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Acute Toxicity of Phenol and Substituted Phenols to the Fathead Minnow

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Water pollution caused by phenolic compounds has many sources: coal and wood distillation, road tars, petroleum refining, chemical and plastics manufacturing, domestic sewage, livestock dips, and wood preservatives. Natural sources, such as plant material decay and leaching from coal and oil deposits, also contribute to water pollution by phenolic compounds. The adverse effects of such pollution on aquatic life fall into three general categories: the direct toxicity of the chemicals to aquatic organisms, the lowering of dissolved oxygen in receiving waters, and the imparting of bad taste and odor to the edible flesh of fish and shellfish.

Because of the wide, high-volume usage of phenolics in our society, it behooves us to be aware of their toxicity in the environment. This awareness must include a knowledge of the effects on freshwater bodies and more specifically, an appreciation of the importance of differences in the physical properties of the water. The variability of the data on some phenolics makes it difficult to separate toxicity values that vary widely from others because of different test methods rather than different physical properties of the water. Data were lacking on some of the compounds we tested, and thus our data contribute significantly to the basic information on these chemicals.

This acute test series was conducted with a variety (12) of phenolic compounds. Test conditions were kept as uniform as possible so that the toxicities of the compounds could be attributed to the differences in toxicity rather than to differences in test conditions. In addition, the tests provide data on the toxic effects in Lake Superior water, which is a major freshwater body in the U.S.A.

MATERIAL AND METHODS

Exposure System. Flow-through acute toxicity studies were conducted with two comparable 2-L/cycle proportional diluters (MOUNT & BRUNGS 1967), each with a dilution factor of 0.6. Each diluter system had five test concentrations and a control, all duplicated, totaling 12 exposure tanks. Diluters were cycled at a rate sufficient to give 10 tank volumes/day. Flow-through acute exposure chambers consisted of all-glass aquaria 30 cm high x 30 cm x 60 cm with a water volume of 41 L. Static reference test

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chambers made of stainless steel and glass were 23 cm x 23 cm x 17 cm high with a water volume of 9 L. A constant 16-h photoperiod was used during all tests. Light was provided by a combination of fluorescent bulbs that produced 48 lumens at the water surface.

Physical and Chemical Conditions. Dilution water in all tests was Lake Superior water which was filtered through sand and then finally filtered by 5-μm cotton rope filters. Water temperature in the flow-through acute bioassays was maintained at 25 ± 2° C and was monitored with a recording telethermometer. Reference test water temperatures for all tests followed ambient room temperatures and varied from 21.6 to 25.4° C.

Dissolved oxygen, pH, hardness, and alkalinity were determined in each concentration at the beginning, middle, and end of each 8-day test according to methods described by the AMERICAN PUBLIC HEALTH ASSOCIATION et al. (1971). Test water for all concentrations in all experiments had a hardness ranging from 43.3 to 48.5 mg/L as $CaCO_3$ and had dissolved oxygen concentrations of 6.2 to 8.2 mg/L. Alkalinities varied with experiments and toxicant concentrations because of the NaOH added to solubilize some toxicants in the stock bottle. The alkalinity for control water, which had no NaOH added, varied from 38.0 to 44.3 mg/L as $CaCO_3$. The test water pH of several tests varied because of the addition of NaOH to the stock bottle used to solubilize some of the compounds. Those chemicals with pH values that varied appreciably from controls were (control to high concentration): pentachlorophenol, pH 7.41 to 8.33; 3-methoxyphenol, pH 7.51 to 8.59; 2,4-dimethylphenol, pH 7.58 to 9.10; 2,6-dimethylphenol, pH 7.29 to 8.61; and 2,4,6-tribromophenol, pH 7.56 to 8.35.

Biological Methods. Laboratory-reared fathead minnows (Pimephales promelas) 30 to 35 days old were used. Fish were reared in a system similar to the exposure systems, with similar diluent water at 25 ± 2° C. Fish in the rearing system were fed live brine shrimp nauplii in excess until 12 to 24 h before testing and were not fed during the 8-day exposures.

