

## CHEMICAL CONTAMINANTS IN DRINKING WATER: MUTAGENIC AND TOXIC EFFECTS

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**Abstract.** This study aimed to estimate the hazards associated with the use of chlorination in a water-work where some episodes of chemical pollution were recorded in the recent years. The methodological approach consisted in twice a year sampling of large volumes of raw and chlorinated water then processed to concentrate the non-volatile organic chemicals. The analyses of water samples indicated high levels of organic matter in chlorinated water, up to 21.17g/l. A complex mixture of chemicals in each of both extracts has been identified by GC-MS technique. Higher levels of 3-chloro-4(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX), a strong mutagen compound, in chlorinated water extract in comparison with the corresponding raw water extract of samples of 2000 year summer have been measured for the first time in Romania. The mutagenicity test Ames results of water extracts were in agreement with the corresponding MX levels. Acute and sub-acute experiments on young fish exposed to gradually increased concentrations of chemicals in water, for different exposure periods have indicated higher lethality percentage for exposure in chlorinated water compared with raw water. A dose-effect relationship between the concentrating factors of chemicals and the fish lethality was also established, statistically significant for acute experiments.

**Key words:** water chemical contamination, chlorination, effect assessment, MX, fish, experimental exposure

**Rezumat.** Acest studiu a avut drept scop estimarea riscurilor asociate clorinării apei la uzina de apă din oraşul Vaslui, unde s-au înregistrat o serie de episoade de poluare chimică în ultimii ani. Metodologia a constat în recoltarea, de două ori pe an, a unor volume mari de apă brută și clorinată, probe care au fost prelucrate pentru concentrarea substanțelor organice nevolatile. Analiza probelor de apă a arătat niveluri crescute ale substanțelor organice în apa clorinată, de până la 21,17g/l. Analizele cantitative și calitative ale extractelor, prin tehnica GC-MS, a identificat, în ambele extracte, amestecuri complexe de substanțe chimice și valori mai mari de 3-cloro-4(diclorometil)-5-hidroxi-2(5H)-furanonă (MX), un compus cu potențial mutagen, în extractul de apă clorinată, în comparație cu cel de apă brută, pentru probele de apă recoltate în vara anului 2000. Rezultatele testului Ames au fost în concordanță cu nivelele corespunzătoare găsite pentru MX. Experimentele acute și subacute pe puiet de pești expuși în concentrații gradat crescute de contaminanți chimici, pentru diferite perioade de expunere, au indicat procente mai mari de letalitate la peștii expuși în apă clorinată comparativ cu apa brută. S-au stabilit, de asemenea, relații doză-efect între factorii de concentrare ai contaminanților chimici și procentele de letalitate a peștilor, relații care au fost statistic semnificative pentru experimentele acute.

**Cuvinte-cheie:** contaminare chimică a apei, clorinare, evaluarea efectelor asupra sănătății, MX, expunere experimentală, pești

## INTRODUCTION

It is well known that the disinfection of drinking water provides protection against microbial diseases but also produces chemical by-products that may pose other types of risks to public health. In this context, the identification and measurement of these risks generated by drinking water from different sources is a realistic goal for public health protection (1).

A preliminary observation is that the possible health risk assessment of this kind of chemical pollution doesn't aim to minimize or to compromise the importance of water disinfection (2). Although the subject of chemical versus microbial risks is not the topic of this paper, we still mention the pioneering work of J.C. Morris (3,4) in this area. Morris showed that, at low levels of chlorination, the chemical risk of drinking water may be initially decrease because the destruction of chemicals by oxidation may more compensate the formation of new hazardous substances. The by-products arising, this chemical risk increases with increasing levels of chlorination.

The review of literature on the chlorination by-products in drinking water points out the features of this human health risk. Most frequently, the exposure data are uncertain because not all compounds of the mixture are known, neither qualitatively nor quantitatively (5). Much attention and concern has been paid to the analysis of genotoxic compounds in drinking water. It is showed that they are rather non-

volatile, quite acid and non-stable at high pH and rather polar. Among these compounds, the most abundant are the volatile chlorinated and/or brominated trihalomethans, usually not recovered in drinking water extracts. Other important groups are halogenated derivatives of acetic acids, acetonitrils, ketones, phenolic compounds. We mention also arsenic, nitrates, bromates, as natural or human-activity-related drinking water contaminants, which are responsible for cancer in rodents or in humans (1). The halogenated hydroxyfuranones, although present at concentrations lower than 0.1 µg/l in drinking water, can be responsible for more than half of Ames test mutagenicity (6-9). The so-called "mutagen X" (MX), the most potent of these chlorohydroxyfuranones, has been submitted to intensive toxicological studies worldwide (10-12) and was identified as a strong carcinogen in rats (13).

