METABOLIC DISPOSITION OF PYRENE BY RAINBOW TROUT (*Salmo gairdneri* Rich.) AND WATER-HYACINTH (*Eichhornia crassipes* Mart. Solm.)

by

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Metabolic Disposition of Pyrene by Rainbow trout (Salmo gairneri)and Water-hyacinth (Eichhornia crassipes)

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ABSTRACT

Pyrene is one of the polycyclic aromatic hydrocarbons (PAH) found in the aquatic environment. However, little is known of the absorption, distribution, metabolism and elimination of pyrene by aquatic species. Much less is known about the environmental effects, particularly temperature and pH, on the toxicokinetics of pyrene. In the present study, rainbow trout (Salmo gairdneri Rich.) and water-hyacinth (Eichhornia crassipess Mart. Solm.) were used as the fish and plant models, respectively to examine the biological fate of pyrene. These studies were performed under static exposure condition; the uptake and disposition experiments were conducted with different temperature and pH whereas the depuration and metabolism experiments were conducted under a constant temperature and pH.

Results of the studies show that both the uptake and distribution processes of 14C-pyrene in fish and water-hyacinth were affected by the exposure temperature and water pH. Pyrene was taken up maximally by rainbow trout and water-hyacinth at 30 °C temperature and water pH 6. The Q10 values of 14C-pyrene by rainbow trout and water-hyacinth were1.5 and 1.6, respectively. Tissue distribution studies showed that radioactivity was distributed unevenly among trout and plant tissues. High levels of 14C-pyrene were found in the bile, liver and intestine whereas low levels of 14C-pyrene were found in gill, muscle, and brain of trout. More than 95 % of the radioactivity absorbed by water-hyacinth was found in the root and less than 1 % in the leaf. At least two major

metabolites of pyrene were found in trout. Peak level of metabolite production was attained after 72 hrs of exposure. No pyrene metabolite was found in the water-hyacinth.

Trout depurated 14C-pyrene faster than water-hyacinth. The mechanism of pyrene depuration from water-hyacinth remains unclear since pyrene might be eliminated from the root cells or simply leached from the outer surface. Nevertheless, the present study confirms previous reports that water-hyacinth absorbs a broad spectrum of organic and inorganic pollutants from water and is a very useful plant for removing toxic chemicals from industrial waste waters, especally in the Tropics.

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PART A

INTRODUCTION

A. Introduction

Polycyclic Aromatic Hydrocarbon (PAHs) are chemicals composed of two or more fused aromatic rings with molecular weights ranging from 166 to greater than 328. PAHs are usually associated with suspended particulate matter in both the atmospheric and aquatic environment (NRCC,1982), because of their low vapor pressures, low water solubilities and high affinity for particle surfaces. Most of the PAHs have been shown to be potent carcinogens. The relative carcinogenicities of PAHs to laboratory animals have been summarized by a publication of the National Academy of Sciences (Appendix 1).

Generally, PAHs are produced during incomplete combustion of fossil fuel (organic matter) at high temperature (e.g. 700 °C). While some higher plants, aquatic algae and microbes are capable of synthesizing PAHs (NRCC, 1983); the biosynthetic rate of PAHs in algae Chlorella sp. is about 67 ug/kg dry algal biomass/year. Ramdahl (1981) and Petter et al.(1981) reported that residential combustion (fuel wood), aluminium smelting, forest fires and transportation (gasoline-powered automobiles) are the largest atmospheric sources of PAHs in the United States. Industrial activities resulting in the production of PAHs include pyrolysis of wood to form charcoal, wood tars and carbon blacks, coke production, gas production from petroleum, coal gasification, production of synthetic alcohol, and oil refinery operation (Andelman and Snorgass, 1972). These anthropogenic PAHs may reach the

aquatic environment from industrial and domestic sewage effluent, surface run off from land, deposition of air borne particulates and spillage of petroleum into water bodies.

Eisenreich et al. (1981) estimated the total input of PAHs to the Great Lakes at approximately 484 ton/year including 24 ton/year for benzo(a)pyrene and 24.8 ton/year for pyrene. Connell and Miller (1982) estimated that about 30,000 to 300,000 ton/year of PAHs entered the world's oceans.

PAHs are partitioned into various compartments (water, sediment, suspended particles, and dissolved organic macro-molecules) of the aquatic environment (Jimenez et al.,1987). However, a wide variety of organic compounds in natural water, such as purines, lactic acid, nitrogen containing organic compounds, acetone, ethanol and dioxane, are capable of increasing the water solubility of PAHs.

The uptake of PAHs by biota is closely related to the physical-chemical properties of these chemicals; the hydrophobicity of PAHs is recognized as the driving force for the partition of PAHs between the aqueous and lipid phases (Bruggeman et al.,1982). For most aquatic plants and animals (both vertebrate and invertebrate), uptake of PAHs occurs directly through the integument (Metcalf, 1977). In more complex fish species, uptake is by the gills. Another route of uptake is ingestion and absorption of materials by the gut.

IL'nitsky et al.(1979) studied the distribution of benzo(a)pyrene among water, sediments, plants, fish, plankton and mollusk exposed to municipal effluents. Benzo(a)pyrene in sediment and biota were found at 100 - 10,000 times the level in water. 14C-anthracene in aquatic organisms also was found at about 1000 times its concentration in water (Gidding et. al., 1977). PAHs level in mussels, Mytilus edulis, collected from Sidney Harbour, Nova Scotia, were found to be 4.12 ug/g wet weight (EPS, 1982). Dunn and Stich (1976a) also reported elevated benzo(a)pyrene concentration (2 - 30 ug/kg) in the mussels collected from the Outer Harbour of Vancouver, B.C. Mackie et al.(1979) also have found pyrene levels in mussels between 2 - 540 ng/g wet tissue.

The accumulation of chemicals by fish is the result of an imbalance between the uptake and elimination processes. Therefore, environmental factors which affect these processes often alter bioaccumulation in fish. For example, an increase in exposure temperature enchances the uptake and accumulation of organic compounds by fish (Powell and Fielder, 1982). A decrease in exposure temperature increases the retention of organic compounds by fish (Collier et al., 1978). Jiminez et al. (1987) found that the uptake of benzo(a)pyrene by bluegill sunfish (Lepomis macrochirus) increases 5.8 fold with a 10 °C increase in acclimation temperature. Kennedy (1989) also has found that an acute temperature change alters the uptake and distribution pattern of benzo(a)pyrene in a marine teleost (toad fish).

Water pH also affects the uptake, distribution and excretion of toxicants by fish. Van der putte (1980) reported that the uptake and distribution of hexavalent chromium by rainbow trout depended on water pH; the highest levels of chromium were found in the gill, liver, kidney and digestive tract of trout exposed to water at pH 7.8. whereas chromium was found mainly in the gills of trout exposed to water at pH 6.5. No information could be found in the literature on the effect of water pH on the uptake and elimination of PAHs by fish.

