LETHAL EFFECTS OF MEXEL® 432 TO COMMON CARP EMBRYOLARVAL STAGES IN RIVER WATER: INFLUENCE OF PHYSICOCHEMICAL PARAMETERS IN SYNTHETIC ISO WATER

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Abstract—The lethal effects of Mexel® 432, an antifouling agent for industrial water circuits, on common carp embryolarval stages in Moselle and Seine River waters and synthetic water were tested in the laboratory. Diluting Mexel 432 in river water resulted in the disappearance of its lethal effects within a few days. In freshly prepared 3-mg/L solutions, 100% mortality occurred within 1 d in synthetic International Standards Organization (ISO) water, compared to 96 h in Seine River water (10-mg/L solutions). No lethal effect was observed after 7 d of exposure in an 8- to 15-d-old 50-mg/L solution in Moselle River water or in a 2-d-old 10-mg/L solution in Seine River water. Inhibition of Mexel 432 toxicity by river waters was not due to calcium concentration, temperature, or pH, because these factors had a negligible influence on the lethal effects of Mexel 432 in synthetic ISO water (ranges, 0.8–800 mg/L, 15–24°C, and 6.9–9.0, respectively). Aldrich Chemical humic acids immediately suppressed the lethal effects of Mexel 432 with an apparent ratio of 1 to 2 mg Mexel 432/mg humic acids. Also, clays and humic acids immediately reduced the concentration of Mexel 432 in semiquantitative colorimetric dosages.

Keywords—Antifouling Embryolarval toxicity test Fish Mexel® 432 Humic acids

INTRODUCTION

Various types of fouling organisms have been quantitatively and qualitatively listed, notably, systematic groups (algae, annelids, bacteria, bryozoans, mollusks, etc.), community structures, attachment and reproductive processes, and intra- and interspecific interactions. Various sites have been inspected, such as the cooling circuit of power stations [1,2], oil platforms [3], harbors or estuarine areas [4,5], ships [6], and oyster culture installations, where fouling induces significant mortality [7].

The settlement, geographic distribution, and succession of fouling communities are influenced by various physicochemical parameters, including temperature, salinity, turbulence, light, ambient nutrient levels, and predation or the nature of the substratum [8–12].

Consequently, control of biofouling requires methods that can be adapted to the diversity of the environmental situations where the fouling is likely to be found. Besides mechanical cleaning, the principal methods to control biofouling are chemical treatments, typically by chlorine or tin and copper compounds. These treatments have the disadvantage of disturbing or impairing the normal development of nontarget organisms [13–15].

Mexel® 432 (Mexel S. A., Cedex, France) is now used in numerous countries (notably France, Hong Kong, Morocco, Poland, and the United States) as an alternative or complementary antifouling and anticorrosion agent (e.g., in cooling systems of industrial circuits such as thermal power plants or in urban air conditioning systems).

Mexel 432 is an off-white, thick, viscous, aqueous emulsion containing fatty amines. Its precise chemical composition remains confidential. It contains traces of nickel but no other heavy metals, halogens, quaternary ammonium, or aromatic compounds. The presence of fatty amines makes its properties nearer those of cationic surfactants. Mexel 432 was registered by the U.S. Environmental Protection Agency.

Fatty amines have been used in a large variety of applications, including film-forming corrosion inhibitors [16], antitrust and antimicrobial additives in water treatment [17], and pesticide adjuvants [18]. Certain fatty amines have been directly tested on nematodes and trematodes [19]. In mammalian cells, xenobiotic or endogenous aliphatic amines may degrade into toxic metabolites [20]. In the presence of composite bacterial communities, degradation in water leads to rapid production of nontoxic compounds before complete degradation [21].

Some data on the toxicity of Mexel 432 to one of the major macrofoulers in freshwaters, the mussel Dreissena polymorpha Pallas, are available in literature [15,22]. However, no information is available on the toxic effects of Mexel 432 on nontarget organisms like fish.

The common carp is one freshwater species recommended by the Organization for Economic and Cooperative Development for early life stage toxicity tests [23]. It is recommended because it is practical to use and an environmentally representative species [24].

Because Mexel 432 is diluted for use in natural waters, the influence of natural waters on its toxicity must be investigated to determine the conditions for use and its mode of action and to evaluate its impact on the environment.

In the present study, we tested, in the laboratory, the lethal effects of Mexel 432 in river water or synthetic International
Gametes were produced by thermal and hormonal treatment of the parents (males and females) according to the procedure of Jaouli and Roubaud [25], that is, maintenance of the breeders in cold water (10°C) from autumn to the test period, followed by 1 or 2 weeks at 20°C with two injections of carp hypophysis extracts under a 13-h-light/11-h-dark photoperiod.

