectively, equivalent to 0.17 and 0.66 of the predicted 48-h LC50 to rainbow trout (I.C. Hart and J.S. Alabaster, pers. comm.).

(80) There exists the possibility of natural fish populations becoming acclimatized to copper as suggested by the observations of Paul (1952) in the Sacramento River (para. 35).

(81) Numerous reports exist in the literature on the use of copper sulphate as an algicide in fish-bearing waters; in general the concentrations applied are higher than those which would be predicted to kill or harm fish, but in practice the amounts actually remaining in solution as the toxic cupric ion and copper carbonate would be small after absorption and complexation by organic matter, and precipitation (para. 82 to 84).

(82) It is clear that considerably more research effort should be given to the analysis of copper ions in natural waters so that a realistic estimate can be made of their potential toxicity to fish. Until this is achieved, water quality objectives based on total copper concentrations will necessarily be more severe than perhaps warranted. All that can be stated at present is that there are no known instances where the toxicity of copper in a field situation is apparently greater than that predicted from laboratory tests.

5. SUMMARY OF DATA ON ALGAE AND INVERTEBRATES

5.1 Algae

(83) Toxic effects of copper on algae are usually thought to be caused by ionic copper (Steeman-Nielsen and Wium-Andersen, 1970) although insoluble copper in contact with algae in culture may also be toxic (Fitzgerald and Faust, 1963). However, under field conditions more copper is required for the treatment of blooms in hard water, where much precipitation of basic copper carbonate occurs, than in soft waters but no precise information is available. Higher concentrations of copper are generally required to kill algae in culture than to control their growth in reservoirs, possibly because in the latter the ratio of copper to algal biomass is greater, the excess chelating capacity lower, and only an algistatic effect is required.

(84) The effects of copper on algae have been reviewed by Whitton (1970a). The growth of most genera is checked at 0.1 to 0.4 mg Cu/l (e.g., Prescott, 1948; Maloney and Palmer, 1956; Whitton, 1970b) but concentrations of 0.4 to 2.0 mg Cu/l are tolerated by some natural populations (Butcher, 1946; Whitton, 1970a; Besch *et al.*, 1972). Copper inhibits photosynthesis (Greenfield, 1942; Steeman-Nielsen *et al.*, 1969; Hassall, 1963) and respiration (Hassall, 1962) of cultured algae, the extent of the response appearing to increase with increase in the concentration of copper per unit of biomass (e.g., McBrien and Hassall, 1967) and with increase in light intensity (Nielson, 1969). Temperature is also important, work by Windle-Taylor (1965) showing that algal growth in a reservoir was more effectively checked at temperatures above 6.5°C than at those below it. Horne and Goldman (1974) also showed that with the blue-green algae *Aphanizomenon* and *Anabaena* nitrogen fixation as well as photosynthesis was inhibited in lake waters on the addition of small amounts of copper(5 to 10 µg/l); however, rates in the lake waters without added copper but already containing either 2 to 3 µg Cu/l or 60 to 70 µg Cu/l were neither inhibited by, nor related to, the indigenous concentration of copper, suggesting that these background levels were largely chelated and that there was little excess chelating capacity available. Chelation can arise from the presence of organic as well as inorganic ligands, some of which might be produced by algae. For example, *Anabaena cylindrica* produces polypeptide in culture able to bind about 0.3 mg Cu/mg total peptide nitrogen (Fogg and Westlake, 1953); in the absence of polypeptide 0.5 mg Cu/l reduced cell movement and 4 mg Cu/l was lethal whereas in its presence the corresponding concentrations were 2.25 and 32 mg/l.

(85) In lakes treated for algal blooms concentrations of copper in the water are not necessarily uniform and soon decline following treatment (Whitton, 1970a) and in practice fish are seldom adversely affected. However, in rivers, polluted continuously by wastes containing copper, the fauna are more severely affected than the flora (Butcher, 1946). At a concentration of 50 µg Cu/l in Lake Orta, fish and zooplankton were absent but algal production on stones was high (D. Calamari, pers. comm.). Thus, levels for the protection of fisheries should not necessarily be based upon those that allow algae to thrive.