Based on these data, our work aimed to identify the chemical pattern of drinking water contaminants, to measure the MX levels in water extracts and to assess their in vitro and in vivo effects, by Ames test of mutagenicity of water sample extracts and, respectively, by acute and sub-acute experiments on fish. An attempt to relate chemical exposure levels with fish lethality was also our purpose.

In this study drinking water was sampled from the water-work Delea of Vaslui town, situated in the North-Eastern Romania. This source of drinking water was selected due both some chemical and biological pollution episodes recorded in the

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recent years and the high frequency of inadequate organic matter and phenolic compound levels found by annual surveys.

The study was carried out during 2000-2001 period.

As raw water, the Delea water-work uses surface water, mainly from Solești lake. About 60,000 inhabitants of Vaslui town use this drinking water and the daily water consumption is about 14,000 cubic meters.

The raw water of this water-work is disinfected with chlorine. The treatment process consists of prechlorination, coagulation, sedimentation, filtration and final chlorination. The prechlorination step is not always carried out and the prechlorine doses are never measured. After the prechlorination, the chlorine level in water is measured, but usually it takes 7-8 days until the results are obtained. For final chlorination, 4-6 kg of chlorine/hour are used for about 14,000 cubic meters of water/day, and 5-7 kg of chlorine when there is no prechlorination step.

In these uncontrolled conditions, the inadequate quality of drinking water had unknown consequences on population health status.

### METHODOLOGICAL APPROACH

Large volumes (over 200 liters) of raw water (RW) of Solești lake and chlorinated water (CW) supplied by Delea water-work were sampled twice a year. The chemical and biological quality parameters of water samples were measured according to national standards.

The next stage was the XAD2 resin adsorption/extraction of water samples to concentrate the non-volatile organic chemicals. The concentrating procedure consisted in the following steps:

- The acidification of water samples by adding 5 ml of concentrated hydrochloric acid (37%) to each 1 liter of water (final pH close to 1).
- The XAD2 column preparation: XAD2 resin was purified by Soxhlet extraction with methanol (8 hours), acetonitrile (8 hours) and with diethyl ether (8 hours); then the resin was suspended in methanol for column preparation (the column dimensions were: high = 6 x inner diameter, namely 10/1.7 cm).
- The water samples were extracted on XAD2 with a rate of 30-50 ml/min, in portions of 10 liters.
- After each portion of 10 liters of water, the elution of the retained compounds was made with 10 ml of diethyl ether. The solvent was maintained for 10 min in contact with the XAD2 resin.
- Ten liters of each water sample were processed using ethyl acetate as eluent solvent.
- The extracts were collected and the final volume of concentrates was noted for each water sample.
- A control extract for XAD2 column and solvents used in the procedure was also prepared by the same procedure using 10 liters of bi-distilled water.

The extracts of water sampled in June 2000 were qualitatively analyzed by gas chromatography-mass spectrometry (GC-MS) technique, using Hewlett

Packard GC-MS 6890-5973 equipment, without sample processing by derivatization techniques. The identification of chemicals was performed based on Willey library. Values over 90% were considered among the identified compounds.

The levels of MX in water sample extracts were measured using also GC-MS. Analyses were performed on a Dani 3800 capillary GC interfaced to a VG7070E MS. The MS was operated in selected ion monitoring (SIM) mode. Based on the results obtained in the analysis, the water sample extracts were selected for effect testing.

The next methodological step of our study was the testing of in vitro and in vivo effects of water extracts.

For in vitro effect, the Ames test for mutagenicity of water extracts was carried out, as a bacterial test using strain TA 100 of *Salmonella typhimurium* without enzymatic activation. Mutation type was basepair mutation. Mutagenicity of water sample extracts was expressed as the slope of the linear part of the dose-response curve. Unit of mutagenicity was the number of netrevertants per liter of water. The detection limit was 100 netrev/l (14).