At the start of each flow-through test, 50 fish were randomly assigned to each of the 12 exposure tanks. Dead fish were counted and removed four times during the first day and twice daily thereafter. Twenty fish were used in each exposure chamber in the static tests. Any methods not previously mentioned followed those specified by the U.S. ENVIRONMENTAL PROTECTION AGENCY, COMMITTEE ON METHODS FOR TOXICITY TESTS WITH AQUATIC ORGANISMS (1975).

Chemical Methods. Eleven of the 12 phenols studied were analyzed daily by using the automated 3-methyl-2-benzothiazolone hydrazone (MBTH) method (FRIESTAD et al. 1969) as modified by GALES (1975). The distillation step was found to be unnecessary and was therefore eliminated. The equipment used included the Technicon[1] autoanalyzer sampler IV, proportioning pump III, colorimeter I with 520-nm filters and a 15-mm flow cell, adjustable range expander, and a Perkin-Elmer 56 recorder. Samples were diluted with

distilled water to bring them into the concentration range of a set of distilled water standards. Concentrations were then calculated on the basis of these standards. The over-all mean percentage fortification recovery and 95% confidence limits for all compounds was 101 $\pm$ 3 (n = 57).

Since the colorimetric reaction upon which the above method depends does not take place appreciably for pentachlorophenol, this compound was analyzed by gas chromatography (F. A. Puglisi, Environmental Research Laboratory-Duluth, Duluth, MN 55804, personal communication). Samples were extracted with 2,2,4-trimethylpentane and derivatized with TRI-SIL/BSA, Formula D[2] before being analyzed by automated gas chromatography. Mean percentage fortification recovery and 95% confidence limits were 93 $\pm$ 4 (n = 4).

Statistical Analysis. The LC50 concentrations were calculated by using the computerized Trimmed Spearman Karber method for estimating median lethal concentrations (HAMILTON et al. 1977).

Threshold indices (T.I.) were derived by using the following manipulation: $\left(\text{T.I.} = \frac{\text{192 h LC50}}{\text{96 h LC50}}\right)$.

RESULTS

The 96-h and 192-h LC50 values and 95% confidence intervals of the phenolic compounds tested in this study are given in Table 1. In addition, threshold indices (T.I.) are given: a value of 1 indicates that no deaths occurred after 96 h, and a value less than 1 indicates that deaths occurred between 96 h and 192 h. Static test LC50 values and their 95% confidence limits are given and arranged to correspond with the flow-through acute test that was conducted at the same time.

The most toxic compound tested was pentachlorophenol with a 96-h LC50 of 0.22 mg/L. The least toxic was 3-methoxyphenol with a 96-h LC50 of 76 mg/L. This compound was 350 times less toxic than pentachlorophenol at 96 h, but it was only 270 times less toxic at 192 h. This difference comes about because the compound was not close to a threshold at 96 h as reflected by a T.I. of 0.75 compared to a T.I. of 0.95 for pentachlorophenol. The second most toxic compound was 4,6-dinitro-o-cresol with a 96-h LC50 concentration of 2.0 mg/L and a 192-h LC50 of 1.5 mg/L and a T.I. of 0.74. Phenol was mid-range in toxicity in the test series with 96-h and 192-h LC50 values of 29 mg/L and 23 mg/L.

The halophenols, except for pentachlorophenol, varied from a 96-h LC50 of 6.6 mg/L for 2,4,6-tribromophenol to 12 mg/L for 2-chlorophenol. Other halophenols tested included

[1] Technicon Instruments Corp., Tarrytown, NY 10591
[2] Pierce Chemicals, Rockford, IL 61105

Table 1. Results of flow-through acute toxicity tests (mg/L), static reference tests (mg/L), and threshold indices (T.I.) with fathead minnows and 12 phenolic compounds