5.2 Invertebrates

a) Acutely lethal values

(86) There is abundant information on the effect of copper on aquatic invertebrates, much of which has been discarded because details of the test conditions, particularly on the water quality, are not given. Water hardness, for example, clearly has a marked effect on the toxicity of copper to invertebrates, as well as to fish, as shown by Boch (1951) for the leach, *Pisicola geometra*. For *Daphnia magna* the 48-h LC50 is 90 µg Cu/l (Mălăcea and Gruia, 1965) at 21°C in water having a hardness of 196 mg/l as $CaCO_3$, and 40 µg Cu/l, as interpolated from the results of Anderson (1944), for tests at 25°C in water having a hardness of 90 mg/l as $CaCO_3$; these values are consistent with those (50 to 100 µg Cu/l) found by Ivekovič (1932) to be acutely toxic to *Daphnia pulex* at 20°C in water having a hardness of 215 mg/l as $CaCO_3$ and are between 0.17 and 0.25 respectively of the corresponding values for rainbow trout. *D. longispina* appears to be more resistant than the other two species of *Daphnia* (Weber, 1932; Deschiens *et al.*, 1964) while *D. hyalina* appears to be quite sensitive (Baudouin and Scoppa, 1974). With *Gammarus pseudolimnaeus* 6 weeks exposure to 280 µg Cu/l at a hardness of 44 mg/l as $CaCO_3$ was fatal and a reduction in survival was evident at 15 µg Cu/l (Arthur and Leonard, 1970).

(87) Some other invertebrates are exceptionally sensitive to copper. The leech, *Piscicola geometra* is adversely affected by a concentration of 8 µg Cu/l at a hardness of 300 mg/l as $CaCO_3$ and is killed within 24 h at 40 µg Cu/l (Boch, 1951). The naid worm, *Chaetogaster diaphanus* and the planktonic crustacean, *Bythotrephes longimanus*, are killed by a concentration of 4 µg Cu/l, both in water having a hardness of about 50 mg/l as $CaCO_3$; for *Planaria gonocephala* the threshold for acute toxicity in water of the same hardness is 4-40 µg Cu/l (Weber, 1932; data on water quality furnished by R. Gächter, pers. comm.). For a number of organisms the lethal concentrations of copper are similar to those of rainbow

trout. Thus, the survival of the snail, Physa integra and Campeloma decisum, at 15°C in water having a hardness of 45 mg/l as $CaCO_3$ was reduced during a 6-week exposure to 15 µg Cu/l and was nil or very low at 28 µg Cu/l (Arthur and Leonard, 1970); for the larval mayfly, Ephemerella subvaria, the 48-h LC50 was 0.32 mg Cu/l at 18°C in water having a hardness of 44 mg/l as $CaCO_3$ (Warnich and Bell, 1969) and for Heptagenia lateralis the 7-d LC50 was about 0.5 mg Cu/l at 10°C in water having a hardness of 109 mg/l as $CaCO_3$ (Liepolt and Weber, 1958); the 60-d LC50 for the worm, Nais communis, was 0.06 mg Cu/l at 20°C in water having a hardness of 320 mg/l as $CaCO_3$, N. variabilis and N. elingis being somewhat more sensitive (Learner and Edwards, 1963).

(88) Other organisms for which there are relevant data appear to be more resistant than trout, e.g., the 48-h LC50 for the stonefly, Acroneuria lycorias, was 8.3 mg Cu/l and the 14-d LC50 of the larvae of the caddis, Hydropsyche betteni, was 32 mg Cu/l at 18°C in water having a hardness of 44 mg/l as $CaCO_3$ (Warnich and Bell, 1969). However, the normal net-building of H. instabilis ceased after exposure to 1 mg Cu/l in water of a hardness of 351 mg/l as $CaCO_3$ (Decamps, 1973). Tubificid worms are less sensitive than the Naid worms, the 8-d LC50 being >8 mg Cu/l in water having a hardness of 50 mg/l as $CaCO_3$ (Weber, 1932).

