# CHEMICALS AFFECTING THE SPAWNING MIGRATION OF ANADROMOUS FISH BY CAUSING AVOIDANCE RESPONSES OR ORIENTATIONAL DISABILITY, WITH SPECIAL REFERENCE TO CONCENTRATIONS IN THE RIVER RHINE

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# SAMENVATTING

Het doel van het project "Ecologisch Herstel Rijn" en het "Rijn Actie Programma" is het herstel van het Rijn-ecosysteem van menselijke ingrepen. Tegen het jaar 2000 moeten populaties van hogere soorten, zoals die van de zalm, zich kunnen ontwikkelen. Als eerste stap wordt gestreefd naar vermindering van de lozing van een aantal prioritaire stoffen. Deze worden geselecteerd door de Internationale Rijn Commissie.
In Nederland zijn recentelijk voor ongeveer 300 verbindingen ecotoxicologische waterkwaliteitsdoelstellingen voorgesteld. Hierbij is gebruik gemaakt van acute en chronische toxiciteits-toetsen met ondermeer algen, kreeftachtigen en vissen (Stortelder et al. 1989). Deze gegevens kunnen een bruikbaar instrument zijn om de noodzaak tot reducties van lozingen te onderbouwen. Echter, bij de vaststelling van deze waterkwaliteitscriteria zijn de effecten van chemische stoffen op de paaitrek van anadrome vissen niet in beschouwing genomen.

De paaitrek is essentieel voor het behoud van populaties van sommige hogere soorten en voor de herbevolking van de Rijn door ondermeer de zalm. Anadrome vissen beginnen hun leven in zoet water, trekken als juvenielen naar voedselgronden in zee en keren als volwassenen terug naar zoet water om te paaien. Van een aantal soorten is bekend dat volwassenen terugkeren naar de paaiplaatsen waar ze als ei gelegd zijn ("homing"). Het reukorgaan is bij zalmachtigen van groot belang voor de oriëntatie tijdens de terugkeer (hoofdstuk 2).
Verontreinigingen kunnen de paaitrek op twee manieren aantasten. Ze kunnnen vermijdingsgedrag veroorzaken: de vissen zwemmen weg naar een schoon gedeelte als reactie op de aanwezigheid van een chemische stof. Daarnaast kan bij trekkende vissen de oriëntatie met het reukzintuig verstoord raken. In beide gevallen zal het aantal vissen dat stroomopwaarts trekt minder zijn dan onder niet-verontreinigde omstandigheden.

Het doel van deze literatuurstudie is vast te stellen of huidige, te verwachten of acceptabele concentraties van prioritaire stoffen of andere verbindingen in de Rijn de paaitrek van anadrome vissen zodanig kunnen verstoren dat de overlevingskansen van vispopulaties aanzienlijk worden verminderd.
Om dit te bereiken wordt een overzicht gegeven van waarnemingen aan de vermijding van verontreinigingen door vis (hoofdstuk 3) en aan functionele en histopathologische schade aan het reukzintuig veroorzaakt door verontreinigingen (hoofdstuk 4).

Er wordt aangetoond dat de reukzintuigen van vissen gevoelig zijn voor lage concentraties van sommige toxische stoffen: in laboratorium experimenten werden tetrachlooretheen, koper,

zink en bepaalde anion-detergenten gemeden. Koper en anion detergenten beschadigden het reukzintuig bij lage concentraties. Deze concentraties benaderden de gemiddelde niveaus in de Rijn bij Lobith en voorgestelde ecotoxicologische waterkwaliteitsdoelstellingen.

De vertaling van deze laboratorium-resultaten naar omstandigheden in het veld zoals die voorkomen in de Rijn is moeilijk, vanwege de beperkte kennis over de werking van een aantal factoren. Een "slechtste situatie" scenario lijkt correct omdat:
(1) onderzoek op dit terrein tot op heden zeer beperkt is geweest. Vissen kunnen gevoelig zijn voor lagere concentraties en/of voor andere verontreinigingen dan tot nu toe beschreven is;
(2) de respons op toxische stoffen additief kan zijn;
(3) zalmachtigen een delicaat reukorgaan hebben dat terugkeer naar hun paaiplaatsen verzekert. Zij zullen toxische stoffen bij lagere concentraties detecteren en vermijden dan andere vissoorten.

Gemiddelde concentraties van contaminanten in de Rijn bij Lobith zijn niet volledig representatief voor de omstandigheden die elders in de rivier voorkomen. De maximum concentraties die gemeten worden bij Lobith (1985) zijn gewoonlijk een factor 1.5 tot 3 maal de gemiddelde concentraties. Veel hogere concentraties kunnen zich voordoen in mengzones van effluenten (zie tabel I) en verder stroomafwaarts, waar de Rijn dichtbevolkte en geïndustrialiseerde gebieden doorkruist. In de Rijn kunnen plaatselijk en tijdelijk concentraties bestaan die niet alleen de drempelwaarden voor vermijding van tetrachlooretheen, koper, zink en anionische detergenten overschrijden maar ook die van chloroform, cadmium, chroom, nikkel en lood (zie tabel I).

Verontreinigingen kunnen ook andere mechanismen voor oriëntatie tijdens de paaitrek van anadrome vis beschadigen: Er worden effecten van verontreinigingen op de normale respons op licht, zoutgehalte, temperatuur en stroming gemeld. Eveneens kan schade aan andere zintuigen (lateraal orgaan en tastzin) optreden.

Met bovengenoemde opmerkingen in gedachten wordt geconcludeerd dat de paaitrek van anadrome vis in de Rijn bij de huidige kwaliteit zeker kan worden belemmerd. Of de overlevingskansen van vispopulaties belangrijk worden aangetast is vooralsnog onduidelijk.

# PREFACE

This report is the result of a literature study performed by order of the Institute for Inland Water Management and Waste Water Treatment (DBW/RIZA). In this study attention is confined to avoidance reactions to chemicals and effects of chemicals on olfactory orientation, which may affect the spawning migration of anadromous fish. Concentrations of chemicals in the River Rhine are compared with concentrations at which effects have been observed. In this way not only concentrations of pollutants occuring in the River Rhine, but also recently proposed dutch water quality objectives, based on ecotoxicological data, are evaluated.

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Utrecht, june 1989.

## SUMMARY

The objective of the project 'Ecological Rehabilitation of the River Rhine' and the 'Rhine Action Plan' is the recovery of the Rhine aquatic ecosystem by the year 2000, from manmade perturbations, so that populations of higher species, such as that of the salmon, might develop. As a first step, priority is given to the reduction of a number of chemicals (prioritary pollutants) selected by the International Rhine Committee (IRC).
Ecotoxicological water quality objectives have recently been proposed in the Netherlands for approximately 300 compounds, using data on acute and chronic toxicity to e.g. algae, crustaceans and fishes (Stortelder et al. 1989). These data may be a usefull instrument in substantiating the desirability of discharge reductions. However, in establishing these water quality objectives, effects of chemicals on the spawning migration of anadromous fish have not been taken into account.

The spawning migration is essential for the preservation of populations of some higher organisms and for the repopulation of the River Rhine by e.g. the salmon. Anadromous fish hatch in freshwater, migrate to feeding grounds at sea as juveniles, and return to freshwater as adults in order to spawn. Of a number of species it is known that adults return to the spawning grounds where they originally hatched (homing). The olfactory organ is of major importance in the orientation of homing salmonids (chapter 2). Pollutants may affect the spawning migration in two ways: they may cause avoidance reactions (respond to the presence of a chemical by moving away from it into a 'clean area'), or they may affect olfactory orientation in homing fish. In both cases the number of fish migrating upstream will be less than under uncontaminated conditions.

The objective of this literature study is to establish whether present-day, expectable or conceivable concentrations of prioritary pollutants or other compounds in the River Rhine can affect the spawning migration of anadromous fishes in such a way that survival chances of fish populations are diminished seriously.
To achieve this, observations on avoidance of contaminants by fish (chapter 3) and on functional and histopathological damage to the olfactory organ caused by pollutants (chapter 4) have been reviewed.

It is demonstrated that fish olfactory systems are sensitive to low concentrations of some chemicals: in laboratory experiments tetrachloroethylene, copper, zinc and certain anionic detergents were avoided, and copper and certain anionic detergents damaged the olfactory organ at concentrations which, as far as data are available, approximate average levels in the River Rhine at Lobith and the proposed ecotoxicological water quality objectives (see table I, p. 4)

The extrapolation of these laboratory findings to field conditions occurring in the River Rhine is difficult, because of a number of modifying factors and limited knowledge on their action. A worst-case treatment seems appropriate since: (1) research has been quite limited and therefore fish may be sensitive to lower concentrations and also to other pollutants than reported so far; (2) the response to chemicals may be additive (which is often the case in toxicity experiments); (3) It is to be expected that salmonids, having exquisite olfaction to secure homing, are among the most sensitive of fish species and will detect and avoid chemicals at lower concentrations than other fish species.

Average concentrations of pollutants in the River Rhine at Lobith are not entirely representative of conditions occurring elsewhere in this river. The maximum concentrations measured at Lobith (1985) are usually a factor 1.5 - 3 times the average concentrations. Much higher concentrations may be encoun-

tered in effluent mixing zones (see table I) and further downstream, where the river Rhine traverses heavily populated and industrialized areas. Locally and temporarily, concentrations in the River Rhine may occur which will not only exceed the threshold concentrations of avoidance reported for tetrachloroethylene, copper, zinc and anionic detergents, but also those of chloroform, cadmium, chromium, nickel and lead.

Pollutants may also affect other orientational mechanisms that may be important in the spawning migration of anadromous fish: Reference is made of effects of pollutants on responses to light, temperature, salinity and flow, and on damage to other sensory organs (lateral line organ and tastebuds).

Considering the remarks mentioned above, it is concluded that the spawning migration of anadromous fishes in the River Rhine may definitely be affected. Whether the survival chances of fish populations are affected seriously, remains unclear.

*Table I*

*Comparison of concentrations of pollutants occurring in the River Rhine: average concentrations at Lobith of dissolved pollutants (1985) and maximum concentrations expected in mixing zones of domestic sewage treatment plant effluents and industrial effluents (DBW/RIZA). An initial dilution of the effluent of 1 : 10 is assumed. In addition proposed dutch water quality objectives for dissolved pollutants (Stortelder et al. 1989) and lowest concentrations reported to elicit avoidance are given.*

| Pollutant | Lobith averages 1985 µg/L | Maxima in mixing zone of domestic sewage µg/L | Maxima in mixing zone of industrial effluent µg/L | Proposed ecotoxicological objective µg/L | Avoidance LOEC µg/L |
|---|---|---|---|---|---|
| Cu | 3.0 | 10 | 300-400 | 1.3 | 0.1 |
| Zn | 18 | 45 | 300-400 | 6.5 | 6.5 |
| PCE* | 0.26 | | | 4.9 | 4 |
| LAS | <50-70 | | | | 0.11 |
| Cd | 0.04 | 1 | 300-400 | 0.025 | 52 |
| Cr | 1.1 | 10 | 300-400 | 2.5 | 28 |
| Hg | 0.01 | 0.03 | | 0.005 | 20 |
| Ni | 3.7 | 10 | 300-400 | 7.5 | 23 |
| Pb | 0.2 | 10 | 300-400 | 1.3 | 26 |
| chloroform | 0.5 - 3.0 | | 400 | 0.6 | 150 |

* PCE = tetrachloroethylene

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# 1 INTRODUCTION

The objective of the project 'Ecological Rehabilitation of the River Rhine' and the 'Rhine Action Plan' is the recovery of the Rhine aquatic ecosystem by the year 2000, from manmade perturbations, so that populations of higher species, such as that of the salmon, might develop. An important element of policy is the reduction of discharge of toxic compounds to biologically safe levels. As a first step priority is given to the reduction of a number of chemicals (prioritary pollutants, see appendix 1) selected by the International Rhine Committee (IRC).
In the Netherlands ecotoxicological water quality objectives have recently been proposed for approximately 300 compounds, using data on acute and chronic toxicity to e.g. algae, crustaceans and fishes (Stortelder et al. 1989). At the proposed concentrations of toxicants, populations of organisms should be able to survive. These water quality objectives may be a usefull instrument in substantiating the desirability of discharge reductions. They will be presented as a point of discussion in the 'derde nota waterhuishouding' (a national watermanagement policy plan), and will eventually be contributed to the IRC.

In establishing these ecotoxicological water quality objectives, effects of chemicals on the spawning migration of anadromous fish have not been taken into account. The spawning migration is essential for the preservation of populations of some higher organisms and for the repopulation of the River Rhine by anadromous fish such as the salmon. Anadromous fish hatch in freshwater, migrate to feeding grounds at sea as juveniles, and return to freshwater as adults in order to spawn. They may even return to the spawning grounds where they originally hatched (homing). The olfactory organ is of major importance in the orientation of homing salmonids (Hasler & Scholz 1983, Stabell 1984). Pollutants may affect spawning migration in two ways: they may cause avoidance reactions, or they may affect olfactory orientation in homing fish.

The objective of this literature study is to establish whether present-day, expectable or conceivable concentrations of prioritary pollutants or other compounds in the River Rhine can affect the spawning migration of anadromous fishes in such a way that survival chances of fish populations are diminished seriously.

In this study attention is confined to avoidance reactions to chemicals and effects of chemicals on olfactory orientation. Although they might affect the spawning migration and the survival chances of fish populations, sublethal effects on the general condition of fishes will not be considered here, since this type of effect has been used in the recent formulation of water quality objectives.

An initial selection of the literature between january 1, 1970 and decembre 30, 1988 was made using the DIMDI datasystem (Biosis and ASFIS-BFA files). However, not all the relevant articles were available on short term. On the other hand, the selection was extended considerably with material that was not mentioned in the initial selection.

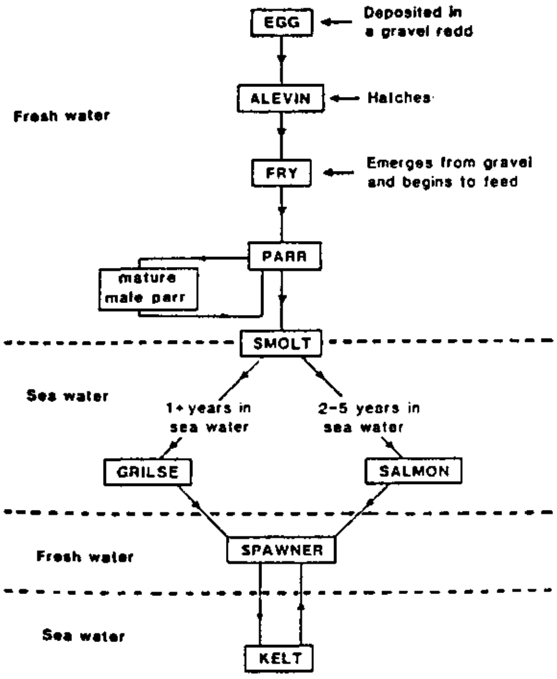
Chapter two is devoted to the spawning migration of anadromous fish, with particular reference to salmonid homing. Chapter three and four review the chemicals that have been observed to cause avoidance reactions and functional or histopathological damage respectively. Conclusions are presented in chapter five.

## 2 THE SPAWNING MIGRATION OF ANADROMOUS FISH

### 2.1 Life history of anadromous fishes

Anadromous fishes are fishes that hatch in freshwater, migrate to feeding grounds at sea as juveniles, and return to freshwater as adults in order to spawn. This behaviour is illustrated by the life history of the atlantic salmon, *Salmo salar* (figure 1). Because of commercial value and conspicuous behaviour, research on anadromous fishes has been focussed primarily on salmonids. Other anadromous fishes include three spined stickleback *(Gasterosteus aculatus)*, american shad *(Alosa sapidissima)*, allis shad *(A. alosa)*, twaite shad *(A. fallax)*, lampreys (e.g. european lamprey, *Lampetra fluviatilis)*, sturgeon *(Acipenser stureo)* and striped bass *(Morone saxatilis)* (Bond 1979, McKeown 1984, Nijssen & de Groot 1987).

*Figure 1*
*The life history of the atlantic salmon (Salmo salar). In rivers with a current of approximately 1 m/s, eggs are deposited in the gravel. After hatching young, or alevins, remain in the gravel and utilize their remaining yolk. Fry emerges from the gravel, begin to feed and develop into parr. The parr-stage can last from one upto eight years. Eventually parr undergo the physiological and behavioural transformation into smolts (smolting or smoltification), which migrate to the feedinggrounds at sea. Some salmon (grilse) return to their homestream after one year in the sea (weight 1.5 - 4 kg). Others return after 2-5 years. Salmon that have survived spawning (kelt) return to sea. (Laird & Needham 1988)*



Before anadromous salmonids migrate from freshwater to seawater, they change from parr into the smolt-stage. In this proces (smolting or smoltification) feeding, metabolism, lipid content, osmoregulation, colouration and social behaviour of the fish change and become more adapted to life at sea (Farmer et al. 1978, McKeown 1984). The migration itself covers long distances, often 2,000 - 4,000 km (Hasler & Scholz 1983). It is evident that the anadromous life strategy involves the investment of tremendous energy. The ability of salmonids to return to the river where they originally hatched (homing ability), is probably important in rendering the anadromous life strategy efficient: homing reduces reproductive wastage (spawning where conditions are unfavorable) and confines spawning to waters proven suitable for survival (Hasler & Sholz 1983). The ability to return to natal areas varies between anadromous species (McKeown 1984). Homing is characteristic of the folowing salmonids: *Oncorhynchus gorbuscha* (pink), *O. keta* (chum), *O. kisutch* (coho), *O. nerka* (sockeye), *O. tschawytcha* (chinook), *Salmo salar* (atlantic salmon) and *S. gairdneri* (rainbow), *S. trutta* (brown) and *S. clarki* (cutthroat trout) (Hasler & Scholz 1983).

## 2.2 Homing ability in salmonids

The homing ability in salmonids is highly developed. Numerous experiments have been performed using marked young salmon. Of the downstream migrants usually 0.5 - 5 % survive until spawning and of these about 95% return to their natal stream, while 5% stray into other streams (Cooper & Hirsch 1982, Hasler & Scholz 1983). According to Stabell (1984), accuracy of homing in salmonids is often underestimated, because no distinction is made between homing experiments with wild and hatchery-reared salmon. In homing experiments with native wild atlantic salmon only 1 - 2% of the total number of recovered fishes was caught in streams other than the homestream.
An experiment performed by Bams (1976) indicates that genetic material influences the homing ability of pink salmon *(Oncorhynchus gorbuscha)*: homing and survival were compared of an introduced pure donor stock and a hybrid stock, created by crossing females from the donor with males from the local stock. After incubation, fry was released and, upon return from the ocean, fish were recaptured. Homing ability of pure donor stock was less than that of hybrids and hybrids did not have the normal accuracy of return to the homestream.
Detailed research on morphological, biochemical and behavioral characteristics revealed that, even within one river, reproductively isolated 'stocks' can be present (reviewed by Stabell 1984 and Smith 1985). Through natural selection these stocks can be adapted to their specific environmental conditions. Accurate homing is essential in maintainig these genetic pools.

## 2.3 Olfactory orientation during upstream migration

The homing migration consists of two main phases: an ocean-phase (open water migration) and a stream-phase (upstream migration in rivers). Because studies have concentrated on freshwater and inshore-aspects of migration, only little is known of orientational cues used during open water migration (Smith 1985).
The movements of migrating atlantic salmon in the Miramichi estuary were studied by Statsko (1975). Ultrasonic tracking revealed only slow progress of the fish. Some of the salmon remained in the estuary for more than two weeks. Elson et al. (1972) compared the upstream progress of atlantic salmon in the Miramichi and Tabusintac River. The overall mean rate of upstream progress in the Miramichi River (0.06 km/h) was considerably lower than in the Tabusintac River (0.21 km/h). Slow progress in an estuary may be caused by pollution, as suggested by Elson et al. (1972), or by a response of anadromous fishes to a gradual change in salinity or a change from open water to a confined channel (suggested by Statsko 1975, During 1989).

It is generally accepted that olfactory cues play an important role in the upstream migration of homing salmonids (Cooper & Hirsch 1982, Hasler & Scholz 1983, Smith 1985, Stabell 1984). However, different theories exist on the exact mechanism of orientation.

The olfactory hypothesis for salmonid homing was originally formulated by Hasler & Wisby in 1951. It has three basic tenets (Cooper & Hirsch 1982):
- Because of local differences in soil and vegetation of the drainage basin, each stream has a unique chemical composition and, thus, a distinctive odour;
- Before juvenile salmon migrate to the sea (as smolts) , they become imprinted to the distinctive odour of their homestream;
- Adult salmon use this information as a cue for homing, when they migrate through the homestream network to the home tributary.

The olfactory hypothesis is supported by a number of studies:

1 Conditioning experiments with several fishspecies demonstrate that fish can discriminate between waters collected from different streams. Impairment of the olfactory organ resulted in loss of discrimination (Hasler & Scholz 1983, Cooper & Hirsch 1982, Smith 1985).

2 Waters from different streams elicit specific EEG-responses from the olfactory organ. In general, water from the homestream results in a stronger EEG-response than water from other streams. (Hara 1970, Smith 1985, Stabell 1984).

3 In general, olfactory impairment, as opposed to impairment of vision, seriously affects salmon homing (Bertmar 1982, Cooper & Hirsch 1982, Hasler & Scholz 1983, Smith 1985). Exceptions, however, were found in which impairment of olfaction did not affect homing (Bertmar 1982). Schooling of sensory impaired with untreated fish may explain this phenomenon.

4 In a number of experiments homing of salmon was studied after transplantation of pre-smolts (not yet imprinted) from one river to a second river. The fish homed to the second river (Cooper & Hirsch 1982, Hasler & Scholz 1983, Stabell 1984). In contrast, transplantation of late-smolt (already imprinted) brown trout *(Salmo trutta)* resulted in homing to the original natal river (Stuart 1959 as cited by Cooper & Hirsch 1982).

5 In several experiments (e.g. Johnson & Hasler 1980), salmon were artificially imprinted to morpholine or phenetylalcohol. These salmon migrated to rivers to which morpholine respectively phenetylalcohol was added (reviewed by Cooper & Hirsch 1982, Hasler & Scholz 1983, Smith 1985, Stabell 1984).

Nordeng (1971) observed migration of arctic char *(Salvelinus alpinus)* to the stream of their parents. Since the fish had grown up in a hatchery from artificially fertilized eggs and had never experienced their parents stream, they could not have been imprinted to homestream odour as postulated by Hasler & Wisby. Nordeng (1971) proposed that the released fish were attracted by pheromones released by their relatives (the pheromone hypothesis).
In view of the above-mentioned 'stocks' within species, these pheromones should be population specific (Hasler & Schulz 1983, Smith 1985, Stabell 1984). Since pheromones can contribute to the homestream odour, both theories do not have to be mutually exclusive (Cooper & Hirsch 1982).

Both theories are confronted with the problem of extremely high dilution rates that eventually reduce the stimulus to below threshold levels. Two theoretical solutions were presented:

- Harden Jones (1968 as cited by Smith 1985) postulated that salmon, on their downstream migration, are imprinted to a sequence of different odour stimuli and cue to each of these odours in turn, on returning to their homestream.
- Nordeng has suggested that juvenile salmonids deposit a pheromone trail while migrating downstream, which serves as an orientational cue to homing adults (Cooper & Hirsch 1982).

As a result of diffusion and turbulence, the odour gradient will be extremely shallow at some distance from the odour source. Although fishes might perceive the homestream or pheromone odour, the odour field itself, as a result of the shallow gradient, does not contain immediate information on the direction of the odour source. Only comparison of odour intensity at different places in the odour field might reveal the direction of the odour source. However, a memory of odour intensity and of position in space are necessaties for such an orientational mechanism. Even then, pinpointing the direction of the odour source will be complicated because of incomplete mixing of water from different sources (Brett & Groot 1963, Kleerekoper 1982). The difference in concentration of homestream odour between incompletely mixed waterbodies contains information on the distance to the odour source (During 1989).

## 2.4 Other orientational cues

Immediate and precise information on the direction of the odour source is available in the direction of waterflow. Harden-Jones (1968 as cited by Hasler & Scholz 1983) proposed that presence of imprinting odour serves as a sign stimulus for the release of positive rheotaxis (swimming against the current), whereas absence of the sign stimulus results in negative rheotaxis (downstream swimming). The behaviour of salmon observed in tagging recapture studies and ultrasonic tracking is in accordance with this theory: When salmon make the wrong choice at a stream junction (overshooting or bypassing) they correct their error by swimming downstream (backtracking) (Hasler & Scholz 1983). This theory of olfactory released rheotaxis also fits the oberved behaviour of salmon in transplantation and artificial imprinting experiments and in research in tidal currents (Cooper & Hirsch 1982, Johnson & Hasler 1980, Hasler & Scholz 1983, Smith 1985).
According to Smith (1985) waterflow is perceived by fish through vision of moving particles or surroundings, touch and the lateral line organ. Smith, who reviews the importance of different stimuli and receptors in the control of fish migration, also suggests perception of flow by acceleration detectors in the inner ear.

This demonstrates that, although olfactory orientation is considered to be of major importance, other orientational cues may also contribute to accurate homing. In figure 2 sensory mechanisms are presented that are involved in integrated orientation.

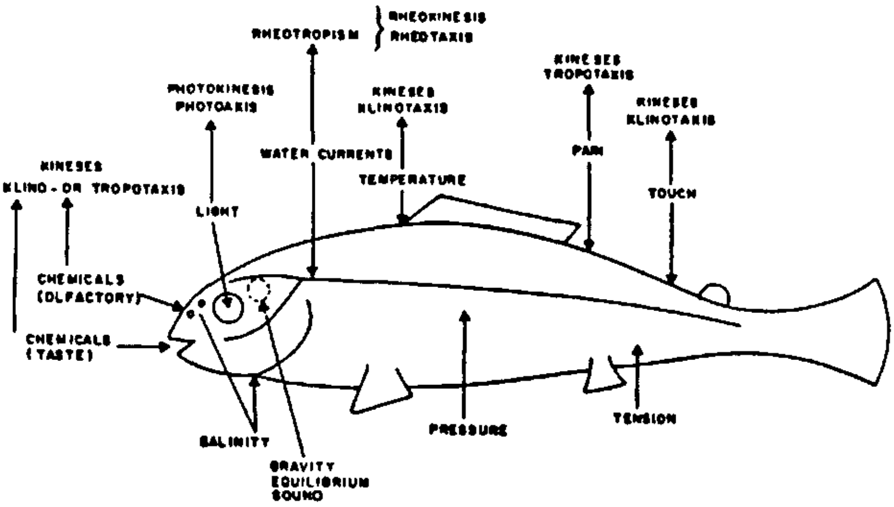


*Figure 2*
*The main stimuli and reactions involved in integrated orientation in migrating fish like baltic trout (Salmo trutta). From Bertmar (1982).*

In addition to olfaction and flow perception, migrating salmonids may use the following senses in orientation: vision (Bertmar 1972), salinity perception (McInerny 1964), temperature perception (Fontaine 1983) and perception of small hydrographic structures consisting of watervolumes of different temperature, salinity and flow (Westerberg 1982 as cited by Stabell 1984 and During 1989). Some fish posess a magnetic compass orientation mechanism (McKeown 1984).

## 2.5 Vulnerability of anadromous life strategy

The migration of anadromous fish is a complex process (e.g. Hasler & Scholz (1983) compare the life history of salmonids with the Krebs cycle.) This complex nature is attended with a vulnerability to disturbances which is only partially comparable to that of freshwater fish. Specific problems are:

Disturbance of osmoregulatory ability.
Since anadromous fish live in fresh water as well as salt water, a very good osmoregulatory ability is required. At sea, the fish drink seawater, retain the free water and excrete NaCl across the gill epithelium. The ionpump with which $Na^+ + K^+$ ATPase appears to be involved, plays an important role in this process (Mehrle & Mayer 1985).
During smolting the juvenile salmonids are prepared for contact with seawater. This proces is characterized by the presence of certain hormones and an increase in $Na^+ + K^+$ ATPase activity (McKeown 1984, Hoar 1976). These parameters have been used in the research on effects of chemicals on smolting of salmonids (Lorz et al. 1978, 1979, Lorz & McPherson 1976, Nichols et al. 1984 and Davis & Shand 1978) (see table 1). In addition, seawater challenge tests (survival in seawater) have been performed, to assess osmoregulatory ability, and seaward migration of exposed and unexposed smolts has been studied (figure 3).
Significant effects on downstream migration of smolts were observed after exposure to copper (Lorz & McPherson 1976 *(Oncorhynchus kisutch,* see figure 3), Davis & Shand 1978 *(O. nerka)* as cited by Mehrle & Mayer 1985), arsenic trioxide (Nichols et al. 1984 *(O. kisutch)*) and the aquatic herbicide diquat (Lorz et al. 1979 *(O. kisutch)*). Copper affected migration at the lowest concentration tested (5 µg/L). A concentration close to the recently proposed water quality objective (1.3 µg/L dissolved copper) and the average concentrations in the River Rhine (3.0 µg/L dissolved Cu). In addition, effects of copper on smolting may be amplified in the presence of other heavy metals: Although exposure to cadmium or zinc did not affect downstream migration, in combination with 10 µg Cu/L a depression of downstream migration was observed that was considerably more severe than after exposure to 10 µg Cu/L alone (Lorz et al. 1978 as cited by DIMDI).
On their return the freshwater, adult anadromous fish are confronted with the transition from sea water to fresh water. No reference was found on osmoregulation during this period.

