W. (1982). Enhanced mercury tolerance
cury binding proteins. *Mar. Pollut. Bull.*

changes in the goblet mucous cells of
ridae: Pisces). *Ecotoxicol. Environ. Saf.*

iemical changes produced by mercuric
–326.
tological and biochemical anomalies in

metal-binding proteins in invertebrates.

ulphate. I. Ion-binding and secondary

phate. II. Molecular weight dependance
as a semi-flexible coil. *J. Biochem.* **83,**

ad and cadmium in skin and mucus of
60, 187–191.
ural alterations in fish epidermal mucus
3, 431–432.
on structure-linked latency of lysosomal

ORUNESU, M. (1980). Rapid induction
Comp. Biochem. Physiol. **67,** 215–218.
1981). Accumulation and detoxication
y of the subcellular distribution in the

rs (S. L. Webb, Ed.), Vol. 11, pp. 729–

f Cadmium. Elsevier, Amsterdam.
iercuric chloride and lead in killi fish
Res. **28,** 364–374.

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Accumulation and Depuration of Chlorinated Phenolics in the Freshwater Mussel (*Anodonta anatina* L.)

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Uptake from ambient water and the depuration of five chlorinated phenolics, two chloroguaiacols (3,4,5-tri- and tetrachloroguaiacol), and three chlorophenols (2,4,6-tri-, 2,3,4,6-tetra-, and pentachlorophenol) were studied in the duck mussel (*Anodonta anatina*). Groups of animals were exposed at four acclimation temperatures (3, 8, 13, 18°C) to four chlorophenolic concentrations (total 6–56 μg/liter). The depuration was monitored for 72 hr. For the analysis of individual chlorophenolics by the GC/ECD technique, the soft tissue of mussels was homogenized, spiked with internal standard, acetylated, and extracted with *n*-hexane. The bioconcentration factors (BCF) (concn. in animal wet wt./concn. in water) were determined for mussel soft tissue. The highest BCF was found for pentachlorophenol (81–461) and the lowest for trichlorophenol (14–125). Neither water temperature nor exposure concentration affected the BCFs. The compounds studied were depurated rapidly and their depuration half-lives ($T_{1/2}$) in soft tissue were generally less than 24 hr.

INTRODUCTION

Bivalve molluscs are widely used as indicator organisms for xenobiotics in marine coastal areas (NAS, 1980; Goldberg *et al.*, 1983; Risebrough *et al.*, 1983). In recent years the Mussel Watch concept has also been applied in freshwater areas. The duck mussel (*Anodonta anatina*), a widely distributed member of the family *Unionidae*, has been tested for bioindicator research in Finland. Both locally occurring chlorinated phenolics, which are discharged primarily by the pulp and paper industry, and organochlorine pesticides have been measured in previous studies (Heinonen *et al.*, 1986; Korhonen and Oikari, 1986; Herve *et al.*, 1988).

Although field surveys have been promising, there is, as far as we know, only one study (Mäkelä and Oikari, 1990) on the kinetics of chlorophenolics in freshwater species. Therefore, some further information on the kinetics of these compounds is needed before a new animal model can be used in routine surveys. On the other hand, because molluscs are poikilothermic animals, water temperature is expected to affect the levels of chemical residues in tissues.

The aim of this study was to measure the uptake of two chloroguaiacols and three chlorophenols from ambient water by the freshwater mussel *A. anatina* at four exposure temperatures and four phenolic concentrations. The mussels were exposed to the chlorinated phenolics only in ambient water, because according to previous studies, the dissolved phase in water is the primary source of the organic contaminants accumulated by mussels (Pruell *et al.*, 1986; Muncaster *et al.*, 1990).

MATERIALS AND METHODS

Animals

Duck mussels (*A. anatina* L.) were obtained by scuba diving from Lake Höytiäinen (uncontaminated environment) in eastern Finland (62°51′N, 29°47′E). Mussels for

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Experiment I, in which the effect of temperature was studied, were collected in late May; mussels for an exposure concentration test (Experiment II) were taken in the beginning of June; and animals for the chlorophenolic elimination experiment (Experiment III) were obtained at the end of October. Mussels were from a shallow bay (2–3 m deep) with an abundant population of *A. anatina* (ca. 6000 mussels/hectare). Each collection included at least 100 individuals. The groups of animals were similar in size and age. Those animals collected in May were maintained in the laboratory for 2 weeks, those taken in June for 6 weeks, and those obtained in October for 23 weeks prior to the experiments.

Animals were transported to the laboratory in 25-liter plastic buckets ($O_2 > 6$ mg/liter) immediately after collection and were maintained in static conditions (12:12 hr photoperiod) without substratum in Joensuu city tap water (unchlorinated). The water temperature in the aquaria (15 × 33 × 55 cm) was 6°C and the water depth about 10 cm. Mussels were fed twice a week with an algae–protozoa culture in which the dominant species were the green algae *Monoraphidium contortum* and *Scenedesmus obliqus.* During the maintenance period mussel mortality was negligible.

The mussels were thermally acclimated for 1 week in clean water prior to use in an experiment. During the last 5 days of the acclimation period they were not fed, and a day before the start of an experiment their shells were scrubbed to remove all debris. The experiments were initiated with mussels that had a shell length of 6–8 cm, a soft tissue weight of 6–10 g, and an age of 8–12 years. All experiments were made in 600-liter stainless steel exposure chambers.

