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Uptake and Body Distribution of Chlorinated Phenolics in the Freshwater Mussel, *Anodonta anatina* L.

PETTERI MÄKELÄ AND AIMO O. J. OIKARI

Department of Biology, University of Joensuu, P.O. Box 111, SF-80101 Joensuu, Finland

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Freshwater mussels (*Anodonta anatina* L.) were exposed to [^{14}C]pentachlorophenol (PCP) and [^{14}C]3,4,5-trichloroguaiacol (CG-3) under laboratory conditions. Uptake and body distribution in mussels as well as total water-soluble metabolites of chlorophenolics in hemolymph and digestive gland were measured. The time course of chlorophenolic accumulation in the mussel soft tissue was followed by analyzing the decrease in the radioactivity in exposure water. The bioconcentration factors (BCFs, activity in animal per activity in water) were measured at steady state for the soft tissue homogenate and separate organs. Both chlorinated phenolics reached a steady-state concentration during the first 24 hr. BCFs in soft tissue ranged from 145 to 342 for PCP and 34 to 125 for CG-3. Accumulations by the digestive gland (hepatopancreas) and kidneys were 2 and 1.3 times greater, respectively, than the average accumulation by the whole soft tissue. The water-soluble fraction of PCP (1–8%) and CG-3 (0.4–2.9%) in separate organs implied only a minor metabolism of chlorophenolics in this animal.

INTRODUCTION

Bivalve molluscs are efficient filter-feeders and they accumulate pollutants, often without any obvious distress, to levels far in excess of those in the hydrosphere. Because of their potential to accumulate environmental pollutants, mussels have been used as indicator organisms for various chemicals (Davis and Pirie, 1980; Goldberg *et al.*, 1983; Risebrough *et al.*, 1983). The mussel watch concept has been employed primarily in marine areas, but in recent years interest in developing a method suitable for freshwater areas has increased in Finland.

The duck mussel (*Anodonta anatina* L.) is widely distributed over much of Scandinavia in a variety of freshwater habitats. The depth tolerance, population density, and biomass of this species are the highest of local freshwater mussels (Haukioja and Hakala, 1974). The duck mussel is large in size, abundant, and therefore easily available. All of these features plus its easy handling are the reasons for choosing *Anodonta* as a bioindicator organism.

A. anatina is a member of the family Unionidae. The specific name *anatina* has been questioned, and some authors have used the name *piscinalis* Nilsson. According to Ellis (1962) the name *anatina* should, however, retain in its familiar and time-honored usage.

Phenolics have been shown to be toxic to aquatic life at mg/liter levels. Furthermore they have the ability to impart taste and odor to drinking water supplies and edible aquatic life at μg/liter levels (Buikema *et al.*, 1979). About 200,000 tons of chlorophenolics are manufactured annually for use as bactericides, insecticides, fungicides, herbicides, and wood preservatives. In addition, chlorophenolic compounds are found in pulpmill effluents and thereby have the potential to contaminate aquatic ecosystems

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(Paasivirta, 1978). In Finland, pulp and paper industries are located primarily on inland waters; thus, a biomonitoring program for freshwater areas is needed.

However, there is no information available about the kinetics and metabolic fate of chlorophenolics in *Anodonta* species. In this study we measured the uptake and bioaccumulation of pentachlorophenol (PCP) and trichloroguaiacol (CG-3) by the duck mussel (*A. anatina*). In addition, the ability of the mussel to metabolize these two chlorophenolics was investigated.

MATERIAL AND METHODS

Animals

Duck mussels were collected by scuba diving from Lake Höytiäinen in Eastern Finland (62°51′N, 29°47′E) at the end of October 1986. Water depth on the collection area was 2–3 m, and the water quality parameters were as follows: temperature 4°C, pH 7.17, color 25 mg Pt/liter, oxygen 12 mg/liter, and electric conductivity 44 mS/m. Mussels were kept in well-oxygenated ($O_2 > 6$ mg/liter) water while being transported to the laboratory.