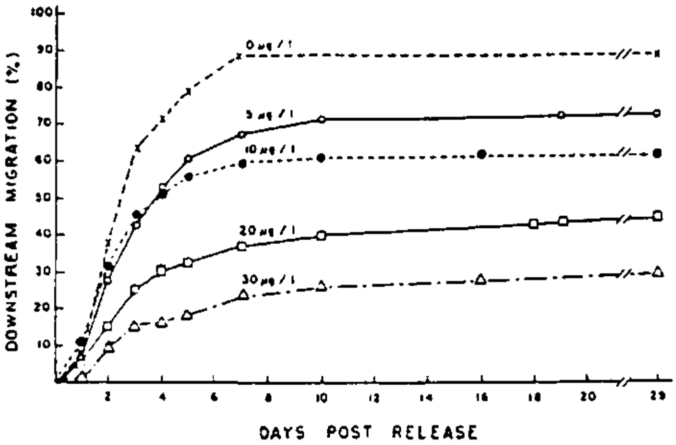


*Figure 3*
*Influence of exposure to copper for 165 days in fresh water (94 mg $CaCO_3$/L hardness) on percent downstream migration of yearling coho salmon (Oncorhynchus kisutch). Each line represents 78 - 172 fish released on June 4, 1975. (From Lorz & McPherson 1976).*

- TABLE 1 -
(effects on smolting)

*Table 1*
*Effects of dissolved pollutants on smolting of coho salmon (Oncorhynchus kisutch): NOEC (No Observed Effect Concentration) and LOEC (Lowest Observed Effect Concentration) are given. Endpoints used: $Na^+$ + $K^+$ ATPase activity (ATPase), survival in sea water (SW-survival), downstream migration (migration) and hormone concentrations (hormone).*

*To enable comparison, for dissolved pollutants, LC50 (concentration lethal to 50% of the test organisms) proposed ecotoxicological water quality objectives (Stortelder et al. 1989) and average concentrations in the River Rhine at Lobith in 1985 (DBW/RIZA) are given. Notes: 1 highest concentration in experiment; 2 lowest concentration in experiment; 3 only concentration in experiment; * threshold concentration of response.*

| COMPOUND | REFERENCE | EXPERIMENTAL CONDITIONS hardness mg CaCO3/L | pH | T °C | time | NOEC ug/L | LOEC ug/L | note | RESPONSE | TOXICITY time | LC50 ug/L | OBJECTIVE ug/L | RHINE 1985 ug/L |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| As2O3 | Nichols et al. 1984 | 70 | 8.1 | 4-14 | 0.5yr | 100 | 300 | | hormons, ATP-ase, migration | | | 12.5 | |
| Cd | Lorz et al. 1978 (in Mehrle & Mayer 1985) | | | | 4d | | 4 | | SW-survival dose-dependent, not migration | | | 0.025 | 0.04 |
| | | | | | | | | | affected migration in presence of 10 ppb Cu | | | | |
| Cr | Lorz et al. 1978 (in Mehrle & Mayer 1985) | | | | 4d | 5 000 | | | SW-survival, migration | | | 2.5 | 1.1 |
| Cu | Lorz & McPherson 1976 | 94 | 7-8 | 11 | 6d | | 5 | 2 | ATP-ase, SW-survival, migration | 96h | 60 - 74 | 1.3 | 3.0 |
| Hg | Lorz et al. 1978 (in Mehrle & Mayer 1985) | | | | 4d | | 50 | | SW-survival dose dependant | | | 0.005 | 0.01 |
| Ni | Lorz et al. 1978 (in Mehrle & Mayer 1985) | | | | 4d | 5 000 | | | SW-survival, migration | | | 7.5 | 3.7 |
| Zn | Lorz & McPherson 1976 | 94 | 7-8 | 11 | 6d | 2 000 | | 1 | ATP-ase, SW-survival | 96h | 4 600 | 6.5 | 18 |
| | Lorz et al. 1978 (in Mehrle & Mayer 1985) | | | | | | | | no effect on migration | | | | |
| | | | | | | | | | affected migration in presence of 10 ppb Cu | | | | |
| Acroleine | Lorz et al. 1979 | 101 | 7.5 | 10 | 6d | 50 | | 1 | ATP-ase, SW-survival | 96h | 68 | | |
| Amitrole-T | Lorz et al. 1979 | 101 | 7.5 | 10 | 14d | 50 000 | | 1 | ATP-ase | 96h | 70 000 | | |
| | | | | | | 1 000 | 25 000 | | SW-survival affected, not osmoregulation | | | | |
| Atrazine | Lorz et al. 1979 | 101 | 7.5 | 10 | 6d | 15 000 | | 1 | ATP-ase | 96h | >15 000 | 0.075 | |
| | | | | | | 8 000 | 15 000 | | SW-survival affected, not osmoregulation | | | | |
| 2,4 D | Lorz et al. 1979 | 101 | 7.5 | 10 | 6d | 200 000 | | 1 | ATP-ase, SW-survival | 96h | >200 000 | | |
| Dicamba | Lorz et al. 1979 | 101 | 7.5 | 10 | 6d | 100 000 | | 1 | ATP-ase, SW-survival | 96h | >100 000 | | |
| Dinoseb | Lorz et al. 1979 | 101 | 7.5 | 10 | 6d | 100 | | 1 | ATP-ase, SW-survival | 144h | 88 | 0.023 | |
| Diquat | Lorz et al. 1979 | 101 | 7.5 | 10 | 4d | | 500 | 2 | ATP-ase, SW-survival, migration | 96h | 30 000 | | |
| Krenite | Lorz et al. 1979 | 101 | 7.5 | 10 | 6d | 200 000 | | 1 | ATP-ase, SW-survival | 144h | >200 000 | | |
| Paraquat | Lorz et al. 1979 | 101 | 7.5 | 10 | 6d | 50 000 | | 1 | ATP-ase | 96h | 76 000 | | |
| | | | | | | 1 000 | 10 000 | | SW-survival affected, not osmoregulation | | | | |
| 2,4,5 T | Lorz et al. 1979 | 101 | 7.5 | 10 | 6d | 7 000 | | 1 | ATP-ase, SW-survival | 144h | >7 000 | | |
| Tordon 22K | Lorz et al. 1979 | 101 | 7.5 | 10 | 6d | 5 000 | | 1 | ATP-ase, SW-survival | 24h | 17 500 | | |
| Tordon 101 | Lorz et al. 1979 | 101 | 7.5 | 10 | 6d | 19 000 | | 1 | ATP-ase, SW-survival, migration | 24h | 20 000 | | |

Blocking of the migrational route by waterworks
According to de Groot (1989), the Haringvliet sluices, the weirs in the Nederrijn/Lek and the Afsluitdijk form barriers for migrating anadromous fish. The construction of fish ladders has been suggested to assure passage of these barriers by migrating fish.

Pollution of waters in the migrational route (subject of this study)
Pollution may cause avoidance reactions and thus disturb migration of anadromous fish to their spawning grounds. Pollution may also affect olfactory orientation in homing anadromous fish as a result of which they will not be able to find their home stream. (Essentially, homing ability may have been impaired in the juvenile stage: imprinting to home stream odour may have been incomplete or may not have occured at all, due to damage of olfactory organs by pollutants.)

# 3 AVOIDANCE OF CONTAMINANTS BY FISH

## 3.1 Introduction

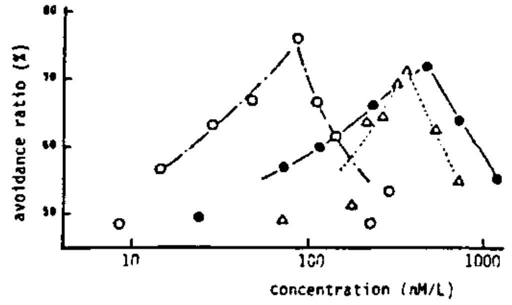
Avoidance-preference responses of fish to aquatic pollutants have been reviewed by a number of authors. However, none of these reviews is specifically related to effects of chemicals on the spawning migration of anadromous fish. In a review by Cherry & Cairns (1982) responses of fishes to different temperatures were emphasized. Beitinger & Freeman (1983) and Giattina & Garton (1983) exclusively reviewed avoidance-preference responses to different chemicals. Also, effects of contaminants on chemoreception (Brown et al. 1982, Hara et al. 1983) and on behaviour in general (Atchinson et al. 1987 and Rand 1985) have been reviewed.

In preference-avoidance studies the ability of organisms to respond to the presence of a chemical (or other stimulant) by moving toward it (attraction, selection or preference) or away from it into a 'clean area' (avoidance) is tested. Preference-avoidance can be the result of a directed or an undirected respons to the chemical: in case of a directed response the chemical is perceived and used as orientational cue for locomotion. On the other hand, the chemical may not be perceived but may influence the activity of the exposed organism and, through an undirected response, cause avoidance or preference behaviour. Hyperactivity enhances the chance of contact with clean water, where recovery to normal activity may occur (avoidance). Induced hypoactivity will usually result in a prolonged contact with the chemical (preference). Judging the various descriptions of behaviour, undirected responses to chemicals seem to be of minor importance in the preference-avoidance experiments reviewed here. However, it will not always be evident whether the observed preference-avoidance behaviour is the result of a directed or an undirected response.

Although the organism is stressed by displacement from its natural habitat, avoidance of a chemical may enhance survival chances, since serious toxic effects are prevented. However some chemicals are avoided at concentrations that are not known to be toxic (Rand 1985). In fact, the ecotoxic action of the compound might just be displacement of organisms and concomitant disruption of the ecosystem.

In this chapter preference-avoidance experiments are reviewed in which animals were given the choice between different concentrations of a chemical.
In addition effects of aquatic contaminants on rheotropism are reviewed. Although in such an experiment the fish is not given a choice between contami-

*Figure 4*
*Avoidance curves by medaka (Oryzias latipes) to monochloramine (● - ●), the anionic surfactant LAS (O - O) and the insecticide fenitrothion (Δ-Δ). Concentrations are guiven in nM/L. From Hidaka & Tatsukawa (1985).*



nated and clean water, reduction of positive rheotaxis or induction of negative rheotaxis is interpreted as an avoidance response of the fish to the chemical: downstream swimming increases the distance to the contaminant source. The relevance of affected positive rheotaxis to the spawning migration of anadromous fish is evident: movement upstream is necessary to reach the spawning grounds. (Olfactory released positive rheotaxis probably is of major importance to homing, as a directional response to homestream or pheromone odour, see § 2.4).
Experiments in which changes in activity were observed on exposure to aquatic pollutants (appendix 2), are not reported on here, unless it was demonstrated that this behaviour resulted in avoidance or preference.

It should be noted that in avoidance behaviour not necessarily a traditional positive concentration-response relationship is encountered: At low concentrations the chemical might not elicit any response. As the concentration increases, an avoidance response can appear, which may be preceded by a preference response (e.g. the response to xyleen by atlantic salmon *(Salmo salar)* as reported by Folmar (1976)). A further increase in concentration does not always result in a more pronounced avoidance response. In fact it may even lead to abolishment of the avoidance response (e.g. figure 4 and 5) and sometimes this is followed by preference for the chemical (e.g. the response of rainbow trout *(Salmo gairdneri)* to copper (Giattina et al. 1982)). Malfunctioning or impairment of olfactory ability may explain this behaviour. At even higher concentrations a new avoidance response may occur when an other type of receptor responds to the chemical (e.g. pain in case of irritation of the skin).
Because of this concentration-response pattern, the absence of an avoidance response at a specific concentration does not necesarily imply that avoidance will not occur at *lower* concentrations. (In toxicity testing toxic effects would mostly be expected to occur only at concentrations *higher* than a concentration where no effect was observed.)

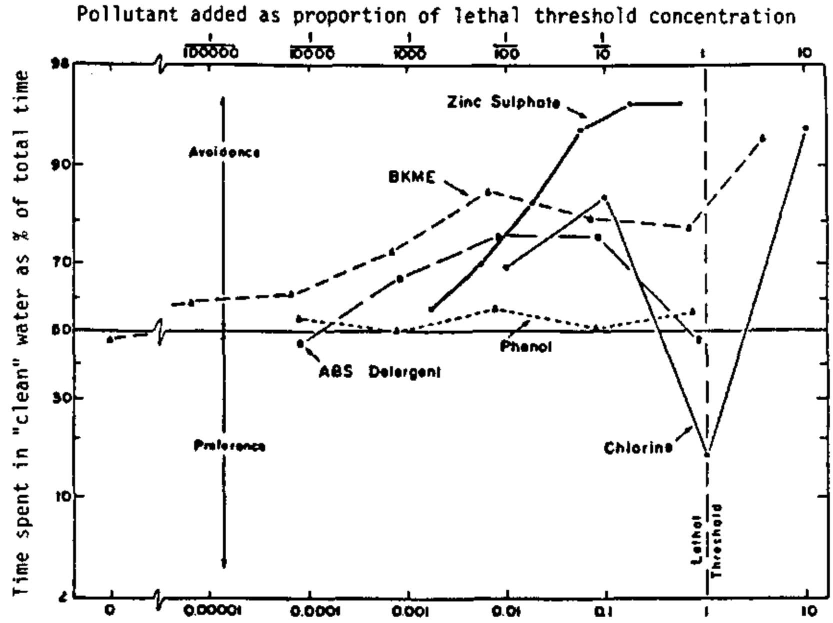


*Figure 5*
*Median avoidance reactions of salmonid fish to various pollutants. Concentration is expressed in terms of toxicity to fish instead of chemical units. Each point is the median of of individual quantitative responses of 5 to 55 fish at that concentration of pollutant. From Sprague & Drury (1969).*

In this chapter observations on preference-avoidance behaviour are summarized by giving the concentration at which no effect was observed (NOEC) and the lowest concentration observed to have effect (LOEC). However, in the references reviewed, these data are not always given. In some cases only the threshold concentration of response is reported. This is generally a concentration between NOEC and LOEC, representing the lowest concentration at which a response is thought to occur. By some authors this threshold is defined as the concentration of the chemical eliciting a response of (e.g.) 50% of the test animals.
Subsequently, these effective concentrations are compared with proposed dutch ecotoxicological water quality objectives and concentrations occuring in the River Rhine. In addition they may be compared with LC50 values: concentrations lethal to 50% of the test organisms within a certain period (e.g. 24, 48 or 96h). In most preference-avoidance experiments fish are exposed to a chemical for only a short time (between ½ - 2½ h). In these experiments fish may be exposed to a concentration that is generally considered lethal (e.g. 24 - 96h LC50). The exposed fish may not be affected at all by the chemical, because of short exposure time. In contrast, the proposed water quality objectives are concentrations, based on ecotoxicological tests, at which populations of organisms should be able to survive for an indefinite time (chronic exposure).

At Lobith concentrations of some pollutants are measured regularly (almost continuously (4 heavy metals) upto approximately once a month). The maximum concentrations measured at Lobith (1985) are normally a factor 1.5 - 3 x the average concentrations (DBW/RIZA). Elsewhere in the river Rhine much higher concentrations may be encountered, e.g. in effluent mixing zones. In addition, the water at Lobith still has to go a long way before it reaches the sea. It will traverse heavily populated and industrialized areas and the concentration of some pollutants will increase along the way.

## 3.2 Modifying factors in preference-avoidance experiments

### 3.2.1 Experimental setup

Steepness of gradient
In avoidance preference experiments many different experimental chambers have been used (figure 6). In some cases the chemical was distributed over a shallow gradient. In most experiments, however, waterbodies differing in chemical concentration were strictly seperated (steep gradient chambers). In steep gradient chambers directional behaviour will be more succesfull, since orientation is easier and favourable conditions are close at hand. Steep gradients are rare in nature, however they are present at the confluence of two water sources (Larrick et al. 1987).

Research of Kleerekoper et al. (1972), Timms et al. (1972) and Westlake et al. (1974) on avoidance of copper by the goldfish *(Carassius auratus)* revealed that directional movement occurred in steep and shallow gradients of copper. In steep gradients avoidance was observed, whereas in shallow gradients preference occurred. So far there is no explanation of this phenomenon.
Giattina et al. (1982) compared the avoidance behaviour of rainbow trout in steep and shallow gradients of copper and nickel. No statistical differences in avoidance behaviour were observed.

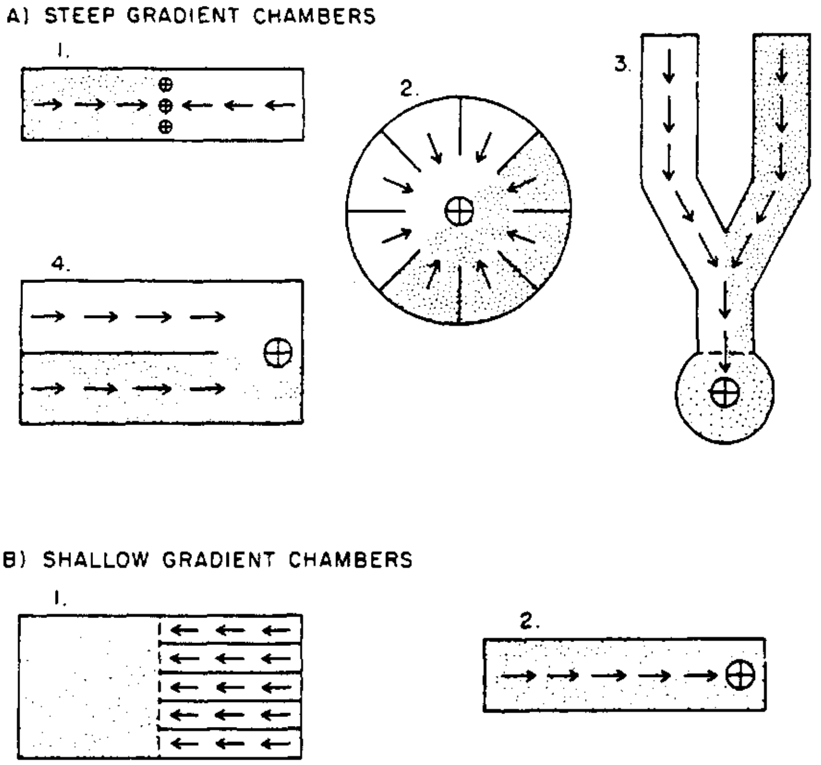


*Figure 6*
*Schematic diagram of basic tank designs as used by different investigators to study preference-avoidance and other locomotor responses to aquatic contaminants (from Giattina & Garton 1983).*
*A. Steep gradient chambers:*
*1. Counter current flow chamber (Giattina et al. 1982, Hall et al. 1982 1984, Jones 1947 1948 1952, Lewis & Livingston 1977, Pinkney et al. 1985, Rehwoldt & Bida 1978, Scherer 1975, Sprague 1964 1968, Sprague & Drury 1969, Tatsukawa & Hidaka 1978).*
*2. "Rosette" apparatus (Dauble et al. 1985, Kleerekoper et al. 1973, Westlake et al. 1974).*
*3. Y-through (Folmar 1976 1978, Folmar et al. 1979, Hansen 1969, Hansen et al. 1972 1973 1974, Kynard 1974, Maynard & Weber 1981, Rehnberg & Schreck 1986).*
*4. Modified fluvarium (Black & Birge 1980, DeGraeve 1982, Hartwell et al 1978A, Hidaka & Tatsukawa 1985, Hidaka et al. 1984 (?), Pedder & Mally 1985, Westlake et al. 1983, Whitman et al. 1982).*
*B. Shallow gradient chambers*
*1. Fluvarium (Hansen 1972).*
*2. Lineair gradient (Schumacher & Ney 1980, Spraggs et al. 1982).*

Hansen and coworkers performed a number of preference-avoidance experiments with steep gradient chambers (Y-maze type). In their articles they stressed the importance of concentration-response relationships: If an increase in concentration does not result in more pronounced avoidance, the organism is unable to detect this increase in concentration. Therefore, Hansen and coworkers reasoned, the testorganism will be able to seek water free of contaminant, but will not be able to distinguish between waters of different concentrations. In their experiments with sheepshead minnows *(Cyprinodon variegatus)* this idea seemed to be confirmed: In contrast to other chemicals tested, 2,4-D elicited increasing avoidance at higher concentrations *and* the test animals consistently prefered lower concentrations of 2,4-D to higher ones (Hansen 1969). Comparable results were obtained in experiments with mosquitofish *(Gambusia af-*

*finis)* and 2,4-D and the organophosphate insecticide Dursban (Hansen et al. 1972).

In other words: organisms may exhibit avoidance responses in steep gradient chambers, however this does not imply that they will effectively avoid chemicals in shallow gradient chambers or under field conditions.

Duration of test

To determine preference-avoidance behaviour generaly three criteria are used: (1) time spent in the test water, (2) number of entries into the test water, and (3) distribution of fish to the test water after a given time interval (Giattina & Garton 1983). In all experiments observations are restricted to a standard test time, the length of which may determine whether or not preference-avoidance is observed:
In experiments of Jones (1948) it was evident that the fish took a progressively longer time to react to lower concentrations of sodiumsulphide. However, as Jones noted, in the end effective avoidance did occur in a 'reaction time' which was always substantially shorter than the survival time.
Hildebrand et al. (1982) compared avoidance of the herbicide glyphosate by rainbow trout after 20 minutes and 1 hour in a Y-maze. Avoidance response was stronger after one hour.
In experiments of Pedder & Maly (1985) with rainbow trout *(Salmo gairdneri)* initial attraction to copper containing water was observed, however this response was reversed to avoidance in 1 to 3 days.

Number of fish in test

Especially in case of schooling fish, it is important whether a single fish or a group was tested in the preference-avoidance experiments. Hall et al. (1982) compared avoidance responses of individual and schooling juvenile atlantic menhaden *(Brevoortia tyrannus).* The latter showed significantly greater avoidance responses than the former.
Kynard (1974) explicitly states use of individual mosquitofish *(Gambusia affinis)* since groups of 2, 4 and 10 exhibited schooling behiour. In contrast, Hall et al. (1984) used groups of atlantic menhaden because of natural schooling behaviour.

### 3.2.2 Differences in sensitivity

Between species

Preference-avoidance responses may vary greatly with fish species:
Birge & Black (1980) reported that rainbow trout were much more sensitive to cadmium, copper and zinc than bluegill sunfish *(Lepomis macrochirus)* and largemouth bass *(Micropterus salmoides)* (cited by Atchinson et al. 1987).
Sprague (1964, 1968) observed differences in sensitivity to zinc between atlantic salmon *(Salmo salar)* and rainbow trout. However, atlantic salmon were less active than rainbow trout and, because of this, the duration of the experiment might be responsible for this difference in response (Sprague 1968).
Largemouth bass was unresponsive, whereas channel catfish *(Ictalurus punctatus)* and goldfish *(Carassius auratus)* prefered 50 µg/L of copper. (Timms et al. 1972)
Differences in sensitivity were also observed between sheepshead minnows *(Cyprinodon variegatus)* and mosquitofish *(Gambusia affinis).* Sheepshead minnows avoided 2,4-D and DDT at lower concentrations than did mosquitofish. Mosquitofish, in contrast to sheepshead minnows, did avoid malathion and sevin, however they did not avoid endrin. (Hansen 1969 and Hansen et al. 1972)

Because homing salmonids cue to highly diluted pheromone or homestream odours, the olfactory organ in salmonids will generally be better developed and more sensitive to chemicals than the olfactory organs of other fish species (see § 4.1). It seems likely that salmonids will be able to detect, and avoid, chemicals at lower concentrations than other fish species.

Within species

Within species, preference-avoidance behaviour may vary with season and developmental stage: E.g. a mixture of aromatic hydrocarbons was avoided by late smolt coho salmon *(Oncorhynchus kisutch)* at significantly lower concentrations than was avoided by early smolt coho salmon (Maynard & Weber 1981).
Often, differences in behavioral disposition will be responsible for the observed variation in response. In this line Maynard & Weber (1981) gave the following explanation: Early smolts are territorial and aggressive and will resist displacement, whereas late smolts are aimed at migration and will more readily accept displacement. With similar reasoning, anadromous fish on their spawning migration will resist avoidance-reactions to chemicals interfering with their migration. Higher concentrations will be needed to elicit an avoidance response than will be needed in these fish without a migrational disposition.
On the other hand sensitivity of the olfactory epithelium may vary with season and developmental stage as a result of hormonal influences (Bertmar 1982, Hara 1967). Higher sensitivity would enhance the ability to avoid aquatic pollutants. In salmonids highest sensitivity of olfactory epithelium should be expected at the imprinting stage (smolting) and during spawning migration.

As a result of varying experiences, two populations of the same species may differ in preference-avoidance behaviour:
Hartwell et al. (1987A en B) compared preexposed and unexposed fathead minnows *(Pimephales promelas)* in their avoidance response to a blend of metals (Cu, Cr, Se and As). Unexposed fathead minnows avoided concentrations higher than 34 - 71 µg total metal/L, whereas fish preexposed to 96 µg total metal/L for 3 months were less sensitive or did not avoid the blend of metals at all.
Kynard (1974) studied avoidance behaviour of susceptible and resistant populations of mosquitofish *(Gambusia affinis)*. Susceptible fish avoided lower concentrations of DDT, endrin and parathion than did resistant fish. (Avoidance to toxaphene did not differ.)

Sprague (1968) reported that rainbow trout *(Salmo gairdneri)*, whether preexposed to 3 or 13 µg Zn/L for 3 weeks, did not differ in avoidance behaviour to zinc (threshold of avoidance being 5.4 µg Zn/L).

On the other hand, preexposure of rainbow trout to chromium for 7 weeks affected avoidance behaviour to chromium. The sensitivity to chromium decreased (increase in threshold of avoidance) with preexposure level. In a seven day recovery period, however, sensitivity of preexposed fish returned to unexposed level (Anestis & Neufeld 1986).

The general effect of preexposure of fish on avoidance seems to be a decrease in sensitivity. In this respect, the slow progress of salmon observed in the Miramichi estuary (Elson et al. 1972, Stasko 1975, see § 2.3) might be due to a gradual overcoming of an avoidance response to aquatic pollutants. As was already mentioned, slow progress in an estuary might be a response of anadromous fish to a gradual change in salinity or a change from open water to a confined channel. At the same time however, it may serve to increase the threshold of avoidance to aquatic pollutants.

### 3.2.3 Abiotic variables

Temperature

Temperature may influence preference-avoidance behaviour: Kleerekoper et al. (1973) using goldfish *(Carassius auratus)* observed avoidance of copper at 21.1°C, however attraction occurred at 21.5°C. Rainbow trout did not differ in avoidance behaviour to zinc, whether exposed at 9.5 or 17.0°C (Sprague 1968). As was demonstrated by Giattina et al. (1981), the threshold of avoidance of total residual chlorine by spotfin shiner *(Notropis spilopterus)* and whitetail shiner *(N. galacturus)* varies with temperature. Annual average temperature in the River Rhine is 12 - 14°C ('t Hoen 1987).

Light regime

Daylength may influence behaviour of fish: Northcote (1958 as cited by Maynard & Weber 1981) observed positive rheotaxis in rainbow trout at 8h daylength and negative rheotaxis at 16h daylength.

pH

Royce-Malmgren & Watson (1987) investigated the effect of acid rain on preference-avoidance behaviour to amino acids. Juvenile atlantic salmon *(Salmo salar)* are attracted to glycine and avoid L-alanine at ph 7.6. Lowering of pH to 5.1 resulted in inversion of this response. This is possibly due to changes in electric charge of these molecules.