Artificial fertilization and incubation were performed according to the methods of Ghillebaert et al. [26]. Approximately 50 oocytes were dispersed with 10 μl of sperm in 20 ml of the test solution on the bottom of the incubator. About 20 min after fertilization, excess sperm were washed out with the test solution, and the fertilized oocytes, which had stuck to the bottom of the incubators, were covered with the same solution. Incubators were placed on an oscillating table (10 oscillations/min). After hatching, the incubators were rinsed with the test solution to eliminate chorion fragments and dead eggs and larvae. The larval incubation was performed without feeding.

In controls kept at 24°C, egg cleavage and epiboly occurred during the first day of incubation, and hatching occurred on the third day. The larval survival rates remained stable at least 7 d. In controls kept at 15°C, hatching occurred after 11 d, and the physiological development rate was reduced by a factor of approx. 3.

**Mexel 432 solutions**

Mexel 432 was provided by Mexel S.A. (Haubourdin Cedex, France) (batch 01/10/92 was used in test 2, and batch 03/11/95 was used in the other biological tests and dosages). The antifouling agent was kept in the dark at room temperature until use. The dilution waters were river waters and standard or modified ISO waters [27], that is, for 1 L of deionized water (resistivity, >1 MΩ), CaCl₂·2H₂O = 294.0 mg/L; MgSO₄·7H₂O = 123.3 mg/L; NaHCO₃ = 63.0 mg/L; and KCl = 5.5 mg/L, pH 7.8. The calcium concentration of the synthetic ISO water was 80 mg/L.

**Exposure**

In tests 1 to 4, the animals were exposed from fertilization to days 7, 9, or 10 of incubation. Tests 5 to 7 were performed on larvae.

Exposure media were renewed daily after control of temperature, pH, and oxygen concentration in each incubator. The physicochemical parameters of the solutions are given in Tables 1 to 5.

**Materials and Methods**

**Biological reagents**

Common carp (Cyprinus carpio L.) breeders were obtained from a fish farm (Les Alevins de Sologne, Vignoux sur Barangeon, France).

Gametes were produced by thermal and hormonal treatment of the parents (males and females) according to the procedure of Jaouli and Roubaud [25], that is, maintenance of the breeders in cold water (<10°C) from autumn to the test period, followed by 1 or 2 weeks at 20°C with two injections of carp hypophysis extracts under a 13-h-light/11-h-dark photoperiod.

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Exposures were performed in 250-ml polyethylene or 100-ml polystyrene incubators. In our experimental conditions, the

**Table 1. Experimental conditions of tests**

<table>
<thead>
<tr>
<th>Test no.</th>
<th>Spawn no.</th>
<th>Life stagea</th>
<th>Incubator</th>
<th>Temperature (°C)</th>
<th>Mexelª 432 concn. (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Typeb</td>
<td>Volume (ml)</td>
<td>Incubation⁶</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>From fertilization (days 0–7)</td>
<td>PE</td>
<td>100</td>
<td>24.4 ± 0.3</td>
</tr>
<tr>
<td>2</td>
<td>A 2</td>
<td>From fertilization (days 0–7)</td>
<td>PE</td>
<td>150</td>
<td>22.8 ± 1</td>
</tr>
<tr>
<td>B C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;2 h</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>From fertilization (days 0–9)</td>
<td>PS</td>
<td>60</td>
<td>24 ± 1</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>From fertilization (days 0–10)</td>
<td>PS</td>
<td>60</td>
<td>24 ± 1</td>
</tr>
<tr>
<td>5</td>
<td>A 5</td>
<td>Larvae (days 7–9)</td>
<td>PS</td>
<td>60</td>
<td>24 ± 1</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>Larvae (days 14–17)</td>
<td>PS</td>
<td>60</td>
<td>24 ± 1</td>
</tr>
<tr>
<td>7</td>
<td>A 4</td>
<td>Larvae (days 14–17)</td>
<td>PS</td>
<td>60</td>
<td>24 ± 1</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Exposure period from end of day x to end of day y (day 0 = fertilization).

ª PE = polyethylene; and PS = polystyrene.

In incubation temperature before exposure.

ª A 10-mg/L solution of Mexel 432 in standard laboratory water aged 8 to 15 d was diluted to a concentration of 1 mg/L by Moselle River water at the time of use (daily renewal of solutions).

Concentrations of Mexel 432 are in a geometric progression ratio of 1/2 in the range of 0.18–6.25 mg/L, 0.25–2 mg/L, etc.
lethality of Mexel 432 was shown to not be significantly dependent on the incubator type and the solution volume (range, 60–150 ml).