Acute and sub-acute experiments on fish species *Carassius auratus Gibelio Bloch* were designed for testing the in vivo effects in order to find out the dose-effect relationships. It was assumed that RW- and CW-extracts contain the same chemicals as RW and CW samples respectively. Ten young fish (length: 8.5-12.5 cm) in each

group were exposed in RW and CW with gradually increased concentrations of organic chemicals: 1-fold, 10-fold, 20-fold and 50-fold the concentration of chemicals normally found in RW and CW, respectively. The concentrating was made by adding adequate quantities of RW- and CW-extracts to RW and CW in aquaria, respectively. The same amounts of diethyl ether as in those ones prepared with water extracts were used as control for each experiment. The fish lethality percentage as an indicator of toxic effect was assessed after different exposure periods: 24 h, 48 h, 72 h, 96 h and 7 days.

The experimental conditions were the following:

Air:

temperature = 20.0-21.5°C  
light intensity = 868-1532 lux

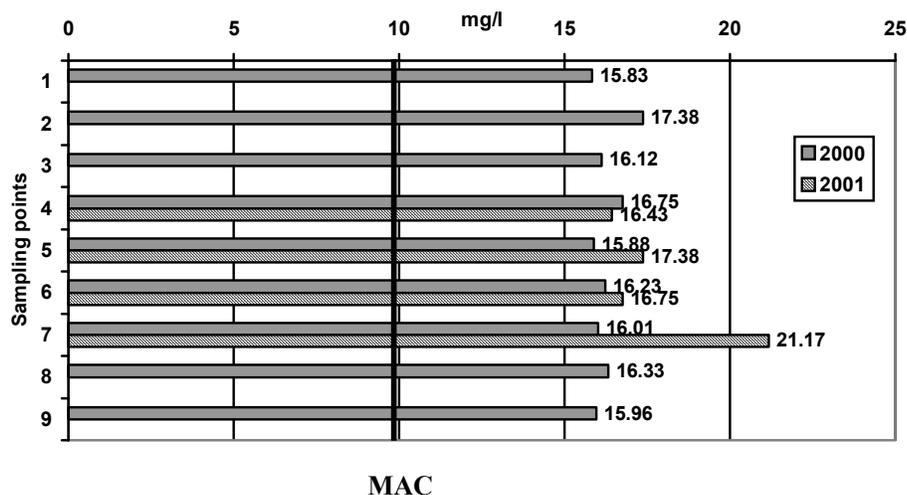
Water:

temperature = 20.0-22.0°C  
total hardness = 18.7°G  
dissolved oxygen = 9.8-10.0 mg/l  
alkalinity 4.0-4.7 ml HCl n/10  
nitrogenous compounds (NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub><sup>-</sup>) = absent  
parasites and organisms indicating an impurity = absent

## RESULTS AND DISCUSSION

The chemical and biological quality of water samples was found in normal ranges, but a significant increase of organic compounds level measured as permanganate consumption up to 21.17 mg/l in CW samples was noted in the study period (fig. 1). The maximum allowable concentration (MAC) for this parameter is 10.0 mg/l.

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**Fig. 1 Organic matter concentrations found in drinking water in different points of distribution network in Vaslui in 2000-2002 period**

Phenolic compounds and anionic detergents levels were in normal range in drinking water, but in high concentrations in raw water, as figure 2 shows. The phenolic compounds maximum concentration found in raw water was 0.083 mg/l. The anionic detergents levels varied between 0.021 and 0.35 mg/l in raw water samples, and lower values (0.0077-0.095 mg/l) in drinking water samples.

The most interesting aspects revealed by biological quality tests were the higher frequencies of:

- ferobacteria (13.04 %) in water samples of distribution network compared with chlorinated water sampled at Delea water-work (7.89%)

- *Giardia sp.cysts* (10.52%) in chlorinated water sampled at water-work in comparison with only 8.69% found in distribution network samples.

These results are consequences of inadequacy of both treatment process of raw water in the Delea water-work and distribution network.

The qualitative GC-MS analysis of water extracts has reliably identified mixtures of chemicals both in CW and RW extracts: 24 chemicals in RW extract and 47 chemicals in CW extract (tables 1 and 2). The identified chemicals have complex structures and further investigation are needed to confirm them using reference standards.