Lee et al. (1972) studied the toxicokinetics of 14C-naphthalene and benzo(a)pyrene in three species of marine fish. All three fish species rapidly accumulated the PAHs, attaining steady state accumulation in about 1 hour. The PAHs appeared to be taken up by the gills and accumulated primarily in the liver and gall bladder. More than 90 % of accumulated 14C-naphthalene was eliminated from the tissues after the fish were placed in 14C-naphthalene-free sea-water for 24 hrs. Accumulated benzo(a)pyrene was released more slowly from the tissues after the fish were placed in benzo(a)pyrene-free sea-water for 24 hrs; about 50 %, 50 %, 90 %, and 20 % of accumulated radioactivity were eliminated by the liver, gut, gill, and flesh, respectively. A significant portion of the excreted radioactivity was in the form of polar metabolites. The bile and urine were the main excretion routes.

PAHs are metabolized by rats and rabbits to quinone, phenol, and trans-dihydrodiol derivatives which are more water-soluble than the parent compounds (Boyland, 1964). Neff (1979) suggested

that PAH metabolites produced by marine and freshwater animals are analogous to those which have been identified in mammals. These metabolites include diols, phenols and quinone derivatives as well as conjugates with sulfate, monosaccharide, glucuronate and glutathione. Lee et al. (1972) reported that benzo(a)pyrene was converted by fish to a compound tentatively identified as a dihydrodiol and naphthalene was metabolized by fish mainly to the corresponding 1, 2-dihydro-1, 2-dihydroxy derivative. Roubal et al. (1977) showed that after intra-peritoneal injection of naphthalene to young coho salmon, the gall bladder contained the highest concentration of metabolites with 1-naphthyl glucuronic acid predominant. However, 1-naphthol was found mostly in the liver, heart, and flesh.

In general, the acute toxicities of PAHs to a particular aquatic animal species increase with increasing molecular weights. However, high molecular weight PAHs such as chrysene, benzo(a)pyrene, and dibenzanthracene have low acute toxicity. Probably, this is because of their extremely low water solubilities. Kennedy (1989) found that pyrene (2 mg/l) was not acutely toxic to rainbow trout. In contrast, naphthalene (0.92 mg/l) is acutely toxic to pink salmon (Thomas and Rice, 1978). There are wide intra-specific differences in the relative toxicities of different PAHs; crustacean are the most sensitive, polychaeta worm intermediate in sensitivity, and fish the most resistant (Neff, 1978). DiMichele and Taylor (1978) have described the effect of different naphthalene concentrations on the histopathology of fish tissues. Naphthalene

caused gill hyperplasia at 2 ug/l concentration. Hemorrhages of the gill filaments were also noted. Focal necrosis, hyperplasia and ischemia in several organs including the pancreas, liver, and brain were found at higher concentrations of naphthalene (2 - 30 mg/l).

The toxic effects of PAHs to aquatic organisms may involve both reversible and irreversible binding of metabolites to the macromolucules. The PAH metabolites may bind reversibly with macromolecules and thereby interfere with essential cellular processes. The PAH metabolites may bind irreversibly to many cellular structure causing long-term damage. Roubal and Collier (1975) conducted in vitro and in vivo studies with coho salmon using spin-labeling spectral techniques. They revealed that PAH were bound primarily to membranes of neural tissues especially the non polar and electron interactive site of the membrane surface. Binding of PAH to the surface of membrane caused perturbation in the membrane surface organization. Many physiological phenomena are dependent directly on the unique properties of biological membranes. For instance, osmotic and ionic exchange between the cell and the extracellular milieu is mediated by the cell membrane and is essential for maintenance of cellular integrity and for such physiological functions as neurotransmission, muscle contraction, and osmoregulation. PAH-induced changes in the surface organization of membranes may interfere with these essential processes. In addition, many enzymes associated with membranes are weakly bound to membrane lipid which is essential for normal enzyme function. If the membrane surface is altered, polar and other

interactions between the membrane and enzymes may alter enzyme activity. Therefore, ability of PAH to alter membrane surfaces may explain in part why these compounds are toxic to living organisms.

Many aquatic animals including rainbow trout have been shown to produce cancer after being exposed to PAHs such as the 5,6-dihydrodiol benz(a)anthracene, 7,12- dimethyl benz(a)anthracene and the 7,8-dihydrodiol benz(a)pyrene (Neff, 1979). Shultz (1983) reported that after repeated exposure of two species of fish (Poeciliopsis lucida and P. monaca) for 5 - 20 hrs to 5 mg/l of an aqueous suspension of 7,12-dimethylbenz(a)anthracene (DMBA), the fish developed liver neoplasms within 7 - 8 months. Also, limphosarcomas were found in the kidney, spleen, pancreatic tissues, heart and liver. Development of epitheliomas was reported in three-spined stickleback (Gasterosteus aculeatus) and bitterling (Rhodeus amarus) after the skin was painted with 3-methylcholanthrene and benz(a)pyrene twice a week for 3 - 6 months (NRCC, 1983).

In contrast, little is known about the uptake, distribution and depuration of PAHs by aquatic plants. Much less is known about the effects of environmental factors on PAH uptake by plants. Therefore, the effects of environmental variables such as temperature and pH on uptake, disposition and depuration of pyrene were conducted in water-hyacinth.

Water-hyacinth (Eichhornia crassipes (Mart.) Solm.) belongs to the family Pontederiaceae, Order Liliales (Hutchinson, 1959). Pontederiacea is found entirely in freshwater of the warmer parts of the world. It is a tropical fresh water plant with beautiful lilac violet flowers, but today is considered, among all the plants, the most troublesome weed of the world. Because of its beauty and a source of environmental problems, it has been called a Demon, Blue Devil, Bengal Terror, Million Dollar Weed, and a Cinderella of the Plant World (Gopal and Sharma, 1981).

Water-hyacinth is found in freshwater ponds, lakes, reservoirs, rivers and irrigation channels and because of its rapid growth in the field, it has become a serious environmental problem. It has a doubling time of 10 to 14 days or 6 - 10 % per day (Wolverton and McDonald, 1975), and produces dry matter up to 197.6 ton/ha/year (Yount and Crossman, 1970). Damages to the environment include a decrease in fish production due to a reduction of phytoplankton as a result of shading and removal of nitrogen, phosphorus, and dissolved oxygen (Sharma et al., 1978; Beasley and Lawrence,1966; Timer and Weldon, 1967). The plant increases the probability of health hazards since it provides both the habitat and food for several harmful animals and other vectors of diseases like malaria, encephalitis, and schistosomiasis (El Fadl et al., 1970; Dassanayke, 1976; Frank, 1976; Koegel et al.,1973; Rao, 1969; Raynes, 1964; and Wilson, 1967). Water-hyacinth also creates problems such as temperature, nutrient and water availability as

results of its high evapotranspiration rate. In the United States, water loss from irrigation channels has cost US $ 39.9 million per year (Benton et al, 1978). In Florida, to control 46.000 acres of water-hyacinth a cost of US $ 5 million per year (Benton et al., 1978). The ratio of evapotranspiration (Et) from water-hyacinth to evaporation from free water surface (E) was different from region to region, ranging from 1.26 - 8.00 (Gopal and Sharma, 1981; Brenzy et al., 1970). The rapid transpirational loss of water may be explained by the stomatal size and inter-stomatal distance. Penfound and Earle, (1947) found that the size of stomatal aperture of water-hyacinth is 12 X 27 μ, which is about twice in both dimensions as compared to that in most other plant. The inter-stomatal distance is eight times the pore aperture.