**Lethal effects of Mexel 432 in river water: Effect of solution aging**

Seine and Moselle River waters were sampled in 20-L polyethylene cans, just upstream of the electric power stations of électricité de France, in Cattenom, and in Nogent. Water was carried to the laboratory and was kept in the dark at 15°C for 8 d before testing began.

Test 1 was performed using Seine River water (Fig. 1). Four days before starting the exposure and then every day, 2 L of a Mexel 432 solution (nominal concentration, 10 mg/L) were prepared. Then, the toxicity of the freshly prepared and 1- to 4-d-old solutions were tested.

Test 2A was performed using Moselle River water (Fig. 1). Eight days before exposure started, 2 L of Mexel 432 solutions with concentrations ranging from 0 to 100 mg/L were prepared. Then, the toxicity of these solutions was tested over 7 d (i.e., the test solutions continued to age until the end of the test).

In test 2B, a 10-mg/L Mexel 432 stock solution in synthetic ISO water was aged 8 to 15 d and diluted to 1 mg/L in Moselle River water at the time of use (just before daily renewal of the solutions).

The results of tests 2A and B were compared with those of test 2C, which was performed using synthetic ISO water. In this test, the Mexel 432 concentrations were in a geometric progression ratio of 1/2 in the range of 0.18 to 6.25 mg/L.

**Lethal effects measurements**

The toxicity of Mexel 432 was measured by its lethal effects, the most generally used criterion in fish early life toxicity tests and the easiest to interpret [23,28]. Dead embryos and larvae were characterized by yolk coagulation and an opaque neutral color of the central nervous system [23,26]. In tests 1 to 4, the total number of eggs (N) was determined in each incubator at the end of the first day of incubation. Fifteen (tests 5 and 6) or 20 (test 7) larvae were introduced into each in-

<table>
<thead>
<tr>
<th>Test no.</th>
<th>Dilution water</th>
<th>Mexel concn. (mg/L)</th>
<th>Toxicity</th>
<th>Duration of exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Moselle River water</td>
<td>0</td>
<td>Survival</td>
<td>1 d</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td>3 d</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td></td>
<td>7 d</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>90/10ºd</td>
<td>1ºd</td>
<td>Survival</td>
<td>1 d</td>
</tr>
<tr>
<td>C</td>
<td>ISO water</td>
<td>0</td>
<td>Survival</td>
<td>1 d</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.2</td>
<td></td>
<td>3 d</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.4</td>
<td></td>
<td>7 d</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ISO waters (tests 3, 5, and 6; Tables 3 and 4) were measred as CaCl₂, 2 H₂O (Prolabo).

**pH.** In test 4 (Tables 1 and 3), solutions buffered at pH 6.9, 7.8, and 9.0 were prepared by adding 10 mM of a pH buffer (Sigma Chemical), 3-[(N-morpholino)-2-hydroxy-propanesulfonic acid, piperazine-N,N’-bis-[2-hydroxy-propanesulfonic acid], and 3-[(1,1-dimethyl-2-hydroxymethyl)amino]-2-hydroxy-propane-sulfonic acid, respectively [26]. pH levels were adjusted by NaOH.

**Temperature and calcium.** In tests 5A and B (Tables 1 and 4), the physiological ages of the larvae at the beginning of exposure were almost identical. In test 5A, the incubators containing the larvae were transferred 8 h before exposure to a thermo-controlled room at 15°C.

In test 6 (Tables 1 and 4), the physiological age of the larvae at the beginning of exposure was approximately the age of 5-d-old larvae incubated at 24°C.

**Humic acids.** In test 7 (Tables 1 and 5), the influence of Aldrich humic acids (batch 0201816, Aldrich Chemical, Milwaukee, WI, USA) was tested in the presence of high (test 7A) or low concentrations (7B) of Mexel 432. The carp were incubated before exposure at 24.4 ± 1.4°C from fertilization to hatching, then at 15°C ± 1 until exposure.

Humic acids (50 mg/L in test 7A and 2 mg/L in test 7B) were introduced before exposure into the freshly prepared solutions of Mexel 432. The physiological age of the larvae at the beginning of exposure was approximately the age of 6- or 7-d-old larvae incubated at 24°C.

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Table 3. Influence of calcium and pH on the embryolarval lethal effects of Mexel in synthetic ISO water.