MAC in drinking water: phenolic compounds – 0.001 mg/l  
 anionic detergents– 0,2 mg/l

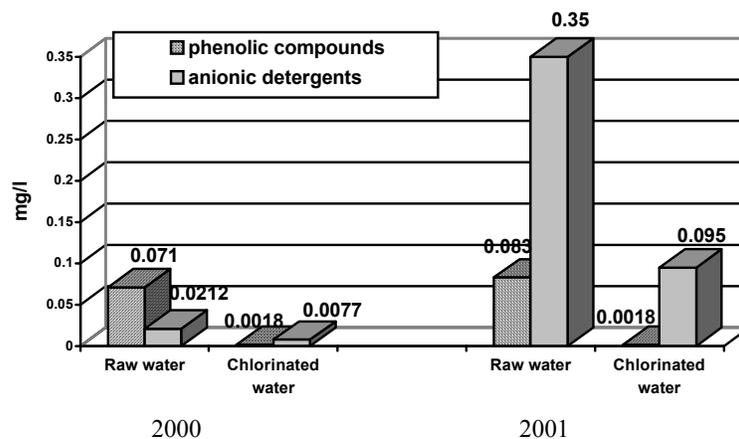


Fig. 2 Organic pollutants levels in RW and CW of Delea water-work in the study period

Table 1. List of organic compounds identified in RW extract sample by GC-MS qualitative analysis

Compounds reliably identified	Library/ID	CAS #	Quality %
	Boric acid,triethyl ester	000150-46-9	90
	Ethane,pentachloro-	000076-01-7	98
	Ethane, hexachloro-	000067-72-1	91
	Undecane	001120-21-4	94
	Dodecane	000112-40-3	95
	Tridecane	000629-50-5	96
	Tetradecane	000629-59-4	96
	2,5-Cyclohexadiene-1,4-dione,2,6-	000719-22-2	98
	Pentadecane	000629-62-9	95
	Hexadecane	000544-76-3	97
	Heptadecane	000629-78-7	94
	Lindane	000058-89-9	99
	Hexadecanoic acid, methyl ester	000112-39-0	95
	Dibutyl phthalate	000084-74-2	94
	Hexadecanoic acid, ethyl ester	000628-97-7	95
	7-Octadecanoic acid, methyl ester	057396-98-2	91
	5-(7a-Isopropyl-4,5-dimethyl-octane	1000193-54-0	95
	1,2'-Binaphthalene-5,5',8,8'-tet	020175-84-2	97
	2-Dodecen-1-yl(-)succinic anhydrid	019780-11-1	95
	Nonacosane	000630-03-5	90
	Cyclotriacontane	100131-19-0	91
	Docosane	000629-97-0	92
	Heptacosane	000593-49-7	93
	Cyclotriacotane	000297-35-9	92

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**Table 2. List of organic compounds identified in CW extract sample by GC-MS qualitative analysis**