In the laboratory, the animals were maintained in black plastic basins (15 × 33 × 55 cm), without substratum, in aerated Joensuu city tap water (unchlorinated, pH 7.2, water depth 10 cm) at 6°C under a 12:12-hr photoperiod with dim light. Mussels were fed twice a week with an algae–protozoa culture in which the dominant species were *Scenedesmus obliqus* and *Monoraphidium contortum*. During the maintenance period, the mortality of mussels was negligible.

The animals used in experiments had an average shell length of 55 mm (SD ± 10), soft-tissue wet weight of 5.2 g (SD ± 1.5), and age of 6–8 years. Both sexes were used.

The mussels for body compartment determinations were dissected immediately after collection; animals for PCP exposure were maintained for 4 weeks and those for CG-3 exposure for 3 months.

Body Compartments and Tissue Indexes

The shell weight, mantle cavity water, and whole soft tissue of six mussels (total weight 10–90 g, length 3–10 cm) were measured. The difference in weights of a mussel with unopened and opened shell valves was considered the mantle cavity water. Particular attention was paid so that no hemolymph was drained out with the mantle cavity water.

In determinations of the organ and tissue percentages (indexes) of the soft tissue, the hemolymph was first absorbed into a tared tissue paper. Separate tissues and organs were then dissected and weighed to the nearest milligram. The mantle, gills, palps, kidney, adductor muscles, and foot were weighed separately. The digestive gland and the stomach, as well as the gonads and the intestine, were handled as one unit. The rest of the animal was weighed together and termed the remainder.

The percentages of body compartments were calculated on the animal total wet weight and the tissue indexes were calculated on the total soft tissue wet weight. The mean values of the tissue indexes were used to estimate the percentages of the chlorophenolic load in separate organs.

Chemicals

Radioactive pentachlorophenol (PCP; sp act, 37 mCi/mmol, radiochemical purity 98%) was obtained from CEA, France. 4,5,6-Trichloroguaiacol (CG-3; sp act, 2.2

mCi/mmol, radiochemical purity over 98%) was from the Wallenberg laboratory, Stockholm, Sweden (Professor G. Wachtmeister, Esther-project). Each toxicant was dissolved in 0.1 *M* NaOH and diluted with deionized water. The stock solutions contained either 3.6 mg PCP/liter or 4.4 mg CG-3/liter.

All chemicals used in analyses were of reagent grade quality.

Exposures and Sampling

Mussels were acclimated to the experimental temperature (13°C) for 1 week and they were not fed during the last 5 days of the acclimation period. One day before the start of an experiment their shells were scrubbed to remove attached debris and they were transferred to clean water.

Three experiments were conducted with PCP. In the first experiment the exposure concentration was 14 μg/liter and in the two following experiments it was 7 μg/liter. Mussels were also exposed to CG-3 in three tests. The CG-3 concentration in the first exposure was 48 μg/liter and in the two others 23 μg/liter. The chlorophenolic solution was added 30 min before the beginning of an exposure. Water pH was kept at 6.5, an average inland water pH in Eastern Finland, and it was readjusted with 0.1 *M* HCl, if required. The water temperature at all exposures was 13°C and the oxygen concentration was maintained over 8 mg/liter.

The mussels were exposed in glass aquaria equipped with a plastic net placed close to the bottom. Three to six animals were transferred to the test water (2000–4000 ml) so that the water/soft tissue ratio was over 100 ml/g. The water was gently circulated by magnetic mixer to maintain a homogeneous concentration.

Because radioactive chlorophenolics were used, the experiments were conducted in a static system with a relatively high biomass load (5.3–9.2 g soft tissue/liter). The time needed to attain steady state was monitored from water instead of directly measuring the soft tissue concentration. Water samples (1 ml) were collected in a geometrical progression of time (0.25, 0.5, 1, 2, 4, 8, 16, 20, 24 hr, and in addition for CG-3, 48 hr) until no net changes in water ^{14}C activity were observed. The intersection of the decrease in radioactivity of the water with a constant level of radioactivity in the water was considered the minimum time needed to reach a steady state. The hydrophilic proportion of ^{14}C activity in the exposure water was determined at the beginning of an exposure and after 24 (PCP) and 48 hr (CG-3).