Water-hyacinth grows under light intensity between 90 - 2000 μE/m2/s (Patterson and Duke, 1979). The optimum temperature of growth is 27 - 30 °C since it ceases to grow below 10 °C or above 40 °C. According to Gopal and Sharma (1981), the optimum water pH for growth is 6.0 - 8.0.

Water-hyacinth is used as a source of animal food, paper pulp and fibre, biogas, and pollution abatement. Several laboratory field studies indicate that water-hyacinth reduces nutrients, BOD, COD, and fecal coliform. It accumulates a large number of heavy metals such as copper, cadmium, nickel, silver, chromium lead, mercury, arsenic and organic pollutants such as phenols, dyes, diphenamid dyes (Dinges, 1976; Wolverton, 1975; Wolverton and McDonald, 1975;

Bingham and Shaver, 1977; Wolverton and McKown, 1976; Wolverton et al., 1975; Wooten and Dodd, 1976; Sutton and Blackburn, 1971). This plant also absorbs mixed pollutants from textile factory wastes (Widiyanto, 1975). Wolverton and MacDonald (1979) estimated that an acre of dense growth of water-hyacinth can treat waste water from 2000 people effectively. The daily uptake rates of organic pollutants by water-hyacinth have been reported by NASA (1984) as follows:

Organic compounds	Concentration		
	Before Exposure	After exposure	%Reduction
Naphthalene (PAH)	4.00	0.71	82.25
Biphenyl	5.27	0.02	99.62
Dimethyl Phthalate	7.48	0.32	95.75
P-Nitrotoluene	10.58	3.52	66.73
Ethyl Benzene	2.80	0.15	94.64
Toluene	2.64	0.23	91.29
P-Xylene	1.06	0.15	85.85
Benzene	3.53	0.18	94.91

This experiment was conducted at the National Space Technology Laboratory (NSTL) in Missisipi. However, the distribution of these organic compounds in water-hyacinth has not been reported.

The effect of PAHs on plants has been described by Van Overbeek and Blondeau (1954). PAHs bind with the plasma membrane of plant cells and increase its permeability to water. They disrupt the structure of grana in chloroplast and interfer with photosynthesis. Acute toxicities of PAHs to plant cells decrease with increasing carbon number. The most toxic PAHs are those in the range of C18 - C14 (C2-benzene to phenanthrene). Boney and Corner (1962) found that low concentrations of several PAHs stimulated growth of sporelings of the marine red algae, Antithamnion plumula. Response of this alga to benzo(a)anthracene derivatives was dose dependent. At concentrations between 10 - 300 ug/l it caused a progressive inhibition of growth. However, pyrene had no effect on the growth of this marine alga. Boney (1974) also reported that PAHs might stimulate, inhibit or not affect growth of the marine algae since growth stimulation was the greatest with chrysene (+58 %) and inhibition was most pronounced with anthracene (-20 %). His studies also showed that the growth-stimulation effect of PAHs was related to an increased rate of cell production by the apical cell cavity.

Because of the characteristics and ubiquity of pyrene in industrial and municipal waste effluents (appendix 2 and 3), ground

water, surface waters, and their sediment and biota, the effects of environmental variables on the uptake, distribution, elimination and metabolism of pyrene by fish and plants are essential to understand and predict the fate of this compound in the aquatic environment. The objective of my thesis is to study the effects of temperature and pH on the uptake, distribution, depuration and metabolism of pyrene by trout and water-hyacinth.

PART B

MATERIALS AND METHODS

B. Materials and Methods

I. Experimental organism and acclimation regimen

1. Fish

Rainbow trout (Salmo gairdnerii Rich.) weighing between 2 - 5 g were obtained from Fraser Valley Trout Hathcery, Vancouver, B.C.. The fish were held in a flow-through fiber glass tank at 6 °C with photoperiod 12 hrs light and 12 hrs dark for at least 3 weeks before being used. Fish were fed once daily with New Age Fish Feed obtained from Moore-Clark Co., Vancouver, B.C.. The fish were not fed for 48 hrs before the experiment.

2. Plant

Water-hyacinth (Eichhornia crassipes (Mart.) Solm.), weighing between 30 - 50 g were purchased from Delta Nursery, Richmond, Vancouver, B.C.. They were imported from Singapore by the Nursery. The plants were acclimated in soil bedded fiber glass containers with 15 cm water at the SFU greenhouse. The plants were kept for at least 30 days before being used. The root of the plants were rinsed with water to release the soil before being transferred to 600 ml beakers for the study. The beakers were placed in a controlled chamber (Sherrer Model No. Cel 3714, Gillet Co., Marshall Mich.) for 48 hrs before the experiment.

3. Chemicals

14C-labelled pyrene (specific activity 56 mCi/mmol) was purchased from Amersham Corporation (Arlington, Heights, IL.). The radiochemical purity of 14C-pyrene as determined by thin-layer chromatography (TLC), was at least 99%. Protosol and Bioflour were obtained from New England Nuclear (NEN), Lachine, Quebec, Ont.. Carbo-Sorb and Permaflour were purchased from United Technologies Co., Mississauga, Ont.

II. Uptake and Disposition of Pyrene by Fish

1. Fish exposure

Trout were maintained in water at 6 °C before the experiment. Groups of 3 fish were exposed to 14C-pyrene at four different combinations of temperature (6 and 16 °C) and pH (6 and 8). Fish were placed individually in a 1-L stoppered Erlenmeyer flasks supplied with a stream of air. The fish were exposed to the test temperature and pH without an acclimatization period, since the aim of this experiment to see the effect of acute temperature change on uptake of 14C-pyrene. Water pH (8 ± 0.2) was maintained with 0.1 N sodium hydroxide solution since the pH of declorinated water was about 6. Because of limited solubility, pyrene was dissolved in 250 ul dechlorinated water containing a drop of Tween 80 (Sigma, St. Louis, Mo.). All flasks were darkened to minimize photodecomposition of pyrene.

2. Sampling and Analysis

Water samples (5.0 ml) were removed from the Erlenmeyer flask at 0, 2, 4, 6, 8 and 24 hrs and an aliquot of the sample was placed in scintillation vials. 14C-radioactivity in the vials was assayed by liquid scintillation counting after the addition of scintillation cocktail (15.0 ml).

After a 24 hrs exposure to 14C-pyrene, fish were sacrificed, rinsed and weighed. The gill, liver, stomach, intestine, swimbladder, brain, kidney, muscle and skin were removed and weighed. The organs were digested in liquid scintillation vials for 24 hrs at 80 °C with 1.0 ml tissue solubilizer (protosol : ethanol, 1 : 1). When the organs were solubilized, scintillation cocktail (15.0 ml) were added. The vials were stored in the dark until counted.

The remaining carcass was weighed, minced and homogenized in 5.0 ml of distilled water using a Polytron Homogenizer (Brinkman Instr. Westbury, N.Y). The homogenate was diluted with 10.0 ml of distilled water. A 1.0 ml aliquot of the homogenate was digested with 1.0 ml tissue solubilizer. The sample was cooled, decolorized with 0.5 ml hydrogen peroxide and counted in a Beckman LS-3801 Liquid Scintillation Counter (LSC) with automatic quench correction.