<table>
<thead>
<tr>
<th>Calcium (mg/L)</th>
<th>pH</th>
<th>L0</th>
<th>LC50 (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>32</td>
<td>9.4</td>
<td>93</td>
<td>1.6 (0.7)</td>
</tr>
<tr>
<td>79</td>
<td>9.4</td>
<td>89</td>
<td>1.6 (0.7)</td>
</tr>
<tr>
<td>151</td>
<td>9.4</td>
<td>87</td>
<td>1.6 (0.7)</td>
</tr>
<tr>
<td>10</td>
<td>9.6</td>
<td>58</td>
<td>1.6 (0.7)</td>
</tr>
<tr>
<td>16</td>
<td>9.6</td>
<td>86</td>
<td>1.6 (0.7)</td>
</tr>
<tr>
<td>1</td>
<td>9.6</td>
<td>96</td>
<td>1.6 (0.7)</td>
</tr>
<tr>
<td>1</td>
<td>9.6</td>
<td>86</td>
<td>1.6 (0.7)</td>
</tr>
<tr>
<td>3</td>
<td>9.6</td>
<td>96</td>
<td>1.6 (0.7)</td>
</tr>
<tr>
<td>1</td>
<td>9.6</td>
<td>86</td>
<td>1.6 (0.7)</td>
</tr>
</tbody>
</table>

* The 95% confidence limits are unreliable.

a ISO = International Standards Organization synthetic water.
b LC50 = median lethal concentration (mg/L).
c The 95% confidence limits are unreliable.
formed in triplicate.

After decantation in the syringe, the organic phase was transferred to a 25-ml volumetric flask. Care was taken to avoid transfer of the interface zone between the aqueous medium and DCE. This process of extraction was repeated three times with 8 ml of DCE, each time in the same syringe containing the aqueous medium. The extracted volume, collected in the volumetric flask, was adjusted to 25 ml with DCE. The solution volume necessary for spectrophotometric measurement (1-cm optic travel spectrophotometer, Shimadzu UV240) was then centrifuged for 5 min at 12,000 g (Ependorf Centrifuge 5414). In the absence of a calibration curve, this method was used only as a semiquantitative method.

**Semiquantitative estimation of Mexel 432 concentration**

The method for semiquantitative estimation of Mexel 432 concentration was adapted from Wang and Langley [30] and A.S. Allonier (personal communication). In an acidic medium, the basic fatty amines of Mexel 432 are ionized and combine with the acidic dye methyl-orange to form hydrophobic colored complexes that are extracted by dichloroethane (DCE) and quantified by colorimetry (Figs. 2 and 3).

Forty milliliters of the test solution was taken into a polypropylene-graduated syringe (Polylabo) as follows:

- 4 ml of pH 3.75 acetic acid–acetate buffer in the presence of a high concentration of KCl to enhance hydrophilicity (acetic acid, 300 ml; sodium acetate, 70 g; KCl, 125 g; brought to 1 L with deionized water), 4 ml of a 0.4-g/L methyl-orange commercial aqueous solution (Prolabo), 8 ml of dichloro-1,2-ethane (99% purity) (Prolabo), and 4 ml of air. The solution was then mixed by vigorous manual shaking for 1 min.

After decantation in the syringe, the organic phase was transferred to a 25-ml volumetric flask. Care was taken to avoid transfer of the interface zone between the aqueous medium and DCE. This process of extraction was repeated three times with 8 ml of DCE, each time in the same syringe containing the aqueous medium. The extracted volume, collected in the volumetric flask, was adjusted to 25 ml with DCE. The solution volume necessary for spectrophotometric measurement (1-cm optic travel spectrophotometer, Shimadzu UV240) was then centrifuged for 5 min at 12,000 g (Ependorf Centrifuge 5414). In the absence of a calibration curve, this method was used only as a semiquantitative method.

**Effects of humic acids or clays on Mexel 432 solutions in synthetic ISO water**

The tests were conducted with Aldrich humic acids, kaolinite (reference 24926, 1995, Prolabo), green clay (Argilets S.A., Mitry Mory, France), and bentonite (reference 21794, 1995, Prolabo).

Test solutions contained 50 mg/L of Mexel 432 and 35 g/L of KCl in deionized water, either alone (Mx50) or in the presence of various concentrations of humic acids (HA) (Figs. 2 and 3), or 50 mg/L of kaolinite (K), bentonite (B), or green clay (Gc) (Fig. 3). The following solutions were made: Mx-HA (50-3.12),..., Mx-HA (50-50), Mx-K (50-50), Mx-B (50-50), and Mx-Gc (50-50). The first value in brackets refers to the concentration of Mexel 432 (mg/L), and the second value refers to the concentration of humic acids or suspended mineral matter. For example, Mx-HA (50-6.25) refers to a test solution containing 50 mg/L of Mexel 432 and 6.25 mg/L of humic acids

### Table 4. Effect of calcium and temperature on the larval lethal effects of Mexel® 432 to synthetic ISO synthetic water.