(89) Some planktonic crustacea, for example, Cyclops strenuus are particularly resistant. The decapod crustacean Orconectes rusticus was killed in 13 days at a concentration of 1 mg Cu/l at 20°C with a hardness of 112 mg/l as $CaCO_3$ (Hubschman, 1967) and Astacus leptodactylus is probably somewhat more sensitive to this concentration (Chaisemartin, 1973); a characteristic delayed mortality of a week or a month after exposure to copper could lead to an underestimation of the sensitivity of this group.

b) Toxicity of organic compounds containing copper

(90) Kapkov (1972), using several molluscan species, investigated complexes of copper with pyridine, α- and β-picoline, and 2,6 lutidine and found them to be more toxic than copper ion alone.

c) Chronically lethal and sub-lethal values

(91) Levels lower than those that are acutely lethal have been shown to be damaging to some invertebrates: exposure of Gammarus pseudolimnaeus from one generation to another showed that no young survived a concentration of 8 µg Cu/l (0.29 of the acutely lethal value) although there were more young produced at 2.8 µg Cu/l than in the controls (Arthur and Leonard, 1970); Biesinger and Christensen (1972) found a 16 percent reduction in reproduction in Daphnia magna after a 3-week exposure to 22 µg Cu/l (approximately 0.5 of the 48-h LC50 estimated by extrapolation from the values given in para. 85). Biesinger et al. (unpublished; cited in National Academy of Sciences, 1973) indicated that the "safe" level for reproduction and growth was 6 µg Cu/l at a hardness of 45 mg/l as $CaCO_3$ which is probably equivalent to about 0.7 of the 48-h LC50.

d) Field observations

(92) Few data are available for situations where copper is the main poison present. In Lake Orvsjøen, Norway, in which the hardness was about 11 mg/l as $CaCO_3$ and the concentrations of copper and zinc were 0.13 and 0.4 mg/l respectively, chironomid larvae and a few planktonic crustaceans were present while Gammarus lacustris, snails, insect life and fish were absent. In the River Skorovasselv, containing 35 µg Cu/l and 150 µg Zn/l, brown trout and salmon (abundant in tributaries), snails and most ephemeroptera were absent but stickleback and other fauna and flora were present (M. Grande, pers. comm.). In streams in North Wales containing copper at between 50 and 99 percent of the total concentration of copper + zinc, each expressed as the fraction of its respective 48-h LC50 to rainbow trout, and in which the biomass of brown trout was markedly reduced and related to the concentrations of copper + zinc, the invertebrate biomass was not affected (Cremer and Warner, pers. comm.).

e) Summary

(93) Invertebrates vary widely in their resistance to copper, a few organisms being at least ten times and Daphnia and Gammarus being about five times as sensitive as rainbow trout (para. 85, 86 and 90) but the majority are either similar to or much more resistant than trout (para. 86 and 87). Thus, while the presence of copper would be expected to produce changes in the species composition this should not necessarily adversely affect the food supply of fish. Indeed, there is no evidence that fisheries in waters containing copper are adversely affected because of a reduction in fish food organisms; on the contrary, reduction in trout biomass has been observed with increase in concentration of copper even though invertebrate biomass is unchanged (para. 90).

6. SUMMARY AND CONCLUSIONS

(94) Copper is commonly present in polluted surface water often together with zinc, cadmium and other poisons, making it difficult to distinguish its contribution to any adverse effects found in fish populations.

(95) Under aerobic aqueous conditions the cupric form of copper that is present tends to form compounds and complexes with carbonate and hydroxide ions, humic and amino acids (para. 2-5) and is also readily adsorbed on to particulate material (para. 7), very little being present as the free ion (Cu^{2+}) except in very pure soft waters low in pH value.

(96) Toxicity to fish seems to be attributable to the combined effect of the inorganic forms of copper, mainly Cu^{2+} and $CuCO_3$, at least in hard borehole water in the pH range 6.5 to 7.5 (para. 6 and 20). However, analytical methods do not enable Cu^{2+} to be measured directly at very low concentrations and therefore in this report, unless otherwise stated, concentrations are expressed as 'soluble' copper, i.e., that which passes through a millipore filter of average porosity 0.45 µ.