Chemical speciation and bioavailability of some pollutants (e.g. heavy metals) is affected by pH. As in the case of L-alanine and glycine, this may result in changes in behavioural response to the chemical.

Annual average pH of the River Rhine at Lobith varies between 7.5 and 7.7 ('t Hoen 1987).

Complexing capacity

Especially heavy metals, but also organic chemicals may form complexes with inorganic (e.g. chloride, carbonate ions) and organic compounds (e.g. humic acids). The degree of complexation of heavy metals will increase with hardness, salinity and organic content of the test water. At the same time ionic activity will decrease. This will affect preference-avoidance behaviour since the availibility of the aquatic pollutant to the organism and to its receptors is altered.

Hardness

Total hardness especially affects the toxic action of heavy metals. In very soft water a certain concentration of a heavy metal may be an order of magnitude more toxic to a fish than in very hard water. This is not only due to changes in the complexing capacity of the water and ionic activity of the metal. Changes in gill permeability, caused by the calcium content of fish, are also considered responsible (Sprague 1985). Likewise, permeability and sensitivity of the olfactory epithelium may vary with hardness.

Total hardness in the River Rhine averages 240 - 250 mg $CaCO_3$/L ('t Hoen 1987), which is much higher than is costumary in preference-avoidance experiments.

### 3.2.4 Conclusions

The preceding paragraphs clearly illustrate that many factor may influence preference-avoidance behaviour. Up to now it is impossible to estimate the influence of each modifying factor on specific experimental results. In view of the limited research in this field, it is not to be expected that this will change in near future. Even today, interlab experiments on acute toxicity (e.g. LC50) show variations of a factor 1.2 upto 10, which can not be explained,

despite the extensive research that has been performed (Sprague 1985).
In establishing concentration levels which threaten the spawning migration of anadromous fish, the best strategy seems to be the use of lowest concentrations eliciting avoidance behaviour (worst-case treatment). Especially since salmonids are expected to be amongst the most sensitive of fish species (see § 3.2.2).

## 3.3 Avoidance in the laboratory

In the following paragraphs, data on avoidance are reviewed of IRC prioritay pollutants (§ 3.3.1), other pollutants (§ 3.3.2) and mixtures of pollutants (§ 3.3.3). Concentrations eliciting an avoidance response are compared to the proposed ecotoxicological water quality objectives and concentrations measured in the River Rhine at Lobith in 1985 (IRC reference year), which are not very different from present-day concentrations (DBW/RIZA). As was already mentioned in § 3.1 average concentrations at Lobith are not entirely representative of concentrations of pollutants occurring in the River Rhine. Additional data on concentrations in effluent mixing zones will therefore be presented, however knowledge on concentrations in effluent plumes is limited.

In most preference-avoidance experiments organisms are exposed to concentrations of dissolved pollutants. Effective levels are therefore compared with concentrations and water quality objectives of dissolved pollutants.

### 3.3.1 Prioritairy compounds

In table 2 preference-avoidance responses of fish to prioritairy compunds are summarized. Only a limited number of compounds (18) has been tested and testing of a compound often was restricted to only one species, using unrealistically high concentrations. In view of this, further research must be expected to result in observations of avoidance at *lower* concentration levels than has been reported so far.

ORGANIC MICROPOLLUTANTS
With the exception of tetrachloroethylene, none of the organic micropollutants tested were reported to elicit avoidance behaviour at concentrations near the proposed water quality objectives and average concentrations in the River Rhine at Lobith in 1985. However, in effluent mixing zones concentrations occur that are much higher than these average concentrations. Concentrations of chloroform have been reported that were 2½ times as high as the LOEC (150 µg/L; the lowest concentration that was tested by Jones (1947)).
Although the avoidance responses to organic micropollutants occurred at relatively high concentration levels, the fish tested must be considered quite sensitive: In most cases avoidance was observed in a short time (usually 10 - 90 minutes), during which the test concentrations did not have any toxic effect.

Benzene was not avoided by green sunfish *(Lepomis cyanellus)*, however only a very high concentration (400 mg/L) was tested (Summerfelt & Lewis 1967). Benzene already elicited an olfactory response at concentrations between 0.2 and 2 mg/L in coho salmon (electrophysiological experiments by Malins et al. 1977) and was suggested to have disruptive effects on olfaction at chronic exposure. Coho salmon avoided benzene at concentrations higher than 1.75 mg/L, while 96h LC50 was 14.1 mg/L (Maynard & Weber 1981).
Water quality objective for benzene (9 µg/L - Stortelder et al. 1989) was much

lower than the reported effective concentrations. In discharges however, concentrations of 25 µg/L - 7 mg/L of benzene occur, which - on initial dilution of 1 : 10 - result in maximum concentrations of 2.5 - 700 µg/L in effluent mixing zones (DBW/RIZA).

Captan did not depress positive rheotaxis in rainbow trout on exposure to 250 and 500 µg/L, however death occurred after some time (van Hooff 1980). Water quality objective for captan is much lower (0.26 µg/L - Stortelder et al. 1989).

Chloroform was prefered by rainbow trout at concentrations higher than 12 mg/L (Black & Birge as cited by Hara et al. 1983), but was avoided by tenspiked stickleback *(Pigosteus pungitus)* at concentrations as low as 150 µg/L (Jones 1947).

The proposed water quality objective for chloroform is 0.6 µg/L (Stortelder et al. 1989). Concentrations of 0.5 - 3 µg/L occur regularly in surface waters and incidentally concentrations of 400 µg/L are measured (DBW/RIZA), which is higher than the effective concentration reported by Jones (1947).

Endosulfan can be detected at concentrations as low as 1 µg/L in an early warning system using positive rheotaxis (BKH 1988). The proposed water quality objective for endosulfan is even lower (0.014 µg/L - Stortelder et al. 1989).

Endrin was avoided by sheepheads minnow *(Cyprinodon variegatus)* at concentarations higher than 1 µg/L (Hansen 1969) and at concentrations higher than 20 µg/L by mosquitofish *(Gambusia affinis)* (Hansen et al. 1972). At 4 µg/L effects on positive rheotaxis were observed (BKH 1988).

Water quality objective for endrin is 0.005 µg/L (Stortelder et al. 1989). Concentrations of 0.01 µg/L of drins in effluents have been reported (DBW/RIZA).

Ethylparathion did not affect positive rheotaxis in rainbow trout at 3 mg/L (van Hooff 1980). The proposed water quality objective is 0.02 µg/L (Stortelder et al. 1989).

A PCB was avoided by pin fish *(Lagodon rhomboides)* and mosquitofish at extremely high concentrations (Hansen et al. 1974). Based on QSAR studies, water quality objectives for PCBs are 0.001 µg/L. At Lobith, in the River Rhine concentrations of this magnitude occur ('t Hoen 1987).

Pentachlorophenol was avoided by medaka *(Oryzias latipes)* at concentrations in the ppb range (Hidaka et al. 1984) and by green sunfish at concentrations in the ppm range (Summerfelt and Lewis 1967). Water quality objective for pentachlorophenol is 0.38 µg/L (Stortelder et al. 1989). Average concentration in the River Rhine, at Lobith, are 0.33 µg/L. No data on concentrations in effluents are available (DBW/RIZA).

Simazine affected positive rheotaxis in rainbow trout at concentrations between 1 and 12.5 mg/L (Dodson & Mayfield 1979B). The proposed water quality objective is much lower (0.38 µg/L - Stortelder et al. 1989). Average concentrations (several years) of simazine in the River Rhine are 0.4 µg/L, with a (measured) maximum of 3.6 µg/L (DBW/RIZA).

Tetrachloroethylene affected positive rheotaxis in rainbow trout at 4 µg/L in an early warning system (BKH 1988). The proposed water quality objective for tetrachloroethylene is 4.9 µg/L (Stortelder et al. 1989). In 1985 concentrations in the River Rhine, at Lobith, were lower (average 0.26 µg/L, maximum 1.0 µg/L - DBW/RIZA).

TBTO (bis(tri-n-butyltin)oxide) was avoided at concentrations higher than 1-25 µg/L by striped bass *(Morone saxatilus)*, atlantic menhaden *(Brevoortia tyrannus)* and mummichog *(Fundulus heteroclitus)* (Hall et al. 1984, Pinkney et al. 1985). Affected positive rheotaxis was observed at concentrations higher than 11.7 µg/L in rainbow trout and *Tilapia rendali* (Chliamovitch & Kuhn 1977).

Water quality objectives for tributyltin compounds are 0.003 µg/L (Stortelder et al. 1989). In the River Rhine concentrations of 0.28 µg/L have been measured (DBW/RIZA).

# - TABLE 2 -
# (IRC prioritairy pollutants)

*Table 2*
*Preference-avoidance responses in the laboratory to dissolved IRC prioritary compounds. The following responses were observed: avoidance (avoid) and preference (pref) usually in a steep, but sometimes in a shallow gradient (shal); affected positive rheotaxis (pos.rheo). Also detection limits of pollutants in early warning systems using positive rheotaxis (EWSdetect) are given. For dissolved pollutants, proposed water quality objectives (Stortelder et al. 1989) and average concentrations at Lobith in the River Rhine in 1985 (DBW/RIZA) are given. In appendix 4 english names of species are listed.*

*Abbreviations: NOEC (No Observed Effect Concentration), LOEC (Lowest Observed Effect Concentration) LC50 (concentration lethal to 50% of the test organisms).*
*Notes: 1 highest concentration in experiment; 2 lowest concentration in experiment; 3 only concentration in experiment; 4 preference observed at lower concentrations; 5 abolished avoidance at high concentrations; * threshold concentration of response.*

| COMPOUND | REFERENCE | TESTORGANISM species | seize | number | EXPERIMENTAL CONDITIONS hardness mg CaCO3/L | pH | T °C | time m | NOEC ug/l | LOEC ug/l | note | RESPONSE | TOXICITY time | LC50 ug/l | ECOTOX OBJECTIVE ug/l | RHINE 1985 ug/l |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Benzene | Summerfelt & Lewis 1967 | Lepomis cyanellus | 10.8 cm | 30 | | 7.2 | 23 | 30 | 400 000 | | 3 | avoid | | | 9 | 0.0 |
| | Maynard & Weber 1981 | Oncorhynchus kisutch | 6 cm | 20-60 | | | 5-17 | 75 | | 1 750 | * | avoid | 96h | 14 ppm | | |
| Captan | van Hooff 1980 | Salmo gairdneri | 200 g | 1 | 250 | 8.0 | 7 | 6h | 250,500 | | | pos.rheo | within test | | 0.26 | |
| Chloroform | Black & Birge 1980 (in Hara et al. 1983) | Salmo gairdneri | | | | | | | | 11 900 | * | pref | | | 0.6 | |
| | Jones 1947 | Pigosteus pungitus | 2.7 cm | 5? | | | 15 | 10 | | 150 | 2 | avoid | | | | |
| | Summerfelt & Lewis 1967 | Lepomis cyanellus | 10.8 cm | 30 | | 7.2 | 23 | 30 | 20 000 | | 3 | avoid | | | | |
| Endosulfan | BKH 1988 | | | | | | | 84h | | 1 | | EWSdetect | | | 0.014 | 0.000 |
| Endrin | Hansen 1969 | Cyprinodon variegatus | 3 cm | 50 | 20%. sal | | 20 | 90 | 0.1 | 1 | 5 | avoid | 24h | 3 | | |
| | Hansen et al. 1972 | Gambusia affinis | 3.3 cm | 50 | | | 20 | 90 | 0.1 - 10 | | | avoid | 24h | 7 | | |
| | Kynard 1974 | Gambusia affinis | | 1 | | | | 15 | | | | | | | | |
| | | susceptible | | | | | | | 20 | 250 | | avoid | 24h | 2 | | |
| | | resistant | | | | | | | 250 | 1 000 | | avoid | 24h | 1 000 | | |
| | BKH 1988 | | | | | | | 84h | | 4 | | EWSdetect | | | | |
| Ethylparathion | van Hooff 1980 | Salmo gairdneri | 200 g | 1 | 250 | 8.0 | 7 | ? | 3 000 | | 3 | pos.rheo | | | 0.02 | |
| PCB Aroclor 1254 | Hansen et al. 1974 | Lagodon rhomboides | 3.5 cm | 50 | 20 %. sal | | | 90 | 480 | 5 700 | | avoid | | | 0.001 | 0.002 |
| | | Cyprinodon variegatus | 3.5 cm | 50 | 20 %. sal | | | 90 | 1 ppb-10 ppm | | | avoid | | | | |
| | | Gambusia affinis | 3.5 cm | 50 | | | | 90 | 100 | 5 700 | | avoid | | | | |
| Pentachlorophenol | Hidaka et al. 1984 | Oryzias latipes | | | 45 | 7.2 | 25 | ?10 | | 42 | *5 | avoid | | | 0.38 | 0.033 |
| | Summerfelt & Lewis 1967 | Lepomis cyanellus | 10.8 cm | 30 | | 7.2 | 23 | 30 | 5 000 | 20 000 | | avoid | ? | 200-600 | | |
| Simazine | Dodson & Mayfield 1979B | Salmo gairdneri | | 1 | 97 | 7.2 | 15 | 25 | | ?1-12.5 ppm | | pos.rheo | 24h | 95 000 | 0.38 | |
| | Princep 80W (form.) | | | | | | | | 1-12.5ppm | | | pos.rheo | | | | |
| TBTO (organotin) | Hall et al. 1984 | Morone saxatilus | | 10 | 10%. sal | 7.7 | 26 | 40 | 15 | 25 | | avoid | | | 0.001 | |
| | | Brevoortia tyrannus | | 1 | | | | | 2.5 | 5.5 | | avoid | | | | |
| | Pinkney et al. 1985 | Fundulus heteroclitus | 7.3 cm | 10 | 11%. sal | 7.4 | 25 | 40 | 1 | 3.7 | | avoid | | | | |
| | Chliamovitch & Kuhn 1977 | Salmo gairdneri | 11 cm | | 350 | 7.7 | 16 | 4d | | 11.7 | 2 | pos.rheo | 24h | 26-30 | | |
| | | Tilapia rendalli | 8.6 cm | | | | 25 | 5d | | 11.7 | 2 | pos.rheo | | | | |
| Tetrachloroetheen | BKH 1988 | Salmo gairdneri | | | | | | 30h | | 4 | | EWSdetect | | | 4.9 | 0.26 |
| Cd | Black & Birge 1980 (in Hara et al. 1983) | Salmo gairdneri | | | 112 | 7.8 | | | | 52 | * | avoid | | | 0.025 | 0.04 |
| | van Hooff 1980 | Salmo gairdneri | 200 g | | 250 | 8 | 7 | ? | 1 - 3 ppm | | | pos.rheo | | | | |
| Cr | Anestis & Neufeld 1986 | Salmo gairdneri | | 18 | 100 | 7.2 | 15 | 35 | | 28 | * | avoid | | | 2.5 | 1.1 |
| | | 7 wk preexposure 10 ug/L | | | | | | | | 71 | * | | | | | |
| | | 100 ug/L | | | | | | | | 220 | * | | | | | |
| | | 300 ug/L | | | | | | | | 170 | * | | | | | |
| | Hadjinicolaou & Spraggs 1988 | Salmo gairdneri | 11 cm | 100 | | 8.3 | 15 | 135 | 15 - 444 | | | avoid | | | | |

## - TABLE 2 -
## (IRC prioritairy pollutants)

| COMPOUND | REFERENCE | TESTORGANISM | | | EXPERIMENTAL CONDITIONS | | | | NOEC | LOEC | | RESPONSE | TOXICITY | | ECOTOX | RHINE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | species | seize | number | hardness mg CaCO3/L | pH | T °C | time m | ug/l | ug/l | note | | time | LC50 ug/l | OBJECTIVE ug/l | 1985 ug/l |
| Cu | Kleerekoper et al. 1972 | Carassius auratus | 30 cm | 7 | | | | | | 11 - 50? | | avoid shal | | | 1.3 | 3.0 |
| | Kleerekoper et al. 1973 | Carassius auratus | 29 cm | 1 | 5 | 8.4 | 21.1 | 24h | | 10 | 3 | avoid shal | | | | |
| | | Carassius auratus | 29 cm | 1 | 5 | 8.4 | 21.5 | 24h | | 10 | 3 | pref shal | | | | |
| | Westlake et al. 1974 | Carassius auratus | 29 cm | 1 | 5 | 8.4 | 21 | 6h | | 5 | 25 | avoid | | | | |
| | Timms et al. 1972 | Carassius auratus | 29 cm | 1 | 5 | 8.4 | 19 | 8h | | ? - 50 | | pref shal | | | | |
| | | Micropterus salmoides | 29 cm | 1 | | | | | ? - 50 | | | pref shal | | | | |
| | | Ictalurus punctatus | 34 cm | 1 | | | | | | ? - 50 | | pref shal | | | | |
| | Jones 1947 | Pigosteus pungitus | 2.7 cm | 5 | | | 15 | 10 | 63 500 | 2.5 g/l | 4 | avoid | | | | |
| | Folmar 1976 | Salmo gairdneri | fry | 10 | 90 | 8 | 11 | 60 | | 0.1 | 2 | avoid | 96h | 140 | | |
| | Giattina et al. 1982 | Salmo gairdneri | 7 cm | 1 | 28 | 7.3 | | 20 | 3.2 | 5.2 | 5 | avoid | | | | |
| | | | | | | | | | 2.8 | 4.4 | 5 | avoid | | | | |
| | Black & Birge 1980<br>(in Giattina & Garton 1983) | Salmo gairdneri | | | 112 | | | | | 70 | •4 | avoid | | | | |
| | Pedder & Maly 1985 | Salmo gairdneri | 15 cm | 20 | 122 | 7.2 | 13 | 96h | | 500 | 2 | avoid | | | | |
| | Hara 1981 (Hara et al. 1983) | Coregonus clupeaformis | | | | | | | | 6.4 | •5 | avoid | | | | |
| | Takayasu & Sotooka 1924<br>(in Hara et al. 1983) | Oncorhynchus masou | | | | | | | | 50 | • | avoid | incip | 44 | | |
| | Sprague 1964 | Salmo salar | 13 cm | 1 | 18 | 7.5 | 18 | 10 | | 2.4 | • | avoid | | | | |
| | Summerfelt & Lewis 1967 | Lepomis cyanellus | 10.8 cm | 30 | | 7.2 | 23 | 30 | 20 000 | | 3 | avoid | | | | |
| | Rehnberg & Schreck 1986 | Oncorhynchus kisutch | 2.9 g | 10 | 31 | 6.7 | 15 | 2.5h | | 6.4 | 2 | pos.rheo | | | | |
| | BKH 1988 | Salmo gairdneri | | | | | | 4h | | 500 | | EWSdetect | | | | |
| Mg | Kamchen & Hara 1980<br>(in Brown et al. 1982) | Coregonus clupeaformis | | | | | | | 2 000 | 20 000 | | avoid | | | 0.005 | 0.01 |
| | Jones 1947 | Pigosteus pungitus | 2.7 cm | 5? | | | 15 | 10 | 20 000 | 60 000 | | avoid | | | | |
| | Summerfelt & Lewis 1967 | Lepomis cyanellus | 10.8 cm | 30 | | 7.2 | 23 | 30 | 20 000 | | 3 | avoid | | | | |
| | Black & Birge 1980<br>(in Atchinson et al. 1987) | Salmo gairdneri | | | 112 | 7.6 | | | | 0.2 | | pref | | | | |
| | Rehnberg & Schreck 1986 | Oncorhynchus kisutch | 2.9 g | 10 | 31 | 6.7 | 15 | 2.5h | | 20 | 2 | pos.rheo | | | | |
| Ni | Hadjinicolaou & Spraggs 1988 | Salmo gairdneri | 11 cm | 100 | | 6.3 | 15 | 135 | 35 | 147 | | avoid | | | 7.5 | 3.7 |
| | Giattina et al. 1982 | Salmo gairdneri | 7 cm | 1 | 28 | 7.3 | | 20 | 21 | 35 | | avoid | | | | |
| | | | | 1 | | | | | 15 | 23 | | avoid shal | | | | |
| Pb | Giattina & Garton 1983 | Salmo gairdneri | | 1 | 29 | | | | | 28 | • | avoid | | | 1.3 | 0.2 |
| | Jones 1948 | Grasostreus aculeatus | | 5 | | 6.8 | 14 | 47 | | 2 000 | 2 | avoid | | | | |
| | | Phoxinus phoxinus | 2.8 cm | 5 | | | | | | 400 | 2 | avoid | | | | |
| | Summerfelt & Lewis 1967 | Lepomis cyanellus | 10.8 cm | 30 | | 7.2 | 23 | 30 | 10-40 ppm | | | avoid | | | | |
| Zn | Black & Birge 1980<br>(in Atchinson et al 1987) | Salmo gairdneri | | | 112 | 7.6 | | | | 47 | • | avoid | | | 6.5 | 18 |
| | Brown et al. 1982 | Coregonus clupeaformis | | | | | | | 46 | 460 | | avoid | | | | |
| | Takayasu & Sotooka 1924<br>(in Hara et al. 1983) | Oncorhynchus masou | | | | | | | | 3 270 | • | avoid | | | | |
| | Jones 1947 | Pigosteus pungitus | 2.7 cm | 5? | | | 15 | 30 | 21 000 | 82 000 | | avoid | | | | |
| | Sprague 1964 | Salmo salar | 13 cm | 1 | 18 | 7.5 | 18 | 10 | | 53 | • | avoid | incip | 580 | | |
| | Sprague 1968 | Salmo gairdneri | 9 cm | 1 | 14 | 7.2 | 17 | 10 | 3.2 | 10 | | avoid | | | | |
| | Summerfelt & Lewis 1967 | Lepomis cyanellus | 10.8 cm | | | 7.2 | 23 | 30 | 40,80 ppm | | 1 | avoid | | | | |
| | Rehnberg & Schreck 1986 | Oncorhynchus kisutch | 2.9 g | 10 | 31 | 6.7 | 15 | 2.5h | | 6.5 | 2 | pos.rheo | | | | |
| | van Hooff 1980 | Salmo gairdneri | 200 g | | 250 | 8 | 7 ? | | 1.5-3 ppm | | 1 | pos.rheo | | | | |

HEAVY METALS

Although preference-avoidance responses of fish to heavy metals have been investigated more extensively, the number of observations is still quite limited. In a number of experiments heavy metals were avoided at concentrations near the proposed water quality objectives and average concentrations in the River Rhine at Lobith (e.g. figure 7). In particular, fish seem to be quite sensitive to copper and zinc (which have been studied most extensively).

In most experiments the water was soft, compared to the River Rhine (240-250 mg $CaCO_3$/L - 't Hoen 1987), and the fish tested probably will be less sensitive to pollutants in the River Rhine. On the other hand, it is doubtfull whether testing has uncovered the lowest concentration at which avoidance may be observed. In addition, concentrations of pollutants in the River Rhine may occur that are much higher than those measured in the sampling program at Lobith (e.g. in effluent mixing zones (table 3) or further downstream) and additive effects may occur in mixtures of pollutants (as will be shown in § 3.3.3, avoidance to copper may occur at even lower concentrations in the presence of zinc and viceversa).

Of twelve fish species, rainbow trout *(Salmo gairdneri)* was most frequently used in these experiments. Next in line was green sunfish *(Lepomis cyanellus)* which was used by Summerfelt & Lewis (1967) in avoidance experiments with unrealistically high concentrations of copper, mercury, lead and zinc (10 - 80 mg/L).

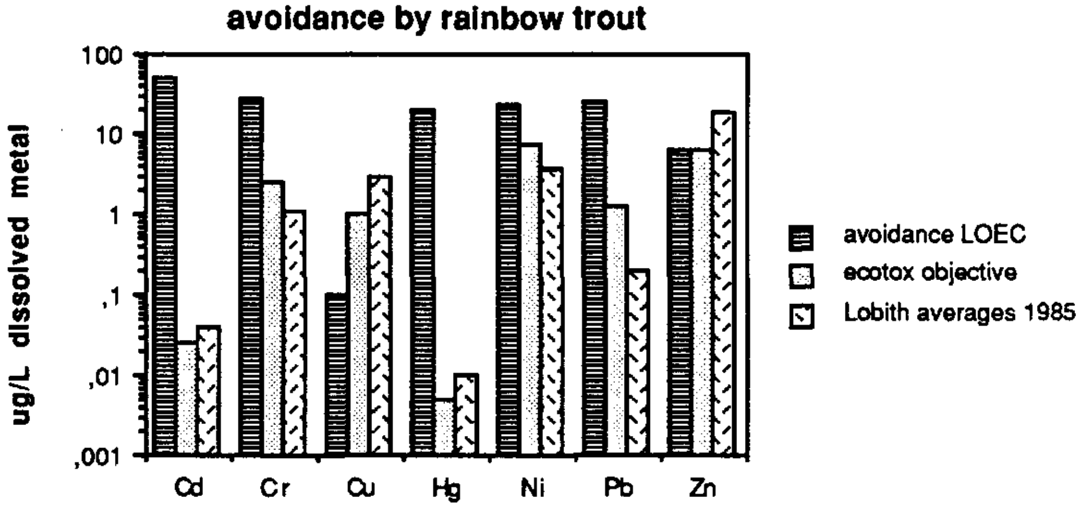


*Figure 7*
*Comparison of avoidance responses of rainbow trout (Salmo gairdneri) to dissolved heavy metals, concentrations proposed as water quality objectives (Stortelder et al. 1989) and average concentrations in the River Rhine at Lobith (dissolved, 1985, DBW/RIZA). Avoidance levels are lowest concentrations reported to have effect (selected from table 2). In case of Zn and Hg positive rheotaxis was affected, the other chemicals were tested in gradient chambers.*

*Table 3*
*Comparison of concentrations of pollutants occurring in the River Rhine: average concentrations of dissolved metals at Lobith (1985) and maximum concentrations expected in mixing zones of domestic sewage treatment plant effluents and industrial effluents (DBW/RIZA). An initial dilution of the effluent of 1 : 10 is assumed. In addition proposed dutch water quality objectives for dissolved metals (Stortelder et al. 1989) and lowest concentrations reported to elicit avoidance are given.*

| Pollutant | Lobith averages 1985 µg/L | Maxima in mixing zone of domestic sewage µg/L | Maxima in mixing zone of industrial effluent µg/L | Proposed ecotoxicological objective µg/L | Avoidance LOEC µg/L |
|---|---|---|---|---|---|
| Cd | 0.04 | 1 | 300-400 | 0.025 | 52 |
| Cr | 1.1 | 10 | 300-400 | 2.5 | 28 |
| Cu | 3.0 | 10 | 300-400 | 1.3 | 0.1 |
| Hg | 0.01 | 0.03 | | 0.005 | 20 |
| Ni | 3.7 | 10 | 300-400 | 7.5 | 23 |
| Pb | 0.2 | 10 | 300-400 | 1.3 | 26 |
| Zn | 18 | 45 | 300-400 | 6.5 | 6.5 |

Cadmium The proposed water quality objective for dissolved cadmium is 0.025 µg/L (Stortelder et al. 1989). Average concentration at Lobith in 1985 was 0.04 µg/L of dissolved Cd. Total Cd averaged 0.14 µg/L with a maximum of 0.22 µg/L (DBW/RIZA). In mixing zones of industrial effluents concentrations as high as 400 µg/L may occur (DBW/RIZA), which is higher than the LOEC reported for rainbow trout by Black & Birge (1980 as cited by Hara et al. 1983): 52 µg/L in relatively soft water (112 mg $CaCO_3$/L).
At high concentrations (1 - 3 mg/L) van Hooff (1980) did not observe any effect on positive rheotaxis of rainbow trout.
Chromium. The proposed water quality objective for dissolved chromium is 2.5 µg/L (Stortelder et al 1989). Average concentrations of dissolved and total chromium in 1985 in the River Rhine at Lobith were 1.1 µg/L and 7.6 µg/L respectively. The highest concentration measured was 13.4 µg/L of total chromium. In mixing zones of industrial effluents concentrations as high as 400 µg/L may occur (DBW/RIZA), which is higher than the LOEC reported by Anestis & Neufeld (1986), 28 µg/L for rainbow trout in relatively soft water (100 mg $CaCO_3$/L).
Hadjinicolaou & Spraggs (1988) did not observe any avoidance by rainbow trout between 15 and 445 µg/L.
Copper The proposed water quality objective for dissolved copper is 1.3 µg/L (Stortelder et al. 1989), whereas concentrations of dissolved and total copper in 1985 in the River Rhine at Lobith averaged 3.0 µg/L and 5.9 µg/L respectively (DBW/RIZA). Avoidance was observed at concentrations approximating these values. Concentrations as high as 400 µg/L may even occur in mixing zones of industrial effluents (DBW/RIZA). The highest concentration of total copper that was measured at Lobith in 1985 was 9.6 µg/L.
Fifteen references report on preference-avoidance experiments with copper. As was already mentioned in § 3.2.1 and 3.2.3, Kleerekoper and coworkers observed preference or avoidance of copper (5 - 50 µg/L in very soft water: 5 mg $CaCO_3$/L) depending on steepness of the copper gradient and temperature (Kleerekoper et al. 1972 and 1973, Timms et al. 1972, Westlake et al. 1974). Avoidance of copper by rainbow trout was observed by Folmar (1976), Black & Birge (1980 as cited by Giattina & Garton 1983), Giattina et al. (1982) and

Pedder & Maly (1985). In the latter experiment relatively high concentrations were used (>500 µg/L) and the NOEC level was not determined. The results of the remaining three experiments vary considerably: Folmar (1976) observed avoidance at concentrations as low as 0.1 µg/L (90 mg $CaCO_3$/L). Black & Birge (1980 as cited by Giattina & Garton 1983) on the other hand reported a threshold of avoidance of 70 µg/L at approximately the same hardness.