3. Depuration by Fish

In this experiment, the fish were exposed to 14C-pyrene at 6 °C water temperature. After a 24 hrs exposure, the fish were removed, rinsed and transferred to pyrene-free water. The fish were kept in pyrene-free water for an additional 6 days. Water samples were taken at 0, 2, 4, 6, 8, 16, 24, 48, 72, 96, 120, and 144 hrs and counted for 14C-radioactivity in a liquid scintillation counter.

III. Metabolism Experiment

1. Fish Exposure

Fish were exposed individually to 14C-pyrene (5 ug/1.375 uCi/L) in 1-L Erlenmeyer flasks supplied with air. Each group of 4 fish was exposed for 2, 4, 8, 16, 48, 72, and 96 hrs. At the conclusion of the exposure period, the fish were removed, rinsed, weighed, and frozen with liquid nitrogen. Water samples were taken for radioactivity counting at zero time and at the end of each exposure.

2. Fish Analysis

Fish were homogenized individually in 5.0 ml of distilled water using a motor-driven Polytron with Power Control PCU-2-110 (Kinematica Gmbh). The homogenate was diluted to 10.0 ml with distiled water. A 1.0 ml aliquot of the homogenate was digested

with 1.0 ml tissue solubilizer (protosol : ethanol, 1 : 3) at 80 °C for 8 hrs in a liquid scintillation vial. The sample was cooled to room temperature. After the addition of 0.5 ml hydrogen peroxide the vials was returned to the oven for an additional 1 hr. The vial was counted for radioactivity in a liquid scintillation counter after the addition of15.0 ml scintillation cocktail.

3. Determination of Metabolites Formed by Fish

An aliquot (3.0 ml) of the homogenate was diluted with 10.0 ml distilled water in a 40.0 ml glass centrifuge tube. The tube was extracted with 15.0 ml ethyl acetate for 15 minutes and centrifuged in a IEC Model K centrifuge at an average speed of 4000 rpm for 15 minutes. The ethyl acetate extract was removed and put into a 20 ml test tube. The procedure was repeated once. The extracts were combined and evaporated under a gentle stream of nitrogen. The residue was reconstituted in 0.5 ml acetone. A 20 ul aliquot of the acetone solution was counted in a LSC.

A 100 ul aliquot of the acetone solution was spotted on a thin layer chromatography (TLC) plate (Whatman K6 Silica gel, 5 x 20 cm, 0.25 mm thickness). The solvent system used for developing the TLC plate was benzene : ethanol (19 : 1, v/v). The TLC plate was marked every 1.0 cm with a pencil from the origin to the solvent front after the plate was removed from the development chamber. The areas corresponding to the Rf of 14C-pyrene and its metabolites were scraped off from the plate and transferred to scintillation

vials. One milliliter of ethanol was added to elute the 14C-pyrene from the silica gel. The vials were shaken and counted for radioactivity after the addition of scintillation cocktail.

The remaining aqueous layer after ethyl acetate extraction was hydrolysed with 0.1 N hydrogen chloride (HCl) for 30 minutes at 80 °C in a waterbath. The mixture was extracted by ethyl acetate and analysed by TLC as described above.

4. Determination of Metabolites in Water Samples

Unconjugated 14C-pyrene metabolites in the water were determined as follows: an aliquot of water samples (250 ml) was extracted with 250 ml ethyl acetate twice. The ethyl acetate extracts were combined and evaporated in a 500-ml round bottom flask, using a rotary flask evaporator (Buchi Rotavapor R.E 120). The liquid residue was transferred to a 15.0-ml glass tube, evaporated under gentle stream of nitrogen gas and redissolved in 0.5 ml acetone. The acetone solution was spotted on a TLC plate and analysed for 14C-pyrene metabolites as described before.

The remaining aqueous layer was hydrolysed by acid at 80 °C for 30 minutes. The aqueous layer was extracted by ethyl acetate and analysed by TLC as described before.

IV. Uptake and Disposition of Pyrene by Plant

1. Plant exposure

These experiments were conducted in a controlled chamber at 4 different combinations of temperature (20 and 30 °C) and pH (6 and 8). Light source originated from 10 x 100 watt flourescent tubes and was equivalent to 110 μmol, m-2, s-1. Light intensity was measured with a light meter (Licor Model Li-185A). Photoperiod cycle was maintained 12 hrs light and 12 hrs dark. Water pH 8 ± 0.2 was maintained with 0.1 N sodium hydroxide.

Each experimental group consist of 4 plants. Each plant was placed in a darkened glass beaker before being exposed to 14C-pyrene (5 ug/1.375 uci/500 ml). The beaker was covered to reduce evaporation.

2. Sampling and Analysis

The plants were exposed for six days (144 hrs). Water samples (2.0 ml) were taken at 0, 8, 16, 24, 48, 72, 96, 120, and 144 hr. Except for the depuration experiment, two additional water samples were taken at 1 hr and 4 hrs. Before sampling, water lost due to evapotranspiration was topped up to the original volume (500 ml) with dechlorinated water. Water was mixed by stirring with a magnetic bar. Radioactivity in the water sample was determined as desribed previously.

At the conclusion of a 144 hrs exposure, the plants were removed. The roots of the plant were rinsed. Each plant was separated into three parts: root, stem, and leaf. They were oven dried at 105 °C for at least 24 hrs before weighing. Each part was reduced to powders with a mortor. About 5 mg of the powdery sample was placed onto a 5.5 cm diameter ashless Whatman filter paper and was burnt in a Tri-Carb B 306 Sample Oxidizer (Packard Co., Downers Grove. IL). The 14-CO2 formed from each sample was trapped by Carbo-Sorb. After the addition of Permaflour, the solution was counted in a liquid scintillation counter.

3. Depuration by Plant

At the conclusion of a 144 hrs exposure, the plants were removed from the beakers. The roots of the plants were rinsed before the plants were transferred to 14C-pyrene-free water. The plants were kept in pyrene-free water for an additional 6 days. Water samples were taken at 0, 4, 24, 48, 72, 96, 120, and 144 hrs. The samples were counted in a liquid scintillation counter as described before.

V. Statistical Analysis

Means and standard deviation of each treatment group were calculated. Statistical significance of the results was analysed by the one-way analysis of variance by Duncan or the Students' t- test. A p value less than 0.05 was considered to be significant.

PART C
RESULTS

C. RESULTS

I. Fish

1. Uptake of 14C-pyrene

Figure 1, 2 and table 1 shows the time course of 14C-pyrene uptake by rainbow trout under different exposure conditions. 14C-pyrene was taken up rapidly by trout from water, especially during the first 8 hours of exposure. The uptake rates were affected by both temperature and pH. Uptake of 14C-pyrene by fish was highest at 16 °C. In the initial 6 hrs, 14C-pyrene uptake by trout was similar at pH 6 and pH 8. However, trout exposed to pH 6 reached a higher steady state level than those at pH 8. At 6 °C water temperature, trout appeared to take up more 14C-pyrene at pH 8 than pH 6.