<table>
<thead>
<tr>
<th>Test no.</th>
<th>Incubation</th>
<th>Exposure</th>
<th>Calcium (mg/L)</th>
<th>Survival (%)</th>
<th>SD</th>
<th>Day</th>
<th>Toxicity (mg/L)</th>
<th>Duration of exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>24 ± 1</td>
<td>15 ± 1</td>
<td>0.8</td>
<td>100</td>
<td>0</td>
<td>2</td>
<td>LC50</td>
<td>1 d</td>
</tr>
<tr>
<td></td>
<td>80*</td>
<td></td>
<td></td>
<td>98</td>
<td>0</td>
<td>2</td>
<td>0.6 ± 0.7</td>
<td>0.5 ± 0.5 ≤ 0.6</td>
</tr>
<tr>
<td>B</td>
<td>24 ± 1</td>
<td>24 ± 1</td>
<td>0.8</td>
<td>100</td>
<td>0</td>
<td>2</td>
<td>LC50</td>
<td>1 d</td>
</tr>
<tr>
<td></td>
<td>80*</td>
<td></td>
<td></td>
<td>96</td>
<td>8</td>
<td>2</td>
<td>0.6 ± 0.7</td>
<td>0.6 ± 0.7 ≤ 0.7</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td></td>
<td></td>
<td>96</td>
<td>8</td>
<td>2</td>
<td>0.6 ± 0.7</td>
<td>0.7 ± 0.7</td>
</tr>
<tr>
<td>C</td>
<td>24 ± 1</td>
<td>24 ± 1</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>3</td>
<td>LC50</td>
<td>1 d</td>
</tr>
<tr>
<td></td>
<td>80*</td>
<td></td>
<td></td>
<td>100</td>
<td>0</td>
<td>3</td>
<td>0.6 ± 0.7</td>
<td>0.6 ± 0.6 ≤ 0.7</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td></td>
<td></td>
<td>100</td>
<td>0</td>
<td>3</td>
<td>0.6 ± 0.7</td>
<td>0.7 ± 0.7</td>
</tr>
</tbody>
</table>

*ISO = International Standards Organization synthetic water.

**Table 5. Effect of Aldrich humic acids on the embryolarval lethal effects of Mexel® 432 in synthetic ISO synthetic water.

<table>
<thead>
<tr>
<th>Test no.</th>
<th>Spawn no.</th>
<th>Survival (%)</th>
<th>SD</th>
<th>Day</th>
<th>Humic acid concn. (mg/L)</th>
<th>Toxicity (mg/L)</th>
<th>Duration of exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4</td>
<td>100</td>
<td>0</td>
<td>2</td>
<td>NOEC</td>
<td>80</td>
<td>1 d</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>LC50</td>
<td>113*</td>
<td>2 d</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>LOC100</td>
<td>160</td>
<td>3 d</td>
</tr>
<tr>
<td>B</td>
<td>98</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>NOEC</td>
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<td>LC50</td>
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<td>2 d</td>
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<td>LOC100</td>
<td>4</td>
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*ISO = International Standards Organization synthetic water.

† LC50 = median lethal concentration; LOC100 = 100% lethality-observed concentration; and NOEC = no-observed-effect concentration.

*The 95% confidence limits are unreliable.
containing 50 mg/L of Mexel 432 and 6.25 mg/L of humic acids. The tests with mineral matters in suspension were performed without replicates, and those with humic acids were performed in triplicate.

Statistical analysis

Biological data were treated at 5% risk by analysis of variance [31] after transformation of the survival rates by the arcsine function. No-observed-effect concentrations (NOECs) were then calculated with the Tukey multiple comparisons test. Statistical analyses were performed with SYSTAT® 5.0 software [32]. Median lethal concentrations (LC50s) were calculated with the trimmed Spearman-Karber program, version 1.5. (U.S. Environmental Protection Agency, Cincinnati, OH, USA).

RESULTS

Survival rates in controls

During continuous-exposure experiments beginning at fertilization (Table 3), the survival rates in controls remained ≥85% after 7 d except in test 2 (Table 2), in which they fell to 55% after hatching (day 3). However, even in this test, they remained relatively stable from hatching to the end of the test (day 7). In every experiment on larvae (Tables 4 and 5), the control mortality rates were <5%.