Experiment I. To determine the effect of temperature on chlorophenolic accumulation in semistatic conditions, four temperatures (3, 8, 13, and 18°C) were used in tests (water/soft tissue ratio > 2 liter/g). In the first part of the experiment chlorophenolic accumulation was analyzed after 3 hours (suggested to represent the initial rate of uptake) and in the second part the bioconcentration factors were determined after 8 days, which represented the steady state (Mäkelä and Oikari, 1990). In different temperatures the total concentration of chlorophenolics, determined by GC, in the water varied between 6 and 8 μg/liter (Table 1).

An "artificial effluent," a mixture of chlorophenolics which imitates kraft pulp mill effluent, was used. The chlorophenolics in the test solution were 2,4,6-trichlorophenol (CP-3) (Merck, chemical purity > 98%), 2,3,4,6-tetrachlorophenol (CP-4) (TCI, purity 98%), pentachlorophenol (PCP) (Fluka AG, purity > 99%), 3,4,5-trichloroguaiacol (CG-3) (Department of Chemistry, University of Jyväskylä, Finland, purity > 99%), and tetrachloroguaiacol (CG-4) (B.C. Research, purity 99%). Sulfate soap (total resin acids 25% and total fatty acids 40% of dry weight; for the composition, see Oikari *et al.* (1984)) was supplied from the analytical laboratory of A. Ahlström Inc., a papermill in Varkaus, Finland. All five phenolics and the sulfate soap were present in the artificial effluent simultaneously.

The chlorophenolics were first dissolved in a small volume (<0.5 ml) of 99% ethanol. To prevent the phenolics from precipitating, a few drops of NaOH (1 *M*) were added before the addition of sulfate soap and dilution water. The stock solution contained 20% CP-3, 25% CP-4, 7% PCP, 20% CG-3, and 27% CG-4 of the total chlorophenolic concentration (37.5 mg/liter) and sulfate soap (2000 mg/liter).

Experiment II. The effect of chlorophenolic concentration on the accumulation at 13°C was measured in a static system (water/soft tissue ratio > 2 liter/g); three concentrations, 6.0, 22, and 56 μg total phenolics/liter of the chlorophenolic mixture

ANALYZED CHLOROPHENOI

Temperature (°C)	CP-3
Experiment I	
3	1.30
8	1.68
13	1.41
18	1.51
Experiment II	
13	1.15
13	4.63
13	10.60
Experiment III	
13	5.22

Note. Abbreviations: CP-3 = 2,4,6-
rophenol; CG-3 = 3,4,5-trichloroguaia
$n = 4$ (for sampling of waters, see un

mentioned above were used (
after 8 days.

Experiment III. Mussels w
water (1.2 liter/g soft tissue/d
in Experiment I (Table 1). T
pump. Elimination of the stud
free dilution water. Mussels v
17, 30, 40, 50, and 72 hr afte

Sampling

In the 3-hr absorption stu
at the beginning and end of t
sampled once in the middle
throughout the test period. V
to be fairly constant at all ex
perature at ±0.1°C of the de

At the end of each exposu
The water was drained out o
then frozen at −20°C.

Analyses

Water samples were analy
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studied, were collected in late
periment II) were taken in the
ic elimination experiment (Ex-
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Oikari, 1990). In different tem-
etermined by GC, in the water

which imitates kraft pulp mill
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lorophenol (CP-4) (TCI, purity
99%), 3,4,5-trichloroguaiacol
skylä, Finland, purity > 99%),
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ap were present in the artificial

ume (<0.5 ml) of 99% ethanol.
s of NaOH (1 *M*) were added
The stock solution contained
3-4 of the total chlorophenolic
g/liter).
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e ratio > 2 liter/g); three con-
f the chlorophenolic mixture

TABLE 1

ANALYZED CHLOROPHENOLIC CONCENTRATIONS (μg/liter) IN EXPOSURE WATERS

Temperature (°C)	CP-3	CP-4	PCP	CG-3	CG-4	TOTCP
Experiment I						
3	1.30	1.86	0.51	0.82	1.63	6.12
8	1.68	2.24	0.81	1.38	2.18	8.29
13	1.41	2.23	0.88	0.98	2.54	8.04
18	1.51	2.26	0.98	1.13	2.29	8.17
Experiment II						
13	1.15	1.21	0.64	1.62	1.40	6.02
13	4.63	6.34	2.13	3.02	5.92	22.04
13	10.60	16.55	5.19	7.36	16.47	56.17
Experiment III						
13	5.22	7.52	4.12	3.02	7.70	27.58

Note. Abbreviations: CP-3 = 2,4,6-trichlorophenol; CP-4 = 2,3,4,6-tetrachlorophenol; PCP = pentachlorophenol; CG-3 = 3,4,5-trichloroguaiacol; CG-4 = tetrachloroguaiacol; and TOTCP = total chlorophenolics. n = 4 (for sampling of waters, see under Methods). Standard deviation was less than 15%.

mentioned above were used (Table 1). The bioconcentration factors were determined after 8 days.