At the end of an exposure animals were weighed individually, the shell adductor muscles were dissected, and the mantle cavity water was drained away. The total soft tissue was homogenized in the two first PCP and CG-3 exposures, and samples from each organ and tissue were collected for radioactivity determinations in the third PCP and CG-3 exposure.

Analytical Techniques

One-milliliter water samples were analyzed to determinate the ^{14}C activity in the exposure water. Metabolites of the chlorinated phenolics in the exposure water were analyzed in acidified (pH 3) water samples (1 ml) by extraction three times with 2 ml *n*-hexane (PCP) or diethyl ether (CG-3). The organic extraction phase (reduced to 1 ml) and the extracted water were analyzed. All samples were counted with a liquid scintillation counter (LSC, LKB-Wallac, Rackbeta) for ^{14}C activity. An external stan-

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Mussel soft tissues were homogenized (Ultra Turrax homogenizer) and frozen at −20°C before analysis. The thawed homogenate (200 mg) was transferred to a glass scintillation vial, 1 ml tissue solubilizer (Luma Solve, Packard Inc.) was added, and each sample was incubated for 24 hr at 50°C. The digested soft tissue homogenate was bleached by H_2O_2 (300 μl), and isopropanol (300 μl) was added to prevent foaming. Samples were counted (LSC) after aging for 24 hr.

Metabolized PCP and CG-3 were analyzed in selected tissues (soft tissue homogenate, hemolymph, digestive gland). Each sample (0.5 g) was diluted with 0.45% NaCl (4.5 ml) and acidified to pH 3 with H_2SO_4, and samples containing PCP were extracted three times with *n*-hexane (2 ml each), whereas samples containing CG-3 were extracted three times with diethyl ether (2 ml). Samples were shaken vigorously for 2 min, followed by a centrifugation (4000 rpm) for 5 min and they were allowed to settle for 5 min. The combined extractor solvent was evaporated to 1 ml under a stream of nitrogen (99.9%) and the ^{14}C activity representing the unmetabolized PCP or CG-3 was counted by LSC. A proportion of the residue liquid containing hydrophilic metabolites was also counted to obtain the total activity in a sample.

Extraction recoveries were determined with samples spiked with ^{14}C-labeled PCP. The soft tissue homogenate was extracted five times as described above. The three first extractions yielded 95% of the total PCP. The fourth and the fifth steps together accounted for 4% of the total PCP activity. The recoveries were taken into consideration when assessing the actual level of hydrophilic metabolites in selected tissues.

The concentrations of chlorinated phenolics in the tissues are given as micrograms per gram of tissue wet weight. The bioconcentration factors (BCFs) were calculated according to the formula: BCF = ^{14}C in animal/^{14}C in water. The BCFs are given only on the basis of the total activity in water, while the difference between the total and the free chlorophenolic concentration in water was small.

RESULTS AND DISCUSSION

Body and Tissue Indexes

The body of *A. anatina* consisted of three nearly equal parts: shell 30%, soft tissues 32%, and mantle cavity water 38% of the total weight (Fig. 1). The hemolymph made

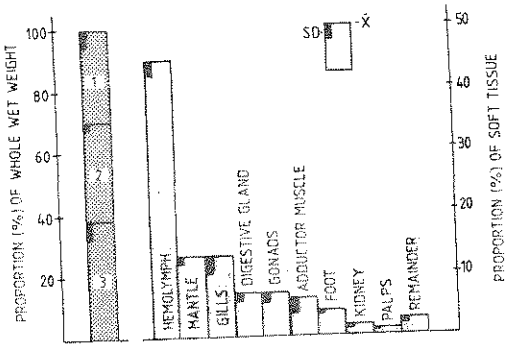
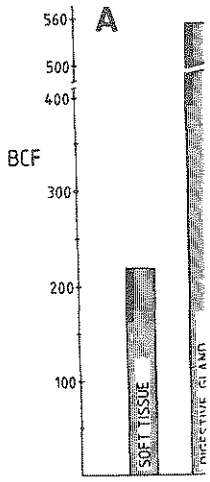