2. Tissue Distribution of 14C-pyrene in rainbow trout

Figure 3, 4 and table 2, 3 show the distribution of radioactivity in the tissues of rainbow trout exposed to 14C-pyrene under different exposure conditions. Radioactivity in the gill, muscle, stomach, and brain were much lower than those in the intestine, kidney, swimbladder, and liver. The highest level of radioactivity was found in the bile.

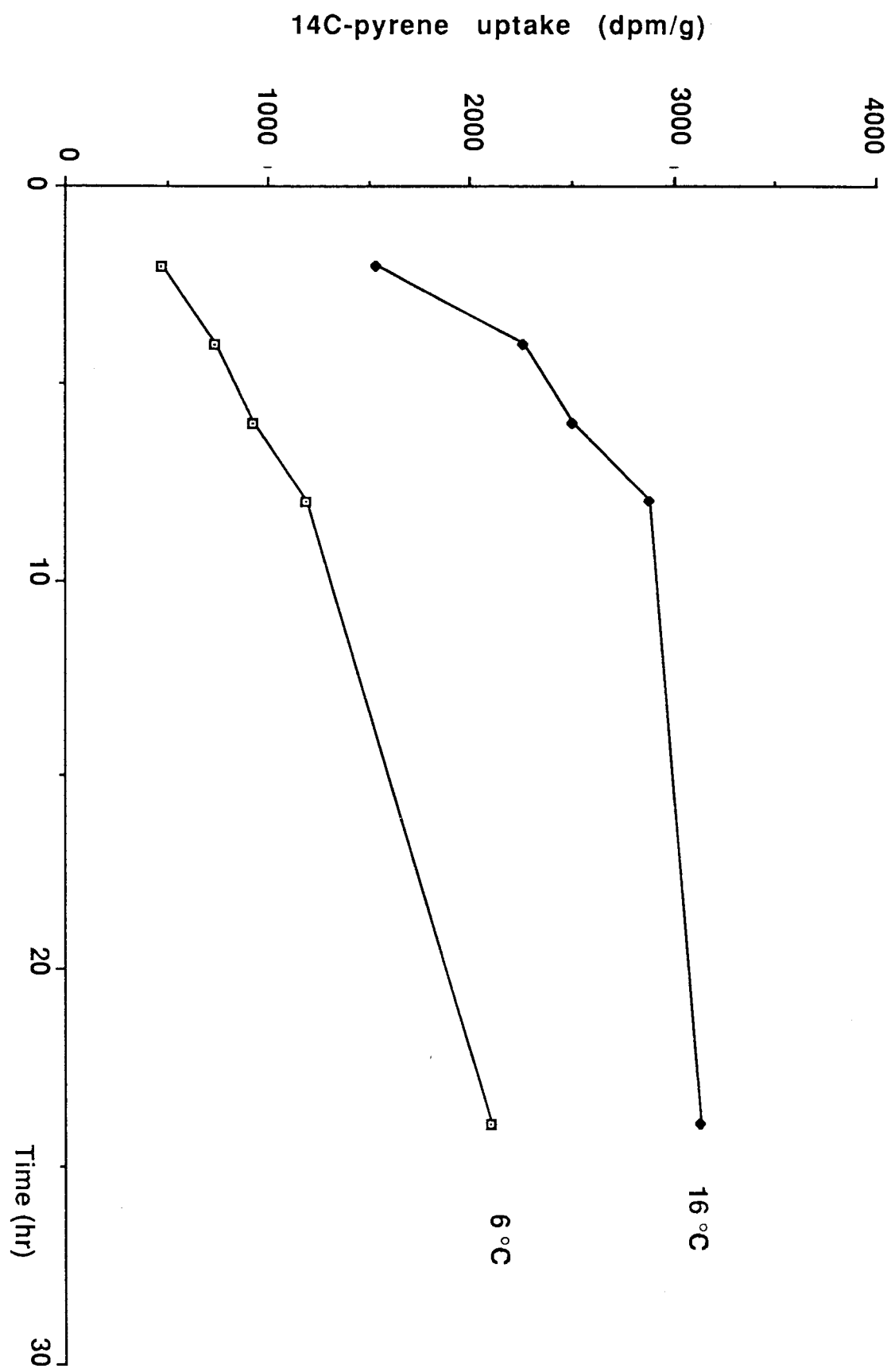

Fig. 1. Time course of 14C-pyrene uptake by rainbow trout exposed to pH 6, at temperature 6°C and 16 °C