	Library/ID	CAS #	Quality %
<b>Compounds reliably identified</b>	1H-Indene, 1-methylene-	002471-84-3	93
	Dodecane	000112-40-3	94
	Nonanoic acid	000112-05-0	95
	Tridecane	000629-50-5	97
	Phthalic anhydride	000085-44-9	91
	1H-Inden-1-one,2,3-dihydro-3,3-di-	026465-81-6	90
	Tetradecane	000629-59-4	98
	Ethanone,1,1'-(1,4-phenylene)bis-	001009-61-6	91
	Quinoline,1,2-dihydro-2,2,4-trimethyl-	000147-47-7	94
	Quinoline,2,4-dimethyl-	001198-37-4	96
	1-Pentadecene	013360-61-7	91
	Pentadecane	000629-62-9	97
	Butylated hydroxytoluene	000128-37-0	98
	Naphtalene,1,4,6-trimethyl-	002131-42-2	92
	Hexadecane	000544-76-3	97
	1-Nonadecene	018435-45-5	95
	Pentadecane,2,6,10-trimethyl	003892-00-0	90
	Heptadecane	000629-78-7	97
	3-Eicosene,(E)-	074685-33-9	98
	Cyclohexane,1,2,3,4,5,6-hexachlor	000608-73-1	91
	Diphenylethyne	000501-65-5	91
	1-Octadecene	000112-88-9	95
	Cyclotetradecane,1,7,11-trimethyl	001786-12-5	97
	Octadecane	000593-45-3	98
	Hexadecane,2,6,10,14-tetramethyl	000638-36-8	96
	1-Docosene	001599-67-3	95
	Tetrapentaacontane,1,4,5-dibromo	1000156-09-4	90
	Nonadecane	000629-92-5	91
	Hexadecene	1000130-87-5	94
	Dibutyl phtahalate	000084-74-2	95
	3-Octadecene	007206-19-1	91
	Pentadecane	000629-62-9	95
	Pyrene	000129-00-0	95
	E-8-Methyl-9-tetradecen-1-ol,acetat	1000130-81-4	91
	Octadec-9-enoic acid	1000190-13-7	90
	Docosane	000629-97-0	93
	[1,2'-Binaphtalene]-5,5',8,8'-tet	020175-84-2	94
	Tricosane	000638-67-5	93
	Cyclotetradecane,1,7,11-trimethyl	001786-12-5	94
	Heptacosane, 1-chloro	062016-79-9	96
	Cyclotriacotane	000297-35-8	92
	Pentacosane	000629-99-2	95
	Bis(2-ethylhexyl)phthalate	000117-81-7	91
Octacosane	000630-02-4	96	
Nonacosane	000630-03-5	98	
1,12-Bis(2-nitrophenoxy)dodecane	1000187-93-0	90	
Ergost-25-ene-3,5,6-triol	056143-28-3	91	

The MX levels and the results of mutagenicity test are indicated in table 3. The water samples of June 2000 had higher levels of MX than those ones of November 2001. In addition, the levels measured for this compound were higher in CW- than those found in RW-extracts.

These results are the first ones in Romania on drinking water. Levels of

MX higher than those found in our study were reported in Finland (15, 16), UK (17) and USA (18,19). Maximum MX levels measured in water samples of Netherlands (20), Spain (21), Japan (22,23), Germany (24) and Russia (25) were lower than those ones reported here.

**Table 3. MX levels in water sample extracts and the results of mutagenity test**

Water sample (sampling period)	MX (ng/l)	Ames test net revertans/l
CW(November 2001)	0.1	<100
RW(November 2001)	<LOQ	<100
CW (June 2000)	23.6	1080
RW (June 2000)	1.0	280
Blank for resin and column	<LOQ	<100

<100 = result is lower than the limit of quantitation in the mutagenity analysis

<LOQ = result is lower than the limit of quantitation in MX – analysis

The water sample with higher MX level showed higher mutagenicity in Ames test.

The range of mutagenicity for different raw or chlorinated water samples tested worldwide is quite large, but, basically, higher values in chlorinated water than their corresponding raw waters were reported (26-28).

Based on these results, the water sample extracts of June 2000 were used in acute and sub-acute experiments on fish (fig. 3).

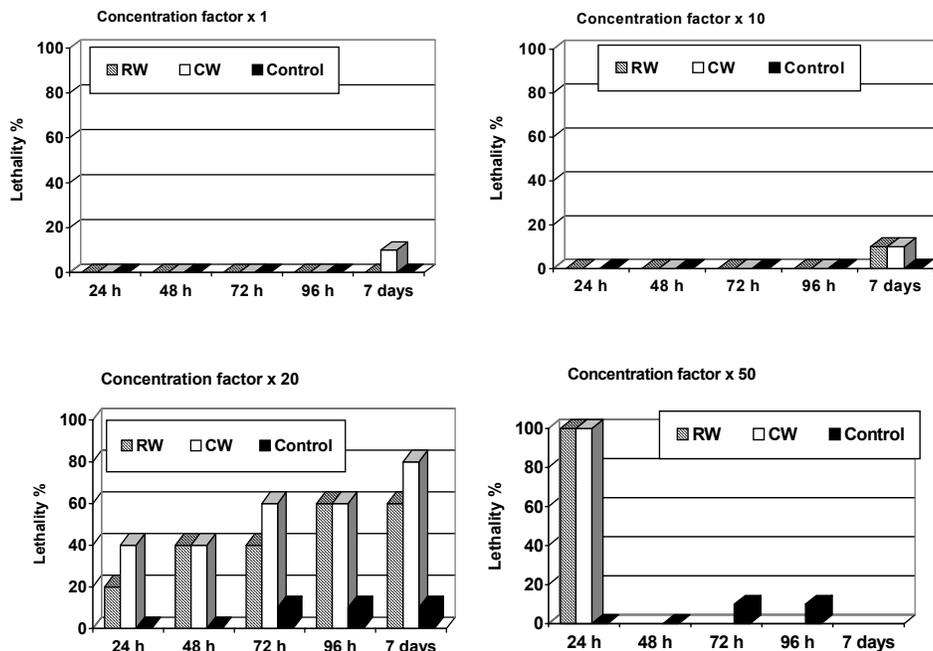
Fish exposure in CW with concentration factor of 1 yielded 10% lethality after 7 days, higher than those