(97) The mode of action of copper on fish is not clear but acutely lethal concentrations damage the gill (para. 10), may affect cell processes (para. 4 and 11) and enzyme activity, cause liver and kidney disorders (para. 55), effects which may also be associated with chronic toxicity and with elevated levels of copper in the tissues (para. 56-58).

(98) Toxicity to aquatic organisms is modified by water quality and, in particular, the lethal toxicity to fish (para. 21 and 22), invertebrates (para. 85) and algae (para. 82) is reduced by increase in water hardness. The relationship is best described for rainbow trout at 15°C, the 7-d LC50 at a total hardness of 10, 50, 100 and 300 mg/l as $CaCO_3$ being 0.024, 0.11, 0.2 and 0.56 mg Cu/l respectively.

(99) Increase in temperature may shorten the time of survival of fish at concentrations that are rapidly lethal yet increase it at lower concentrations; however, the LC50 is decreased by reduction in temperature (para. 15 and 17), the value for rainbow trout at 6°C being 0.3 of that at 15°C. There is little information on the effect of pH but at a value of 5.6 the time of survival, though not the threshold concentration, may be lower than at 7.5 (para. 20). Toxicity is increased by reduction in dissolved oxygen (para. 18) and decreased by chelating agents such as EDTA and NTA (para. 25), humic acids (para. 26) and amino acids (para. 27); thus organo-copper complexes appear to have little, if any, acute toxicity. Toxicity of total copper is reduced but that of the soluble copper is increased in the presence of organic and inorganic solids (para. 29).

(100) The early larval stages of some fish tend to be the most sensitive period in the life cycle (para. 31 to 34).

(101) While acclimation to copper has not been observed in the laboratory there is one report suggesting that acclimatization might have occurred in a polluted river (para. 35).

(102) Data from short-term tests in hard water show that some fish species are more sensitive to copper than salmonids. For example, the 96-h LC50 values for stone loach, rudd, common carp and perch are 0.8, 0.7, 0.7 and 0.3 respectively of the corresponding value for rainbow trout. Tench also appear to be more sensitive than trout. For pike and eels the values are about six and eight times respectively that of trout (para. 51). Tests lasting several weeks show that the LC50 values for stone loach and goldfish are about 0.6 and 0.24 respectively of that of trout (para. 54). No reliable data for comparing the sensitivity of trout and other species are available relating to soft water, to the young stages, or to sub-lethal effects.

(103) A significant (24 percent) mortality was found among rainbow trout kept for 17 weeks at 0.6 of the 96-h LC50 (para. 52). Salmon fry were unwilling to feed at 0.4 of the 21-d LC50 (para. 31) and growth of rainbow trout was adversely affected over a period of 17 weeks at concentrations in excess of 0.1 of the 96-h LC50 (para. 66). These values are close to those adversely affecting the growth of brook trout and the survival, growth and reproduction of some North American species (para. 63 to 65 and 67); no comparable data, however, are available for the growth and reproduction of European species.

(104) Data on behavioural changes induced by exposure to copper are fragmentary and difficult to relate to field conditions. There is some evidence that depression of the response of chemo-receptors, increase in cough frequency, respiration (para. 68) and activity, and reduction in feeding (para. 69) observed at sub-lethal concentrations are only transitory. Laboratory studies have demonstrated avoidance by Atlantic salmon of concentrations of copper in soft water of 0.05 of the 7-d LC50 but

under field conditions adult migrating fish were turned back only by much higher concentrations equivalent to 0.35 to 0.43 of the combined LC50 of copper and zinc (para. 70). A concentration of 0.01 mg Cu/l in soft water (close to the 96-h LC50 to rainbow trout) was avoided by goldfish under laboratory conditions unless the temperature was slightly higher than the acclimation temperature, when it was attractive (para. 70).

(105) Aquatic algae and invertebrates vary widely in their resistance to copper but under field conditions are generally less severely affected than fish (para. 84 and 86). While the presence of copper may alter the invertebrate species composition this should not necessarily affect the food supply of fish and there is no evidence that fisheries in water containing copper have been adversely affected because of a reduction in their food.