FIG. 1. Wet weight proportion of body compartments (1, shell; 2, soft tissue; 3, mantle cavity water) as well as tissues and organs indexes in *Anodonta anatina* (n = 6).

up 40% of the soft tissue. The gills and the mantle were the largest organs, and they were about 10% of the soft tissue weight each (Fig. 1).

In general, the difference in the percentage of body compartments of the *Unionidae* bivalves seemed to be rather small (Dietz, 1974; Hammen, 1979; Kluytman *et al.*, 1983). According to Haukioja and Hakala (1974) the mantel cavity water in *Anodonta piscinalis* (*anatina*) living in southwest Finland was 28.7%, about 10% less than that in our animals. However, it is difficult to explain the difference, because Haukioja and Hakala (1974) did not describe how they measured the mantle cavity water.

The soft tissue of blue mussels (*Mytilus edulis*) was divided among the different organs as follows: gills 3–4%, adductor muscles 4–7%, mantel 13–25%, and the remainder 60–75% (Zandee *et al.*, 1980). Our data on *A. anatina* were very similar to those of the blue mussel; however, the relative size of the gills was three times greater in *Anodonta.*



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Chlorophenolics in Exposure Water

In the first experiment at 14 μg/liter PCP the behavior of mussels changed; i.e., their foot was distended and it was not retracted normally after the animal was touched. At a lower concentration (7 μg PCP/liter) no behavioral toxicity was observed. An equilibrium level of PCP in water (1.8 ± 0.1 μg/liter, SD) in two separate experiments was reached in 4 and 16 hr.

In an experiment where the initial exposure concentration of CG-3 was 48 μg/liter, a steady-state concentration (31 μg/liter) in water was achieved in 16 hr. However, this exposure concentration was noted to be similarly toxic to *A. anatina* as was 14 μg PCP/liter; therefore the two other CG-3 exposures were carried out at about half of this concentration. The initial concentration of CG-3 in the two other experiments was 23 μg/liter; the equilibrium level in water (15 ± 2.8) μg/liter was reached in 22–25 hr.

In conclusion, the pentachlorophenol and trichloroguaiacol accumulated rapidly into the mussels, reaching steady state in 24 hr. This is in accordance with the observations of McLeese *et al.* (1984), who showed that the steady state for PCP in *Mytilus edulis* was achieved in 24 hr.

The water samples extracted at the end of an exposure gave a hydrophilic fraction of 13–16% for PCP and 2–10% for CG-3. While the hydrophilic fraction of the chlorinated phenolics at the beginning of the exposures was negligible (<0.2%), these elevated values must represent either chlorophenolics which were metabolized and excreted by the mussel or those biotransformed by microbes in the water.

Bioaccumulation of Chlorophenolics

The bioconcentration factor of pentachlorophenol in the soft tissue varied from 145 to 342. The values for separate organs and tissues differed clearly (Fig. 2A). The BCF values were highest in the digestive gland (447–637), and somewhat lower in the kidneys (300–326). The high lipid content of the digestive gland, as described by Stickle (1975) for the snail *Thais lamellosa,* may largely explain the high chlorophenolic accumulation of this organ. All other tissues and organs, excluding the hemolymph, accumulated PCP fairly similarly (BCF = 117–227). The PCP concentration in the hemolymph was very low (BCF = 2.6–21). The digestive gland accounted for 31% of