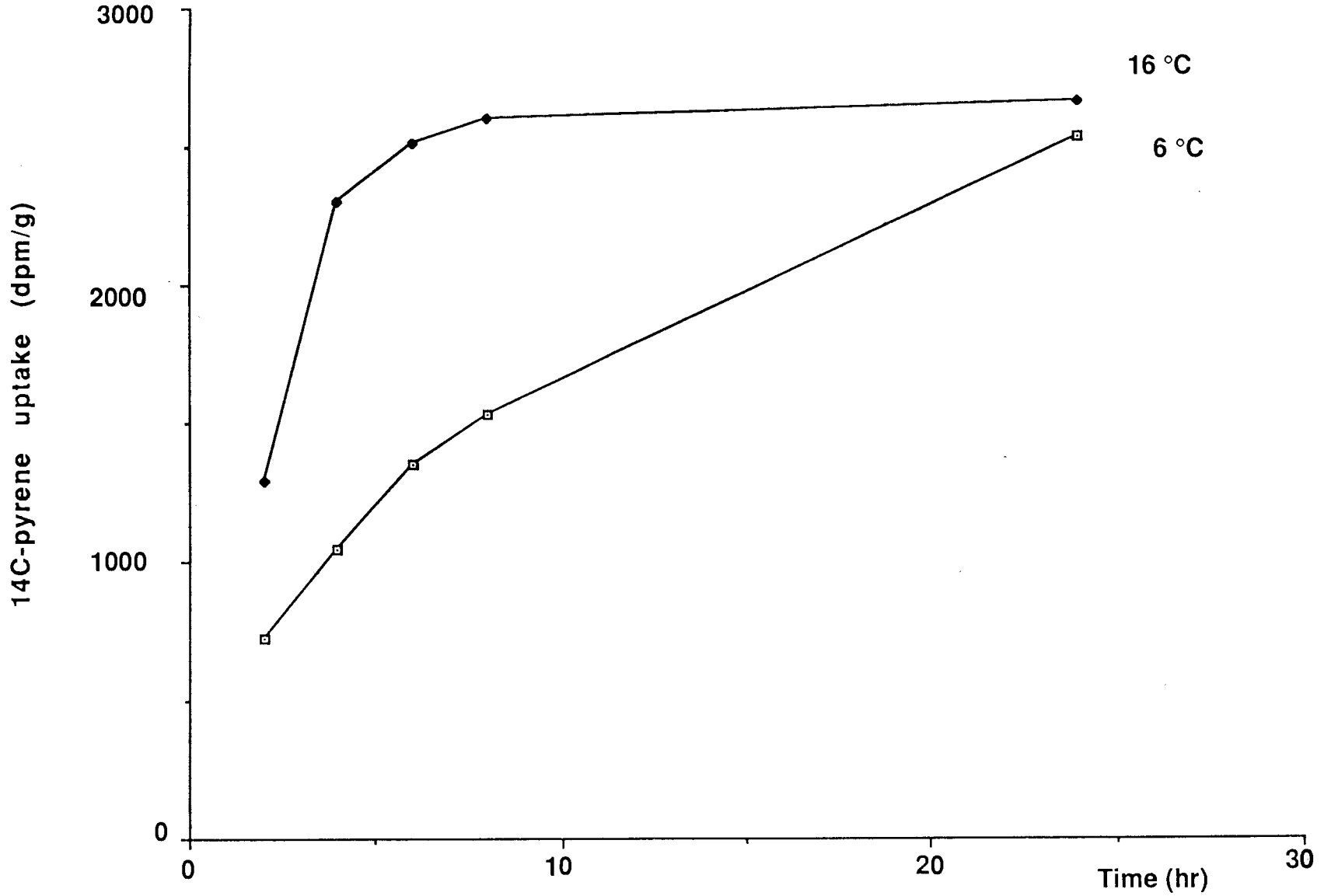

Fig. 2. Time course of 14C-pyrene uptake by rainbow trout exposed to pH 8, at temperature 6 °C and 16 °C

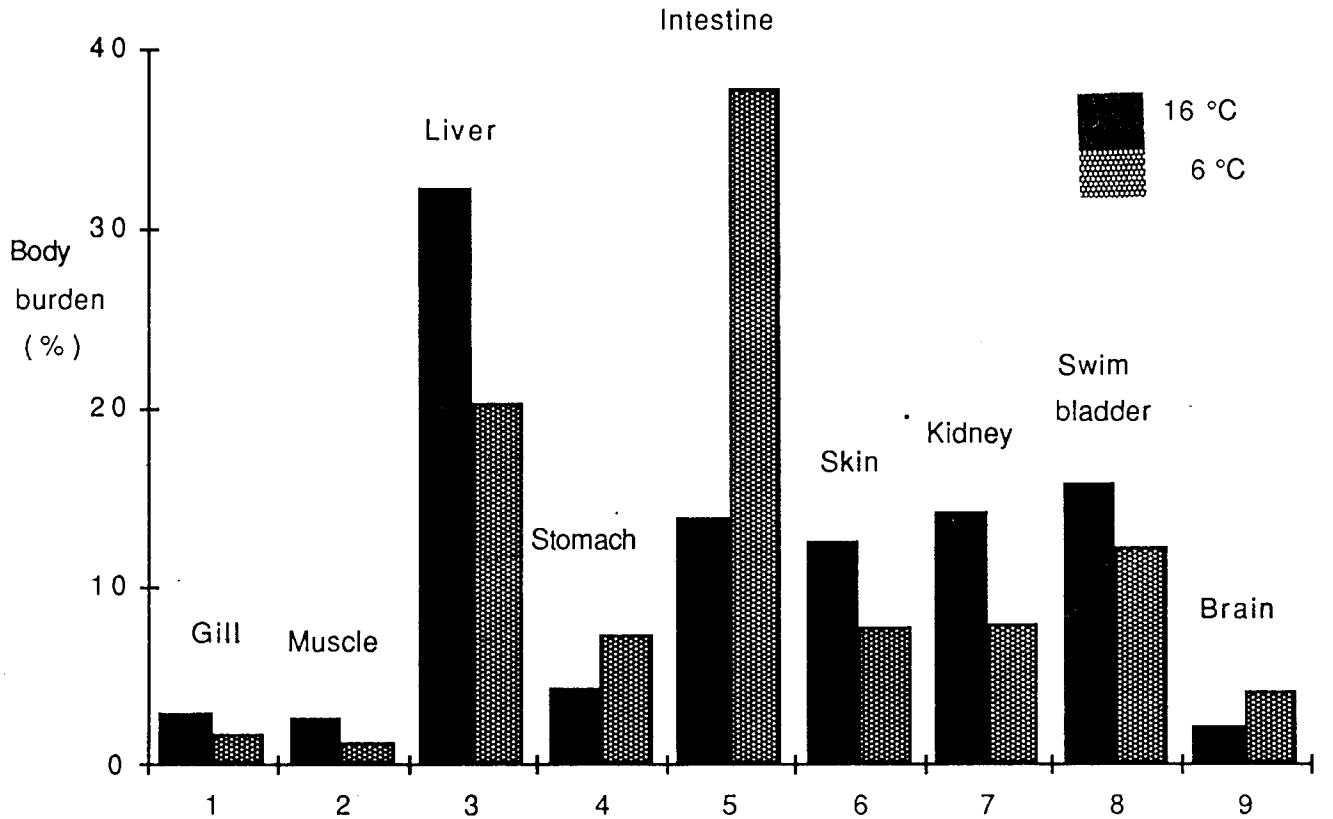

Fig. 3. Distribution of radioactivity in the tissues of rainbow trout exposed to pH 6 at temperature 6 °C and 16 °C