resulted in fish 7 days-exposure in RW (0%).

Fish exposure in CW with concentration factor x 20 yielded higher lethalties: 40%, 60% and 80% after 24h-, 72 h- and 7 days-exposures, respectively, in comparison with the lethalties of 20%, 40% and 60% recorded in fish exposed in RW with the same concentration factor and for similar experimental periods.

These experimental results confirm the in vitro findings for mutagenicity and both are in agreement with the MX levels measured in the two water samples.

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**Fig. 3** Lethality percentage of *Carassius auratus* – *Gibelio Bloch* treated with gradually increased concentrations of chemicals in RW and CW during experimental period

An attempt was made for an dose-effect relationship based on fish lethality percentage as indicator of toxic effect of gradually increased concentrations of chemicals in CW and RW, for acute (24 h) and sub-acute (7 days) experimental exposure period. The results are indicated in figure 4. The relationships were statistically significant in acute exposure to CW and RW.

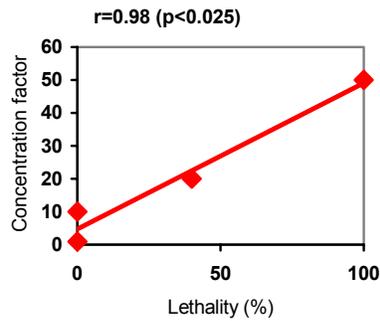
### CONCLUSIONS

1. High levels of organic pollution in chlorinated water are the consequence of inadequacy of water treatment procedures.
2. Complex mixtures of chemicals in both raw and chlorinated water samples were identified.
3. For the first time, in our country, the levels of strong mutagenic chlorinated compound MX were measured. Higher values were found for chlorinated water, results strengthened by mutagenicity test.
4. Also, the lethality percentage of experimentally - exposed fish in gradually -increased concentrations of chemicals was higher in chlorinated water. The dose-effect relationships were statistically significant for acute exposures (24 h)

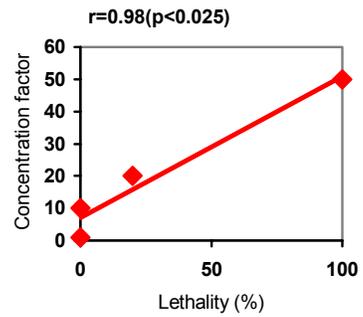
in raw and, respectively, chlorinated water.

5. Our results point out the necessity of further investigations on

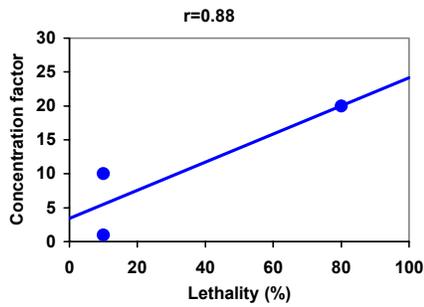
chemical risk arises from the use of surface water as sources for drinking water in Romania.



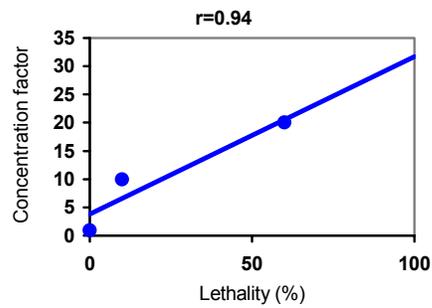
24 h-exposure in CW



24 h-exposure in RW



7 days-exposure in CW



7 days-exposure in RW

**Fig. 4 Exposure-effect relationship in acute and subacute experiments on fish**

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