Compound	96-h LC50		192-h LC50		Threshold Index 192-h LC50 / 96-h LC50	48-h LC50 Static Tests
	A[a]	B[a]	A[a]	B[a]		
Pentachlorophenol	0.22 (0.21-0.23)[b]	0.23 (0.21-0.23)[b]	0.20 (0.19-0.21)[b]	0.22 (0.21-0.23)[b]	0.95	7.9 (6.4-9.7)[b]
4,6-dinitro-o-cresol	2.2 (1.9-2.4)	1.9 (1.7-2.1)	1.7 (1.7-1.8)	1.3 (1.1-1.4)	0.74	8.6 (7.5-9.9)
2,4,6-tribromophenol	6.5 (5.0-8.3)	6.8 (4.7-9.8)	4.5 (3.8-5.4)	4.9 (4.2-5.6)	0.71	10 (7.7-13)
2,4-dichlorophenol	8.3 (7.6-9.0)	8.2 (7.4-8.8)	6.5 (6.1-7.0)	6.5 (5.9-7.0)	0.78	8.4 (7.5-8.4)
2,4,6-trichlorophenol	9.7 -[c]	8.6 (7.6-9.9)	5.8 (5.3-6.4)	6.4 (5.6-7.2)	0.67	7.7 (7.2-8.2)
2-chlorophenol	11 (9.7-13)	13 (11-16)	6.3 (6.0-6.7)	6.3 (6.1-6.6)	0.51	9.7 (7.0-13)
2,4-dinitrophenol	17 (16-17)	17 (16-18)	16 (15-17)	16 (15-17)	0.95	7.3 (6.4-8.3)
2,4-dimethylphenol	17 (16.1-17.9)	17 (16-18)	14 (13-15)	13 (12-15)	0.82	9.5 (8.4-11)
2,6-dimethylphenol	>27	>27	21 (20-22)	23 (21-26)	-[d]	6.7 (6.2-7.2)
Phenol	28 (23-34)	29 -[c]	22 (21-23)	23 (22-24)	0.78	8.3 (7.1-9.7)
4-nitrophenol	59 (56-63)	62 (57-67)	49 (46-53)	50 (46-54)	0.82	10 (9.6-11)
3-methoxyphenol	77 (70-85)	75 (70-81)	55 (49-62)	60 (54-65)	0.75	8.7 (7.9-9.5)

[a] Duplicate tests.
[b] 95% confidence limits.
[c] Confidence limits not calculated because 50% mortality was first reached at 96 h.
[d] Threshold index not calculated because a 96-h LC50 was not ascertained.

2,4-dichlorophenol with 96-h and 192-h LC50 values of 8.2 mg/L and 6.5 mg/L and 2,4,6-trichlorophenol LC50 values of 9.2 mg/L and 6.1 mg/L for 96 h and 192 h, respectively.

Two nitrophenols were tested: 4-nitrophenol had 96-h and 192-h LC50 values of 61 mg/L and 50 mg/L compared to 96-h and 192-h LC50 values of 17 mg/L and 16 mg/L for 2,4-dinitrophenol.

Two structural isomers of dimethylphenol tested had quite different toxicities. The compound 2,4-dimethylphenol had a 96-h LC50 of 17 mg/L, but a 96-h LC50 was not attained with 2,6-dimethylphenol with a high concentration of 27 mg/L in the test. The 192-h LC50 values for 2,4-dimethylphenol and 2,6-dimethylphenol were 14 mg/L and 22 mg/L, respectively.

The 12 static 2,4-dichlorophenol 48-h LC50 values ranged from 6.7 mg/L to 10 mg/L with a mean LC50 concentration for all tests of 8.6 mg/L $\pm$ 1.1 mg/L (Table 1). Since the test fish for each static reference test and each corresponding flow-through acute test came from the same stock, we were able to ascertain if a batch of fish was severely weakened from disease or were more or less sensitive to 2,4-dichlorophenol for some unexplained reason.

The course of mortality was plotted for each test (Fig. 1). The compound 2,4-dinitrophenol, for example, has a nearly flat curve and from the T.I. of 0.95 (Table 1) it is apparent that it is near a mortality threshold. In contrast, 2,4,6-trichlorophenol has apparently not reached a threshold, as indicated by the steep slope of the line from day 7 to day 8 and a T.I. of 0.67.