Experiment III. Mussels were exposed at 13°C for 4 days in continuously flowing water (1.2 liter/g soft tissue/day) to the chlorophenolic mixture 28 μg/liter described in Experiment I (Table 1). The exposure stock solution was added with a peristaltic pump. Elimination of the studied compounds was monitored for 72 hr in contaminant-free dilution water. Mussels were sampled at the end of the exposure and at 2, 5, 12, 17, 30, 40, 50, and 72 hr after the animals were transferred into clean water.

Sampling

In the 3-hr absorption study, water samples for chlorophenol analyses were taken at the beginning and end of the exposure; but in all other experiments water was also sampled once in the middle of an exposure and for a cumulative sample twice a day throughout the test period. Water quality was measured at each sampling and found to be fairly constant at all exposures: pH 7.1 ± 0.2, oxygen > 10 mg/liter, and temperature at ±0.1°C of the desired value.

At the end of each exposure, the mussels were weighed (nearest 0.01 g) and opened. The water was drained out of the mantle cavity, and the soft tissue was weighed and then frozen at −20°C.

Analyses

Water samples were analyzed by the method of Voss *et al.* (1981) using a gas chromatograph (GC, 5890A, Hewlett–Packard) with a silica capillary column SE-30 (Nor-

dibond) and EC-detector. The temperature in the injector was 260°C and in the detector 325°C. The oven temperature rose at a rate of 8°C per minute from 80 to 260°C. The gas flow rates for helium (carrier) and argon–methane were 1 and 35 ml/min, respectively.

Newly thawed soft tissue was homogenized in an Ultra-Turrax homogenizer, frozen (−20°C), and thawed again to complete the breakdown of tissue structures. Each mussel was analyzed individually in Experiments I and II, but in Experiment III the soft tissues of two mussels were pooled. Lipid content was determined by 12-hr Soxhlet extraction (chloroform–methanol, 1:1).

Because there are no specific procedures for chlorophenolic analyses of mussel soft tissue, the method of Voss *et al.* (1981) was applied with the following modifications. Homogenized soft tissue (0.5 g) was diluted with 4.5 ml distilled water. The pH was adjusted to 7 with 1 *N* NaOH, and an internal standard 2,6-dibromophenol (BP-2, Fluka AG, purity 98%) was added. The chlorophenolics were acetylated by shaking for 2–3 min in Teflon-caped glass tubes containing acetic acid anhydride (2 ml) in 5.2 *M* K_2CO_3 (200 μl) buffered solution. The phases were then allowed to settle for 5 min. Acetylated phenols were extracted (2 min) three times with 2 ml *n*-hexane. The combined organic phases were centrifuged for 5 min (4000 rpm) and again allowed to settle for 5 min before hexane was separated. One milliliter of the combined extracts was evaporated to a small volume (10 μl) under a N_2 stream and a 1- to 2-μl subsample was injected into the GC.

Extraction efficiencies for chlorophenols in mussel tissue were determined by using duck mussels exposed to ^{14}C-labeled pentachlorophenol (14 μg/liter, for 24 hr), as described in detail by Mäkelä and Oikari (1990). Samples were extracted five times with hexane (as described above) and analyzed with both a gas chromatograph and a liquid scintillation counter (LSC, Wallac 1217 Rackbeta). PB-2 and lindane were used as internal standards. The first three extractions gave 97% of the total PCP analyzed by GC. The same extract gave 93% of the total counts of ^{14}C-PCP by LSC. This was considered to be a sufficient degree of recovery.

The amount of acetic acid anhydride affected chlorophenol acetylation. Maximal yields were gained with 2–3 ml of acetic acid anhydride per 0.5 g of soft tissue homogenate. Acetylation time (5–30 min) did not affect the degree of acetylation, nor did K_2CO_3 concentration (200–1000 μl/0.5 g of tissue) as long as the pH remained above 10.

Calculations

Bioconcentration factors (BCF) were calculated as the concentration of individual chlorophenolics in the mussel soft tissue (based on wet weight) divided by the average concentration ($n = 4$) of the same compound analyzed from the exposure water.

Q_{10} values, which represents the uptake rate differences caused by a 10°C increase in temperature, were calculated according to Schmidt-Nilsen (1986),

$$Q_{10} = \frac{K_{T+10}}{K_T}, \tag{1}$$

where

K_T uptake at temperature T (°C)
K_{T+10} uptake rate at temperature $T + 10$°C.

One compartment model a
and depuration of chlorinated
applied to describe eliminatio

where

C concentration of chlorop
C_0 residue concentration at
K_d first-order rate constant f
t time (hr).

The depuration half-lives f

where K_d is the elimination-r

The slope of the straight lin
by using a simple linear least-s
groups were analyzed using
statistical tests were made by
computer.

Effects of Water Temperatur

The initial uptake rate for
differed significantly ($P < 0.$
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CG-4 at 3°C and highest for C
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In most cases, pentachlo
461) of the phenolics studied
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constant at all exposures (6

One compartment model and first-order kinetics were used to describe the uptake and depuration of chlorinated phenolics by *A. anatina*. The following equation was applied to describe elimination of these xenobiotics from the soft tissue,

$$\ln C = \ln C_0 - K_d t, \tag{2}$$

where

C concentration of chlorophenol in the animal (μg/g)
C_0 residue concentration at the start of the depuration period
K_d first-order rate constant for depuration (hr^{-1})
t time (hr).