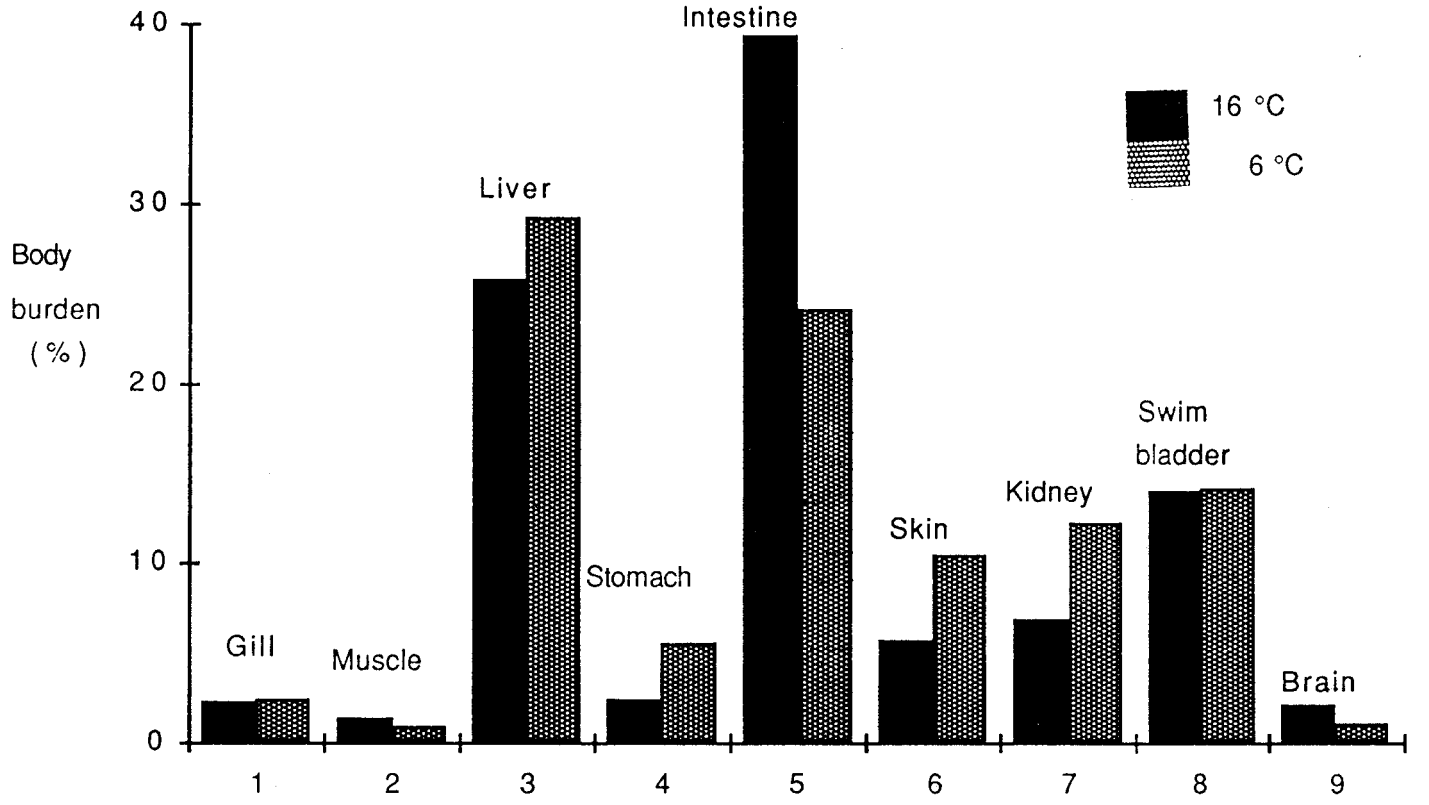

Fig. 4. Distribution of radioactivity in the tissues of rainbow trout exposed to pH 8, at temperature 6 °C and 16 °C

3. Metabolism of 14C-pyrene

Figure 5, 6 and table 4 shows the time course of unchanged pyrene concentration in water (PW) and fish (PF) and the time course total metabolite (PM) formed after exposing trout to 14C-pyrene. Pyrene concentration in water decreased with exposure time. In contrast, the concentration of unchanged pyrene in fish and the total metabolites formed increased with exposure time. Apparently, 14C-pyrene uptake by fish depleted the amount of unchanged pyrene present in water. Figure 7 and table 5 shows the concentration of metabolites in fish and water at each time point. Metabolite concentration in water was about 2-fold of those in the fish with the exception of the initial 4 hr of exposure.

Figure 8, 9 and table 6 shows the TLC profiles of ethyl acetate extractable metabolites in fish and water, respectively. At least two major metabolic products of 14C-pyrene were formed by the fish.