Other salmonids also avoided copper: In very soft water (18 mg $CaCO_3$/L), threshold of avoidance for atlantic salmon *(Salmo salar)* was 2.4 µg Cu/L (Sprague 1964). Threshold of avoidance for masou salmon *(Oncorhynchus masou)* was 50 µg/L (Takayasu & Sotooka 1924 as cited by Hara et al. 1983).

Positive rheotaxis was depressed in coho salmon *(O. kisutch)* at 6.4 µg/L in very soft water (31 mg $CaCO_3$/L - Rehnberg & Schreck 1986) and in rainbow trout at 500 µg/L (BDH 1988).

Lake whitefish *(Coregonus clupeaformis)* was reported to avoid copper at concentrations higher than 6.4 µg/L (Hara 1981 as cited by Hara et al. 1983).

Mercury The proposed water quality objective for dissolved mercury is 0.005 µg/L (Stortelder et al. 1989). At Lobith the average concentration in 1985 was 0.01 µg dissolved Hg/L, but concentrations as high as 0.19 µg/L total Hg were measured (DBW/RIZA). Avoidance was only observed at higher concentrations.

Rehnberg & Schreck (1986) reported depressed positive rheotaxis in coho salmon at concentrations as low as 20 µg/L. At a lower concentration (0.2 µg/L) preference for mercury was observed in rainbow trout (Black & Birge 1980 as cited by Hara et al. 1983). Summerfelt & Lewis (1967), Jones (1947) and Kamchen & Hara (1980 as cited by Hara et al. 1983) performed tests at extremely high concentrations (in the mg/L range). In two out of three cases avoidance was observed. Other senses then olfaction are probably involved in these avoidance responses: Experiments with coho salmon demonstrated complete blocking of electrical response of the olfactory bulb to a stimulus after seconds of exposure to concentrations of this magnitude (Sutterlin & Sutterlin 1971, Hara 1972).

Nickel The proposed water quality objective for dissolved nickel is 7.5 µg/L and average concentrations at Lobith in 1985 were 3.7 µg/L and 4.8 µg/L of dissolved and total nickel respectively. The highest concentration of total nickel measured at Lobith in 1985 was 9.2 µg/L. Concentrations as high as 400 µg/L may occur in mixing zones of industrial effluent (DBW/RIZA), which is higher than reported effective levels.

Avoidance experiments with rainbow trout by Hadjinicolaou & Spraggs (1988) and Giattina et al. (1982) agree fairly well. Reported thresholds of avoidance are 35 - 147 µg/L and 21 - 35 µg/L (in very soft water, 28 mg $CaCO_3$/L) respectively.

Lead. Summerfelt & Lewis (1967) and Jones (1947) tested high concentrations of lead. Jones observed avoidance behaviour at concentrations higher than 2000 µg/L *(Gastereosteus aculeatus)* and 400 µg/L *(Phoxinus phoxinus)* but did not determine NOEC levels. Threshold of avoidance for rainbow trout was much lower (26 µg/L in very soft water 29 mg$CaCO_3$/L - Giattina & Garton 1983).

The proposed water quality objective for dissolved lead is 1.3 µg/L (Stortelder et al. 1989) whereas concentrations of dissolved and total lead in 1985 in the River Rhine averaged 0.2 µg/L and 4.2 µg/L respectively, with a maximum of 7.1 µg total Pb/L (DBW/RIZA). In mixing zones of industrial effluents concentrations as high as 400 µg/L may occur, which exceeds the LOEC reported for rainbow trout.

Zinc. Water quality objective for dissolved zinc is 6.5 µg/L (Stortelder et al. 1989), whereas concentrations of dissolved and total zinc in 1985 in the River Rhine averaged 18 µg/L and 51 µg/L respectively. The maximum concentration measured that year was 100 µg/L of total Zn. Concentrations as high as 400 µg/L may occur in the mixing zone of industrial effluents (DBW/RIZA). Avoidance was observed at concentrations approximating the water quality objective.

In general salmonids seem to be quite sensitive to zinc. Two thresholds of avoidance were reported for rainbow trout: 3.2 - 10 µg/L (Sprague 1968) and 47 µg/L (Black & Birge 1980 as cited by Atchinson et al. 1987) in very soft and soft water (14 and 112 mg $CaCO_3$/L respectively). However, no effect on positive rheotaxis was observed at 1.5 mg Zn/L (van Hooff 1980). Atlantic salmon avoided zinc concentrations higher than 53 ug/L in very soft water (18 mg $CaCO_3$/L - Sprague 1964) whereas threshold of avoidance for masou salmon was reported to be much higher (3.27 mg/L - Takayasu & Sotooka 1924 as cited by Hara et al. 1983). Rehnberg & Schreck (1986) reported depressed positive rheotaxis in coho salmon at concentrations as low as 6.5 µg/L in very soft water (31 mg $CaCO_3$/L).
Non-salmonids were tested at extremely high concentrations by Jones (1947) and Summerfelt & Lewis (1967).

### 3.3.2 Other pollutants

In table 4 **data** on avoidance of a large number of non-prioritairy compounds are summarized. On avoidance of chlorine and chlorinated effluent is not reported here (see data given by Giattina et al. 1983 and Beitinger & Freeman 1983, appendix 3).
It is difficult to compare these data with concentrations in the River Rhine since these are often unknown. Ecotoxicological water quality objectives have not been formulated for most pollutants. In addition, testing was usually restricted to one concentration with only one species. Because of this, effective levels are compared with toxicity values. Of the compounds listed only the most interesting will be discussed here. Further research may show sensitivity of fish to lower concentrations and other pollutants than have been tested so far.

A number of compounds elicited avoidance responses at sublethal concentrations e.g.: acrolein, toluene, xylene, cyanide, the organochlorine insecticide 2,4-D, the organophosphate pesticides Diazinon, Dursban, Fenitrothion (= MEP or sumithion) and IBP, the carbamate NAC, the insect growth regulator Dimilin, and all detergents tested. Of these compounds especially the detergents seem to pose a threat to the spawning migration of anadromous fish, because of their widespread use.

Cyanide was tested by Summerfelt & Lewis (1967 - avoidance) and van Hooff (1980 - rheotaxis). LOECs are just below the lethal threshold reported by van Hooff (1000 µg/L).
Toluene was avoided at concentrations higher than 1.4 - 1.8 mg/L by coho salmon *(Oncorhynchus kisutch)* (Maynard & Weber 1981), whereas effects of toluene on positive rheotaxis in rainbow trout were observed at 12 µg/L (BKH 1988). Maynard & Weber reported 96h LC50 values between 6.4 and 8.1 mg/L.
Xylene (1,4 dimethylbenzene) was avoided by atlantic and coho salmon at concentrations higher than 100 and 680 µg/L respectively, whereas 96h LC50 were 10 and 13.5 mg/L (Folmar 1976, Maynard & Weber 1981).
2,4-D Proposed water quality objective for 2,4-D is 11 µg/L (Stortelder et al 1989). In all experiments avoidance occurred at higher concentrations; threshold concentrations of response were 1 mg/L or lower. Sheepshead minnows *(Cyprinodon variegatus,* Hansen 1969) and medaka *(Oryzias latipes,* Hidaka et al. 1984) seem to be more sensitive than rainbow trout (Folmar 1976) and mosquitofish *(Gambusia affinis,* Dodson & Mayfield 1979A), however different forms of 2,4-D were used.

# - TABLE 4 -
## (non-prioritairy pollutants)

*Table 4*
*Preference-avoidance responses in the laboratory to dissolved non-prioritary compounds. The following responses were observed: avoidance (avoid) and preference (pref) usually in a steep, but sometimes in a shallow gradient (shal); affected positive rheotaxis (pos.rheo). Also detection limits of pollutants in early warning systems using positive rheotaxis (EWSdetect) are given. For dissolved pollutants, proposed water quality objectives (Stortelder et al. 1989) and average concentrations at Lobith in the River Rhine in 1985 (DBW/RIZA) are given. In appendix 4 english names of species are listed.*

*Abbreviations: NOEC (No Observed Effect Concentration), LOEC (Lowest Observed Effect Concentration) LC50 (concentration lethal to 50% of the test organisms).*
*Notes: 1 highest concentration in experiment; 2 lowest concentration in experiment; 3 only concentration in experiment; 4 preference observed at lower concentrations; 5 abolished avoidance at high concentrations; * threshold concentration of response.*

| | COMPOUND | | REFERENCE | TESTORGANISM species | seize | EXPERIMENTAL CONDITIONS hardness mg CaCO3/L | pH | T °C | time m | NOEC (ug/L) | LOEC (ug/L) | note | RESPONSE | TOXICITY LC50 (ug/L) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AS | Laurylsulfaat, | Na | Hidaka & Tatsukawa 1985 | Oryzias latipes | 2.7 cm | 45? | 7.2? | 25 | 20 | | 7.1 | * | avoidance | |
| | (anionic) | | Tatsukawa & Hidaka 1978 | Plecoglossus altivelis | 10 cm | | 7.7 | 25 | | | 6.4 | * | avoidance | |
| | | | formulation* | | | | | | | | 4 | * | avoidance | |
| | | | Zlotkin & Gruber 1984 | Negaprion brevirostris | 7.2 cm | | | | | | 200 | 2 | avoidance | 3-6ppm |
| | | | Thompson & Hara 1978 | Coregonus clupeaformis | 15 cm | 78 | 7.5 | 11 | 10 | 10 - 10 000 | | 4 | avoidance | |
| | | Li | Zlotkin & Gruber 1984 | Negaprion brevirostris | 7.2 cm | | | | | | 200 | | avoidance | 3-6ppm |
| LAS | Lineair laurylbenzeen | | Hidaka & Tatsukawa 1985 | Oryzias latipes | 2.7 cm | 45? | 7.2? | 25 | 20 | | 14 | *5 | avoidance | |
| | sulfonate | | Tatsukawa & Hidaka 1978 | Plecoglossus altivelis | 10 cm | | 7.7 | 25 | | | 1.5 | * | avoidance | |
| | (anionic) | | formulation* | | | | | | | | 0.11 | * | avoidance | |
| ABS | Branched laurylbenzeen | | Hidaka & Tatsukawa 1985 | Oryzias latipes | 2.7 cm | 45? | 7.2? | 25 | 20 | | 14 | * | avoidance | |
| | sulfonate | | Tatsukawa & Hidaka 1978 | Plecoglossus altivelis | 10 cm | | 7.7 | 25 | | | 11 | * | avoidance | |
| | (anionic) | | Sprague & Drury 1969 | Salmo gairdneri | 11 cm | 15 | 7.9 | 17 | 10 | 10 | 100 | 5 | avoidance | |
| AES | Polyoxyethylene lauryl-ethersulfate | | Hidaka & Tatsukawa 1985 | Oryzias latipes | 2.7 cm | 45? | 7.2? | 25 | 20 | | 25 | *5 | avoidance | |
| --- | Polyethoxylated octyl fenol (Triton X-100) (nonionic) | | Zlotkin & Gruber 1984 | Negaprion brevirostris | 7.2 cm | | | | | | 6 000 | * | avoidance | 36 ppm |
| --- | Ethoxylated sorbitanmono-laurate (Tween20) (nonionic) | | Zlotkin & Gruber 1984 | Negaprion brevirostris | 7.2 cm | | | | | | 10 000 | * | avoidance | 100 ppm |
| --- | Lauryltrimethylammonium-bromide (cationic) | | Zlotkin & Gruber 1984 | Negaprion brevirostris | 7.2 cm | | | | | | 3 000 | * | avoidance | 60 ppm |
| PHC | Shark repellent | | Zlotkin & Gruber 1984 | Negaprion brevirostris | 7.2 cm | | | | | | 800 | * | avoidance | 16 ppm |
| zeep | Laurate | Na | Hidaka & Tatsukawa 1985 | Oryzias latipes | 2.7 cm | 45? | 7.2? | 25 | 20 | | 35 | * | avoidance | |
| | Palmitate | Na | Hidaka & Tatsukawa 1985 | Oryzias latipes | 2.7 cm | 45? | 7.2? | 25 | 20 | | 285 | *5 | avoidance | |
| | Stearate | Na | Hidaka & Tatsukawa 1985 | Oryzias latipes | 2.7 cm | 45? | 7.2? | 25 | 20 | | 135 | * | avoidance | |

* article is in japanese; probably a commercially available formulation has been used

# - TABLE 4 -
# (non-prioritairy pollutants)

| COMPOUND | REFERENCE | TESTORGANISM | | | EXPERIMENTAL CONDITIONS | | | | NOEC | LOEC | | RESPONSE | TOXICITY | | ECOTOX | RHINE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | species | seize | number | hardness | pH | T | time | | | note | | time | LC50 | OBJECTIVE | 1985 |
| | | | | | mg CaCO3/L | | °C | m | (ug/L) | (ug/L) | | | | (ug/L) | (ug/L) | (ug/L) |
| Acetic acid | Summerfelt & Lewis 1987 | Lepomis cyanellus | 10.8 cm | 30 | | 7.2 | 23 | 30 | 20 000 | | 3 | avoid | | | | |
| | Wildish et al. 1977 | Clupea harengus | | 9-13 | | | | 90 | 1 220 | | 3 | avoid | | | | |
| Aceton | Summerfelt & Lewis 1967 | Lepomis cyanellus | 10.8 cm | 30 | | 7.2 | 23 | 30 | 0.2-1 g/L | | | avoid | ? | 14 g/L | | |
| | Wildish et al. 1977 | Clupea harengus | | 9-13 | | | | 90 | | 2 430 | 3 | avoid | | | | |
| ACP (teargass) | Summerfelt & Lewis 1987 | Lepomis cyanellus | 10.8 cm | 30 | | 7.2 | 23 | 30 | 10 | 50 | | avoid | 24h | 1 100 | | |
| | | Lepomis megalotis | | | | | | | | 1 000 | 3 | avoid | | | | |
| | | Lepomis microlophus | | | | | | | | 1 000 | 3 | avoid | | | | |
| | | Notemigonus crysoleucas | | | | | | | | 1 000 | 3 | avoid | | | | |
| Acrolein | Folmar 1976 | Salmo gairdneri | | 10 | 90 | 8.0 | 11 | 60 | 10 | 100 | | avoid | 24h | 140 | | |
| Acrylamides | Spraggs et al. 1982 | Salmo gairdneri | | | | | | | | | | avoid | | | | |
| | acrylamide monomeer | | | | | | | | 4 000 | 8 000 | 4 | avoid | 48h | 14 000 | | |
| | poly acrylamide | | | | | | | | 660? | 800 | 4 | avoid | 48h | 400 | | |
| | dimethyldiallylammdichlor | | | | | | | | 0.3-4mg/L | | | avoid | 48h | 900 000 | | |
| | polymeer | | | | | | | | 400 | 500 | | avoid | 48h | 200 | | |
| Alcohol | Jones 1947 | Pigosteus pungitus | 2.7 cm | 5? | | | 15 | 10 | | 1% | 2 | avoid | | | | |
| Ammonia | Summerfelt & Lewis 1987 | Lepomis cyanellus | 10.8 cm | 30 | | 7.2 | 23 | 30 | 1 700 | 8 500 | | avoid | | | | |
| | Jones 1948 | Gasostreus aculeatus | 2.8 cm | 5 | soft | 10 | 14 | 15 | 18 | 180 | | avoid | | | | |
| Aquatol K | Folmar 1976 | Salmo gairdneri | | 10 | 90 | 8.0 | 11 | 60 | 0.1-10ppm | | | avoid | 96h | 150 000 | | |
| As2O3 | Summerfelt & Lewis 1987 | Lepomis cyanellus | 10.8 cm | 30 | | 7.2 | 23 | 30 | 5 - 20ppm | | | avoid | | | 12.5 | |
| Benthiocarb | Hidaka et al. 1984 | Oryzias latipes | | | 45 | 7.2 | 25 | ?10 | | 522 | * | avoid | 48h | 4 400 | | |
| BCBC | Summerfelt & Lewis 1967 | Lepomis cyanellus | 10.8 cm | 30 | | 7.2 | 23 | 30 | 20 000 | | 3 | avoid | | | | |
| Carbaryl | see Sevin | | | | | | | | | | | | | | | |
| Chlordane | Summerfelt & Lewis 1967 | Lepomis cyanellus | 10.8 cm | 30 | | 7.2 | 23 | 30 | 1 000 | 5 000 | | avoid | | | 0.00078 | |
| Cresol | Jones 1951 (o- en p-) | Phoxinus phoxinus | | 1 | soft | 6.7 | 18 | 15 | 0.01% | 0.02% | | avoid | | | | |
| | Summerfelt & Lewis 1967 | Lepomis cyanellus | 10.8 cm | 30 | | 7.2 | 23 | 30 | 20 000 | | 3 | avoid | | | | |
| Cryoliet | Summerfelt & Lewis 1967 | Lepomis cyanellus | 10.8 cm | 30 | | 7.2 | 23 | 30 | 20 000 | | 3 | avoid | | | | |
| Cyanide | Summerfelt & Lewis 1967 | Lepomis cyanellus | 10.8 cm | 30 | | 7.2 | 23 | 30 | 500 | 1 000 | | avoid | ? | 1 000 | | |
| | van Hooff 1980 | Salmo gairdneri | 200 g | 1 | 250 | 8.0 | 7 | 75 | 100 | 400 | | pos.rheo | | | | |
| Cymene thiocyanate | Summerfelt & Lewis 1967 | Lepomis cyanellus | 10.8 cm | 30 | | 7.2 | 23 | 30 | 10 000 | 20 000 | | avoid | | | | |
| 2,4 D DMA-salt | Folmar 1976 | Salmo gairdneri | | 10 | 90 | 8.0 | 11 | 60 | 100 | 1 000 | | avoid | 96h | 100 000 | 11 | |
| BEE-ester | Dodson & Mayfield 1979A | Salmo gairdneri | 21 cm | 1 | | 7.5 | 15 | 24h | | 2 000 | 2 | pos.rheo | | | | |
| BEE-ester | Hansen 1969 | Cyprinodon variegatus | 3 cm | 50 | 20%. sal | | 20 | 90 | 10 | 100 | | avoid | | | | |
| BEE-ester | Hansen et al. 1972 | Gambusia affinis | 3.3 cm | 50 | | | 20 | 90 | 100 | 1 000 | | avoid | 24h | 7 000 | | |
| Na-salt | Hidaka et al. 1984 | Oryzias latipes | | | 45 | 7.2 | 25 | ?10 | | 177 | *5 | avoid | 48h | >40 000 | | |
| Dalapon | Folmar 1976 | Salmo gairdneri | | 10 | 90 | 8.0 | 11 | 60 | 100 | 1 000 | | avoid | 96h | 340 000 | | |
| DDT | Hansen 1969 | Cyprinodon variegatus | 3 cm | 50 | 20%. sal | | 20 | 90 | 1 | 5 | 5 | avoid | 24h | 6 | 0.00025 | |
| | Hansen et al. 1972 | Gambusia affinis | 3.3 cm | 50 | | | 20 | 90 | 10 | 100 | | avoid | 24h | 42 | | |
| | Kynard 1974 | Gambusia affinis | | 1 | | | | | | | | | | | | |
| | | susceptible | | | | | | 15 | 1 000 | 10 000 | | avoid | 24h | 20 | | |
| | | resistant | | | | | | 15 | 0.1-10 000 | | 1 | avoid | 24h | 100 | | |
| | BKH 1988 | | | | | | | | | 50 | | EWSdetect | | | | |
| Diazinon | Hidaka et al. 1984 | Oryzias latipes | | | 45 | 7.2 | 25 | ?10 | | 98 | *5 | avoid | 48h | 5 300 | | |
| Dimilin | Granett et al. 1978 | Salmo salar | 15 cm | 1 | | | 10 | 10 | | 10 | | avoid | | >80 ppm | | |
| Dinitro-o-cresol | van Hooff 1980 | Salmo gairdneri | 200 g | | 250 | 8.0 | 7 | 12h? | 250-300 | | | pos.rheo. within test | | | 0.30 | |
| Dioctyl phtalate | Black & Birge 1980 | Salmo gairdneri | | | | | | | | 112 400 | * | prefer | | | | |
| Diquat | Folmar 1976 | Salmo gairdneri | | 10 | 90 | 8.0 | 11 | 60 | 0.1-10 ppm | | 1 | avoid | 98h | 12 000 | | |
| | Dodson & Mayfield 1979B | Salmo gairdneri | | 1 | 97 | 7.2 | 15 | 25 | | ?500-5000 | | pos.rheo | 24h | 90 000 | | |
| | as Reglone A (formul) | | | | | | | | | ?500-5000 | | pos.rheo | 24h | 90 000 | | |

# - TABLE 4 -
## (non-prioritairy pollutants)

| COMPOUND | REFERENCE | TESTORGANISM species | seize | number | EXPERIMENTAL CONDITIONS hardness mg CaCO3/L | pH | T °C | time m | NOEC (ug/L) | LOEC (ug/L) | note | RESPONSE | TOXICITY time | LC50 (ug/L) | ECOTOX OBJECTIVE (ug/L) | RHINE 1985 (ug/L) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dursban | Hansen 1969 | Cyprinodon variegatus | 3 cm | 50 | 20%. sal | | 20 | 90 | 50 | 100 | 5 | avoid | 24h | > 1 000 | | |
| | Hansen et al. 1972 | Gambusia affinis | 3.3 cm | 50 | | | 20 | 90 | 10 | 100 | | avoid | 24h | 4 000 | | |
| Ethaanethiol | Summerfelt & Lewis 1967 | Lepomis cyanellus | 10.8 cm | 30 | | 7.2 | 23 | 30 | 20 000 | | 3 | avoid | | | | |
| Ethylbenzene | BKH 1988 | Salmo gairdneri | | | | | | 30h | | 26 | | EWSdetect | | | | |
| Fenitrothion | Hidaka et al. 1984 | Oryzias latipes | | | 45 | 7.2 | 25 | ?10 | | 73 | *5 | avoid | 48h | 3.8-7ppm | 0.05 | |
| | formulation | | | | | | | | | 102 | *5 | avoid | | | | |
| | Scherer 1975 | Carassius auratus | 10 cm | 1 | | 7.8 | 15 | 10 | | 10 | * | avoid | 10d | >100 | | |
| Formaline | Jones 1947 | Pigosteus pungitus | 2.7 cm | 5? | | | 15 | 10 | | 1 000 000 | 2 | avoid | | | | |
| | Summerfelt & Lewis 1967 | Lepomis cyanellus | 10.8 cm | 30 | | 7.2 | 23 | 30 | 0.03-1 g/L | | | avoid | | | | |
| Glyphosate MON 0573 | Folmar et al. 1979 | Salmo gairdneri | | | 90 | 8.0 | 11 | 60 | 0.1-10ppm | | | avoid | | | | |
| MON 0573 | Folmar 1976 | Salmo salar | | 10 | 90 | 8.0 | 11 | 60 | 0.1-10ppm | | | avoid | 96h | 50 000 | | |
| MON 02139 | Hildebrand et al. 1982 | Salmo gairdneri | 5.5 cm | | | 6.8 | 13 | 60 | 30 000 | 40 000 | | avoid | 96h | 55 000 | | |
| IBP | Hidaka et al. 1984 | Oryzias latipes | | | 45 | 7.2 | 25 | ?10 | | 174 | *5 | avoid | 48h | 3 700 | | |
| Lindane | Summerfelt & Lewis 1967 | Lepomis cyanellus | 10.8 cm | 30 | | 7.2 | 23 | 30 | 20 000 | | 3 | avoid | | | | |
| | BKH 1988 | Salmo gairdneri | | | | | | 45 | | 60 | | EWSdetect | | | | |
| Malathion | Hansen 1969 | Cyprinodon variegatus | 3 cm | 50 | 20%. sal | | 20 | 90 | 10 - 1 000 | | | avoid | 24h | 300 | 0.023 | |
| | Hansen et al. 1972 | Gambusia affinis | 3.3 cm | 50 | | | 20 | 90 | 5 | 50 | 5 | avoid | 24h | 2 000 | | |
| | van Hooff 1980 | Salmo gairdneri | 200 g | 1 | 250 | 8.0 | 7 | 75 | 5 000 | | 1 | pos.rheo | | | | |
| Maleic acid | Summerfelt & Lewis 1967 | Lepomis cyanellus | 10.8 cm | 30 | | 7.2 | 23 | 30 | 20, 40ppm | | | avoid | | | | |
| Menthol | Summerfelt & Lewis 1967 | Lepomis cyanellus | 10.8 cm | 30 | | 7.2 | 23 | 30 | 40 000 | | 3 | avoid | | | | |
| Naphtalene 1-methyl | Summerfelt & Lewis 1967 | Lepomis cyanellus | 10.8 cm | 30 | | 7.2 | 23 | 30 | 40, 60 ppm | | | avoid | | | | |
| 2-Nitrobenzene, | | | | | | | | | | | | | | | | |
| 1,4-dichloro | Summerfelt & Lewis 1967 | Lepomis cyanellus | 10.8 cm | 30 | | 7.2 | 23 | 30 | | 1 000 | 2 | avoid | 24h | 6 500 | | 0.09 |
| Parathion | Kynard 1974 | Gambusia affinis | | | | | | | | | | | | | | |
| | | susceptible | | | | | | 15 | 20 | 200 | | avoid | 24h | 20 | | |
| | | resistant | | | | | | 15 | 200 | 1 000 | | avoid | 24h | 2 000 | | |
| Phenol | DeGraeve 1982 | Salmo gairdneri | | 1 | 710 | 8.0 | 12 | 96h | 3 200 | 6 500 | | avoid | 96h | 8.9ppm | 1.7 | |
| | Hasler & Wisby 1950 | Hyborhynchus notatus | | | | | | | | 0.5 | | avoid | | | | |
| | (in Hara et al. 1983) | | | | | | | | | | | | | | | |
| | Jones 1951 | Phoxinus phoxinus | | 1 | soft | 6.7 | 18 | 15 | 0.0004-0.04% | | | avoid | | | | |
| | Sprague & Drury 1969 | Salmo gairdneri | 11.3 cm | | 15 | 7.3 | 17 | 10 | 0.001-10ppm | | | avoid | ? | 13.6ppm | | |
| | Summerfelt & Lewis 1967 | Lepomis cyanellus | 10.8 cm | 30 | | 7.2 | 23 | 30 | 20 000 | | 3 | avoid | | | | |
| | Stott & Buckley 1979 | Phoxinus phoxinus | population | | | | 9 | 8h | 4-10ppm | | | avoid | | | | |
| Phenylaceticacid | Summerfelt & Lewis 1967 | Lepomis cyanellus | 10.8 cm | 30 | | 7.2 | 23 | 30 | 5 000 | | 3 | avoid | | | | |
| NAC | Hidaka et al. 1984 | Oryzias latipes | | | 45 | 7.2 | 25 | ?10 | | 248 | *5 | avoid | 48h | 2.8-10ppm | | |
| Quinaldine | Summerfelt & Lewis 1967 | Lepomis cyanellus | 10.9 cm | 30 | | 7.2 | 23 | 30 | 5 - 20ppm | | | avoid | 24h | | | |
| Quinine sulphate | Summerfelt & Lewis 1967 | Lepomis cyanellus | 10.8 cm | 30 | | 7.2 | 23 | 30 | 20 000 | | 3 | avoid | 24h | | | |
| Quinoline | Summerfelt & Lewis 1967 | Lepomis cyanellus | 10.8 cm | 30 | | 7.2 | 23 | 30 | 20 000 | | 3 | avoid | 24h | | | |
| Se | BKH 1980 | Salmo gairdneri | | | | | | | | 100 000 | | EWSdetect | | | | |
| Sevin | Hansen 1969 | Cyprinodon variegatus | 3 cm | 50 | 20%. sal | | 20 | 90 | 0.1 - 10ppm | | | avoid | 24h | 2 800 | | |
| | Hansen et al. 1972 | Gambusia affinis | 3.3 cm | 50 | | | 20 | 90 | 1 000 | 10 000 | | avoid | 24h | >10 000 | | |
| | van Hooff 1980 ("carbaryl") | Salmo gairdneri | 200 g | 1 | 250 | 8.0 | 7 | 75 | 1 500 | | 1 | pos.rheo | | | | |
| Sulphide | Jones 1948 | Gasostreus aculeatus | 2.8 cm | 5 | soft | 6.8 | 14 | 47 | | 3 040 | 1 | avoid | | | | |
| | Summerfelt & Lewis 1967 | Lepomis cyanellus | 10.8 cm | 30 | | 7.2 | 23 | 30 | 300 - 1000ppm | | | avoid | | | | |
| Thanite | Summerfelt & Lewis 1967 | Lepomis cyanellus | 10.8 cm | 30 | | 7.2 | 23 | 30 | 1 000 | 2 000 | | avoid | 6h | <500 | | |
| Toluene | Maynard & Weber 1981 | Oncorhynchus kisutch | presmolt | 20-60 | | | 12 | 75 | 900 | 1 800 | | avoid | 96h | 6.4-8.1ppm | | |
| | | | smolt | | | | 8 | 75 | | 1 400 | 2 | avoid | | | | |
| | BKH 1988 | Salmo gairdneri | | | | | | 6d | | 12 | | EWSdetect | | | | |
| Toxaphene | Kynard 1974 | Gambusia affinis schoon | | 1 | | | | 15 | 50 | 250 | | avoid | 24h | 10 | | |
| | | belast | | | | | | 15 | 50 | 250 | | avoid | 24h | 800 | | |
| Trichloroaceticacid | Folmar 1976 | Salmo salar | | 10 | 90 | 8.0 | 11 | 60 | 10 - 1 000 | | 1 | avoid | 96h | 10ppm | | |
| Xylene -p | Folmar 1976 | Salmo salar | | 10 | 90 | 8.0 | 11 | 60 | 10 | 100 | 4 | avoid | 96h | 100 | | |
| -o | Maynard & Weber 1981 | Oncorhynchus kisutch | presmolt | 20-60 | | | 8 | 75 | | 680 | * | avoid | 96h | 13.5ppm | | |

Fenitrothion was avoided by goldfish *(Carassius auratus)* at concentrations higher than 10 µg/L (Scherer et al. 1975) and by medaka *(Oryzias latipes)* at concentrations between 73 and 147 µg/L (Hidaka et al. 1984). The 48h LC50 for medaka was 3.8 - 7.0 mg/L.