DISCUSSION

An abundance of literature dealing with toxicity to many organisms is available for some of the phenolics tested. This discussion will concern itself, however, only with that literature dealing with the fathead minnow.

Similar test methods, conditions, and fish stocks make the data in this toxicity test series comparable. Some toxicants, however, were solubilized with sodium hydroxide to make the stock chemical solution. Thus the pH varied in some of the tests, and according to HOLCOMBE et al. (1980) the toxicity of some phenolics may be decreased as pH increases. The chemicals with pH values that varied from controls were: pentachlorophenol; 3-methoxyphenol; 2,4-dimethylphenol; 2,6-dimethylphenol; and 2,4,6-tribromophenol. Since the pH increased at each succeedingly higher concentration, it is likely that the LC50 values for these compounds may be higher than they would be at a lower pH.

ADELMAN & SMITH (1976) conducted sixteen 96-h tests with fathead minnows exposed to pentachlorophenol and found a mean 96-h LC50 of 0.21 mg/L. RUESINK & SMITH (1975) found 96-h LC50 values in tests run at 15 and 25° C of 0.34 mg/L and 0.21 mg/L,

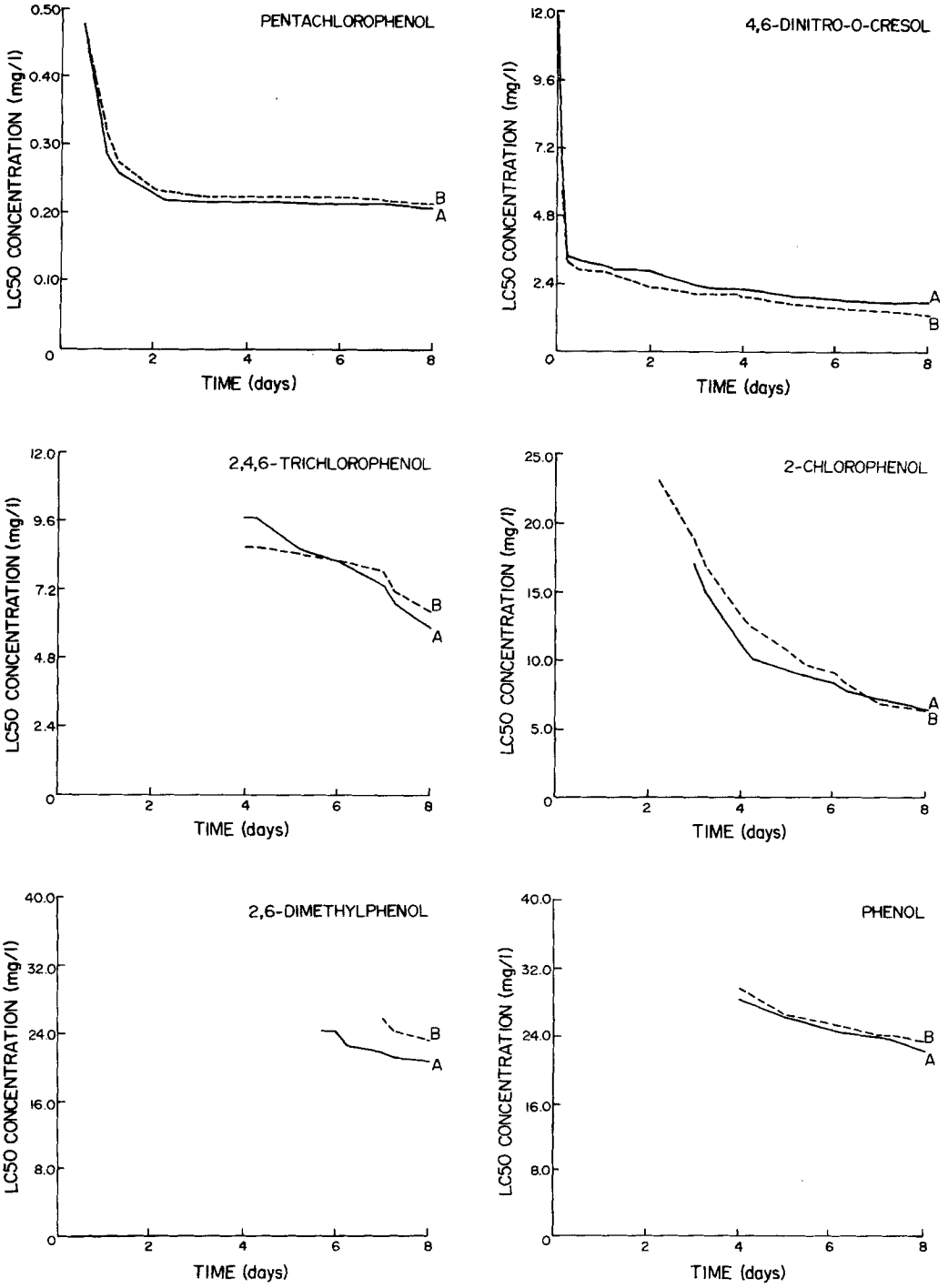
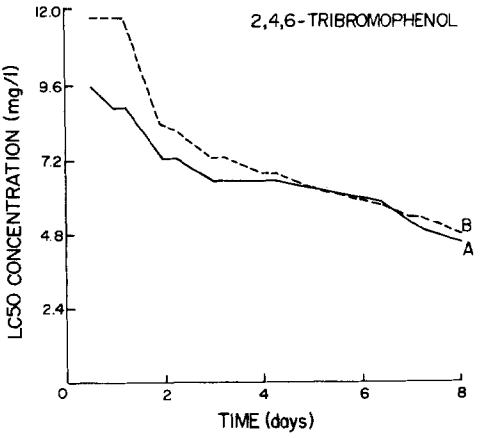
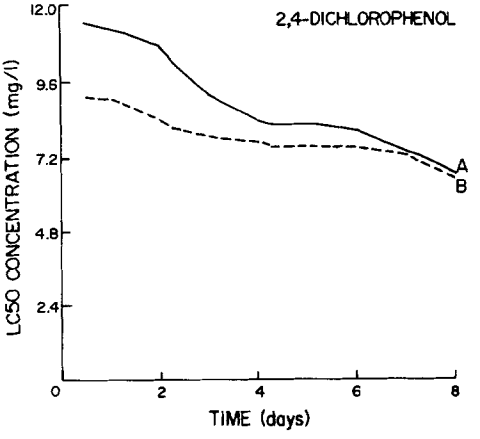
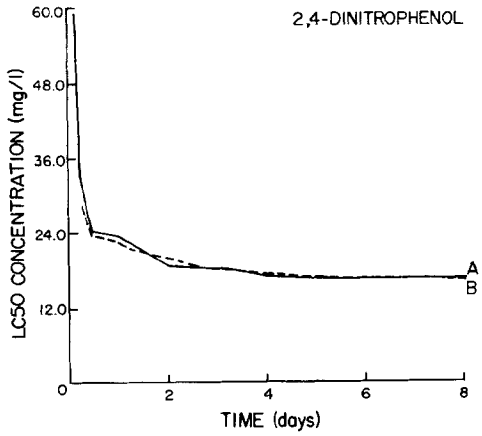
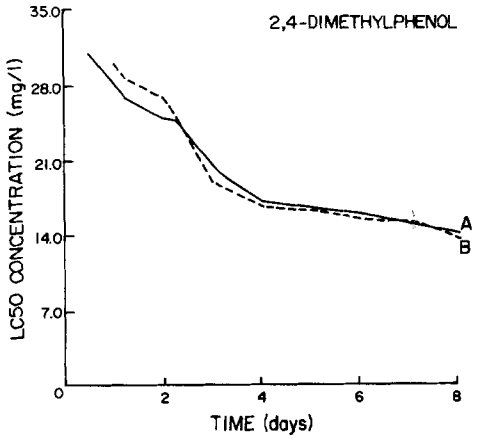