Control survival rates in Seine River water (95, 94, and 89% at days 1, 3, and 7, respectively) were not significantly different from rates in synthetic ISO water (Fig. 1). However, the Moselle River water appeared to be toxic to common carp early life stages (Table 2). The survival rate in Moselle River water after 7 d of exposure (36%; Table 2) was significantly
Lethal effects of Mexel 432 in river water: Effect of solution aging

Seine River water. During an exposure to a 1-h or 1-d-old 10-mg/L Mexel 432 solution (test 1, Fig. 1), no lethal effect was observed until hatching (day 3), and 100% mortality was observed at the end of day 4. In contrast, no lethal effect was observed during a 7-d exposure to a 2- or 4-d-old solution.

Moselle River water. The lethal effects of Mexel 432 in solutions aged 8 to 15 d in Moselle River water was low (test 2A, Table 2); the LC50 was 67.8 mg/L after 7 d of exposure. In the 8- to 15-d-old 10-mg/L solution in ISO water, which was diluted to 1 mg/L in Moselle River water at the time of use (test 2B, Table 2), significant mortalities were observed after 7 d of exposure.

Lethal effects of freshly prepared solutions of Mexel 432 in synthetic ISO water

In continuous-exposure experiments beginning at fertilization (Tables 2 and 3), the LC50 was >2 mg/L after 1 d of exposure and decreased to 1.1 or 0.6 mg/L after 3 to 5 d of exposure. Then it remained stable for at least 4 d.

This stability of the LC50 was also observed at a similar level (0.6 or 0.7 mg/L) during direct exposure of developed larvae regardless of the preexposure or exposure temperatures (tests 5A and B and 6, Table 4 [calcium concentration, 80 mg/L]).

Influence of calcium and temperature on the lethal effects of Mexel 432 in synthetic water

In continuous-exposure experiments beginning at fertilization (test 3, Table 3), no clear effect of a low calcium concentration (20 mg/L) was observed in synthetic ISO water.

During direct exposure of larvae (tests 5 and 6, Table 4), regardless of the preexposure or exposure temperature, little or no variations of the lethal effects of Mexel 432 were observed in the ranges of nominal calcium concentrations (0–800 mg/L) and temperatures (15–24°C).

Influence of pH on the lethal effects of Mexel 432 in synthetic ISO water

In continuous-exposure experiments beginning at fertilization, control survival rates in ISO water without buffers were 97, 96, 94, and 77% after 1, 3, 7, and 10 d of incubation, respectively. In addition, no lethal effect was observed in the buffered waters at pH 6.9, 7.8, and 9.0 (test 4, Table 3). Mexel 432 appeared to be slightly more toxic with increasing pH. For example, the LC50 (after 10 d) was 0.6, 0.5, and 0.4 mg/L at pH 6.9, 7.8, and 9.0, respectively.

Influence of humic acids on the lethal effects of Mexel 432 in synthetic ISO water

During direct exposure of larvae, humic acids had no lethal effect up to a concentration 50 mg/L. In the presence of high concentrations of Mexel 432, 50 mg/L of humic acids drastically reduced its toxicity (test 7A, Table 5); the LC50 (at 2 d) was 113 mg/L. Similarly (test 7B), in the presence of low concentrations of Mexel 432, 2 mg/L of humic acids suppressed the toxicity of 2 mg/L of Mexel 432. The LC50 (3 d) was 2.8 mg/L, and 100% mortality was observed with 4 mg/L, compared to only 1 mg/L in pure ISO water.

Influence of suspended matter and humic acids on Mexel 432 solutions

After extraction of DCE in acidic medium and in the presence of a high concentration of KCl (Figs. 2 and 3), the absorbency spectrum of Mexel 432 fatty amines–methyl-orange complexes in the organic phase showed two peaks (at wavelengths λ = 354 and 414 nm).

With the deionized water (control solutions), a very weak coloration of the DCE extract was observed. In the presence of humic acids, this coloration was not changed. During DCE extraction without Mexel 432, the humic acids were concentrated on droplet surfaces at the interface between the aqueous solution and DCE. In the presence of Mexel 432, the color intensity of the humic acids at this interface was increased, but the diameter of the droplets as well as the color of the aqueous supernatant were strongly reduced. Symmetrically, the coloration of the DCE phase was reduced with increasing concentrations of humic acids mixed with Mexel 432. The absorbencies attributed to the Mexel 432 solutions decreased sharply with increasing concentrations of humic acids, especially at 354 nm (Fig. 2).

In similar conditions (i.e., acidic medium and high concentrations of KCl), suspended mineral matters reduced the quantity of extractable Mexel fatty amines–methyl-orange complexes (Fig. 3). The least important effect was observed with kaolinite and the most important with bentonite. The absorbency reduction was more important at 354 nm than at 414 nm.