(106) Measurements of the toxicity of sewage effluents, except in soft water free from organic matter and suspended solids, and natural waters show that acute toxicity is less than that predicted from laboratory tests in clean water, probably because of the presence of non-toxic complexes and insoluble precipitates (para. 24 to 29 and 73 to 76). This may partly explain why natural fish populations have been found where the measured concentrations of copper approach the values found lethal in laboratory tests. Even in the presence of other poisons moderate populations of brown trout have been found where the annual 50 and 95 percentile values were 0.17 and 0.38 of the 48-h LC50 to rainbow trout, and some non-salmonid species have been reported where the corresponding values were 0.17 and 0.66 respectively (para. 77). These values would be somewhat higher expressed as fractions of the median threshold concentration rather than as fractions of the 48-h LC50.

(107) There is a clear need for the development of more refined analytical techniques to measure the chemical states of soluble copper compounds at low concentrations in natural waters to enable better comparisons to be made between laboratory and field data.

7. TENTATIVE WATER QUALITY CRITERIA

(108) There are fairly extensive data on the toxicity of copper to salmonid fish but in general toxicity under field conditions is less than that predicted from laboratory tests except in water low in organic matter and suspended solids; there are no comparable data for non-salmonid species. Furthermore, there are virtually no field observations that indicate unequivocally the concentrations of copper that are not inimical to fish populations or fisheries. This is mainly because of deficiencies in the analytical data on water quality, an absence of quantitative data on the size and structure of the fish populations, and the fact that other poisons are frequently present with copper. Again, the best information relates to salmonid species, only meagre qualitative data on the presence of fish being available for other species.

(109) Since the concentrations of copper in fresh water fluctuate both seasonally and within shorter time intervals, and fish populations are likely to be adversely affected by particular levels at particular times of the year, some account should be taken of this in water quality criteria for fish. However, in the absence of data on the precise effects of copper on fish populations the variations in concentrations of copper are arbitrarily expressed (as in the Working Party's reports on dissolved oxygen and zinc) as the annual percentile distribution. It is possible, however, that peak values could be more damaging in the winter than in the summer if, as found in laboratory tests with rainbow trout, copper is more toxic at low temperatures (the 6-d LC50 at 6°C was 0.3 of that at 15°C for rainbow trout) and because the sensitive young stages of salmonid species are present at low temperatures.

(110) Field and laboratory data for which analyses are given only for concentrations of total copper can be misleading because much of the copper may be present as insoluble particles or may be adsorbed on to particulate organic or inorganic matter; analyses of the various forms of copper would be the most relevant, but methods are not available for the very low concentrations important in soft water. The criteria are therefore expressed as "soluble" copper, i.e., the portion that passes through a millipore filter having an average porosity of 0.45 µ, although it is recognized that this may also lead to errors because it could include non-toxic cupro-organic complexes of low toxicity.

(111) Considering all the data available, it is proposed that the criteria should be based upon an annual percentile distribution of water quality expressed as the 50 and 95 percentile concentrations of soluble copper, and it is suggested that these should be 0.05 and 0.2 of the threshold LC50 respectively. Such criteria must take into account the hardness of the water. Table I shows the 50 and 95 percentiles for rainbow trout for different hardness values for which there are well-founded data, and these criteria may be generally applicable to other salmonid species.

Table I Approximate maximum annual 50 and 95 percentile concentrations of soluble copper for rainbow trout. Adjustments can be made for the presence of organic matter, low temperature, harmful substances and other species (see para. 111)

Water hardness (mg/l as $CaCO_3$)	50 percentile (μg Cu/l)	95 percentile
10	1.0*	5.0*
50	6.0	22.0
100	10.0	40.0
300	28.0	112.0

* Higher values occurring naturally where fish are present may indicate the predominance of soluble organo-copper complexes

(112) The presence of organic matter might allow the values in Table I to be increased perhaps up to 3-fold. The values should be decreased to allow for low temperatures (para. 108) and for the presence of other poisons, and also adjusted to allow for the different sensitivity of various species of fish (para. 101).

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