The depuration half-lives for chlorophenolics were computed using the equation,

$$T_{1/2} = \ln 0.5/K_d, \tag{3}$$

where K_d is the elimination-rate constant calculated in Eq. (2).

The slope of the straight line describing the depuration was fitted to the data points by using a simple linear least-squares regression. Statistical differences between exposure groups were analyzed using Duncan's multiple range test. The curves were fit and statistical tests were made by the SAS program (SAS Institute, 1985) on a VAX-785 computer.

RESULTS

Effects of Water Temperature and Exposure Concentration

The initial uptake rate for total chlorophenolics correlated with temperature and differed significantly ($P < 0.05$) between exposures at 3 and 13°C, 3 and 18°C, and 8 and 18°C. Uptake rates over 3 hr varied from 33 to 254 ng/g hr^{-1}, being lowest for CG-4 at 3°C and highest for CP-4 at 13°C (Fig. 1). For individual phenolics a significant difference ($P < 0.05$) in uptake rates for PCP, CP-4, and CG-3 was detected between 3 and 13°C, for PCP between 8, 13, and 18°C, and for CG-3 between 8 and 18°C. The effect of temperature on uptake was not, however, monotonous. The inverse order of temperatures for CP-3 possibly approaching a nonlinear phase of uptake might have been due to elimination that had already started at higher temperatures. However, this was not possible to show in our experimental procedure. The reason why the uptake rates of PCP and CG-3 were lowest at 8°C is unclear, but a period without filtration activity is out of question while the same effect should be visible also for other compounds at the same temperature.

The Q_{10} values varied from 0.88 to 2.73 and were highest for CG-4 and lowest for CP-3 (Table 2). For all compounds except CP-3 and CP-4, the values were higher between 8 and 18°C than between 3 and 13°C.

After 8 days, when the steady state was reached accumulation of any chlorophenolics was not correlated with temperature. As expected, the chlorophenolic concentration in exposure water did correlate ($P < 0.05$) with the chlorophenols in soft tissue.

In most cases, pentachlorophenol had the highest bioconcentration factor (156–461) of the phenolics studied (Table 3). At 3°C, however, the BCF of CP-4 was highest (133) and at 18°C the BCF for CG-3 was highest (107). The bioconcentration of CP-3 was always lowest (BCF = 31–125). The BCF for total chlorophenolics was fairly constant at all exposures (67–172). In the concentration experiment (Experiment II),

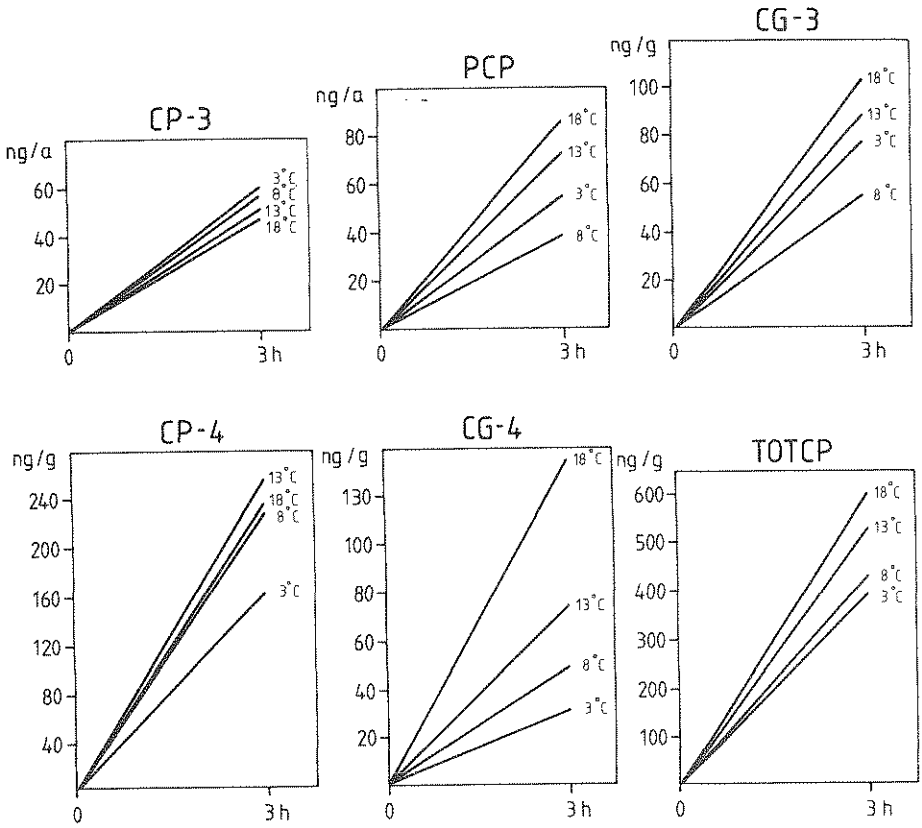


FIG. 1. Accumulation of trichlorophenol (CP-3), tetrachlorophenol (CP-4), pentachlorophenol (PCP), trichloroguaiacol (CG-3), tetrachloroguaiacol (CG-4), and total chlorophenolics in *A. anatina* during 3 hr at different temperatures (n = 8). For abbreviations, see Table 1.

however, it was somewhat higher at exposure concentrations of 6 and 56 μg/liter than at any other exposure. The BCFs for total chlorophenolics were highest (105) at 8°C and lowest (67) at 18°C. Although less lipophilic, CP-4 and CG-3 were more concentrated (BCF = 76–156 and 103–237, respectively) than CG-4 (BCF = 45–154; Table 3).