The proposed water quality objective for fenitrothion is much lower (0.05 µg/L - Stortelder et al. 1989) and concentrations have not been reported to exceed this objective (DBW/RIZA).

Detergents Although only limitid data are available on avoidance of detergents, it seems evident (table 4) that fish are extremely sensitive to detergents and will avoid relatively low concentrations compared to toxicity data. The repelling capacity of detergents was demonstrated by Zlotkin & Gruber (1984). Hungry lemon sharks *(Negaprion brevirostris)* were offered food, however, as they held on to baite, a substance was injected into their mouths. Although the fish were highly motivated, some substances totally abolished feeding response and resulted in avoidance of the feeding site.

The anionic detergent laurylsulphate, AS, repelled sharks at lower concentrations than the known shark repellent PMC (an extract of the red sea flatfish *(Parachirus marmoratus)*). NOEC values for AS were not determined by Zlotkin & Gruber. Hidaka & Tatsukawa (1985) and Tatsukawa & Hidaka (1978) reported threshold concentrations of avoidance of AS by medaka and ayu *(Plecoglossus altivelis)* of 4 - 8 µg/L. Lake whitefish *(Coregonus clupeaformis)* on the other hand did not avoid AS at concentrations of 10 µg/L up to 10 mg/L (Hara & Thompson 1978). Zlotkin & Gruber (1984) reported LC50 values of AS for killifish *(Floridicthyes carpio)* of 3 - 6 mg/L.

In Germany (Schöberl et al. 1988) and the Netherlands (DBW/RIZA), the most widely used anionic detergent is lineair laurylbenzene sulfonate (LAS). The threshold concentrations of avoidance reported by Hidaka & Tatsukawa (1985) and Tatsukawa & Hidaka (1978) range between 0.11 and 14 µg/L, whereas 24-96h LC50 values of four fish species range between 3.2 and 9.2 mg/L (Schöberl et al. 1988). and invertebrate 48h LC50 range between 1.7 to 270 mg/L (Lewis & Suprenant 1983) . Misra et al. (1985) reported higher sensitivity of *Cirrhina mrigala* (fish fingerlings) to LAS: LC50 values of 20 µg/L were reported as well as behavioral changes and histological damage to the gills after exposure to 5 µg/L LAS.

Medaka, ayu and rainbow trout also avoid the anionic detergent ABS at low concentrations. AES and three kinds of soap were avoided by medaka (Hidaka & Tatsukawa 1985). Two nonionic and one cationic detergent repelled lemon shark at high, but still sublethal concentrations (in the ppm-range, Zlotkin & Gruber 1984).

Concentrations of total anionic detergents do not exceed 100 µg/L. It is estimated that approximately 50-70% of the anionic detergents in surface waters is LAS (DBW/RIZA).

The responses of fish to five other compounds are noteworthy:

DDT was avoided by sheepshead minnows *(Cyprinodon variegatus)* and mosquitofish *(Gambusia affinis)* at concentrations near the 24h LC50 (6 and 100 µg/L respectively) (Hansen 1969, Hansen et al. 1972). The proposed water quality objective is several orders of magnitude lower (0.25 ng/L - Stortelder et al. 1989). Incidentally concentrations as high as 0.01 µg/L have been measured (DBW/RIZA).

Dinitro-*o*-cresol did not affect positive rheotaxis in rainbow trout at 250 and 300 µg/L, however death occurred in 12 - 19h (van Hooff 1980). The proposed water quality objective is 0.30 µg/L (Stortelder et al. 1989), but discharges containing 2 - 3 mg/L have been reported (DBW/RIZA).

Lindane was not avoided by green sunfish *(Lepomis cyanellus)* at 20 mg/L (Summerfelt & Lewis 1967). At 60 µg/L (2 - 3 times LC50) however, lindane was detected in an early warning system using positive rheotaxis of rainbow trout (BKH 1988).

Malathion was not avoided by sheepshead minnows at sublethal to highly toxic concentrations (Hansen 1969), whereas mosquitofish avoided 50 µg/L and higher concentrations (Hansen et al. 1972). A highly toxic concentration of 5 mg/L did not affect positive rheotaxis in rainbow trout (van Hooff 1980). (24h LC50 range between 300 and 2000 µg/L.)
Proposed water quality objective for malathion is much lower (23 ng/L - Stortelder et al. 1989). Concentrations of malathion in the River Rhine are generally below detection limit (10 ng/L), however a concentration of 40 ng/L has been reported (DBW/RIZA).
Phenol The proposed water quality objective for phenol is 1.7 µg/L (Stortelder et al. 1989). Using rainbow trout Sprague & Drury (1969) did not observe avoidance to phenol at concentrations between 1 µg/L and 10 mg/L. However Hasler & Wisby (1950) report an effective concentration of 0.5 µg/L (as cited by Hara et al. 1983).

### 3.3.3 Mixtures

In the River Rhine organisms are exposed to mixtures of pollutants, whereas in the experiments reported on so far, organisms were exposed to a single contaminant at a time.

The most common form of mixture *toxicity* is additive action of contaminants. Greater than additive (synergistic) or less than additive (antogonistic) action of contaminants are exceptions (Marking 1985). In assessing water quality objectives additive effects of chemicals have been taken into account: it is assumed that PAKs, monocyclic aromatics, volatile halogenated hydrocarbons and chlorobenze compounds have a common toxic mechanism and will be additive in their toxic action. Also for metals an additive action is observed (Enserink et al. in prep.).

As was stressed in § 3.1 *preference-avoidance* behaviour does not follow the traditional positive concentration-response relationship mostly encountered in toxicological experiments. In addition, preference and avoidance are contrairy responses. What the effect will be of a combination of contaminants on avoidance preference behaviour is therefore difficult to predict. Although only little is known on avoidance of mixtures of compounds, research so far illustrates that additive and more than additive avoidance responses to mixtures of pollutants may occur (table 5):

Hadjinicolaou & Spraggs (1988) investigated avoidance reactions of rainbow trout to the effluent of a representative metalplating industry, containing chromium, iron and nickel. Avoidance responses to the mixture as well as the individual metals were investigated. As is clearly illustrated by figure 8, nickel is the most important factor in the avoidance response. Between the threshold of avoidance of the mixture (64 µg total metal/L or 1:700 LC50) and 296 µg total metal/L (1:150 LC50), the avoidance response was initially more, but later on less than the sum of the responses to the individual metals.

Sprague (1964) examined avoidance behaviour of atlantic salmon to a mixture of copper and zinc. Whereas threshold concentration of avoidance for zinc and copper seperately were 53 and 2.4 µg/L respectively, threshold concentration of avoidance for the mixture was 7.1 µg total metal/L (6.7 µg Zn and 0.4 µg Cu /L). At low concentrations the avoidance response to the mixture was stronger than the added responses to the metals seperately.
The observations of Wildish et al. (1977) and Maynard & Weber (1981) may be examples of less than additive responses to chemicals. However, it is not clear whether experiments with a mixture and those with its components do not

differ in experimental conditions (e.g. bioavailability of compounds may have been influenced).
Wildish et al. (1977) examined avoidance behaviour of herring *(Harengus clupeaformis)* to pulpmill effluent. Threshold of avoidance of the effluent was 2.5 - 2.9 mg/L. At this concentration the effluent contained 1.2 - 1.9 mg/L lignosulfonate. When tested seperately this compound was already avoided at concentrations higher than 340 µg/L. Together with data on avoidance of other compounds, this indicated, according to the authors, a less than additive response of fish to the mixture.

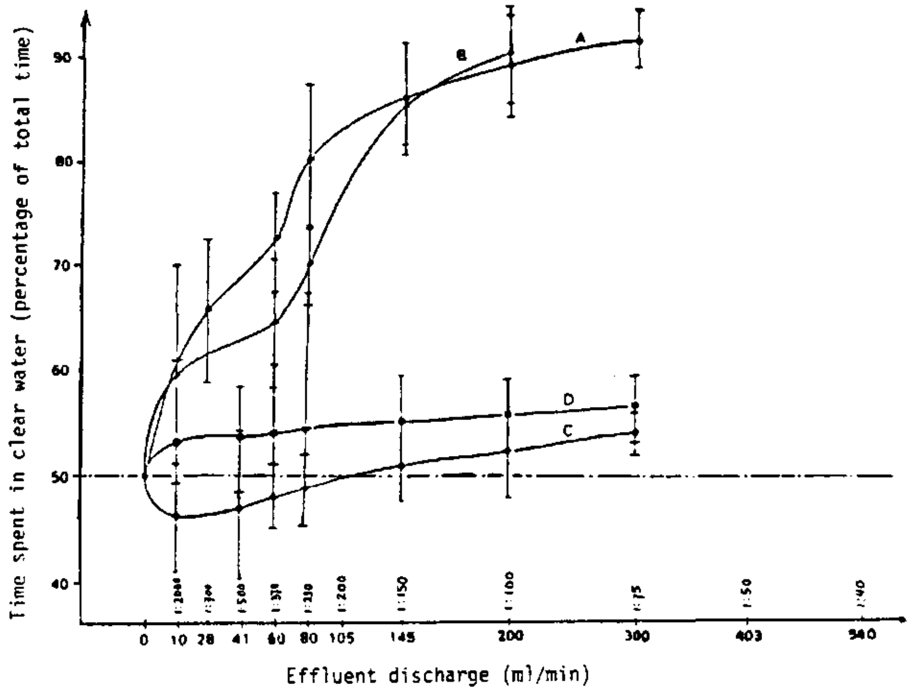


*Figure 8*
*Avoidance by rainbow trout (Salmo gairdneri) of a metalplating effluent and its compounds: A industrial effluent, B nickel, C chromium, D iron. Effluent discharge is given in ml/min as well as in toxic unit (quotient of concentration and 96h LC50 (60% effluent). For threshold of avoidance values and experimental conditions see table 5 (From Hadjinicolaou & Spraggs 1988.)*

Maynard & Weber (1981) also observed a less than additive avoidance response of coho salmon to a mixture of aromatic hydrocarbons (saltwater soluble fraction of Prudhoe Bay oil). Threshold of avoidance of this mixture was approximately equal to 96h LC50, whereas individual components were avoided at sublethal concentrations.

A number of avoidance experiments with mixtures have been performed in which the components were not tested seperately.
- Hartwell et al. (1987A and B) tested avoidance of a mixture of heavy metals (representative for a fly ash slurry at a coalfired powerplant) by unexposed and preexposed fathead minnows *(Pimephales promelas).* Not previously exposed fish avoided sublethal concentrations.
- Bleached craft mill effluent was avoided at sublethal concentrations by pinfish *(Lagodon rhomboides)* and gulf killifish *(Fundulus grandis)* (Lewis & Livingstone 1977), whereas response of atlantic salmon was somewhat indefinite (Sprague & Drury 1969).

- TABLE 5 -
(mixtures)

*Table 5*
*Preference-avoidance responses in the laboratory to mixtures of dissolved chemicals. For dissolved pollutants, proposed water quality objectives (Stortelder et al. 1989) and average concentrations at Lobith in the River Rhine in 1985 (DBW/RIZA) are given as well. In appendix 4 english names of species are listed.*

*Abbreviations: NOEC (No Observed Effect Concentration), LOEC (Lowest Observed Effect Concentration) LC50 (concentration lethal to 50% of the test organisms).*
*Notes: 1 highest concentration in experiment; 2 lowest concentration in experiment; 3 only concentration in experiment; 4 preference observed at lower concentrations; 5 abolished avoidance at high concentrations; * threshold concentration of response.*

| COMPOUND | REFERENCE | TEST ORGANISM species | seize | number | EXPERIMENTAL CONDITIONS hardness mg $CaCO_3$/L | pH | T °C | time m | | NOEC (ug/L) | LOEC (ug/L) | note | RESPONSE | TOXICITY time | LC50 (ug/L) | ECOTOX OBJECTIVE (ug/L) | RHINE 1985 (ug/L) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| effluent: | Hadjinicolaou & Spragge | Salmo gairdneri | 8 cm | 100 | | 8.3 | 15 | 135 | eff1: | | 0.088% | * | Avoidance of ) 63.8 ug TM/L (17 ug Cr/L, | 96h | 60% | | |
| 20 ppm Cr | 1988 | | | | | | | | Cr: | 15-444 | | | 3.4 ug Fe/L, 43 ug/L Ni. Ni is most important | | (=44.4) | | |
| 4 ppm Fe | | | | | | | | | Fe: | | 20 | * | factor causing avoidance. At threshold more | | | Cr 2.5 | 1.1 |
| 50 ppm Ni | | | | | | | | | Ni: | 35 | 147 | | than additive effect, at higher concentration | | | Fe | |
| | | | | | | | | | | | | | less than additive. | | | Ni 7.5 | 3.7 |
| mixture proportions: | Hartwell et al. 1987A | Pimephales promelas | | 5 | 84 | 7.5 | | 15 | | | | | | | | | |
| Cu 1 | | not pre-exposed | | | | | | | | | 31 ppb TM | * | Avoidance. No effect of season observed. Pre- | 96h | LC01: | | |
| Cr 0.58 | | pre-exposed to 98 ug TM/L | | | | | | | | | | | exposure results in higher threshold of avoi- | | 10% | Cu 1.3 | 3 |
| As 1.85 | | 3 months | | | | | | | | | 98 ppb TM | *4 | dance. Metals were added as $Cu_2O$, $K_2CrO_7$, | | (=500) | Cr 2.5 | 1.1 |
| Se 0.38 | | 6 months | | | | | | | | | 393 ppb TM | *4 | $As_2O_3$ and $SeO_2$. Threshold 31 ppb TM = 8.1 ug | | | As 12.5 | |
| | | 9 months | | | | | | | | 735 | | | Cu/L, 4.7 ug Cr/L, 15 ug As/L and 3.1 ug Se/L | | | Se | |
| | | 12 months | | | | | | | | highly variable | | | | | | | |
| mixture: | Sprague 1964 | Salmo salar | 12.5cm | 1 | 18 | 7.5 | 18 | 10 | mix: | 4.8 TM | 17 TM | | Avoidance. Threshold 7.1 ug TM/L containing | incip | 22 Cu + | Cu 1.3 | 3.0 |
| Cu | | | | | | | | | Cu | | 2.4 | * | 0.4 ug Cu/L and 6.7 ug Zn/L. At low concen- | | 290 Zn | Zn 6.5 | 18 |
| Zn | | | | | | | | | Zn | | 53 | * | trations the effect is more than additive. | | | | |
| Bleached Kraft Mill | Lewis & Livingstone 1977 | Lagodon rhomboides | 7.7 cm | 1 | 30 %. sal | 7.8 | 29 | 4h | | 0.01% | 0.1% | | Avoidance. | 96h | 10% | | |
| effluent | | Fundulus grandis | 4.8 cm | 3 | | | | | | 0.01% | 0.1% | | | | | | |
| Bleached Kraft Mill | Sprague & Drury 1969 | Salmo salar | 11.3 cm | | 15 | 7.3 | 17 | 10 | | 1 000 | 10 000 | | Avoidance. Threshold of avoidance is 15% SKME | | 15% | | |
| effluent | | | | | | | | | | | | | Response is indefinite. | | | | |
| Pulp Mill effluent | Wildish et al. 1977 | Clupea harengus | | 9-13 | | | | 90 | | | | | | | | | |
| components tested | | lignosulfonate | | | | | | | | 180 | 340 | | Avoidance. Threshold of effluent avoidance is | | | | |
| seperately | | humic acid | | | | | | | | 150 | 200 | | 2.5 - 2.9 ppm with 1.2 - 1.9 ppm lignosulfo- | | | | |
| | | D-glucose | | | | | | | | 1 100 | | 3 | nate. In effluent avoidance occures at a con- | | | | |
| | | fructose | | | | | | | | 1 120 | | 3 | centration higher than with seperately tested | | | | |
| | | abietit acid | | | | | | | | 590 | | 3 | compounds (less than additive response). | | | | |
| | | dihydroabietic acid | | | | | | | | 1 550 | | 3 | | | | | |
| | | Tall oil | | | | | | | | 1 440 | | 3 | | | | | |
| | | furfural | | | | | | | | 1 160 | | 3 | | | | | |
| | | catechol | | | | | | | | 1 190 | | 3 | | | | | |
| | | acetic acid | | | | | | | | 1 220 | | 3 | | | | | |
| | | formic acid | | | | | | | | 1 250 | | 3 | | | | | |
| | | acetone | | | | | | | | | 2 430 | 3 | | | | | |

# - TABLE 5 -
## (mixtures)

| COMPOUND | REFERENCE | TEST ORGANISM<br>species | seize | number | EXPERIMENTAL CONDITIONS<br>hardness<br>mg CaCO3/L | pH | T<br>°C | time<br>m | NOEC<br><br>(ug/L) | LOEC<br><br>(ug/L) | Note | RESPONSE | TOXICITY<br>time | LC50<br>(ug/L) | ECOTOX<br>OBJECTIVE<br>(ug/L) | RHINE<br>1985<br>(ug/L) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Oil refinery effl. | Westlake et al. 1983 | Salmo gairdneri | 6.4 cm | 190 | | | | 60 | 30% | | 1 | Avoidance. Physiological threshold value was | 96h | 100% | | |
| 32 ppm N | | | | | | | | | | | | estimated at 6%. Activity was not influenced. | | | | |
| 16 ppm oil grease | | | | | | | | | | | | | | | | |
| 31 ppb fenol | | | | | | | | | | | | | | | | |
| <40 ppb Sulf. | | | | | | | | | | | | | | | | |
| 52 ppm solids | | | | | | | | | | | | | | | | |
| Prudhoe Bay Oil | Maynard & Weber 1981 | Oncorhynchus kisutch | 8 cm | 20-80 | | | 5-17 | 75 | | | | | | | | |
| Salt watersoluble: | | effluent | presmolt | | | | | | | 3 710 | * | Avoidance. Mixture is not avoided as strongly | 96h | | | |
| toluene 57% | | | late smolt | | | | | | | 1 890 | 2 | as seperately tested components (less than | | 3.6 ppm WSF | | |
| o-xylene 16% | | toluene | presmolt | | | | | | | 1 990 | * | additive effect). Season or developmental | | | | |
| benzene 6% | | | early smolt | | | | | | | 1 400 | 2 | state influence avoidance response. | | 8.4-8.1 ppm toluene | | |
| triesthbenz 6% | | o-xylene | presmolt | | | | | | | 680 | * | | | | | |
| p-xylene 5% | | benzene | presmolt | | | | | | | 1 750 | * | | | 13.5 ppm xylene | | |
| m-xylene 5% | | | | | | | | | | | | | | 14.1 ppm benzene | | |
| ethylbenz. 2% | | | | | | | | | | | | | | | | |
| Coal liquid: | Dauble et al. 1985 | Pimephales promelas | 6 cm | 38 | | | 12 | 2d | 1.7 ppm phenols | | * | Avoidance | ? | 6.25 ppm | | |
| phenols 80% | | | | | | | | | | | | | | | | |
| arom. amines | | | | | | | | | | | | | | | | |
| HCs | | | | | | | | | | | | | | | | |
| Volcanic ash | Whitman et al. 1982 | Oncorhynchus tschawytscha | adult | 1 | | | 13 | 10 | | 350 ppm | 3 | Avoidance. Preference of home-water was depressed. | | | | |

- Effluent from a petroleum refinery was not avoided by rainbow trout (Westlake et al. 1983) whereas fathead minnows avoided the water soluble fraction of coal liquid at sublethal concentrations (Dauble et al. 1985).
- Whitman et al. (1982) investigated the influence of suspended volcanic ash on homing behaviour of adult chinook salmon *(Oncorhynchus tschawytscha)*. In laboratory experiments, addition of ash to homewater (350 mg/L) diminished preference for homewater and reduced upstream movement in the testing apparatus.

## 3.4 Avoidance under field conditions

### 3.4.1 Introduction

Although in limited numbers, field observations were reported that definetely show avoidance of chemicals by fish. Accounts of avoidance are given in which, unfortunately, the description of environmental conditions such as toxicant concentration and hardness are lacking (Saunders 1969, Abban & Saman 1980, Kelso 1977). In addition there is a number of observations on the distribution and movements of fish that may very well be explained by avoidance of chemicals (see § 3.4.3).
Most valuable are well documented cases of avoidance. Especially those experiments with which observations in laboratory *and* field can be compared, since these may clarify whether observations on avoidance in the laboratory may be used to predict behaviour under field conditions.

In only four cases laboratory experiments have been performed next to field observations. In the Northwest Miramichi River, the spawning migration of atlantic salmon was affected due to copper and zinc pollution. Effective levels were higher under field than under laboratory conditions. (Sprague 1964, Sprague et al. 1965, Saunders & Sprague 1967). However, field observations agreed better with laboratory observations in the other three studies, using a metal mixture representative of a fly ash slurry (Hartwell et al. 1987A and B), heated chlorinated water (Giattina et al. 1981) and a Water Soluble Fraction (WSF) of Prudhoe Bay crude oil (Weber et al. 1981, Maynard & Weber 1981).

In some of the 'field' studies reported here, natural conditions may not have occurred, due to the experimental procedures. E.g. the natural speciation of heavy metals will not always have occurred: In experiments of Geckler et al. (1976), Sutterlin & Gray (1973) and Hartwell et al (1987b), heavy metals were added *during* the field experiment and physico-chemical equilibria with compounds of the natural water may not have been achieved. Because of this, the metals may have been more available and may have caused avoidance responses at lower concentrations than would have been the case under more natural conditions. In contrast, Sprague and coworkers reported on heavy metal pollution that actually occurred (Sprague 1964, Sprague et al. 1965, Saunders & Sprague 1969).

Because of some modifying factors, avoidance responses to chemicals in the River Rhine may be expected to occur at concentrations higher than those reported in laboratory studies (see also § 3.2):
(1) The hardness and complexing capacity of River Rhine water will lower the bioavailibility. This will result in higher threshold concentrations of avoidance, as compared to laboratory data.
(2) Under field conditions the behavioural disposition of fish will be different from that of fish under laboratory conditions. Migrating anadromous fish will be highly motivated to move upstream, and will not as easily be displaced as

laboratory fish lacking this drive.
(3) Under field conditions fish may have adapted to the presence of a pollutant and may consequently be less responsive than not-previously exposed fish used in the laboratory.
(4) In laboratory experiments steep gradient chambers have been used, in which orientation is easier and favourable conditions are close at hand. In nature steep gradients are rare (they are present at the confluence of two water sources e.g. effluent mixing zones).
Sprague & Drury (1969) suggested that fish under field conditions effectively avoid chemicals at concentrations that, in the laboratory, would result in 90% time spent in clean water.

On the other hand a worst-case treatment seems appropriate since: (1) research on avoidance has been quite limited and therefore fish may be sensitive to lower concentrations and also to other pollutants than have been reported so far; (2) the response to chemicals may be additive (which is often the case in toxicity experiments - see § 3.3.3); (3) It is to be expected that salmonids, having exquisite olfaction to secure homing, are among the most sensitive of fish species and will detect and avoid chemicals at lower concentrations than other fish species (§ 3.2.2).

Under natural conditions repopulation of the River Rhine by atlantic salmon will occur through 'straying': a small percentage of the salmon in adjacent rivers will not home accurately and may enter the River Rhine (see § 2.2). In the River Rhine homestream or pheromone odour is not present, therefore straying salmon entering this river may be less motivated to move upstream than they would have been in their homestream. Consequently they may avoid chemicals at lower concentrations.

It should be noted that the avoidance responses observed in field experiments are not always the result of a choice by fish between more and less contaminated water (directed response). E.g. the observations of Sprague and coworkers on depressed upstream migration by atlantic salmon *(Salmo salar)* in the Miramichi River, may also result from affected olfactory orientation (see chapter 4).

### 3.4.2 Observations definitely resulting from avoidance

In general, the effective concentrations reported in the limited number of field studies are higher than the average concentrations in the River Rhine (table 6). Even if the limited number of observations do not underestimate sensitivity of fish to chemicals, it is not unconcievable that concentrations occur (e.g. of heavy metals in effluent mixing zones) that may temporarily affect the spawning migration of anadromous fish.

HEAVY METALS
In evaluating chronic toxicity of copper to bluntnose minnows *(Pimephales notatus)* Geckler et al. (1976, as cited by Atchinson et al. 1987) added copper to a natural river (120 µg Cu/L, which is slightly more than chronic laboratory LOEC). Most fish avoided the heavily polluted areas and spawning of bluntnose minnows was restricted to concentrations below 35 - 77 µg/L. Fish production of the stream was affected.
In an outdoor test apparatus Sutterlin & Gray (1973) demonstrated affected preference for homewater by atlantic salmon, when this contained 44 µg Cu/L (incipient lethal level according to Sprague 1964).