DISCUSSION

Biological test validity

In every biological test, pH, oxygen concentration, and temperature, measured before daily renewal of the incubation medium, remained compatible with values for normal embryo-larval development [26,29].

Except in test 2, the control survival rates were better than required in standardized carp early life toxicity tests (80% at hatching and 75% before yolk resorption) [23].

In test 2C (Table 2), control survival rates in synthetic ISO water declined before hatching but later remained relatively stable, which represents a reliable criterion of the quality of the spawns and incubation conditions [26].

Mexel 432 toxicity in natural waters: Influence of solution aging

In synthetic ISO water, Mexel 432 is a relatively toxic product for carp early life stages, particularly in subchronic tests; the NOEC (7 d) may be as low as 0.5 mg/L (Table 3). Preliminary data showed that the toxicity of Mexel 432 in pure waters remained almost unchanged during at least 2 weeks of conservation.

In river waters such as those from the Seine or Moselle River, Mexel 432 toxicity was considerably lower, particularly after the solution had been aged (Fig. 1 and Table 2).

In the Seine River water (test 1), the reduction of toxicity was first due to a rapid or immediate inhibition, since in a 10-mg/L solution the lethal effect appeared after 3 d of exposure, compared to 100% mortality within 1 d with a concentration of 3.1 mg/L in the synthetic ISO water (Table 2). Further aging was then likely to suppress completely the lethal activity of the 10-mg/L solution within 2 d. A similar reduction of the Mexel 432 solution activity in time occurred in the tested...
Moselle River water, since after 8 to 15 d of aging of the solution, the LC50 (7 d) was as high as 67.8 mg/L. However, no important immediate inhibition was then observed, since in test 2B and C, the lethal activity of freshly prepared 1-mg/L solutions was not very different in the ISO water (Table 2C) or in a 90%/10% (v/v) Moselle River/synthetic ISO water (Table 2B). Other experiments will be necessary to verify whether this difference between Seine and Moselle River waters in the time required to suppress Mexel 432 toxicity could be explained by differences in their suspended or dissolved organic contents.

Our observations are consistent with the data of A.S. Allonier and M. Khalanski (personal communication), which distinguished, after Microtox® tests, a variable (immediate demand) followed by a progressive inhibition. This inhibition was considered to result, at least in part, from bacterial degradation. In parallel, for a given nominal Mexel 432 concentration, the disappearing percentage of Mexel 432 in solution in the Seine River water was strongly dependent on the season of water sampling (A.S. Allonier, personal communication).

**Mexel 432 toxicity in synthetic ISO waters: Influence of physicochemical parameters**

Under our conditions, Mexel 432 toxicity was similar if the exposure was performed in a polyethylene incubator with 150 ml of test solution (test 2C, Table 2) or in a polystyrene incubator with 60 ml of test solution (test 3; Table 3).

Our results confirm the tolerance of carp embryo and larva of variations in calcium concentration, temperature, pH, and humic acids concentration [28,29]. Indeed, no difference in survival was observed in larvae at 2 or 3 d with conditions in the ranges of 0 to 800 mg/L for calcium concentration, 15 to 24°C for temperature, 6.9 to 9.0 for pH, and 0 to 64 mg/L for humic acids. Most fish embryos and larvae encounter and tolerate a great diversity of environmental situations in natural waters. For this reason, multifactorial evaluation of the toxic risk linked to a xenobiotic could a priori appear to be indispensable [33]. Moreover, fouling is likely to develop in a wide diversity of environmental situations, consequently inducing the use of antifouling agents in the same situations. However, within the limits of our tests, none of the calcium, temperature, and pH physicochemical parameters exerted an important influence on Mexel 432 toxicity on carp early life stages (Tables 3 to 5). The only observed effect was a slight increase in toxicity at basic pH, which could have been due to an ionization rate change of its fatty amines content. In contrast, Mexel 432 toxicity was strongly inhibited by humic acids (Table 5) at an apparent ratio of 1 to 2 mg Mexel 432/mg humic acids, at concentrations of humic acids widely observed in natural water [34]. For example, in Moselle River water, the suspended matter content varied between 10 and 80 mg/L during the spring and summer of 1992 [8]. The existence of an immediate demand of Mexel 432 in natural waters (Fig. 1) was in agreement with the immediate detoxicant effect observed with humic acids. Among fishes, a similar protective effect of humic acids was observed on carp larva [33] and, with other toxicants, on diverse developmental stages of different fish species [35–37]. However, the Aldrich humic acids used in our study are not representative of most of the humic acids present in an aquatic medium because their binding affinities and capacities are quite different from those in many natural waters [38].