TABLE 2

Q_{10} VALUES FOR THE UPTAKE RATE OF CHLORINATED PHENOLICS AFTER 3 hr EXPOSURE IN 7.6 μg/liter TOTAL CHLOROPHENOLIC CONCENTRATION

Temperature (°C)	CP-3	CP-4	PCP	CG-3	CG-4	TOTCP
3–13	0.88	1.55	1.32	1.32	2.26	1.32
8–18	0.86	1.02	2.15	1.85	2.73	1.41

Note. For abbreviations, see Table 1.

BIOCONCENTRATION FACTORS B AND TOTCP IN *A. anatin*

T	C	n	CP-3	C
Experiment I				
3	7.6	8	38	1
8	7.6	8	38	1
13	7.6	8	33	1
18	7.6	8	31	
Experiment II				
13	6	5	125	1
13	22	5	30	
13	56	3	68	1

Note. The standard deviation was 3(water (μg/liter); n, number of animals

Depuration

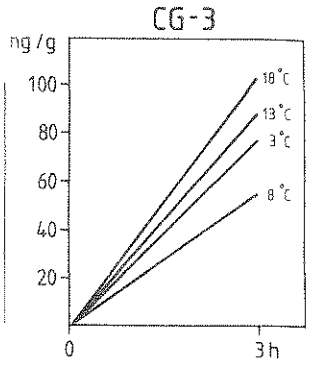
Chlorophenolics were relea portion of the body burden b contaminants released over t CP-4, PCP, CG-3, CG-4, an eliminated from soft tissue in 4. The depuration half-lives v

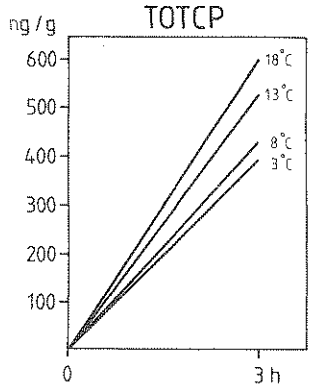
The rate constant for dep chlorophenolics it varied fro burden was eliminated by th a 4-day exposure at 28 μg/lit

BCF VALUES AT THE BEGINNI RATE CONSTANTS (K_d), AND (*A. anatina*) TRANSF TO 28 μg/liter

Toxicant
CP-3
CP-4
PCP
CG-3
CG-4
TOTCP

Note. The pH of the exposure w tissue was 1.2% of the wet weight.





l (CP-4), pentachlorophenol (PCP),
phenolics in *A. anatina* during 3 hr

ions of 6 and 56 μg/liter than
ics were highest (105) at 8°C
nd CG-3 were more concen-
an CG-4 (BCF = 45–154;

NOLICS AFTER 3 hr EXPOSURE
NCENTRATION

-3	CG-4	TOTCP
32	2.26	1.32
35	2.73	1.41

TABLE 3

BIOCONCENTRATION FACTORS BASED ON WET WEIGHT FOR CP-3, CP-4, PCP, CG-3, CG-4, AND TOTCP IN *A. anatina* SOFT TISSUE AND THE AVERAGE LIPID CONTENT (WET WEIGHT %)

T	*C*	*n*	CP-3	CP-4	PCP	CG-3	CG-4	TOTCP	Lipids (%)
Experiment I									
3	7.6	8	38	138	111	103	45	84	1.8
8	7.6	8	38	154	156	108	85	105	1.7
13	7.6	8	33	137	162	142	47	93	1.8
18	7.6	8	31	76	81	108	53	67	1.8
Experiment II									
13	6	5	125	156	305	237	146	172	1.9
13	22	5	30	94	263	128	79	98	1.5
13	56	3	68	125	461	155	154	157	1.8

Note. The standard deviation was 30–60% of the mean. T, temperature (C°); C, concentration in exposure water (μg/liter); n, number of animals. For abbreviations, see Table 1.

Depuration

Chlorophenolics were released rapidly from *A. anatina* soft tissue, the main proportion of the body burden being depurated within 12 hr. The average percentages of contaminants released over this period were 100, 30, 52, 40, 58, and 40% for CP-3, CP-4, PCP, CG-3, CG-4, and total chlorophenolics, respectively. Thus, they were eliminated from soft tissue in the following order: CP-3 > CG-4 > PCP > CG-3 > CP-4. The depuration half-lives were less than 24 hr.

The rate constant for depuration (K_d) was highest for CP-3 (0.45); for the other chlorophenolics it varied from 0.03 to 0.06 (Table 4). The total chlorophenolic body burden was eliminated by the mussels at an average rate constant (K_d) of 0.042 after a 4-day exposure at 28 μg/liter.