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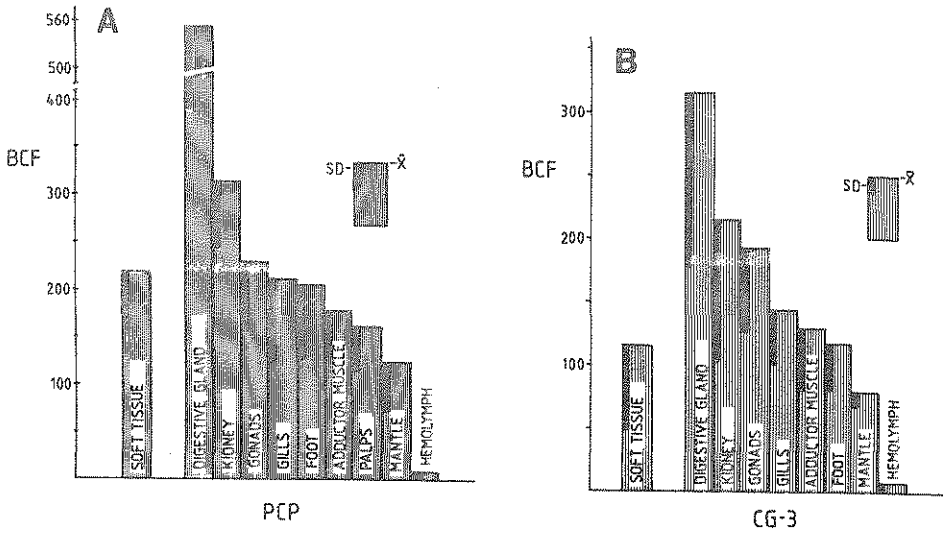

FIG. 2 (A. and B). Bioaccumulation of ^{14}C-labeled pentachlorophenol (PCP, A) and 3,4,6-trichloroguaiacol (CG-3, B) by mussel (*Anodonta anatina*) soft tissue and organs (n = 6). BCF, concn in tissue (wet wt)/concn in water after 24 and 48 hr of exposure to PCP and CG-3, respectively.

the PCP body burden, while the gills accounted for 21% and foot and mantle for 13% each (Table 1).

The BCF for trichloroguaiacol was 34–125 in the total soft tissue. Similarly to PCP, the BCF values for CG-3 were highest in the digestive gland (BCF = 275–324) and kidneys (BCF = 189–211). The accumulation into the hemolymph was very low (BCF = 3.5–15). The bioconcentration factors for the other organs and tissues varied between

TABLE 1

PERCENTAGES OF THE TOTAL BODY BURDEN OF CHLOROPHENOLICS IN SEPARATE ORGANS AND TISSUES IN *A. anatina* AFTER 24 hr OF EXPOSURE TO PENTACHLOROPHENOL AND 48 hr OF EXPOSURE TO 3,4,6-TRICHLOROGUAIACOL

Tissue	Percentage of body burden	
	PCP	CG-3
Digestive gland	31 ± 4.6	25 ± 5.4
Gills	21 ± 4.6	21 ± 3.6
Foot	13 ± 1.3	18 ± 3.5
Mantel	13 ± 3.3	13 ± 5.7
Add. muscles	8.8 ± 1.9	9.4 ± 3.2
Kidneys	2.6 ± 0.2	2.5 ± 0.2
Palps	2.4 ± 0.2	N.A.
Hemolymph	1.8 ± 0.3	4.2 ± 4.2

Note. Steady state concentrations in water were 1.8 and 15 μg/liter, respectively. Mean ± SD (n = 6).
N.A., not analyzed.

70 and 183 (Fig. 2B). The body burden of CG-3 in mussels was divided much more alike than that of PCP; the digestive gland accounted for the most (25%), followed by the gills (21%) and foot (18%, Table 1).

The experimental system used in the present study can be criticized due to the potential accumulation of physiological metabolites and other excretory products in water, which may have some negative effect on the animals. However, excluding the experiments at the highest PCP and CG-3 concentrations (PCP 14 μg/liter, CG-3 48 μg/liter), the behavior of the mussel seemed to be normal during the 24–48-hr experimental period.