# - TABLE 6 -
# (field observations)

*Table 6*
*Preference-avoidance responses under field conditions. For dissolved pollutants, proposed water quality objectives (Stortelder et al. 1989) and average concentrations at Lobith in the River Rhine in 1985 (DBW/RIZA) are given as well. In appendix 4 english names of species are listed.*

*Abbreviations: NOEC (No Observed Effect Concentration), LOEC (Lowest Observed Effect Concentration) LC50 (concentration lethal to 50% of the test organisms).*
*Notes: 1 highest concentration in experiment; 2 lowest concentration in experiment; 3 only concentration in experiment; 4 preference observed at lower concentrations; 5 abolished avoidance at high concentrations; * threshold concentration of response.*

| COMPOUND | REFERENCE | TESTORGANISM species | seize | number | EXPERIMENTAL CONDITIONS hardness mg CaCO3/L | pH | T °C | time m | NOEC (ug/L) | LOEC (ug/L) | note | RESPONSE | TOXICITY time | LC50 (ug/L) | ECOTOX OBJECTIVE (ug/L) | RHINE 1985 (ug/L) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cu | Geckler et al. 1978<br>(Atchinson et al. 1987) | Pimephales notatus | | | | | | | | 120 | 3 | Spawning restricted to areas containing less than 35 - 77 ug Cu/L. Fish production of stream was affected. | chronic | LOEC<br>115 | 1.3 | 3 |
| Cu | Sutterlin & Gray 1973 | Salmo salar | adult | 8-12 | | | 15 | 12h | | 44 | 3 | Avoidance. Preference for home-water was affected. | incip | 44 | 1.3 | 3 |
| mixture proportions: | Hartwell et al. 1987B | Pimephales promelas | | 5 | | | | | | | | Avoidance. In artificial stream comparable to steep gradient chamber (lab). In other season (71 ppb) and in field experiment (74 ppb) threshold of avoidance are higher, possibly due to higher hardness and turbidity. Pre-exposure strongly effects avoidance. | | | | |
| Cu 1 | | artificial stream | | | 74 | 7.2 | | 15 | | 34 ppb TM | * | | | | Cu 1.3 | 3 |
| Cr 0.58 | | natural stream | | | 100-140 | | | | | 74 ppb TM | * | | | | Cr 2.5 | 1.1 |
| As 1.85 | | pre-exposed to 98 ppb | | | | | | | 1 470 | | | | | | As 12.5 | |
| Se 0.38 | | TM for 3 months | | | | | | | | | | | | | | |
| effluent: | Sprague et al. 1969A | Salmo salar | adult | | | | | | | | | Avoidance. Affected spawning migration was observed at concentrations > 8.4 - 10.3 ppb Cu and 105 - 109 ppb Zn. Migration was blocked at 260 ppb TM (19.2 Cu and 240 Zn). In lab. avoidance threshold is considerably lower (7.1 ppb TM). | incip | 22 Cu + | Cu 1.3 | 3.0 |
| Cu | in field | | | | 20 | | 18 | | | 113-139 TM | | | | 290 Zn | Zn 6.5 | 18 |
| Zn | in laboratory Cu | | | 1 | 18 | 7.5 | 18 | 10 | | 2.4 | * | | | | | |
| | Zn | | | | | | | | | 53 | * | | | | | |
| | Cu + Zn | | | | | | | | | 0.5 Cu 6.7 Zn | | | | | | |
| | (Sprague 1984) | | | | | | | | | | | | | | | |
| Nabam<br>Endrin | Saunders 1969 | Salmo salar<br>Salvelinus fontinalis | juv. | | | | | | | | | Mass mortalities, unseasonal movement downstream and unusual distribution resulting from accidental spillage. | | | | |
| Abate | Abban & Samman 1980 | 15 species | | | | | | | | 50-100 | 3 | Fewer numbers and species caught after aerial application than before. Virtually no fish were killed. | 24h | 1-200 ppm | | |
| Mixture:<br>m-, p-xylene 23 %<br>o-xylene 5 %<br>toluene 57 %<br>ethylbenzene 7 % | Weber et al. 1981 | Oncorhynchus kisutch<br>O. gorbuscha<br>O. keta<br>O. tschawytscha | adult | | | | | | | 3 200 | * | Avoidance. Number of upstream migrants (99% O. kisutch) was affected. Whether reproductive succes was influenced is not clear. (Alternative routes were open for movement upstream.) | | | | |
| Paper & Pulp Mill effluent | Kelso 1977 | Catostomus commersoni | | | | | | | | 15% | 35 | Avoidance. Only at concentrations lower than 15%. Despite avoidance aggregation occured at effluent plume, possibly due to food availability. | | | | |
| Chlorination | Schumacher & Ney 1980 | Salmo gairdneri | 25 cm | 10 | | | 20 | 60 | | 50 ug/l TRC | * | Avoidance. Observed in power plant discharge canal on single dose chlorination. | | | | |
| | Giattina et al. 1981 | several species | | | 68 | 7.8 | 12-30 | 10 | | | | Avoidance. "In most cases, laboratory determined avoidance concentrations predicted accurately the TRC conc. that would elicit avoidance behaviour under natural field conditions." | | | | |
| | | Notropis spilopterus | field | | | | < 18 | | | 210 TRC | | | | | | |
| | | | | | | | 24-29 | | | 180 TRC | | | | | | |
| | | | | | | | 30-34 | | | 120 TRC | | | | | | |
| | | N. galacturus | field | | | | < 18 | | | 110 TRC | | | | | | |
| | | | | | | | 24-29 | | | 180 TRC | | | | | | |
| | | | | | | | 30-34 | | | 40 TRC | | | | | | |
| Volcanic ash | Whitman et al. 1982 | Oncorhynchus tschawytscha | adult | 20 | | | 13 | 7d | 850 ppm | | 3 | Avoidance. Homing was compared of pre-exposed and unexposed fish. | | | | |

In both experiments effective values compare favourably with those reported in laboratory experiments and are lower than concentrations occuring in effluent mixing zones (table 3). In both cases hardness was not stated.

As was already mentioned in § 3.3.3, Hartwell et al. (1987a en b) performed avoidance experiments with a metal mixture representative of a fly ash slurry (containing $Cu_2O$, $K_2Cr_2O_7$, $SeO_2$ and $As_2O_3$). This was done using fathead minnows *(Pimephales promelas)* in a steep gradient chamber of the fluvarium type (Hartwell et al. 1987a), an artificial and a natural stream (Hartwell et al. 1987b). The threshold concentrations of avoidance for naive (not previously exposed) fish did not differ much (31, 34 and 74 µg total metal/L respectively). The somewhat higher threshold of avoidance in the field (19.4 µg Cu/L, 11.3 µg Cr/L, 36 µg As/L and 7.4 µg Se/L) could be the result of higher hardness (100 - 140 mg $CaCO_3$/L, which is still quite soft compared to the River Rhine) and turbidity under field conditions. In effluent mixing zones in the River Rhine concentrations may occur that are much higher than reported effective concentrations.

A classic example of avoidance behaviour under field conditions is the interference of copper and zinc pollution with the spawning migration of atlantic salmon, as observed by Sprague and coworkers (Sprague 1964, Sprague et al. 1965, Saunders & Sprague 1969). Due to metal mining activity, the Northwest Miramichi River was polluted with varrying levels of Zn and Cu (annual averages range between 5.8 - 18.5 µg Cu/L and 72 - 231 µg Zn/L). At salmon counting fences it was observed that 10 - 22% of the ascending salmon returned downstream during four years of pollution compared to 1-3% before pollution started (Sprague et al. 1965). Of the fish returning downstream 31% reascended and it was estimated that 8 - 15 % of the spawners did not reach the spawning grounds as a result of pollution (Saunders & Sprague 1967). Threshold concentration of avoidance was estimated at 113 - 139 µg total metal/L, consisting of 8.4 - 10.3 µg Cu/L and 105 - 129 µg Zn/L, whereas threshold of avoidance in the laboratory was much lower (7.1 µg total metal/L - Sprague 1964). Total blocking of migration occurred at 260 µg total metal/L (19.2 ppb Cu and 240 ppb Zn).
Water in this experiment was very soft (18 - 20 mg $CaCO_3$/L) and in the much harder River Rhine the response of salmon is expected to be less vehement. In addition reported effective levels are higher than average concentrations in the River Rhine at Lobith. Yet the spawning migration of salmon in the River Rhine may be affected: In mixing zones of industrial effluent concentrations of 300 - 400 µg/L of copper and zinc may occur in the River Rhine (DBW/RIZA). This exceeds the concentrations of copper (upto 20 x) and zinc (upto 2 x) which have *completely blocked* the spawning migration in the Northwest Miramichi River.

ORGANIC MICROPOLLUTANTS
Unusual distribution and unseasonal movement downstream, towards the estuary, of brook trout *(Salvelinus fontinalis)* and atlantic salmon was observed by Saunders (1969) after accidental spillage of nabam and endrin into the River Mill. These responses strongly suggest avoidance behaviour. Concentrations of contaminants are not given, but must have been high since mass mortalities were reported.
Apparently the organophosphate insecticide Abate was avoided by fish in the River Oti in Ghana. In the 24 hours after aerial application fewer numbers and species were caught than in the 24 hour period preceding application. Virtually no fish were killed. Expected concentrations 300 m downstream of the aerial application point were 50 - 100 µg/L, whereas 24h LC50 values for fish range between 1 and 200 mg/L. (Abban & Saman 1980)

Effects of Prudhoe Bay crude oil on salmonid homing was investigated by Malins et al. (1977) and Weber et al. (1981). Malins et al. did not observe any effect of 14 upto 250 µg Water Soluble Fraction/L on the number of homing chinook salmon *(Oncorhynchus tschawytscha)* entering a fish ladder. Weber et al. (1981) observed decreased numbers of upstream migrants (mainly coho salmon *(O. kisutch)*) at concentrations of 3.2 mg WSF/L in a fish ladder. Whether the number of spawners reaching spawning gounds was affected is not clear, since alternative routes were open for movement upstream. In a laboratory experiment threshold of avoidance of a slightly differing mixture of hydrocarbons was not very different (3.7 mg/L - Maynard & Weber 1981). In 1985 average concentrations of oil in the River Rhine at Lobith were much lower (0.03 mg/L - 't Hoen 1987).

Kelso (1977) studied the distribution and movement of fish in the Nipigon Bay in relation to a paper and pulp mill effluent. Near the effluent plume aggregation of fish and species dominance by white and longnose suckers *(Catostomus commersoni, C. catostomus)* was observed. Elsewhere yellow perch *(Perca flavescens)* dominated. Radiotelemetric research with white suckers demonstrated avoidance at low concentrations (<15%) of discharge. Despite this avoidance response, aggregation of the community occurred at the discharge site, presumably in response to increased food availability.

Brett & McKinnon (1954) performed field experiments with 54 different compounds. The number of coho and chinook salmon moving up a fish ladder was counted, as a compound was poured into the path of the salmon. Concentrations of compounds (e.g. formalin, pulp mill effluent, soap, ethyl laurate, analiine, nitrobenzene, phenol) were not determined. The only compounds affecting number of migrants were dilute solutions of mamalian skin rinses.

CHLORINATION

Schumacher & Ney (1980) established avoidance by rainbow trout of a single dose chlorination in a discharge canal. Giattina et al. (1981) compared laboratory and field avoidance of fish in heated chlorinated water. Laboratory determined avoidance in most cases accurately predicted concentrations of TRC that would elicit avoidance under natural field conditions.

### 3.4.3 Phenomenon possibly resulting from avoidance

Cairns & Cherry (1983) compared fish populations up- and downstream of a fly ash settling basin. In the influence zone Shannon Weaver diversity index was lower than in the upstream region (1.52 *vs* 2.20). Although more fish were caught in the influenced zone, more species were observed and most species were more abundant in the upstream region (suggesting avoidance of fly ash by a number of fish). The authors conclude that responses of fish are difficult to predict because of associated complex variables (e.g. temperature, pH, ash particles and heavy metal content may vary considerably).

Kalabina (1935) studied the distribution of organisms in two rivers polluted with phenol. The distribution of fish species was restricted to concentrations of less than 0.2 mg/L phenol, whereas fish were able to survive at 10 - 15 mg/L. This suggests avoidance of phenol, however also other compounds in the effluent may be responsible as well as effects of effluent on food availability.

Heavy pollution by domestic and industrial effluent from the city of Hamburg is considered responsible for the decline of characteristic fish populations in the River Elbe (Wilkens & Koehler 1977). According to the authors, the highly polluted zone also prevents upstream migration of several anadromous fish (e.g. lampreys *(Lampetra fluviatilis, Petromyzon marinus)* and sea trout *(Salmo trutta)*). Whether avoidance of chemicals plays a role of importance, is not clearly stated. During the last century sturgeon *(Acipenser sturio)*, allis shad *(Alosa alosa)*, atlantic salmon and houting *(Coregonus oxyrhynchus)* have disappeared from the River Elbe (Wilkens & Koehler 1977).

Paterson & Nursall (1975) studied the distribution of organisms in the North Saskatchewan River, which receives domestic and industrial effluent from its south bank. Benthic variety along the north side of the river was much higher and fish stayed to this side of the river. Several fish species were abundant in the North Saskatchewan River at Edmonton, but not downstream from the city (e.g. lake chub *(Couesius plumbeus)*, emerald shiner *(Notropis atherinoides)* river shiner *(N. blennius)* and sauger *(Stizostedion vitreum)*. These fish are also found in the South Saskatchewan River and downstream from its confluence with the North Saskatchewan River. The authors suggest that these species were excluded between Edmonton and its confluence as a result of severe pollution during the early 1950s and have not become re-established (data were collected in 1964-65). Avoidance of effluents may also explain this distribution of fish.

Elson et al. (1972) studied the movement of adult atlantic salmon in the Miramichi estuary, which recieves domestic and industrial effluents. As was already mentioned (§ 2.3), the overall mean rate of upstream progress in the Miramichi River (0.06 km/h) was considerably lower than in the nearby, much cleaner Tabusintac River (0.21 km/h). Elson et al. (1972) suggested that this slow progress was caused by avoidance of pollution. In a number of cases upstream moving salmon returned downstream on contact with rapidly increasing pollution.

# 4 FUNCTIONAL AND HISTOPATHOLOGICAL DAMAGE TO THE OLFACTORY ORGAN

## 4.1 The olfactory organ

The outline of the olfactory organ is described by Smith (1985) and Bertmar (1982): The olfactory epithelium is situated in the olfactory chamber which, through nares or nostrils, has contact with the environment (figure 9). The olfactory epithelium is arranged in a structure of primary and sometimes secondary lamella (e.g. salmonids - Bertmar 1982), the olfactory rosette. The surface area of the olfactory rosette is usually larger in species to which olfaction is more important (Smith 1985). The olfactory mucosa can be highly organized, as is illustrated by research of Bertmar (1982) on *Salmo trutta* (figure 10). Bertmar distinguishes 19 different celltypes, including phagocytic and immunological cells that protect the epithelium against foreign intruders. Research of Evans et al. (1982) and Graziadei & Monti-Graziadei (1978) (as cited by Hara et al. 1983), has shown a potential for turnover and regeneration of olfactory receptor cells and olfactory neurons.

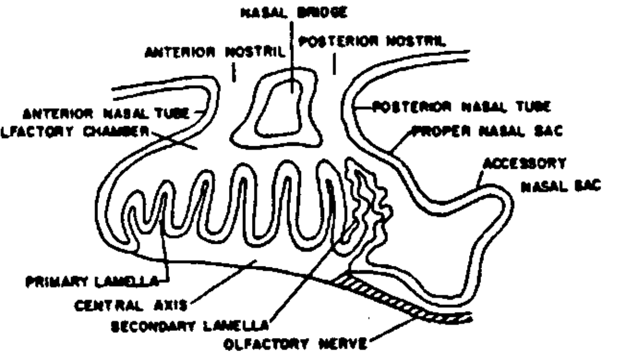


*Figure 9*
*Diagram of the olfactory organ of Baltic trout (Salmo trutta) in saggital section. The secondary folding is shown only in the posterior lamella. (From Bertmar 1982)*

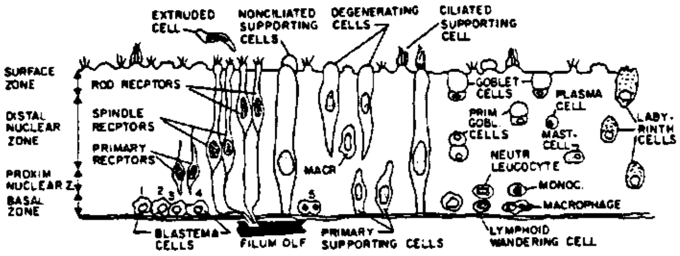


*Figure 10*
*Diagram of the sensory epithelium in adult Baltic trout (Salmo trutta) in transverse section. (From Bertmar 1982)*

Fish olfactory systems can be extremely sensitive: e.g. thresholds of detection of morpholine and ß-phenetylalcohol are dilutions of 1 : $10^9$ and 1: $3*10^{18}$ respectively (for conditioned *Oncorhynchus kisutch* and *Anguila anguila* - Smith 1985). Sensitivity may vary within species during development and season (Smith 1985, Hara 1967).

Detection of changes in chemical composition of the medium is enhanced by increased flow of water through the chamber. This can be achieved by several mechanisms: (a) ciliary movement; (b) pumping of water back and forth by an olfactory sac (figure 9); and (c) arrangement of the nostrils so that forward movement forces water over the olfactory epithelium.

## 4.2 Functional damage

### 4.2.1 Aberrant behaviour

*In a number of cases aberrant behaviour was observed which was caused by functional damage to the olfactory organ, after exposure to pollutants (table 7). Copper and the detergent lineair alkylbenzenesulfonate (LAS) affected the functioning of the olfactory organ at relatively low concentrations compared to those measured (Cu) or estimated (LAS) in the River Rhine. The effective concentration of copper (6.4 µg/L) approximates the proposed ecotoxicologicial water quality objective (1.3 µg dissolved Cu/L - Strotelder et al. 1989).*
*If concentrations of copper and detergents are as effective in the River Rhine, migration of anadromous fish could be hampered even at average concentrations, since orientational ability will be seriously affected. If this is not so, disorientation may occur in effluent mixing zones, where higher than average concentrations exist. However, contact will usually be temporary, allowing for recovery and renewed migration upstream.*
*Research has been quite limited in this field. It should be expected that test organisms are susceptible to lower concentrations and to a variety of other pollutants than those reported below.*

#### Heavy Metals

Coho salmon *(Oncorhynchus kisutch)* normally avoid L-serine, however in the presence of 6.4 µg Cu/L or 20 µg Hg/L avoidance behaviour does not occur. Upto 650 µg/L zinc did not have this effect (Rehnberg & Schreck 1986).

Whitefish *(Coregonus clupeaformis)* exposed to 36 or 50 (nominal?) µg Hg/L for 1 - 2 weeks, did not show preference for foodextract, in contrast to untreated fish. The preference response of untreated fish was abolished after cauterization of the nares. It was concluded that the reaction to foodextract was olfactory mediated. (Kamchen & Hara 1980 as cited by Brown et al. 1982).

Adult zebrafish *(Brachydario rerio)* are normally attracted to pheromone containing donor water. After exposure to zinc (5 mg/L for 9 days) this preference response was no longer present (Bloom et al. 1978).

The effective concentration reported for copper by Rehnberg & Schreck (6.4 µg/L) approximates the proposed water quality objective (1.3 µg/L dissolved Cu - Stortelder et al. 1989) and average dissolved concentrations measured in the River Rhine at Lobith in 1985 (3.0 µg/L - DBW/RIZA). Rehnberg and Schreck (1986) used experimental conditions (low hardness (31 mg $CaCO_3$/L), little complexing factors, pollutant freshly added as $CuSO_4$) under which the metal copper will be more potent a toxicant than under the conditions prevailing in the River Rhine. For this reason it is to be expected that effects of copper in the River Rhine on olfaction in fish will occur at concentrations higher than reported by Rehnberg & Schreck. On the other hand, the authors did not test concentrations lower than the reported effective concentration, nor did they test other species which may have been even more sensitive. Since concentrations occur in mixing zones of industrial effluents which are much higher than average (upto 400 µg/L - DBW/RIZA) functional damage to the olfactory organ may occur in the River Rhine and, consequently, the spawning migration of anadromous fish may be affected. However, contact will usually be temporary, allowing for recovery (§ 4.4) and renewed migration upstream.

Effective levels reported for zinc and mercury exceed water quality objectives and average concentrations in the River Rhine.

# - TABLE 7 -
# (aberrant behaviour)

*Table 7*
*Effects of dissolved pollutants on olfactory mediated behaviour in fish. For dissolved pollutants, proposed water quality objectives (Stortelder et al. 1989) and average concentrations at Lobith in the River Rhine in 1985 (DBW/RIZA) are given as well. In appendix 4 english names of species are listed.*

*Abbreviations: NOEC (No Observed Effect Concentration), LOEC (Lowest Observed Effect Concentration) LC50 (concentration lethal to 50% of the test organisms).*
*Notes: 1 highest concentration in experiment; 2 lowest concentration in experiment; 3 only concentration in experiment; * threshold value of response.*

| COMPOUND | REFERENCE | SPECIES name | size | EXPERIMENTAL CONDITIONS hardness mg CaCO3/L | pH | T °C | time | NOEC (ug/L) | LOEC (ug/L) | note | RESPONSE | ECOTOX OBJECTIVE (ug/L) | RHINE 1985 (ug/L) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cu | Rehnberg & Schreck 1986 | Oncorhynchus kisutch | 2.9 g | 31 | 6.7 | 15 | 2.5h | | 6.4 | 2 | Inhibition of avoidance to L-serine | 1.3 | 3.0 |
| Hg | Rehnberg & Schreck 1986 | Oncorhynchus kisutch | 2.9 g | 31 | 6.7 | 15 | 2.5h | | 20 | 2 | Inhibition of avoidance to L-serine | 0.005 | 0.01 |
| | Kamchen & Hara 1980 (in Brown et al. 1982) | Coregonus clupeaformis | | | | | 7-14d | | 37 - 50 | | Abolished preference for food extract | | |
| Zn | Bloom et al. 1978 | Brachydario rerio | | 50? | 7.0 | 24 | 9d | | 5 000 | 3 | Abolished attraction to pheromone containing water | 8.5 | 18 |
| | Rehnberg & Schreck 1986 | Oncorhynchus kisutch | 2.9 g | 31 | 6.7 | 15 | 2.5h | 650 | | 1 | Inhibition of avoidance to L-serine | | |
| LAS | Bardach et al. 1965 | Ictalurus natalis | 300 g | 65 | 9.1 | 21 | 25d | | 1 000 | 3 | Impaired feeding behaviour, hyperactivity | | |
| | Olsen & Hogland 1985 | Salvelinus alpinus | 18 cm | | 8.0 | 9 | 6h | | 20 | 2 | Reduced attraction between juveniles | | |
| ABS | Bardach et al. 1967 | Ictalurus natalis | 300 g | 65 | 9.1 | 21 | 28d | | 1 000 | 3 | Impaired feeding behaviour, hyperactivity | | |
| | Foster et al. 1966 | Jordanella floridae | adult | | | | 4d | | 10 000 | 2 | Affected feeding behaviour | | |
| TritonX-100 | Kasumyan & Paschenko (1982) | Ctenopharyngodon idella | | | | | | | 0.5% | 3 | Depressed defense reaction on release of alarm pheromone | | |
| Oil | Malins et al. 1977 | Oncorhynchus tschawytscha | | | | | 16h | 5 - 101 | | | Affected homing on field release after exposure to water soluble fraction. | | 30 |
| | | O. kisutch | | | | | 1-2d | 0.5 - 256 | | | | | |
| Volcanic ash | Whitman et al. 1982 | Oncorhynchus tschawytscha | adult | | | | 7d | 650 mg/L | | 3 | Affected homing on field release. | | |
| pH | Royce-Malmgren & Watson 1987 | Salmo salar | 18 cm | | 7.6 | 10 | 15 | | | | Attraction to glycine, avoidance of L-alanine | | 7.5 - 7.7 |
| | | | | | 5.1 | | | | | | indifferent to glycine,attracted to L-alanine | | |
| | Lemly & Smith 1985 | Pimephales promelas | | 117 | 6.5 | 20 | 72h | | | | Response to chemical feeding stimulus | | |
| | | | | | 6.0 | | | | | | response abolished | | |

### Petroleum hydrocarbons

Malins et al. (1977) investigated whether exposure to the Water Soluble Fraction of Prudhoe Bay crude oil (containing petroleum hydrocarbons like toluene, benzene and xylene) affected homing ability of salmonids. No effect on homing was observed after 14 - 18h exposure of chinook salmon *(Oncorhynchus tschawytscha)* to 5 - 101 µg/L WSF and after 26 - 45h exposure of coho salmon *(O. kisutch)* to 0.5 - 256 µg/L model oil. However both experiments were performed during abnormal weather conditions, accompanied by low recoveries of both control and pre-exposed animals. This may have obscured effects of exposure on (olfactory) orientational ability .

### Volcanic Ash

Whitman et al. (1982) performed similar experiments with adult chinook salmon. Seven day exposure to 650 mg/L of volcanic ash did not affect homing ability. Again low recoveries were observed.

### Detergents

Bardach et al. (1965) observed impaired feeding behaviour in yellow bulheads *(Ictalurus natalis)* exposed to 1 mg/L lineair alkylbenezenesulphonate (LAS, 25 days) or 1 mg/L branched-chain alkylbenezenesulphonate (ABS, 28 days). Although histological examination at this concentration did not reveal damage to the olfactory organ, the fish could not locate food pellet smell. In detergent-free water recovery occured, which was not completed after 6 weeks.

Foster et al. (1966) observed effects on feeding behaviour in flagfish *(Jordanella floridae)* after exposure with 10 mg ABS/L or more for 4 days.

Olsén & Högland (1985) observed reduction of olfactory mediated attraction between juveniles of atlantic charr *(Salvelinus alpinus)* by the detergent LAS. Exposure with 20 µg LAS/L for 6 hours resulted in a 30% reduction in attraction. However, the effect seemed to be partially reversible: exposure to 1 - 2 mg/L for 6 hours resulted in completely depressed attraction whereas exposure over 96 hours resulted in only partial reduction of attraction.

On release of an alarm pheromone the white amur *(Ctenopharyngodon idella)* displays a defense reaction. After brief treatment of the olfactory epithelium with 0.5% solution of the detergent Triton X-100, sensitivity to the alarm pheromone was affected. Sensitivity was restored in 4 days (Kasumyan & Paschenko 1982).

Concentrations of total anionic detergents in the River Rhine do not exceed 100 µg/L. It is estimated that approximately 50-70% of the anionic detergents in surface waters is LAS (DBW/RIZA). A maximum concentration of 50 µg/L LAS exceeds the effective concentration reported by Olsén & Högland (20 µg/L). As was already stressed in chapter 3 (avoidance), detergents may pose a threat to the spawning migration of anadromous fish. The limited data on concentrations of detergents occuring in surface waters and effects these may have on aquatic organisms, must be seen as a serious lack of knowledge.

### pH

Royce-Malmgren & Watson (1987) investigated the effect of acid rain on olfactory mediated behaviour. Juvenile atlantic salmon *(Salmo salar)* are attracted to glycine and avoid L-alanine at ph 7.6. On lowering pH to 5.1 the response to both amino acids was changed in indifference and attraction respectively. This change was reversible with pH. The authors conclude that acidification may seriously affect recognition of olfactory cues during the spawning migration.

Exposure of fathead minnows *(Pimephales promelas)* to pH 6.0 (72 hours) abolished behavioural response to chemical feeding stimuli, which did occur at ph 6.5 or higher (Lemly & Smith 1985).

Acidification is not a major problem in the River Rhine: during the perion 1980 - 1985 pH averaged 7.5 - 7.7 ('t Hoen 1987).

### 4.2.2 Electrophysiological experiments

Functional damage to the olfactory organ by a number of pollutants was observed in electrophysiological experiments (table 8). In these experiments EEG-response of the olfactory bulb to a standard stimulant (e.g. L-serine, L-alanine, foodextract) is measured (figure 11), and depression of this response is observed on addition of, or after exposure to, varying concentrations of toxicants (figure 12). Effective concentrations were low for copper (8 µg/L), silver (3 µg/L) and the anionic detergent lauryl sulfate (AS, 100 µg/L). The effective level for copper was close to average concentrations of dissolved copper in the Rhine at Lobith (3.0 µg/L in 1985) and proposed water quality objective (1.3 µg/L dissolved Cu). Translation of these data to behaviour of fish in the River Rhine is difficult.
Research has been quite limited in this field and it should be expected that test organisms may be susceptible to lower concentrations and a variety of other pollutants than those reported below.