TABLE 4

BCF VALUES AT THE BEGINNING OF THE DEPURATION PERIOD, FIRST-ORDER DEPURATION RATE CONSTANTS (K_d), AND DEPURATION HALF-LIVES ($T_{1/2}$) FOR FRESHWATER MUSSEL (*A. anatina*) TRANSFERRED TO CLEAN WATER AFTER 4-DAY EXPOSURE TO 28 μg/liter TOTAL CHLOROPHENOLIC CONCENTRATION

Toxicant	BCF	K_d (hr)	$T_{1/2}$
CP-3	141	0.450	1.5
CP-4	49	0.030	23.0
PCP	150	0.060	11.5
CG-3	128	0.046	15.0
CG-4	74	0.073	10.0
TOTCP	74	0.042	16.5

Note. The pH of the exposure water was 7.1. For abbreviations, see Table 1. The lipid content in soft tissue was 1.2% of the wet weight.

DISCUSSION

When mussels are used as indicators of water quality, the degree to which they accumulate xenobiotics is often expected to be constant and not very dependent on environmental conditions. This assumption may not necessarily be valid and must be ascertained in changing environmental conditions if a new animal model is introduced.

In earlier studies (Korhonen and Oikari, 1986; Mäkelä and Oikari, 1990) it has been shown that a steady state of chlorinated phenolics in soft tissue is reached in less than 4 days. Based on this knowledge a steady state was assumed to be achieved in all experiments (but excluding, of course, the 3-hr exposure in Experiment I). In addition, no changes in mussel behavior were observed; therefore the data obtained in this study can be considered very representative of the normal chlorophenolic kinetics in *A. anatina*.

Uptake of Chlorophenolics

At higher exposure temperatures the uptake rates of CP-4, PCP, CG-3, and CG-4 increased. The Q_{10} values, especially those for CG-4, were significantly dependent on temperature. In the case of CP-3, however, the 3-hr experimental period probably was too long for determining the initial uptake rates, while the steady state was reached at all temperatures. Depuration analyses of CP-3 ($t_{1/2}$ = 1.5 hr) also support this conclusion.

The concentrations of chlorinated phenolics in the mussels were directly proportional to the exposure concentration determined by correlation analysis. However, certain differences in BCFs were noted at different temperatures. The lipid content in mussel soft tissue did not explain the differences in accumulation between the temperature exposures; these differences might be due to the sex of the animal and/or the different reproductive states in early and late summer.

Boryslawskyj *et al.* (1987) reported that in the freshwater clam *Spaerium corneum* temperature was positively correlated with dieldrin accumulation. In our experiments accumulation of chlorophenols was not correlated with temperature in the steady state, even though it was correlated with the 3-hr uptake rates.

Bioconcentration of Chlorophenolics

The bioconcentration factors for phenolics varied between 100 and 200, except for CP-3, for which the BCF averaged about 30. The BCF values found in this study were similar to those (20–400) determined in our earlier studies (Korhonen and Oikari, 1986; Mäkelä and Oikari, 1990) and to those reported for resident mussels in Rainy River (Metcalfe and Hayton, 1989).

The bioconcentration factors for chlorophenolics did not differ statistically according to water temperature (Table 3), and in general, they were relatively independent of exposure concentration over a wide range of concentration, 6–56 μg/liter.

Renberg *et al.* (1985) found that more organochlorine compounds accumulate in animals collected recently than in those maintained for a long period in the laboratory. In the present study no such effect was detected. The mussels maintained the longest (23 weeks) and the shortest (2 weeks) period in the laboratory had very similar BCFs and those animals maintained for 6 weeks had the highest BCF values. This might be

because of different season; the gr
and the differing group was expo

The bioconcentration factors f
with log $P_{o/w}$ only for CP-3 and
despite the fact that their log P
related to log $P_{o/w}$ values were ve
fish (Hawker and Connell, 1986)
than the values calculated acco
blue mussel (Table 5).

In addition, the data of Zaroo
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close to the values of 60 and 17
(Folke *et al.*, 1983). Therefore w
good estimates of BCF values f

Elimination of Chlorophenolics

The linear relationship betwe
mussels (*A. anatina*) and time
follows first-order kinetics. The
the rapid uptake shown earlie
relationship between chloroph
the 72-hr depuration period w
contaminants reported by sev
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The chlorophenolic half-live
determined by Xie (1984) in b
(1979), however, reported a 2-
about four times longer than
study were also at the same lev

ACIDITY AND LIPO
MEASURED

Toxicant	p*Ka*[a]
CP-3	6.0
CP-4	5.4
PCP	5.3
CG-3	8.0
CG-4	6.0

Note. For abbreviations, see Table
[a] Xie (1984).
[b] Calculated according to the equa

because of different season; the groups with similar BCFs were exposed in early summer and the differing group was exposed in late summer.

The bioconcentration factors for chlorophenolics in *A. anatina* were in accordance with log $P_{o/w}$ only for CP-3 and PCP; more CP-4 and CG-3 than CG-4 accumulated, despite the fact that their log $P_{o/w}$ values are lower. The measured log BCF values related to log $P_{o/w}$ values were very similar to those for other molluscs, daphnids, and fish (Hawker and Connell, 1986). In any case, over all, the measured BCFs were lower than the values calculated according to the Hawker and Connell (1986) model for blue mussel (Table 5).