The bioaccumulation of 4,5,6-trichloroguaiacol was somewhat lower than that of pentachlorophenol, which agrees with their lipophilic properties (log $P_{o/w}$: CG-3 = 4.10 and PCP = 5.85; Xie, 1984). The bioconcentration factors determined for PCP and CG-3 in *A. anatina* in this work were of the same magnitude as those in the few previous works done with this species (Korhonen and Oikari, 1986; Oikari *et al.*, 1987), as well as those done with fish and other bivalves (Saarikoski and Viluksela, 1982; Hawker and Connell, 1986).

Metabolites of Chlorinated Phenolics in Tissues

The hydrophilic fraction of the total radioactivity measured in the mussel soft tissue was 8% or less for PCP and about 2 to 3% for CG-3 (Table 2). The values for hemolymph were somewhat lower, 2 and 1% for PCP and CG-3, respectively. Almost all of these values were, however, below the 5% error caused by incomplete recovery in extraction of tissues (see Materials and Methods).

In our experiments, *A. anatina* seemed to have only very limited capacity for biotransformation of chlorinated phenolics. However, the clam (*Tapes philippinarum*) has been shown to be able to transform PCP to a bound form, which was hydrolyzed back to PCP (Kobayashi, 1977). It was also found that even when there was water-soluble PCP in the exposure water, the PCP in the tissues of the clam was not conjugated. Engelhardt *et al.* (1985) expected that chlorophenol biotransformation might be noticeable in the digestive gland, where benzo[*a*]pyrene monooxygenation *in vitro* has been observed in several species of molluscs (Anderson and Angel 1986; Stegeman 1985).

TABLE 2

THE HYDROPHILIC FRACTION (%) OF PENTACHLOROPHENOL (PCP) IN 24 hr OF EXPOSURE AND 3,4,6-TRICHLOROGUAIACOL (CG-3) IN 48 hr OF EXPOSURE IN *A. anatina* SOFT TISSUE HOMOGENATE AND SEPARATE ORGANS

Toxicant	*n*	Soft tissue	Hemolymph	Digestive gland
PCP	3	8.0 ± 0.3	3.8 ± 8.4	N.A.
PCP	3	3.3 ± 1.0	1.0 ± 0.2	1.0 ± 0.2
PCP	3	3.0 ± 3.0	2.2 ± 0.8	1.0 ± 0.1
CG-3	3	2.9 ± 1.2	0.4 ± 0.5	N.A.
CG-3	3	2.0 ± 0.1	0.0	N.A.
CG-3	6	N.A.	2.8 ± 4.1	1.7 ± 0.4

Note. Mean ± SD. N.A., not analyzed.

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organisms. *Ecotox*
KLUYTMAN, J. H., E
(1983). Anaerobic
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According to the present data, however, the metabolism of chlorophenolics in *A. anatina* was probably similar to the metabolism of PCP in *T. philippinarum*, while the amount of metabolites in animals was low and some metabolites were present in the exposure water. Our results also suggest, according to the negligible fraction of the hydrophilic chlorophenolics in the digestive gland, that the biotransformation activity in this organ is very low.

CONCLUSIONS

We have demonstrated the utility of *Anodonta anatina* as a bioindicator organism for chlorinated phenolics in this study. The accumulation of the chlorinated phenolics in this species is comparable to that in other molluscs and fish. Similarly, the time needed to reach a steady state is short (<24 hr) and roughly equal to that of fish. Diffusion of the parent compound, probably through the gills, appears to play a major role in the elimination of chlorophenolics. The similarities in chlorophenolic bioconcentration between mussel and fish make *A. anatina* a bioindicator organism preferable to fish, because mussels are much easier to collect, handle, transport, and incubate than fish.

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REFERENCES

ANDERSON, R. S., AND ANGEL, A. (1986). Biotransformation of benzo[*a*]pyrene by *Mercenaria mercenaria* and *Crassostrea virginica*. In *Aquatic Toxicology and Environmental Fate* (T. M. Poston and R. Purdy, Eds.), Vol. 9, pp. 241–251. American Society For Testing and Material, Philadelphia.