*Figure 11*
*Experimental setup for electrophysiological recording of olfactory response to a standard stimulant in absence or presence of toxicant solution. The standard stimulant (ST) or toxicant (TX) solutions are pored into the nares (N) through glass capilaries (C) adjusted by a micromanipulator (M). Response to a stimulus is measured with recording electrodes (E). (modified from Hara et al. 1983)*

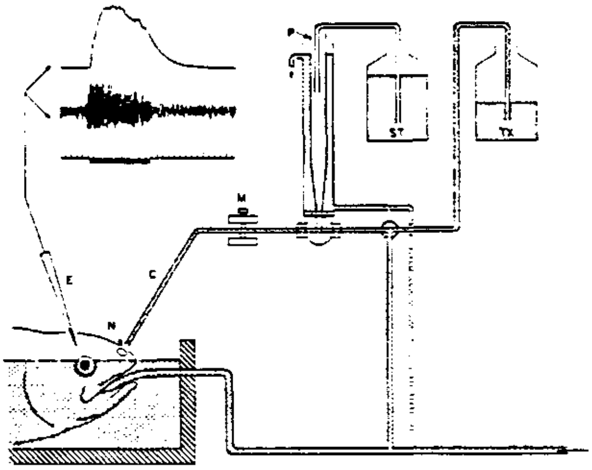
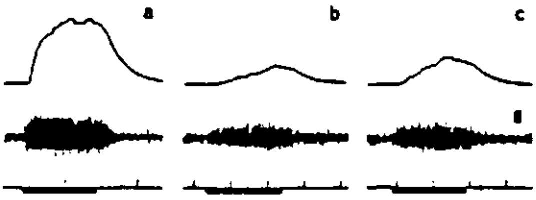


*Figure 12*
*Electrical responses recorded from the olfactory bulb of rainbow trout (Salmo gairdneri) when nares were stimulated with L-serine (10 µM) before (a), during (b) and after exposure to 5 µM mercuric chloride (1 mg/L). The upper tracing of each pair is the integrated response of the lower. Duration of stimulation is indicated by heavy lines below each record. Time scale: each division is 5 sec. (From Brown et al. 1982)*



## HEAVY METALS

A large part of this electrophysiological research was done by T.J. Hara and coworkers. In Brown et al. (1982) results of their research on toxicity of heavy metals to olfaction in *Salmo gairdneri* were presented. Copper, mercury and silver affect olfaction at sublethal concentrations (LOECs (lowest concentrations observed to have effect) are 12.7, 80 and 3 µg/L respectively), whereas cadmium, zinc, lead, cobalt and nickel depress olfaction only at levels exceeding lethal values (Brown et al. 1982 - figure 13).

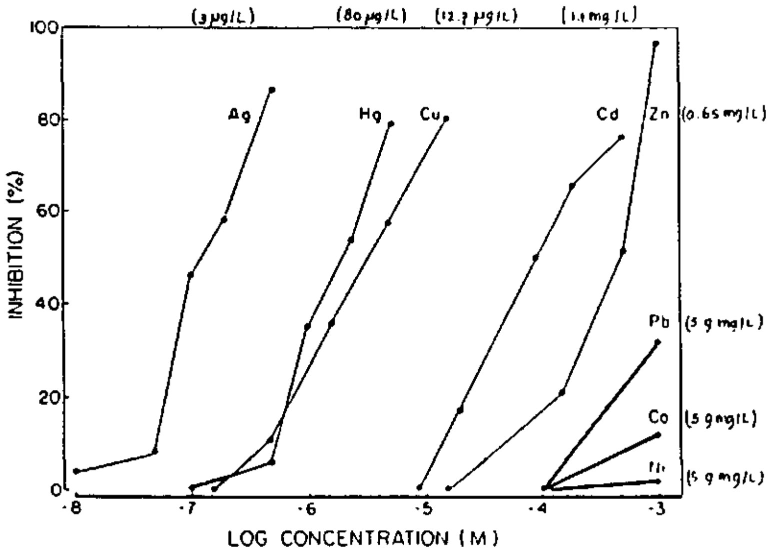


*Figure 13*
*Inhibition of the olfactory bulbar response to L-serine (10 µM) when the nares of rainbow trout (Salmo gairdneri) were exposed to various concentrations of heavy metals. Inhibition was determined from the average response (n = 5) to L-serine in the 10-min period following 30-min treatment with metals. Between brackets LOECs (lowest concentrations observed to have effect) are given. Hardness and pH are not specified. (From Brown et al. 1982.)*

The lowest copper concentration reported to affect bulbar response to L-serine was 8 µg/L (2h exposure - Hara et al. 1976), which is close to average concentrations reported in the River Rhine at Lobith in 1985 (3.0 µg/L - DBW/RIZA) and the proposed water quality objective (1.3 µg/L - Stortelder et al. 1989). In the same experiment 50 µg Cu/L for 2 hours resulted in a depression of the response to approximately 55% of the original level. Complete recovery of olfactory response was not achieved, even after rinsing with dechlorinated tapwater for another 2 hours. The water used in this experiment was soft (90 mg $CaCO_3$/L) compared to the River Rhine (240 - 250 mg $CaCO_3$/L, 't Hoen 1987) and a higher concentration of copper may be needed before a similar effect on the olfactory organ will occur in the River Rhine. Higher concentrations however do occur (upto 400 µg/L in the mixing zone of industrial effluent - DBW/RIZA).

The effective concentration of mercury was much higher than the proposed water quality objective and the average concentrations in the River Rhine. For silver these data are not available.

Thompson & Hara (1977 - as cited by Hara et al. 1983) compared the toxicity of natural lake water, polluted with heavy metals from mining and smelting activities, with that of artificial lake water with a similar heavy metal content. The natural lake water depressed the olfactory response of arctic charr *(Salvelinus alpinus)* to a lesser extent than did the artificial water. Differences in metal speciation may explain this phenomenon.

- TABLE C -
(electrophysiology)

*Table 8*
*Effects of dissolved pollutants on electrical response of the olfactory bulb to a standard stimulant. For dissolved pollutants, proposed water quality objectives (Stortelder et al. 1989) and average concentrations at Lobith in the River Rhine in 1985 (DBW/RIZA) are given as well. In appendix 4 english names of species are listed.*

*Abbreviations: NOEC (No Observed Effect Concentration), LOEC (Lowest Observed Effect Concentration) LC50 (concentration lethal to 50% of the test organisms).*
*Notes: 1 highest concentration in experiment; 2 lowest concentration in experiment; 3 only concentration in experiment; * threshold value of response.*

| COMPOUND | REFERENCE | SPECIES |  | EXPERIMENTAL CONDITIONS |  |  |  | NOEC | LOEC |  | RESPONSE | ECOTOX | RHINE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  |  | name | seize | hardness mg CaCO3/L | pH | T °C | time | (ug/L) | (ug/L) | note |  | OBJECTIVE (ug/L) | 1985 (ug/L) |
| Ag | Brown et al. 1982 | Salmo gairdneri |  |  |  |  | 30m |  | 3 | * | depression response to L-serine |  |  |
| Cd | Brown et al. 1982 | Salmo gairdneri |  |  |  |  | 7d | 50 | 150 |  | depression response to L-serine | 0.025 | 0.04 |
|  |  |  |  |  |  |  | 30m |  | 1 100 | 3 | depression response to L-serine |  |  |
| Co | Brown et al. 1982 | Salmo gairdneri |  |  |  |  | 30m |  | 5 900 | 3 | depression response to L-serine |  |  |
| Cu | Hara 1972 | Onchorhynchus kisutch | 22cm |  |  | 15 | 10s |  | 16 000 | 3 | inhibition response to L-serine for >20m | 1.3 | 3.0 |
|  |  | O. nerka |  |  |  |  | 12h |  | 100 | 3 | inhibition response to L-serine |  |  |
|  | Hara et al. 1978 | Salmo gairdneri | 23cm | 90 | 7.7 | 13 | 2h |  | 8 | * | depression response to L-serine |  |  |
|  | Brown et al. 1982 | Salmo gairdneri |  |  |  |  | 30m |  | 12.7 | * | depression response to L-serine |  |  |
| Hg | Sutterlin & Sutterlin 1971 | Salmo salar | 17cm |  |  |  | brief |  | 20 000 | 3 | inhibition response to 10 aminoacids for >1hr | 0.005 | 0.01 |
|  | Hara 1972 | Onchorhynchus kisutch | 22cm |  |  | 15 | 10s |  | 27 000 | 3 | inhibition response to L-serine for <20 - 30m |  |  |
|  |  | O. nerka |  |  |  |  | 3d |  | 100 | 3 | depression response to L-serine |  |  |
|  | Hara et al. 1978 | Salmo gairdneri | 23cm | 90 | 7.7 | 13 | 2h | 50 | 100 |  | depression response to L-serine |  |  |
|  | Brown et al. 1982 | Salmo gairdneri |  |  |  |  | 30m |  | 80 | * | depression response to L-serine |  |  |
|  |  |  |  |  |  |  | 3d |  | 100 | 3 | depression response to L-serine |  |  |
|  |  |  |  |  |  |  | 7d | 50 | 150 |  | depression response to L-serine |  |  |
|  | Kamchen & Hara 1980<br>(as cited by Brown et al. 1980) | Coregonus clupeaformis |  |  |  |  | ? |  | 100 | * | electrophysiological effect |  |  |
| methyl-mercury | Huve & Bagot 19.. | Salmo gairdneri |  |  |  |  |  | 1 000? | 10 ng/L |  | general depression of olfactory response<br>(toxicant elicits a specific response) |  |  |
| Ni | Brown et al. 1982 | Salmo gairdneri |  |  |  |  | 30m |  | 5 900 | * | depression response to L-serine | 7.5 | 3.7 |
| Pb | Sutterlin & Sutterlin 1971 | Salmo salar | 17cm |  |  |  | brief | 207 000 |  | 3 | response to 10 aminoacids | 1.3 | 0.2 |
|  | Brown et al. 1982 | Salmo gairdneri |  |  |  |  | 30m |  | 20 700 | * | depression response to L-serine |  |  |
| Zn | Brown et al. 1982 | Salmo gairdneri |  |  |  |  | 30m |  | 650 | * | depression response to L-serine | 6.5 | 18 |
|  | Cancalon 1980 | Ictalurus punctatus |  |  |  | 21 | short |  | 20 g/L | 3 | 70% depression response to alanine<br>recovery in 6 to 7 days |  |  |
| AS | Hara & Thompson 1978 | Coregonus clupeaformis | 13cm | 78 | 7.5 | 11 | 15m |  | 100 | 2 | depression response to L-serine or food extr. |  |  |
| LAS, ABS | Sutterlin et al. 1971<br>(as cited by Olsen & Hogland 1985) | Salmo salar |  |  |  |  | 15s |  | 1 000? | 2 | depression response to L-alanine |  |  |
| Triton X-100 | Cancalon 1980 | Ictalurus punctatus |  |  |  | 21 | short |  | 0.03-0.1% |  | inhibition of response to alanine<br>recovery within next hour |  |  |
| atrazine | Huve & Bagot 19.. | Salmo gairdneri |  |  |  |  |  | 1 000? | 10 ng/L |  | general depression of olfactory response<br>(toxicant elicits a specific response) | 0.075 |  |
| benzene | Malins et al. 1977 | Oncorhynchus kisutch |  |  |  |  | 25s | 0.2-2 mg/L |  |  | depression response to L-serine<br>(toxicant elicits response) | 9 | 0.0 |
| ethylparathion | Huve & Bagot 19.. | Salmo gairdneri |  |  |  |  |  | 1 000? | 10 ng/L |  | general depression of olfactory response<br>(toxicant elicits a specific response) | 0.02 |  |
| lindane | Huve & Bagot 19.. | Salmo gairdneri |  |  |  |  |  | 1 000? | 10 ng/L |  | general depression of olfactory response<br>(toxicant elicits a specific response) |  |  |
| naphtalene | Malins et al. 1977 | Oncorhynchus kisutch |  |  |  |  | 25s | 0.2-17 mg/L |  |  | depression response to L-serine<br>(toxicant elicits response) |  |  |
| di- trimethyl-<br>naphtalene | Huve & Bagot 19.. | Salmo gairdneri |  |  |  |  |  | 38<br>238 |  |  | effect on response to 3 aminoacids<br>(no olfactory response observed) |  |  |
| Oil, water souluble | Maynard & Weber 1981 | Oncorhynchus kisutch | 8 cm |  |  | 5-17 | 20m | 4 000 |  | 3 | depression response to L-serine<br>(toxicant elicits olfactory response) |  | 30 |
|  | Malins et al. 1977 | Oncorhynchus kisutch |  |  |  |  | 25s | 1.8-20 mg/L |  |  | depression response to L-serine<br>(toxicant elicits response) |  |  |

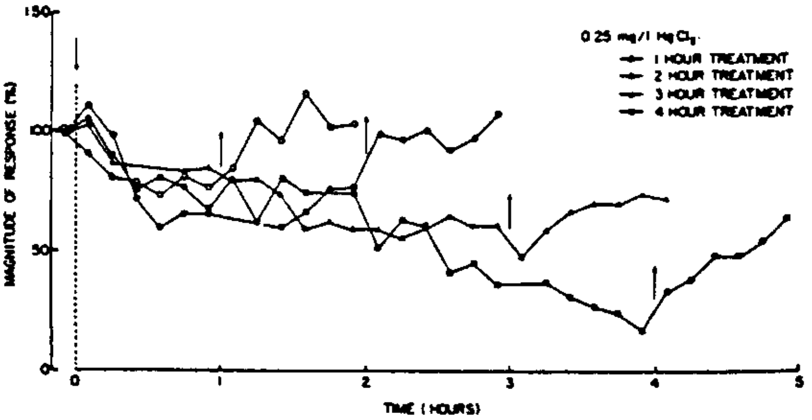


*Figure 14*
*Effect of duration of treatment on the olfactory bulbar response of rainbow trout (Salmo gairdneri) to L-serine (10µM). The nares of fish were treated with 250 µg Hg/L for 1, 2, 3 and 4 h respectively. The magnitude of response is represented as a percentage of the control response before treatment. (Hardness 90 mg CaCO3/L, pH 7.5 - 7.8) From Hara et al. (1976).*

In general, longer exposure further reduces the response to L-serine and prolongs the time needed for recovery (Hara et al. 1976, Hara & Thompson 1978, Brown et al. 1982). Although this effect of exposure time was observed in experiments with mercury (Hara et al. 1976, figure 14), threshold values of response do not seem to decrease with increasing exposure time: 30-min, 3-hour or 7-days LOEC values do not differ much (table 8). Comparison of data, however, is open to question because experimental conditions are not specified and may have influenced results.

ORGANIC MICROPOLLUTANTS

Even at the lowest concentration they tested (100 µg/L for 15 min.), Hara & Thompson (1978) observed effects of the detergent laurylsulfate (AS) on olfactory response. Olsén & Högland (1985) refer to research by Sutterlin et al. (1971) on the effects of 21 different detergents. It is estimated that concentrations of anionic detergents in the River Rhine do not exceed 100 µg/L (DBW/RIZA). Whether this is also true for mixing zones of effluents is not known.

In contrast to the heavy metals and detergents tested, the synthetic compounds atrazine, lindane and ethyl-parathion (upto 1 mg/L or more) did not depress olfactory response, but elicited a specific and reproducable signal with a distinct spectral signature. Threshold of response to these compounds was approximately 10 ng/L (Huvé & Bagot). A mixture of monocyclic aromatics (toluene, xylene, benzene) was also reported to elicit electrical response (Maynard & Weber 1985). In this experiment exposure to 4 mg/L for 20 min did not depress response to L-serine. Naphtalene, benzene and saltwater soluble Prudhoe Bay crude oil also elicit EEG response and do not depress olfactory response to amino acids (Malins et al. 1977). Because of the nature of the response, Malins et al. (1977) suggest it to be non-specific irritant effects in the olfactory epithelium, which might have a more disruptive effect on chronic exposure.

Pollutants which elicit olfactory response, may compete for sites on the chemoreceptor membranes and in this way mask biologically relevant chemical stimuli (Hara et al 1983). No reference was found which clearly illustrates this effect. Simultaneous stimulation of the olfactory epithelium with L-serine (0.5 mg/L) and naphtalene (0.75 mg/L) resulted in an EEG response which was characteristic of the L-serine concentration used (Malins et al. 1977).

Which mechanisms are responsible for the depression of electrical response to standard stimulants, remains unclear. Detergents have been suggested to remove mucus, denaturalize proteins and alterate membrane permeability and transport (Hara & Thompson 1978). Heavy metals may bind strongly to receptorsites or enzymatic components (Sutterlin & Sutterlin 1971). Exposure to copper and cadmium result in a reduction in total number of L-serine bindingsites in the olfactory rosette *(in vivo)*, which parallels the observed depression of olfactory response (Brown et al. 1982). On the other hand, the mechanism of mercury intoxication may be impairment of the central nervous system (Brown et al. 1982).

Obscurities concerning effect of exposure time and experimental conditions hinder the comparison of the data in table 8 with field conditions or water quality objectives. Of the (few) compounds tested especially copper, detergents like laurylsulfate (AS) and possibly silver, may pose a threat to the physiology of the olfactory organs of fishes in the River Rhine.
It is difficult to assess the impact these physiological effects will have on orientation and survival chances of fish. Concentrations of pollutants instantly abolishing electrical response to amino acids obviously represent unfavourable conditions. However, these concentrations are not quite concievable in the River Rhine and it is unclear to what extent partial reduction of electrical response affects the orientational ability of fish and, ultimately, their survival chances.

## 4.3 Structural damage

In a limited number of histopathological studies, damage to the olfactory apparatus was observed (table 9). Of the heavy metals studied, silver, copper and mercury are the most toxic. Because structural damage in general is preceded by functional damage, histopathology is not a very sensitive tool for assessment of toxicity. E.g. Bardach et al. (1965) observed impaired feeding behaviour of yellow bulheads *(Ictalurus natalis)* after exposure to the detergents ABS and LAS, whereas no histopathological damage was observed. In this respect it is not surprising that LOECs from table 9 are well above present-day concentrations in the River Rhine and water quality objectives.

The most sensitive assay was probably used by Brown et al. (1982). With Baker's acid hematein, staining was achieved of phospholipids (which are highly localized in the receptor neurons of the olfactory mucosa). Exposure to a number of heavy metals for two weeks resulted in a reduction of the number of stained neurons. In animals subsequently placed under control conditions, the number of stained neurons returned to pre-exposure levels in 12 weeks. Other histopathological damage was not observed. In Brown et al. (1982) this research is presented as a qualitative example of histopathological damage to olfaction, unfortunately LOEC and NOEC values are not reported.

*Table 9*
*Histopathological damage of the olfactory organ after exposure to several dissolved toxicants. For dissolved pollutants, proposed water quality objectives (Stortelder et al. 1989) and average concentrations at Lobith in the River Rhine in 1985 (DBW/RIZA) are given as well. In appendix 4 english names of species are listed.*

*Abbreviations: NOEC (No Observed Effect Concentration), LOEC (Lowest Observed Effect Concentration) LC50 (concentration lethal to 50% of the test organisms).*
*Notes: 1 highest concentration in experiment; 2 lowest concentration in experiment; 3 only concentration in experiment; * threshold value of response.*

| COMPOUND | REFERENCE | SPECIES name | EXPERIMENTAL CONDITIONS seize | hardness mg CaCO3/L | pH | T °C | time | NOEC (ug/L) | LOEC (ug/L) | note | RESPONSE | ECOTOX OBJECTIVE (ug/L) | RHINE 1985 (ug/L) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ag | Gardner 1975 | Fundulus heteroclitus | adult | 20 %. sal | | 20 | 96h | | 50 | 2 | Widespread degeneration of epithelial lining and sustencular epithelium. Neurosensorycells apically necrotic. | | |
| Cd | Gardner & Yevich 1970 | Fundulus heteroclitus | 8cm | 32 %. sal | | 20 | 48h | 50 000 | | 1 | Olfactory epithelium intact (Gardner 1976). | 0.025 | 0.04 |
| | Gardner 1975 | Fundulus heteroclitus | adult | 20 %. sal | | 20 | 48h | 65 000 | | 3 | Olfactory epithelium intact. | | |
| | | | | | | | 1yr | 560 | | 3 | Olfactory epithelium intact. | | |
| | Brown et al. 1982 | Salmo gairdneri | | | | | 14d | | 270? | | Reduction of number of receptor neurons. | | |
| Cu | Gardner & Laroche 1973 | Menidia menidia | adult | 20 %. sal | | 20 | 6h | | 500 | 2 | Architecture of olf. epithelium destroyed. | 1.3 | 3.0 |
| | | Fundulus heteroclitus | adult | | | | 24h | | 500 | 2 | Architecture of olf. epithelium destroyed. | | |
| | Brown et al. 1982 | Salmo gairdneri | | | | | 14d | | 150? | | Reduction of number of receptor neurons. | | |
| Hg | Gardner 1975 | Fundulus heteroclitus | adult | 20 %. sal | | 20 | 96h | 250 | 500 | | Widespread cellular degeneration and necrosis | 0.005 | 0.01 |
| | Brown et al. 1982 | Salmo gairdneri | | | | | 14d | | 480? | | Reduction of number of receptor neurons. | | |
| Zn | Gardner 1975 | Fundulus heteroclitus | adult | 20 %. sal | | 20 | 96h | 70 000 | | 3 | Olfactory epithelium intact. | 6.5 | 18 |
| | Cancalon 1980 | Ictalurus punctatus | | | | | short | | 20 g/L | 3 | Degeneration of receptor cells. Recovery is vissible after 6 days | | |
| LAS | Bardach et al. 1965 | Ictalurus natalis | 300 g | 65 | | 21 | 28d | | 4 000-5 000 | | Decrease in number receptorcells, thickening of border of cells. | | |
| | Olsen (unpublished) (as cited by Olsen & Hogland 1985) | Salvelinus alpinus | 16 cm? | | | | 4d | 2 000 | | 3 | No damage to surface of olfactory epithelium observed with scanning E.M.. | | |
| ABS | Bardach et al. 1965 | Ictalurus natalis | 300 g | 65 | | 21 | 28d | | 4 000-5 000 | | Decrease in number receptorcells, thickening of border of cells. | | |
| TritonX-100 | Cancalon 1980 | Ictalurus punctatus | | | | 21 | short | | 0.03-0.11% | | loss of cilia and microvili of olfactory epithelium, recovery in 2 - 3 days | | |
| Naphtalene | DiMichelle & Turner 1978 | Fundulus heteroclitus | 8 cm | 15%. sal | 7.6 | 20 | 15d | 2 | 20-200 | | Necrosis of neurosensory cells. | | |
| Oil | Gardner 1975 | Menidia menidia | adult | 28 %. sal | | | 7d | | | | | | 30 |
| | | whole crude oil | | | | | | | 140 | 3 | Hyperplasia and cell degeneration | | |
| | | salt water insoluble | | | | | | | 560 | 3 | Vasodialtion of submucosa | | |
| | | salt water soluble | | | | | | | 167 | 3 | Metaplasia | | |
| | Solangi & Overstreet 1982 | Menidia beryllina | adult | 18 %. sal | | 25 | | | | | | | |
| | | whole crude oil | | | | | 7d | 5 000 | 100 000 | | Hyperplasia of sustencular epithelium and | | |
| | | | | | | | 30d | | 5 000 | 2 | cell necrosis | | |
| | | water soluble fraction | | | | | 7d | 5% | 50% | | | | |
| | | | | | | | 30d | | 5% | 2 | | | |
| | | Trinectes maculatus | adult | 18 %. sal | | 25 | | | | | | | |
| | | whole crude oil | | | | | 38-60 | 5 000 | 100 000 | | Necrosis of sustencular epithelium | | |
| | | water soluble fraction | | | | | 30-60 | 5% | 50% | | Hyperplasia, necrosis sustencular epithelium | | |

## 4.4 Recovery

In the River Rhine, fish temporarily make contact with high concentrations of pollutants in effluent mixing zones (see table 3 in § 3.3.1). Whether this type of exposure will seriously affect the spawning migration of anadromous fish, depends strongly on the time needed for recovery (after contact) and the frequency of contact with effluent plumes. Knowledge on frequency of high concentration discharges, and in general on concentrations of pollutants in effluents, is quite limited (DBW/RIZA).

Examples of recovery of damaged olfaction have been given throughout this chapter. The time needed for recovery varies with the severity of damage, which is related to:
- type of chemical (mechanism and velocity of intoxication);
- dose (concentration, availability and duration of exposure);
- sensitivity of the organism.

In case of masking of natural odours by pollutants, recovery in unpolluted water will be swift since the olfactory organ itself is unaffected. On the other hand recovery will be slow when pollutants strongly bind to receptorsites.

The experiments reported on vary considerably in the three factors mentioned above, and in only a fraction of the experiments reference is made of the time needed for recovery. As a consequence, knowledge on recovery is scant.
In general the time needed for recovery is longer than the exposure time, e.g.:
- After two weeks of exposure of rainbow trout to admittedly high concentrations of heavy metals, 12 weeks were needed for morphological recovery of histopathological damage (Brown et al. 1982, § 4.3).
- More than 6 weeks were needed for recovery of feeding behaviour of *Ictalurus natalis* after 3½-4 weeks of exposure to 0.5 mg/L of the detergents LAS or ABS (Bardach et al. 1965, § 4.2.1).
- After brief treatment (10-15 seconds) with high concentrations of pollutants, a relatively long time (>20 min) is needed for recovery of electrical response of the olfactory bulb (§ 4.2.2).

The behaviour of atlantic salmon in the Northwest Miramichi River has already been described (§ 3.4.2). Saunders & Sprague (1967) reported that a part of the ascending salmon returned downstream as concentrations of copper and zinc rose in the river. It is not clear whether this behaviour is due to active avoidance of pollution or to loss of orientational ability. It was also observed that approximately one third of the fish that had returned downstream, reascended the River. This may have been due to recovery of functional damage.

## 4.5 Effects on other orientational mechanisms

In this report attention has been focussed on effects of pollutants on olfactory orientation. However, pollutants may also affect other orientational mechanisms that are important in the spawning migration of anadromous fish (see § 2.4):
- Lesions in other sensory organs have been observed e.g. in the tastebuds (Bardach et al. 1965, DiMichele & Taylor 1978) and in the lateral line organ (DiMichelle & Taylor 1973, Eisler & Gardner 1973).
- Responses to light and temperature (directed locomotor orientation) may be altered after exposure to sublethal concentrations of heavy metals and pesticides (Rand 1985, Murty 1986). E.g. temperature preference was altered by exposure to copper (*Carassius auratus* - Kleerekoper et al. 1973) and various chlorinated hydrocarbons (*Salmo salar, Salvelinus fontinalis* - Peterson 1973).
- Flowperception was altered in goldfish *(Carassius auratus)*, bluegill sunfish *(Lepomis macrochirus)* and largemouth bass *(Micropterus salmoides)* after

exposure to parathion (Rand et al. 1975, Rand 1977A, B).
- Salinity selection in mosquitofish *(Gambusia affinis)* was affected by DDT (Hansen 1972).

# 5 CONCLUSIONS

## Avoidance experiments

1. In preference-avoidance studies the ability of organisms to respond to the presence of a chemical (or other stimulant) by moving toward it (attraction, selection or preference) or away from it into a 'clean area' (avoidance) is tested. Laboratory experiments have shown that :

- The IRC prioritairy pollutants tetrachloroethylene, copper and zinc were avoided by fish at concentrations near the proposed dutch ecotoxicological water quality objectives and average concentrations in the River Rhine at Lobith (table 10).
- Some anionic detergents (e.g. LAS, lineair alkylbenzene sulfonate) were avoided at concentrations of 0.1 - 20 µg/l. Concentrations occurring in the River Rhine are estimated at maximally 100 µg/L of total anionic detergents of which 50 - 70% is LAS. In view of their widespread use, anionic detergents may pose a serious threat to the spawning migration of anadromous fish.
- Fish seem to be less sensitive to other pollutants. However, research has been limited. Furthermore, in the River Rhine concentrations of pollutants may occur that are much higher than the average concentrations at Lobith.

2. Avoidance of chemicals has been observed under field conditions. The number of observations is limited and in only four cases laboratory experiments have been performed next to field observations. In the Northwest Miramichi River, the spawning migration of atlantic salmon was affected due to copper and zinc pollution. Effective levels were higher under field than under laboratory conditions. However, field observations in the other three studies agreed better with laboratory observations.