In addition, the data of Zarooria *et al.* (1985) suggest that log BCF can be estimated, within an order of magnitude, for marine species using freshwater mussels for a minimum of 71% of the chemicals having a log $P_{o/w}$ from 1.61 to 6.50. Our results support this conclusion while BCFs obtained for CP-4 (49–156) and PCP (81–461) were very close to the values of 60 and 170 measured for the same compounds in blue mussel (Folke *et al.*, 1983). Therefore we can conclude that freshwater mussels offer reasonably good estimates of BCF values for marine species or visa versa.

Elimination of Chlorophenolics

The linear relationship between the logarithms of depuration of chlorophenolics in mussels (*A. anatina*) and time in uncontaminated water indicated that depuration follows first-order kinetics. The fast average reduction in body burden, together with the rapid uptake shown earlier (Mäkelä and Oikari, 1990), accounts for the direct relationship between chlorophenolics in soft tissue and the exposure water. During the 72-hr depuration period we did not detect the two-phase elimination kinetics of contaminants reported by several investigators with Kepone and petroleum hydrocarbons (Morales-Almo and Haven, 1983; Broman and Ganning, 1985).

The chlorophenolic half-lives found in this study (1.5–23 hr) were similar to those determined by Xie (1984) in blue mussels from brackish water (10 hr–7 days). Ernst (1979), however, reported a 2- to 3-day half-live for PCP in blue mussels, which is about four times longer than our finding. Half-lives of lake mussels in the present study were also at the same level as those reported for nonylphenol (7 hr), aminokarb

TABLE 5

ACIDITY AND LIPOPHILICITY OF CHLOROPHENOLICS, AND THEIR MEASURED AND CALCULATED BCFs IN *A. anatina*

Toxicant	p*Ka*[a]	log $P_{o/w}$[a]	BCF measured	BCF[b] calculated
CP-3	6.0	3.69	14–125	75
CP-4	5.4	4.34	49–156	268
PCP	5.3	5.08	81–263	1129
CG-3	8.0	4.18	97–236	196
CG-4	6.0	4.76	45–154	606

Note. For abbreviations, see Table 1.

[a] Xie (1984).

[b] Calculated according to the equation log BCF = 0.844 log $P_{o/w}$ − 1.235 (Hawker and Connell, 1986).

(9–15 hr), and 585 oil (0.3 days) in the blue mussel (McLeese *et al.*, 1980) and for 3-trifluoro-methyl-4-nitrophenol in *Anodonta* sp. (2 hr; Buikema and Herricks, 1978). Kepone, which is more lipophilic than the chlorophenolics studied here, also has a longer half-life, 3–10 days (Morales-Alamo and Haven, 1983).

CONCLUSIONS

The BCFs for chlorinated phenolics can differ two- to threefold from one exposure group of animals to another. The values are so constant, however, that the contamination level in water can be back-calculated by tissue analyses within quite a large range of concentrations, more or less irrespective of the temperature or concentration of phenolics during exposure. The compounds studied were eliminated rapidly, and their depuration half-lives in soft tissue were generally less than 24 hr.

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REFERENCES

BORYSLAWSKYJ, J. M., CARROOD, A. C., PEARSON, J. T., AND WOODHEAD, D. (1987). Rate of accumulation of dieldrin by freshwater filter feeder: *Spherium corneum. Environ. Pollut.* **43**, 3–13.

BROMAN, D., AND GANNING, B. (1985). Bivalve mollusks (*Mytilus edulis*) and (*Macoma baltica*) for monitoring diffuse oil pollution in a Baltic archipelago. *Ambio* **14**, 23–28.

BUIKEMA, A. L., AND HERRICKS, E. E. (1978). Effects of pollution on freshwater invertebrates. *J. Water Pollut. Control Fed.* **50**, 1637–1648.

ERNST, W. (1979). Factors affecting the evaluation of chemicals in laboratory experiments using marine organisms. *Ecotoxicol. Environ. Saf.* **3**, 90–98.

FOLKE, J., BIRKLUND, J., SOVENSEN, A. K., AND LUND, V. (1983). The impact on ecology of polychlorinated phenols and other organics dumped at the bank of a small marine inlet. *Chemosphere.* **12**, 1169–1181.

GOLDBERG, E. D., KOIDE, M., HODGE, V., FLEGAL, A. R., AND MARTIN, J. (1983). U.S.A. Mussel Watch 1977–1978 results on trace metals and radio nuclides. *Estuarine Coastal Shelf Sci.* **16**, 69–94.

HAWKER, D. W., AND CONNELL, D. W. (1986). Bioconcentration of lipophilic compounds by some aquatic organisms. *Ecotoxicol. Environ. Saf.* **11**, 184–197.

HEINONEN, P., PAASIVIRTA, J., AND HERVE, S. (1986). Perifyton and mussel in monitoring chlorohydrocarbons and chlorophenols in watercourses. *Toxicol. Environ. Chem.* **11**, 191–201.

HERVE, S., HEINONEN, P., PAUKKU, R., KNUUTILA, M., KOISTINEN, J., AND PAASIVIRTA, J. (1988). Mussel incubation method for monitoring organochlorine pollutants in watercourses: Four-year application in Finland. *Chemosphere* **17**, 1945–1961.