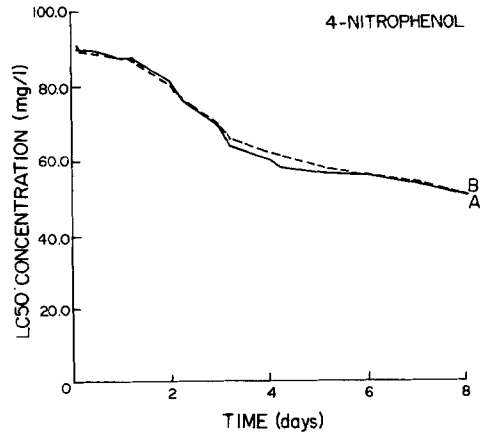
FIGURE 1: LC50 mortality plots for fathead minnows in duplicate tests (A and B) of 12 phenolic compounds.











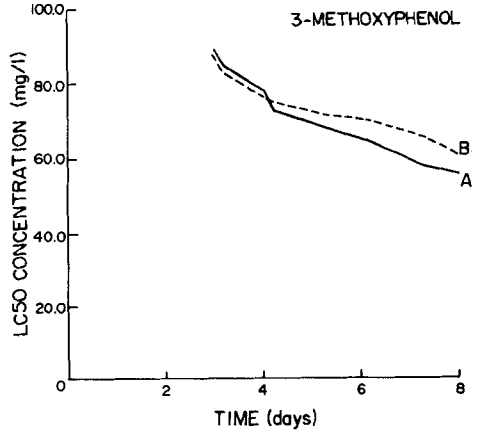


FIGURE 1: (Continued)

respectively. In another test with pentachlorophenol, CARDWELL et al. (1976) determined a 96-h LC50 of 0.29 mg/L and a 14-day LC50 of 0.15 mg/L. The 96-h LC50 value of 0.22 mg/L for pentachlorophenol from this study compared favorably with other 96-h values in the literature. However, the 14-day LC50 of 0.15 mg/L (CARDWELL et al. 1976) does not correspond, because the 8-day value of 0.21 and T.I. value of 0.95 for this study indicates a near-threshold condition.

Values found in the literature for other chlorophenols include two for 2-chlorophenol and one for 2,4,6-trichlorophenol. PICKERING & HENDERSON (1966) found 96-h LC50 values of 14 mg/L and 12 mg/L for 2-chlorophenol in hard and soft water, respectively, using static tests with unmeasured concentrations. The present study yielded a 96-h LC50 concentration of 12 mg/L for 2-chlorophenol, which compares very favorably with their softwater value. The U.S. ENVIRONMENTAL PROTECTION AGENCY (1972) found a 96-h LC50 value for 2,4,6-trichlorophenol of 0.60 mg/L, which varied widely from the value of 9.2 mg/L from the present study.

The U.S. ENVIRONMENTAL PROTECTION AGENCY (1978) conducted an embryo-larval test on 2,4-dimethylphenol and found a MATC of 1.5 to 3.2 mg/L. The present study yielded a 192-h LC50 of 14 mg/L with a T.I. of 0.82, which indicated that a mortality threshold had not been achieved at 192 h. The mortality curve for 2,4-dimethylphenol (Fig. 1) was on a downward slope, which would bring the LC50 closer to the MATC with increased exposure time.

PICKERING & HENDERSON (1966) in static tests with unmeasured concentrations found 96-h LC50 values of 34 mg/L and 32 mg/L for phenol in soft and hard water, respectively. RUESINK & SMITH (1975) found 15 and 25° C 96-h LC50 values of 36 and 24 mg/L. MATTSON et al. (1976) conducted a static phenol test with unmeasured concentrations and found a 96-h LC50 value of 32 mg/L. A phenol flow-through test with measured concentrations conducted by the U.S. ENVIRONMENTAL PROTECTION AGENCY (1978) produced a 96-h LC50 value of 68 mg/L. JENKINS (1964) determined a 24-h LC50 value of 65 mg/L. The phenol 96-h LC50 value of 29 mg/L from the present study with fathead minnows compares favorably with that of RUESINK & SMITH (1975), who also conducted flow-through tests using adult fathead minnows but was much lower than the 96-h value determined by the U.S. ENVIRONMENTAL PROTECTION AGENCY (1978).

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