3. The extrapolation of the laboratory findings to field conditions occurring in the River Rhine is difficult, because of a number of modifying factors and the limited knowledge on their action (see § 3.2). Avoidance responses to chemicals in the River Rhine may be expected to occur at concentrations higher than those reported in laboratory studies:

- The hardness and complexing capacity of River Rhine water will lower the bioavailability of most pollutants. This will result in higher threshold concentrations of avoidance, as compared to the laboratory data reported here.
- Under field conditions the behavioural disposition of fish will be different from that of fish under laboratory conditions. Migrating anadromous fish will be highly motivated to move upstream, and will not as easily be displaced downstream.
- Under field conditions fish may have adapted to the presence of a pollutant and may consequently be less responsive, than not-previously exposed fish used in the laboratory.
- In laboratory experiments steep gradient chambers have been used, in which orientation is easier and favourable conditions are close at hand. In nature steep gradients are rare (they are present at the confluence of two water sources e.g. effluent mixing zones).

4. Despite of this, the best strategy to establish concentration levels which threaten the spawning migration of anadromous fish seems to be the use of lowest observed concentrations eliciting avoidance behaviour (worst-case treatment). Sensitivity of fish to chemicals should not be underestimated.

- Pollutants that have been tested were often tested at high concentrations, using only one species. It is not very likely that these experiments have uncovered the lowest concentrations of chemicals that elicit avoidance responses. Fish may also avoid compounds that have not been tested so far.

- Mixtures of pollutants are present in the River Rhine. Although only little is known on avoidance of mixtures, research so far demonstrates that mixtures of pollutants may be avoided at concentrations lower than those observed in experiments testing pollutants seperately. (Additive toxicity often occurs and has been taken into account in assessing the proposed ecotoxicological water quality objectives.)
- It is to be expected that salmonids, having exquisite olfaction to secure homing, are among the most sensitive of fish species and will detect and avoid chemicals at lower concentration than other fish species.

5. Average concentrations of pollutants in the River Rhine at Lobith are not entirely representative of conditions occurring elsewhere in this river. The maximum concentrations measured at Lobith (1985) are usually a factor 1.5 - 3 times the average concentrations (DBW/RIZA). Locally, much higher concentrations may be encountered, e.g. in effluent mixing zones (table 10). In addition, the water at Lobith still has to go a long way before it reaches the sea. It will traverse heavily populated and industrialized areas and the concentration of some pollutants will increase along the way. In effluent mixing zones, zinc and copper may occur at concentrations that are a factor 1 (for Zn) to 20 (for Cu) times as high as the concentration reported to have completely blocked the spawning migration of atlantic salmon in the (very soft) Northwest Miramichi River. Locally and temporarily, concentrations in the River Rhine may occur of chloroform, cadmium, chromium, nickel and lead that exceed reported effective levels as well. Concentrations of other pollutants in effluent mixing zones are not known, however these could also be higher than effective levels.

*Table 10*
*Comparison of concentrations of pollutants occurring in the River Rhine: average concentrations at Lobith of dissolved pollutants (1985) and maximum concentrations expected in mixing zones of domestic sewage treatment plant effluents and industrial effluents (DBW/RIZA). An initial dilution of the effluent of 1 : 10 is assumed. In addition proposed water quality objectives for dissolved pollutants (Stortelder et al. 1989) and lowest concentrations reported to elicit avoidance are given.*

| Pollutant | Lobith averages 1985 µg/L | Maxima in mixing zone of domestic sewage µg/L | Maxima in mixing zone of industrial effluent µg/L | Proposed ecotoxicological objective µg/L | Avoidance LOEC µg/L |
|---|---|---|---|---|---|
| Cu | 3.0 | 10 | 300-400 | 1.3 | 0.1 |
| Zn | 18 | 45 | 300-400 | 6.5 | 6.5 |
| PCE* | 0.26 | | | 4.9 | 4 |
| LAS | <50-70 | | | | 0.11 |
| Cd | 0.04 | 1 | 300-400 | 0.025 | 52 |
| Cr | 1.1 | 10 | 300-400 | 2.5 | 28 |
| Hg | 0.01 | 0.03 | | 0.005 | 20 |
| Ni | 3.7 | 10 | 300-400 | 7.5 | 23 |
| Pb | 0.2 | 10 | 300-400 | 1.3 | 26 |
| chloroform | 0.5 - 3.0 | | 400 | 0.6 | 150 |

* PCE = tetrachloroethylene

Functional and histopathological damage of the olfactory organ.

6. At concentrations approximating those measured (or estimated) in the River Rhine, the pollutants copper and the anionic detergents LAS and AS (lauryl sulfate) may cause serious damage to the olfactory organ. This is demonstrated by behavioural and electrophysiological studies. However, untill now the number of studies has been quite limited and generally high experimental concentrations were used. Further research may demonstrate that the olfactory organs of fish are susceptible to lower concentrations and to other pollutants than have been reported so far. Effects of chemicals on the olfactory organ generally seem to occur at concentrations higher than the effective levels reported in avoidance experiments.

7. Histopathology is not a very sensitive tool for the assessment of toxicity, because structural damage in general is preceded by functional damage. In this respect it is not surprising that effective concentrations of histopathological experiments are well above present-day concentrations in the River Rhine and water quality objectives.

8. Pollutants may also affect other orientational mechanisms that may be important in the spawning migration of anadromous fish: Reference is made of effects of pollutants on responses to light, temperature, salinity and flowvelocity, and on damage of other sensory organs (lateral line organ and tastebuds).

Considering the remarks mentioned above, it is concluded that the spawning migration of anadromous fishes in the River Rhine may definitely be affected. Whether the survival chances of fish populations are affected seriously remains unclear.

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## PUBLICATIES EN RAPPORTEN VAN HET PROJECT "ECOLOGISCH HERSTEL RIJN"

no. 1 - 1988 Ecological rehabilitation of the river Rhine: a proposal for a Netherlands research programme. (DBW, RIVM, RIVO).

no. 2 - 1988 Fish and their environment in large european river ecosystems; the Dutch part of the river Rhine. W.G. Cazemier, Science de l'Eau 7, 95-114 (1988). (RIVO).

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no. 4 - 1988 Impact of biological activity on detritus transported in the lower river Rhine: an excercise in ecosystem analy. W. Admiraal en B. van Zanten, Freshwater Biology 20, 215-225 (1988). (RIVM).

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no. 6 - 1988 Environmental stress in five aquatic ecosystems in the floodplain of the river Rhine. W. Admiraal, E.D. de Ruyter van Steveninck en H.A.M. de Kruijf. The Science of the Total Environment 78, 59-75 (1988). (RIVM).

no. 7 - 1989 Bioaccumulation in yellow ell (Anguilla anguilla) and perch (Perca fluviatilis) from the Dutch branches of the Rhine-mercury, organochlorine compounds and polycyclic aromatic hydrocarbons. F. van der Valk, H. Pieters en R.C.C. Wegman. (RIVO).

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no. 9 - 1989 Ecologisch herstel Rijn - beleid en onderzoek. Symposium-verslag 26 mei. E.C.L. Marteijn (red.) (DBW).

no. 10 - 1989 Summary of results and conclusions from the first phase (1988-1989) of the Netherlands research programme "Ecological Rehabilitation Rhine". J.A.W. de Wit, W. Admiraal, C. van der Guchte and W.G. Cazemier. (DBW).

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no. 17 - 1990 Chemicals affecting the spawning migration of anadromous fish by causing avoidance responses or orientational disability, with special reference to concentrations in the River Rhine. T.C. van Brummelen. (DBW)

Aanvragen/requests:
(DBW): Institute for Inland Water Management and Waste Water Treatment, P.O. Box 17, 8200 AA Lelystad, The Netherlands.
(RIVM): National Institute for Public Health and Environmental Protection, P.O. Box 1, 3720 BA Bilthoven, The Netherlands.
(RIVO): Netherlands Institute for Fishery Investigations, P.O. Box 68, 1970 AB IJmuiden, The Netherlands.

# APPENDIX 1
# IRC PRIORITAIRY POLLUTANTS

IRC prioritairy pollutants and dutch ecotoxicological waterquality objectives for dissolved pollutants, proposed by Stortelder et al. (1989).

| Pollutant: | Water Quality Objective (µg/L): | Pollutant: | Water Quality Objective (µg/L): |
|---|---|---|---|
| Aldrin | 0.0015 | Chloroform | 0.6 |
| Dieldrin | 0.0019 | PCB 28 | 0.001 |
| Endrin | 0.0050 | 52 | 0.001 |
| Isodrin | 0.0022 | 101 | 0.001 |
| Endosulfan | 0.014 | 118 | 0.001 |
| Chloronitrobenzene: | | 138 | 0.001 |
| mono | 12 | 153 | 0.001 |
| penta | 0.37 | 180 | 0.001 |
| Trichlorobenzene | 0.43 | Dichlorovos | 0.002 |
| Hexachlorobenzene | 0.0004 | Azinphos-m | 0.015 |
| Hexachlorobutadies | 0.12 | Bentazon | - |
| Pentachlorophenol | 0.38 | Tributyltin | |
| Trichloroethylene | 2.2 | oxide | 0.001 |
| Tetrachloroethylene | 4.9 | compounds | 0.003 |
| Chloroanilines | | Triphenyltin | |
| 2-mono | 22.5 | compounds | 0.008 |
| Parathion methyl | 0.20 | Tetrabutyltin | |
| ethyl | 0.02 | compounds | - |
| Benzene | 9 | Trifluralin | 0.24 |
| 1,1,1 Trichloro- | | Fention | 0.02 |
| ethane | - | Atrazine | 0.075 |
| 1,2 dichloro- | | Simazine | 0.38 |
| ethane | 103 | Chlorotoluene | |
| Mercury | 0.005 | -2 | - |
| Cadmium | 0.025 | -4 | - |
| Chromium | 2.5 | Mevinfos | 0.0045 |
| Copper | 1.3 | Triazofos | 0.030 |
| Nickel | 7.5 | MCPA | 0.16 |
| Zinc | 6.5 | Captan | 0.26 |
| Lead | 1.3 | Thiram | 0.018 |
| Tetrachlorocarbon | 16.6 | | |

# APPENDIX 2
# OBSERVATIONS OF OTHER BEHAVIOURAL EFFECTS

*Other behavioural effects observed after exposure to several dissolved toxicants.*
*Abbreviations: NOEC (No Observed Effect Concentration), LOEC (Lowest Observed Effect Concentration) LC50 (concentration lethal to 50% of the test organisms).*
*Notes: 1 highest concentration in experiment; 2 lowest concentration in experiment; 3 only concentration in experiment; * threshold value of response.*

| COMPOUND | REFERENCE | TESTORGANISM | | EXPERIMENTAL CONDITIONS | | | | NOEC | LOEC | note | RESPONSE |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | species | seize | hardness mg CaCO3/L | pH | T °C | time | (ug/L) | (ug/L) | | |
| Aldrin | Juneja & Mahajan 1984 | Channa punctatus | 24 g | 196 | 7.7 | 20 | 6h | | 25 | 2 | hyperactivity |
| DDT | Besch et al. 1977 | Cyprinus carpio | yearling | | | | 2-12h | | 50 | | swimming performance affected |
| | Ellgaard et al. 1977 | Lepomis macrochirus | 2.5 cm | | | 22 | 14d | | 0.008 | 2 | hyperactivity |
| | Davy et al. 1969 | Carassius auratus | 24 cm | 5 | 8.4 | 21 | 20h | | 10 | 3 | exploratory pattern affected |
| | Weis & Weis 1974 | Carassius auratus | | | | | 24h | | 100 | | disruption in schooling behaviour |
| Dimilin | Ellgaard et al. 1979 | Gambusia affinis | | | 8.5 | 22 | | | 200 | | temporary hyperactivity |
| Fenitrothion | Bull & McInerny 1974 | Onchorynchus kisutch | juv | | | | | | 230-480 | * | different types of behaviour affected, coughing frequency increases |
| | Symons 1973 | Salmo salar | juv | | | | 18h | | 100 | 2 | territoriality, swimming affected |
| Methroprene | Ellgaard et al. 1979 | Gambusia affinis | | | 8.5 | 22 | | 200 | | 3 | activity |
| | | Carassius auratus | | | | | | 200 | | 3 | activity |
| Parathion | Rand 1977B | Lepomis macrochirus | 16.5 cm | | | 21 | 24h | | 10 | 3 | response to foododour and flow |
| | | Micropterus salmoides | 30 cm | | | 21 | 24h | | 25 | 3 | are affected |
| | Rand 1977A | Carassius auratus | 27.5 cm | | | 21 | 24h | | 330 | 3 | response to foododour and flow |
| | Rand et al. 1975 | Carassius auratus | 27.5 cm | | | 21 | 24h | | 330 | 3 | are affected |
| Pentachlorophenol | Webb & Brett 19973 | Oncorhynchus nerka | juv | | 8.8 | 15 | | 50 | | 1 | swimming performance |
| | | | | | | | | | 1.74 | * | growth rate affected |
| | | | | | | | | | 1.8 | * | food conversion affected |
| Cd | Ellgaard et al. 1983 | Lepomis macrochirus | 5 cm | 105 | 8.5 | 22 | 14d | | 100 | | hyperactivity |
| Cr | Ellgaard et al. 1978 | Lepomis macrochirus | 5 cm | 105 | 8.5 | 8.5 | 14d | | 50? | | hyperactivity |
| Cu | Steele 1983 | Arius felis | | | | | | | 5 | 2 | orientation affected, 5-50 ppb hypo- 100 & 200 ppb hyperactive |
| | Waiwood & Beamish 1978 | Salmo gairdneri | 7 cm | 30 | | 12 | 10d | | 30 | * | swimming performance affected modifying action of pH and hardness |
| | Scarfe et al. 1982 | Lagodon romboides | 15 cm | 31%. sal | | 23 | 72h | | 100 | 3 | locomotor behaviour affected |
| | | Micropogon undulatus | 19 cm | | | | | | 100 | 3 | locomotor behaviour affected |
| | | Archosargus probatocephalus | | | | | | | 100 | 3 | locomotor behaviour affected |
| | | Arius felis | 27 cm | | | | | | 100 | 3 | locomotor behaviour affected |
| | Koltes 1985 | Menidia menidia | 9.9 cm | 30% sal | | 11-24 | | | 0-100 | | increased schooling: closer, less turning, higher swimming speed |
| Zn | Ellgaard et al. 1978 | Lepomis macrochirus | 5 cm | 105 | 8.5 | 8.5 | 14d | | 100? | | hyperactivity |

# APPENDIX 3
# AVOIDANCE OF CHLORINATED COMPOUNDS

Data reported by Giattina & Garton (1983) and Beitinger & Freeman (1983):

*Lowest total residual chlorine concentrations (arranged in increasing order from most sensitive species to least sensitive species) causing avoidance in freshwater and saltwater tests as reported for a variety of fish species and testing procedures.*

| Species | Avoidance Concentration (mg/L) | Reference |
|---|---|---|
| | Freshwater | |
| *Salmo gairdneri*<br>(rainbow trout) | 0.01 | Sprague & Drury (1969) |
| *Oncorhynchus kisutch*<br>(coho salmon) | 0.05-0.10 | Cherry *et al.* (1979) |
| *Micropterus punctulatus*<br>(spotted bass) | 0.05-0.20 | Cherry *et al.* (1977 b) |
| *Notropis rubellus*<br>(rosyface shiner) | 0.05-0.20 | Cherry *et al.* (1977 b) |
| *Catostomus commersoni*<br>(white sucker) | 0.10-0.20 | Cherry *et al.* (1979) |
| *Cyprinus carpio*<br>(carp) | 0.10-0.21 | Cherry *et al.* (1979) |
| *N. cornutus*<br>(common shiner) | 0.11-0.21 | Cherry *et al.* (1978) |
| *N. galacturus*<br>(whitetail shiner) | 0.11-0.22 | Giattina *et al.* (1981) |
| *N. spilopterus*<br>(spotfin shiner) | 0.21 | Giattina *et al.* (1981) |
| *Ictalurus punctatus*<br>(channel catfish) | 0.20-0.41 | Cherry *et al.* (1979) |
| | Marine | |
| *O. kisutch*<br>(coho salmon) | 0.002 | Stober *et al.* (1980) |
| *Morone americana*<br>(white perch) | 0.02 | Meldrim *et al.* (1974) |
| *Lagoden rhomboides*<br>(pinfish) | 0.02-0.04 | Cripe (1979) |
| *Menidia menidia*<br>(Atlantic silverside) | 0.06 | Meldrim & Fava (1977) |
| *Chromis punctipinnis*<br>(blacksmith) | 0.08-0.10 | Hose & Stoffel (1980) |
| *Cymatogaster aggregata*<br>(shiner perch) | 0.175 | Stober *et al.* (1980) |
| *Morone saxatilis*<br>(striped bass) | 0.29 | Middaugh *et al.* (1977) |

A = avoidance
S = selection = preference

Behavioral responses of fishes to water-borne chemicals.

| cals | Species | Size | Temp., °C | Concentration | Behavior | Reference |
|---|---|---|---|---|---|---|
| E<br>esidual chlorine RC) | *Salmo gairdneri* | 7.7-14.8 cm TL | 17 ± 0.2 | 0.01, 1.0 mg/L | A | Sprague & Drury (1969) |
| | | | | 0.1 mg/L | S | Sprague & Drury (1969) |
| | *Morone saxatilis* | 24 days | 18 ± 1 | 0.79-0.82 mg/L | A | Middaugh *et al.* (1977) |
| | | | | 0.29-0.32 mg/L | A | Middaugh *et al.* (1977) |
| | | | | 0.16-0.18 mg/L | NA | Middaugh *et al.* (1977) |
| Total, chloramine, free residual chlorines (TRC, CRC, FRC) and hypochlorous acid (HOCL) | *Cyprinus carpio* | | 6-30 | minimum | | |
| | | | | 0.104 mg/L TRC @ 12°C | A | Larrick (1977) |
| | | | | 0.056 mg/L CRC | A | Larrick (1977) |
| | | | | 0.049 mg/L FRC | A | Larrick (1977) |
| | | | | 0.019 mg/L HOCL | A | Larrick (1977) |
| | | | | maximum | | |
| | | | | 0.212 mg/L TRC @ 24°C | A | Larrick (1977) |
| | | | | 0.074 mg/L CRC | A | Larrick (1977) |
| | | | | 0.138 mg/L FRC | A | Larrick (1977) |
| | | | | 0.035 mg/L HOCL | A | Larrick (1977) |
| | | | | 0.025 mg/L TRC @ 24°C | S | Larrick (1977) |
| | | | | 0.050 mg/L TRC @ 18° & 24°C | S | Larrick (1977) |
| | *Ictalurus punctatus* | | 6-30 | minimum | | |
| | | | | 0.205 mg/L TRC @ 30°C | A | Larrick (1977) |
| | | | | 0.097 mg/L CRC | A | Larrick (1977) |
| | | | | 0.108 mg/L FRC | A | Larrick (1977) |
| | | | | 0.037 mg/L HOCL | A | Larrick (1977) |
| | | | | maximum | | |
| | | | | 0.403 mg/L TRC @ 24°C | A | Larrick (1977) |
| | | | | 0.184 mg/L CRC | A | Larrick (1977) |
| | | | | 0.219 mg/L FRC | A | Larrick (1977) |
| | | | | 0.066 mg/L HOCL | A | Larrick (1977) |
| | | | | 0.50 mg/L TRC @ 24°C | NA | Larrick (1977) |
| | | | | HOCL minimum: | | |
| | | | | 0.015 mg/L @ 30°C | A | Larrick (1977) |
| | | | | HOCL maximum: | | |
| | | | | 0.017 mg/L @ 24°C | A | Larrick (1977) |
| | *Notemigonus crysoleucas* | | 6-30 | minimum | | |
| | | | | 0.199 mg/L TRC @ 18°C | A | Larrick (1977) |
| | | | | 0.112 mg/L CRC | A | Larrick (1977) |
| | | | | 0.086 mg/L FRC | A | Larrick (1977) |
| | | | | 0.027 mg/l HOCL | A | Larrick (1977) |
| | | | | maximum | | |
| | | | | 0.395 mg/L TRC @ 24°C | A | Larrick (1977) |
| | | | | 0.255 mg/L CRC | A | Larrick (1977) |
| | | | | 0.139 mg/L FRC | A | Larrick (1977) |

| Chemicals | Species | Size | Temp., °C | Concentration | Behavior | [illegible] |
|---|---|---|---|---|---|---|
| | | | | 0.045 mg/L HOCL | A | Larric[illegible] |
| | | | | 0.025 mg/L TRC 12° & 18°C | S | Larrick ([illegible] |
| | | | | 0.050 mg/L TRC 18°, 24°, & 30°C | S | Larrick (1977) |
| | | | | HOCL minimum 0.067 mg/L @ 24°C | A | Larrick (1977) |
| | | | | HOCL maximum 0.077 mg/L @ 30°C | A | Larrick (1977) |
| | *Pimephales promelas* | | 18 | 0.115 mg/L TRC | A | Larrick *et al.* (1978) |
| | | | | 0.079 mg/L CRC | A | Larrick *et al.* (1978) |
| | | | | 0.036 mg/L FRC | A | Larrick *et al.* (1978) |
| | | | | 0.022 mg/L HOCL | A | Larrick *et al.* (1978) |
| Chloramine & free residual Chlorine | *Rhinichthyes atratulus* | | 21 | 0.07 & 0.17 mg/L (chloramines) | A | Fava & Tsai (1978) |
| | | | | 0.07, 0.21 & 0.47 mg/L (free chlorine) | A | Fava & Tsai (1978) |
| Total residual oxidants (TRO) | *Menidia menidia* | | many | multifactorial design significant factors:<br>1. salinity<br>2. salinity & pH<br>3. temperature<br>4. temperature & pH | A | Meldrim & Fava (1977) |
| Total residual oxidants (TRO) in seawater | *Chromis punctipinnis* | 40-100 mm | 15, 20, 24 | 0.08-0.10 ppm | A | Hose & Stoffel (1980) |
| | *Cymatogaster aggregata* | 1-3 mon | 12, 16, 20 | 2 μg/L @ 12°C | S | Stober *et al.* (1980) |
| | | | | 0, 10, 25, 50, 100 μg/L 12°C | NA | Stober *et al.* (1980) |
| | | | | 175, 250, 500 μg/L 12°C | A | Stober *et al.* (1980) |
| | | | | 0.2 μg/L 16° & 20°C | NA | Stober *et al.* (1980) |
| | | | | 10, 25, 50, 100 μg/L 16° & 20°C | S | Stober *et al.* (1980) |
| | | | | 175, 250, 500 μg/L 16° & 20°C | A | Stober *et al.* (1980) |
| | *Oncorhynchus kisutch* | 1 year | 12, 16 20 | 2, 10, 25, 50, 100, 250, 500 μg/L 12°C | A | Stober *et al.* (1980) |
| | | | | 10, 25, 100 μg/L 16°C | A | Stober *et al.* (1980) |
| Chlorinated primary sewage effluent | *C. aggregata* | 74.6 mm TL | 10.3 ± 0.5 | 1, 5, 10 v/v % | S | Dinnel *et al.* (1979) |
| | | | | 15 & 20 v/v % | A | Dinnel *et al.* (1979) |

# APPENDIX 4
## NAMES OF FISH SPECIES: LATIN - ENGLISH

| Latin name: | English name: |
|---|---|
| Acipenser sturio | sturgeon |
| Alosa alosa | allis shad |
| Alosa fallax | twaite shad |
| Alosa sapidissima | american shad |
| Archosargus probatocephalus | sheepshead |
| Arius felis | sea catfish |
| Brevoortia tyrannus | atlantic menhaden |
| Carassius auratus | goldfish |
| Catostomus catostomus | longnose suckers |
| Catostomus commersoni | white sucker |
| Clupea harengus | herring |
| Compostoma anomalum | stoneroller |
| Coregonus clupeaformis | lake whitefish |
| Cyprinodon variegatus | sheepshead minnow |
| Cyprinus carpio | carp |
| Fundulus grandis | gulf killifish |
| Fundulus heteroclitus | mummichog |
| Gambusia affinis | mosquitofish |
| Gasterosteus aculeatus | three-spined stickleback |
| Hyborhynchus notatus | bluntnose minnow |
| Ictalurus natalis | yellow bullheads |
| Ictalurus punctatus | channel catfish |
| Lagodon rhomboides | pinfish |
| Lampetra fluviatilis | european lamprey |
| Latin name: | English name: |
| Lepomis cyanellus | green sunfish |
| Lepomis macrochirus | bluegill sunfish |
| Lepomis megalotis | longeared sunfish |
| Lepomis microlophus | redear sunfish |
| Menidia berylliana | tidewater silverside |
| Menidia menidia | atlantic silverside |
| Micropogon undulatus | atlantic croaker |
| Micropterus dolomieni | smallmouth bass |
| Micropterus punctulatus | spotted bass |
| Micropterus salmoides | largemouth bass |
| Morone saxatilus | striped bass |
| Negaprion brevirostris | lemon shark |
| Notemigonus crysoleucas | golden shiner |
| Notropis atherinoides | emerald shiner |
| Notropis cornotus | common shiner |
| Notropis galacterus | whitetail shiner |
| Notropis rubellus | rosyface shiner |
| Notropis spilopterus | spotfin shiner |
| Oncorhynchus gorbuscha | pink salmon |
| Oncorhynchus keta | chum salmon |
| Oncorhynchus kisutch | coho salmon |
| Oncorhynchus masou | masou salmon |
| Oncorhynchus nerka | sockeye salmon |
| Oncorhynchus tchawytscha | spring salmon |
| Oryzias latipes | medaka |
| Osmerus mordav | smelt |

| | |
|---|---|
| Perca flavescens | yellow perch |
| Phoxinus phoxinus | minnow |
| Pigosteus pungitius | ten-spined stickleback |
| Pimephales notatus | bluntnose minnow |
| Pimephales promelas | fathead minnow |
| Plecoglossus altivelis | ayu |
| Salmo gairdneri | rainbow trout |
| Salmo salar | atlantic salmon |
| Salmo trutta fario | brown trout |
| Salmo trutta trutta | seatrout |
| Salvelinus alpinus | atlantic charr |
| Salvelinus fontinalis | brook trout |
| Thymallus thymallus | grayling |
| Tilapia rendalli | |
| Trinectes maculatus | hogchoker |

# APPENDIX 5
# NAMES OF CHEMICALS

In the articles to which is referred, the following names were used.

| COMPOUND | EQUIVALENT NAME |
|---|---|
| 2,4,5 T | 2,4,5 trichlorophenoxyaceticacid |
| 2,4-D | 2,4 dichlorophenoxyaceticacid |
| ABS | branched alkylbenzenesulfonate |
| ACP | a-chloroacetophenone |
| Acrolein | acrylaldehyde |
| Amitrole T | 3-amino-s-triazole + ammoniumthiocyanate |
| Aquatol K | dipotassium salt of endothal |
| AS | laurylsulfate |
| Atrazine | 2-chloro-4-ethylamino-6-isopropylamino s-triazine |
| BCBC | n-butycarbitol thiocyanate |
| enthiocarb | s-p-chlorobenzyldiethylthiocarbamate |
| loroform | trichloromethane |
| Dalapon | 2,2 dichloropropionicacid |
| Diazinon | diethyl 2-isopropyl-4-methyl-6-pyrimidinilphosphorothionate |
| Dicamba | 3,6-dichloro-a-anisicacid |
| Dimilin | contains diflubenzuron |
| Dinoseb | 2-sec-butyl-4,6-dinitrophenol |
| Diquat | 6,7 dihydrodipiridi[1,2-a; 2'-1'-c]pyrazinediium-ion |
| DNOC | dinitro-o-cresol |
| Endosulfan | thiodam |
| Ethanethiol | ethylmercaptan |
| Fenitrothion | dimethyl 4-nitro-m-tolyl phosphorothionate |
| Fenitrothion | sumithion |
| Glyphosate | n-phosphonomethylglycine |
| IBP | s-benzyl diisopropyl phosphorothiolate |
| Krenite | contains ammoniumethylcarbomylphosphonate |
| LAS | lineair alkylbenzenesulfonate |
| NAC | 1-naphtyl methylcarbamate |
| Paraquat | 1,1'-dimethyl-4-4'bipyridiniumion |
| PCE | tetrachloroethylene |
| PCP | pentachlorophenol |
| Sevin | carbaryl |
| Simazine | 2 chloro-4,6bis(ethylamino-s-triazine |
| TBTO | bis(tri-n-butyltin)oxide |
| TCA | trichloroaceticacid |
| TCE | trichloroethylene |
| Thanite | isobornylthiocyanoacetate |
| Tordon 22K | 4-amino-3,5,6 trichloropicolinicacid |
| Triton X-100 | polyethoxylated octylphenol |
| Xylene | 1,4 dimethylbenzene |