KORHONEN, M., AND OIKARI, A. (1986). Bioconcentration of chlorophenolic compounds by freshwater mussel, *Anodonta piscinalis. Univ. Joensuu Fac. Math. Nat. Sci. Rep. Ser.* **8**, 64–65.

MÄKELÄ, P., AND OIKARI, A. O. J. (1990). Uptake and body distribution of chlorinated phenolics in the freshwater mussel *Anodonta anatina* L. *Ecotoxicol. Environ. Saf.* **20**, 354–362.

MCLEESE, D. W., ZITKO, V., AND SERGEANT, D. B. (1980). Uptake and excretion of aminocarb, nonylphenol, and pesticide diluent 585 by mussel (*Mytilus edulis*). *Bull. Environ. Contam. Toxicol.* **24**, 575–581.

METCALFE, J. L., AND HAYTON, A. (1989). Comparison of leeches and mussels as biomonitors for chlorophenol pollution. *J. Great Lakes Res.* **15**, 654–668.

MORALES-ALMO, R., AND HAVEN, D. S. (1983). Uptake of Kepone from sediment suspensions and subsequent loss by the oyster *Crassostrea virginica. Mar. Biol.* **74**, 187–201.

MUNCASTER, B. W., HEBERT, P. D. N., AND LAZAR, R. (1990). Biological and physiological factors affecting the body burden of organic contaminants in freshwater mussels. *Arch. Environ. Contam. Toxicol.* **19**, 25–34.

National Academy of Sciences (NAS) (
sored by the Environmental Studies B
DC.

OIKARI, A., NAKARI, T., AND HOLMBC
(KME): Residues of toxicants, and e

PRUELL, R. J., LAKE, J. L., DAVIS, W
contaminants by blue mussels (*Mytil*
Biol. **91**, 497–507.

RENBERG, L., TARKPEA, M., AND LIND
for bioconcentration studies. I. Desi
chlorine compounds. *Ecotoxicol. Er*

RISEBROUGH, R. W., DE LAPPE, B. V
REGUEIRO, G. J. A., NOLLA, B. A.,
concept in studies of the distributio
Pollut. Bull. **14**, 181–187.

SAS Institute (1985). *SAS Users Guid*

SCHMIDT-NILSEN, K. (1986). Physiolo
and Environment, pp. 227–229. Ca

VOSS, R. H., WEARING, J. T., AND W
of chlorinated phenolic in pulp mi
Pollutants in Water (L. H. Keith, E

XIE, T. M. (1984). *Investigation of Cl*
thesis, Department of Analytical an

ZAROORIA, G. E., HELTSCHE, J. F.,
species using structure–activity mo

National Academy of Sciences (NAS) (1980). *The International Mussel Watch: Report of a Workshop Sponsored by the Environmental Studies Board.* Commission on Natural Resources Council, NAS, Washington DC.

OIKARI, A., NAKARI, T., AND HOLMBOM, B. (1984). Sublethal actions of simulated kraft pulp mill effluents (KME): Residues of toxicants, and effects on blood and liver. *Ann. Zool. Fenn.* **21,** 45–53.

PRUELL, R. J., LAKE, J. L., DAVIS, W. R., AND QUINN, J. G. (1986). Uptake and depuration of organic contaminants by blue mussels (*Mytilus edulis*) exposed to environmentally contaminated sediment. *Mar. Biol.* **91,** 497–507.

RENBERG, L., TARKPEA, M., AND LINDEN, E. (1985). The use of the bivalve *Mytilus edulis* as a test organism for bioconcentration studies. I. Designing a continuous-flow system and its application to some organochlorine compounds. *Ecotoxicol. Environ. Saf.* **9,** 171–178.

RISEBROUGH, R. W., DE LAPPE, B. W., WALKER, W., SIMONEIT, B. R. T., GRIMATT, J., ALBAIGES, J., REGUEIRO, G. J. A., NOLLA, B. A., AND FERNANDEZ, M. M. (1983). Application of the Mussel Watch concept in studies of the distribution of hydrocarbons in the coastal zone of the Ebro delta, Spain. *Mar. Pollut. Bull.* **14,** 181–187.

SAS Institute (1985). *SAS Users Guide: Statistics.* SAS Institute, Cary, NC.

SCHMIDT-NILSEN, K. (1986). Physiological effects of temperature change. In *Animal Physiology, Adaption and Environment,* pp. 227–229. Cambridge Univ. Press, New York.

VOSS, R. H., WEARING, J. T., AND WONG, A. (1981). A novel gas chromatographic method for the analysis of chlorinated phenolic in pulp mill effluents. In *Advances in Identification and Analysis of Organic Pollutants in Water* (L. H. Keith, Ed.), Vol. 2. Ann Arbor Science, Ann Arbor, MI.

XIE, T. M. (1984). *Investigation of Chlorophenolic Compounds from the Paper and Pulp Industries.* Ph.D. thesis, Department of Analytical and Marine Chemistry. University of Göteborg, Sweden.

ZAROORIA, G. E., HELTSCHE, J. F., AND JONSON, M. (1985). Estimation of bioconcentration in marine species using structure–activity models. *Environ. Toxicol. Chem.* **4,** 3–12.