BUIKEMA, A. L., MCGINNISS, M. J., AND CAIRNS, J. (1979). Effects of pollution on freshwater invertebrates. *J. Water Pollut. Control Fed.* **50**, 1637–1648.

DAVIS, I. M., AND PIRIE, J. M. (1980). Evaluation of mussel watch *Mytilus* project for heavy metals in Scottish coastal waters. *Mar. Biol.* **57**, 87–94.

DIETZ, T. H. (1974). Body fluid composition and aerial oxygen consumption in the freshwater mussel, *Ligumia subrostrata* (Say.): Effects of dehydration and anoxic stress. *Biol. Bull.* **147**, 560–572.

ELLIS, A. E. (1962). British freshwater bivalve molluscs with key and notes for the identification of the species. *Synapses Brit. Fauna* **13**, 34–35.

ENGELHARDT, F. R., GILFILLAN, E. S., BOEHM, P. D., AND MAGEAU, C. (1985). Metabolic effects and hydrocarbon fate in arctic bivalves exposed to dispersed petroleum. *Mar. Environ. Res.* **17**, 245–249.

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.

HAMMEN, C. S. (1979). Metabolic rates of marine bivalve molluscs determined by calorimetry. *Comp. Biochem. Physiol. A* **62**, 955–959.

HAUKIOJA, E., AND HAKALA, T. (1974). Vertical distribution of freshwater mussel (*Pelecypoda, Unionidae*) in southwestern Finland. *Ann. Zool. Fenn.* **11**, 127–130.

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

KLUYTMAN, J. H., DE BONT, A. M. T., KRUITWAGEN, E. C. J., RAVESTEIN, H. J. L., AND VEENHOF, P. R. (1983). Anaerobic capacities and anaerobic energy production of some mediterranean bivalves. *Comp. Biochem. Physiol.* **75B**, 171–179.

KOBAYASHI, K. (1977). Metabolism of pentachlorophenol in fish. In *Pentachlorophenol* (K. R. Rao, Ed.), pp. 89–105. Plenum, New York.

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

MCLEESE, D. W., ZITKO, U., AND PETERSON, M. (1984). Structure–lethality relationship for phenols, anilines, and other aromatic compounds in shrimps and clams. *Chemosphere* **13**, 53–57.

OIKARI, A., PETÄNEN, T., AND MÄKELÄ, P. (1987). Bioconcentration of chlorophenolics in a freshwater mussel, *Anodonta piscinalis. Acta Univ. Ouluensis, Ser. D* **157**, 45–46.

PAASIVIRTA, J. (1978). Chlorophenolics—Poisons, possible poisons. *Kem.–Kemi* **9**, 367–370.

RISEBROUGH, R. W., DE LAPPE, B. W., WALKER, W., SPRINGER, A. M., FIRESTONE-GILLIS, M., LANE, J., SISTEK, W., LETTERMAN, E. F., SHORPSHINE, J. C., WICK, R., AND NEWTON, A. S. (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.

SAARIKOSKI, J., AND VILUKSELA, M. (1982). Relation between physiochemical properties of phenols and their toxicity and accumulation in fish. *Ecotoxicol. Eviron. Safety* **6**, 501–512.

STEGEMAN, J. J. (1985). Benzo(a)pyrene oxidation and microsomal enzyme activity in the mussel (*Mytilus edulis*) and other bivalve molluscs species from the Western North Atlantic. *Mar. Biol.* **89**, 21–30.

STICKLE, W. B. (1975). The reproductive physiology of the intertidal prosobranch *Thais lamellosa* (Gmelin). II Seasonal changes in biochemical composition. *Biol. Bull.* **148**, 448–460.

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

ZANDEE, D. I., KLUYTMANS, J. H., ZURBURG, W., AND PIETERS, H. (1980). Seasonal variation in biochemical composition of *Mytilus edulis* with reference to energy metabolism and gametogenesis. *Neth. J. Sea Res.* **14**, 1–29.