**Mexel 432 solution analysis**

Mexel 432 solution analysis in the presence of Aldrich humic acids and suspended matters (Figs. 2 and 3) showed that these materials are likely to link strongly enough with the Mexel fatty amines in acidic medium and in the presence of high concentrations of KCl to render nonextractable their complexes with methyl-orange in DCE. Thus, the inhibitory effect of these humic acids could be attributed to a reduction of its bioavailability by complexation. The absorbency peaks in the visible spectrum of these complexes suggest that among the components of Mexel 432, the complex related to the toxicity absorbs light at 354 nm. The confirmation of this hypothesis could lead to the definition of a more specific dosage method of the toxic fraction in the Mexel 432 complex mixture than the present method.

In colorimetric analysis conditions, the adsorption of Mexel fatty amines by humic acids was associated with a concentration of the complexes Aldrich humic acids–Mexel fatty amines–methyl-orange at the interface between the aqueous medium and DCE. However, the physicochemical conditions for this observation or for the dosages (acidic medium, presence of high concentration of KCl and methyl-orange) were very far from the conditions of an eventual complexation of Mexel 432 in natural waters.

In pure deionized water (at neutral or weakly basic pH and without the presence of methyl-orange or KCl), humic acids also form complexes with Mexel 432. This is shown by the preliminary observation that the absorbency of centrifuged humic acid solutions directly measured in water was progressively reduced by addition of increasing concentrations of Mexel 432. Measurement of Mexel n-octanol/river water partition coefficients ($K_{ow}$) and eventual concentration at the water surface could provide better data about the relations between suspended or dissolved organic matters and the true immediate bioavailability of Mexel 432 in the medium. The existence of an apparent ratio of complexation (1 mg Aldrich humic acids/1 or 2 mg Mexel 432) will have to be confirmed, especially in the case of low Mexel 432 concentrations with various types of humic acids, which are generally weak adsorbants compared to Aldrich humic acids [38]. In each case, it would also be necessary to verify whether the humic acids–Mexel 432 complexes are likely to dissociate, as observed with hexachlorobiphenyl [39], which could make the toxicant bioavailable again. However, according to Van Ginkel et al. [21], in the presence of composite bacterial communities, an initial oxidation of the fatty amines in water leads to a rapid production of nontoxic compounds before complete degradation.

**CONCLUSION**

In synthetic ISO water, the lethality thresholds of Mexel 432 are relatively low (approx. 2 mg/L after 24 h of exposure and 0.5 mg/L after 7 d). However, this embryolarval lethality is strongly reduced when the agent is dissolved in natural waters and is marked by both an immediate (within 1 h) and a progressive (over several days) reduction.

This reduction is not due to temperature, pH, or calcium concentration. On the contrary, Aldrich humic acids induce a drastic and immediate reduction of Mexel 432 lethality. In dosages (in acidic and high-ionic-strength medium), the concentration of Mexel 432 is reduced in the presence of humic acids or clays.

From a methodological point of view, the demonstration of an inhibitory power of suspended mineral waters or organic
Lethal effects of Mexel® 432 on common carp embryos and larvae

Matters on Mexel 432 toxicity points out one of the major limitations of laboratory aquatic toxicity tests when they are performed in semistatic exposure conditions or with exogenous feeding. This limitation is especially obvious in the case of hydrophobic and amphiphilic molecules, which are likely to adsorb strongly on a wide diversity of substratums. For example, in semistatic tests, the presence of carp larvae strongly increased the percentage of disappearance of the insecticide deltamethrin, a very hydrophobic molecule, in the medium [33]. Therefore, laboratory flow-through tests or rapid renewal semistatic tests will be necessary to confirm the actual level of Mexel 432 toxicity in pure waters.

The complexation of Mexel 432 with suspended mineral or dissolved organic matters may explain the observed variations in the time of its toxic effect and the rapidity of its disappearance in river waters at different periods of the year. Indeed, concentrations of suspended or dissolved matter are widely changing parameters, especially in rivers in which the problem of biofouling proliferation exists. In practice, use of Mexel 432 could be limited in highly charged waters. A precise adjustment of Mexel 432 treatments to the water quality and to the treated hydraulic circuit characteristics will be necessary to optimize the anticrocorrosion and antifouling treatments themselves and to simultaneously minimize the toxicity of Mexel 432 on nontarget organisms in the environment. For this purpose, a method for rapid field determination of the immediate demand is under study.

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