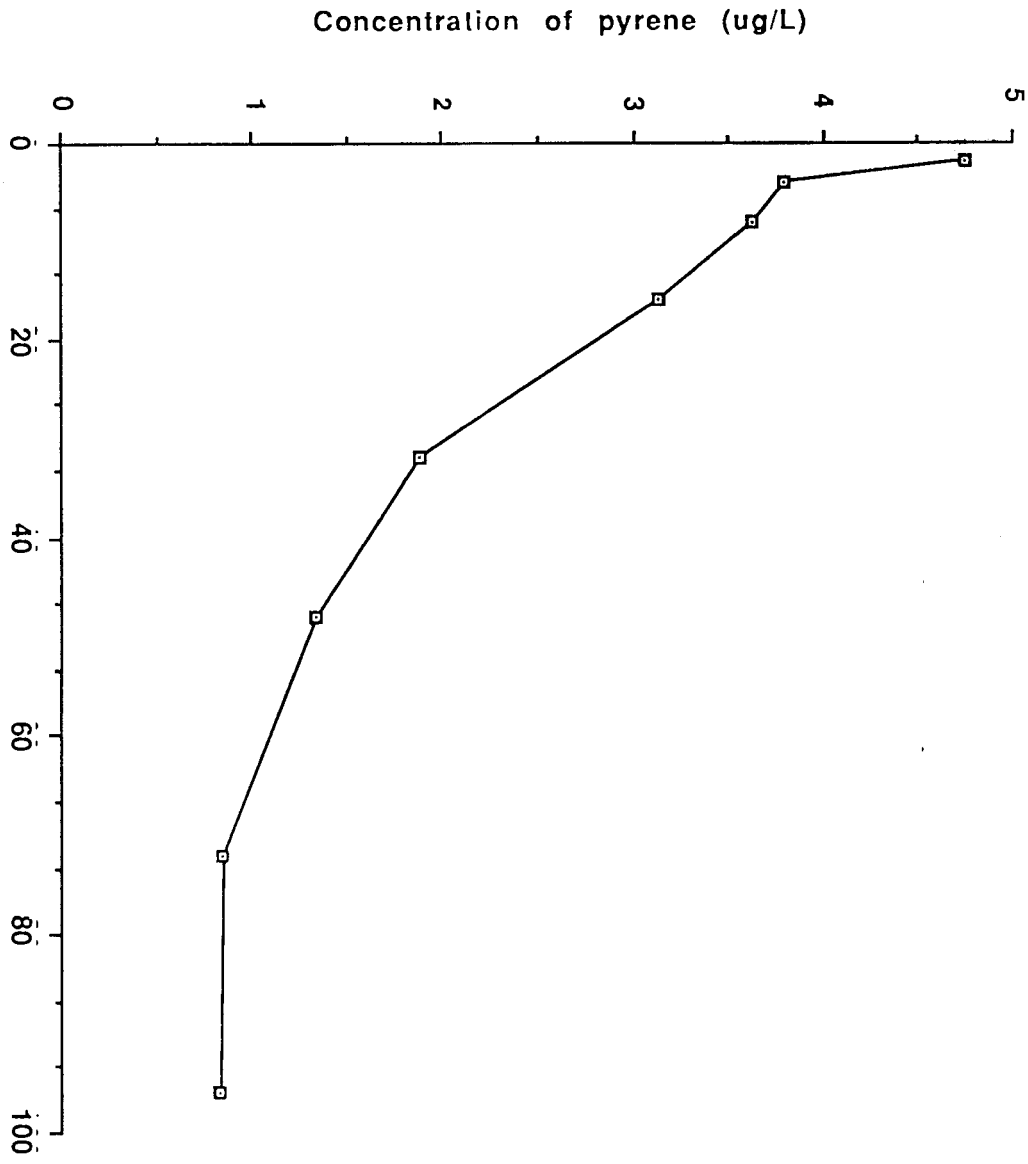

Fig. 5. Concentration of pyrene in water after period of time exposure

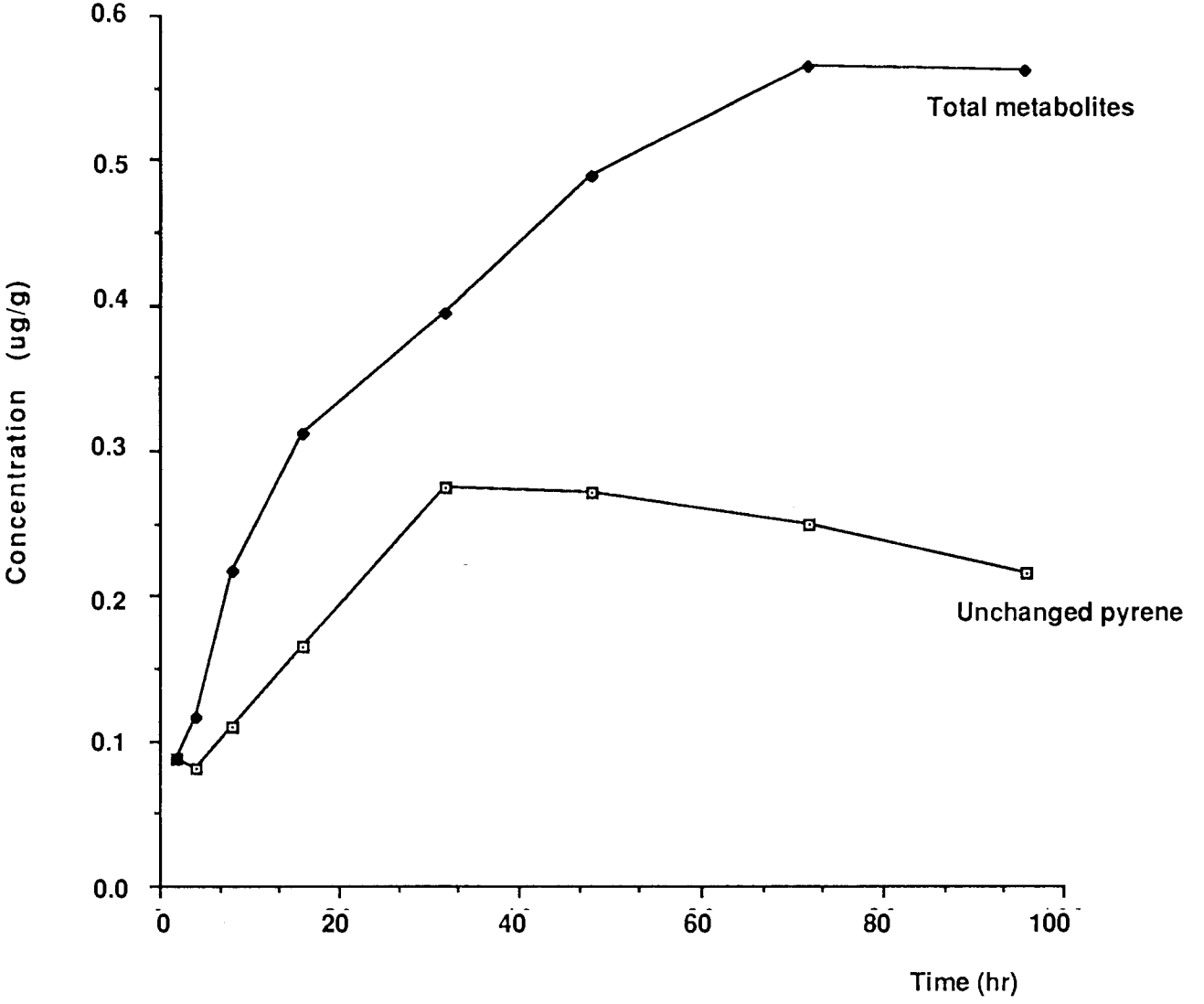

Fig. 6. Concentration of unchanged pyrene in fish and total metabolites formed after exposure

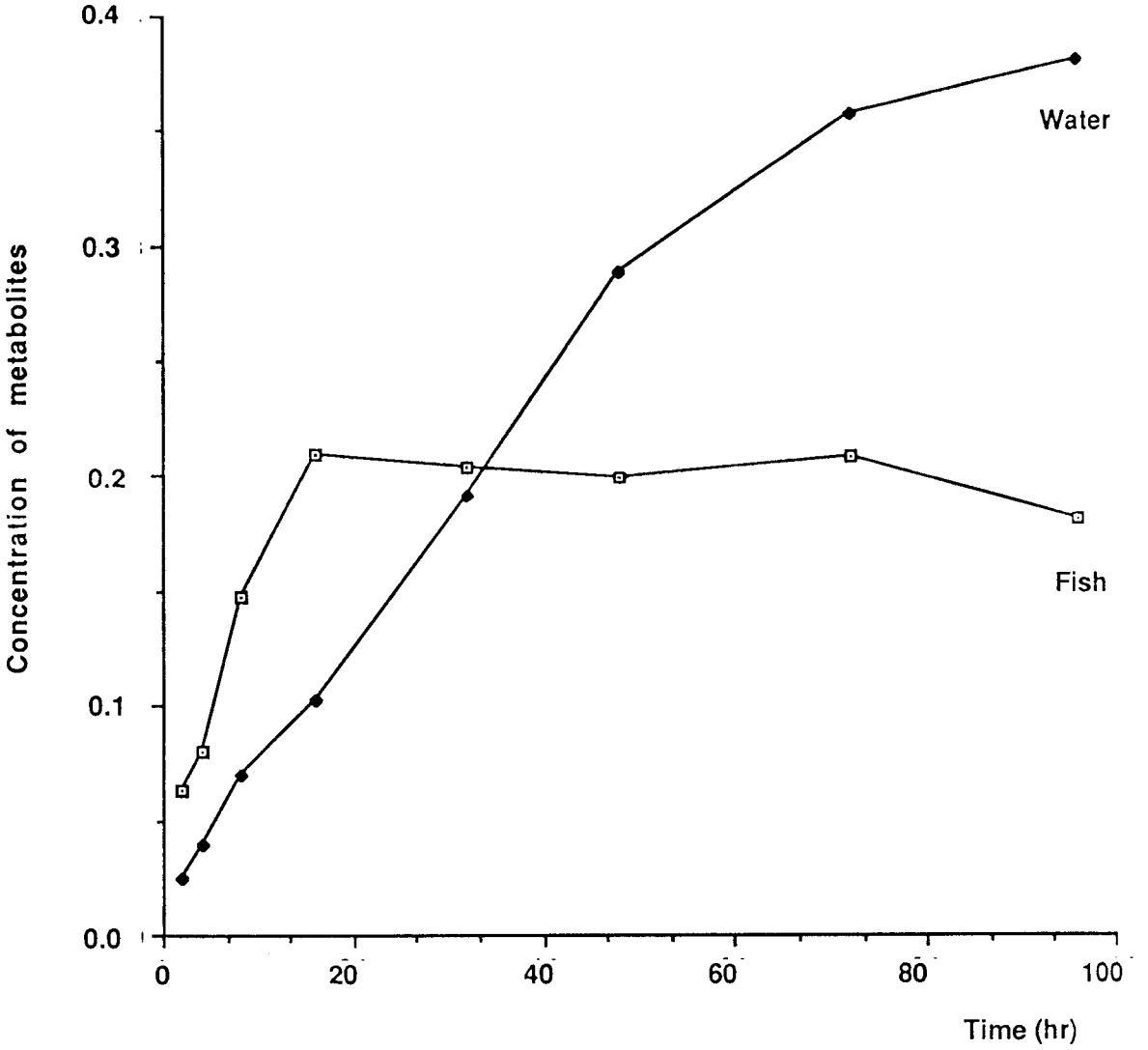

Fig. 7. Metabolites distribution in fish (ug/g) and water (ug/L) after period of time exposure

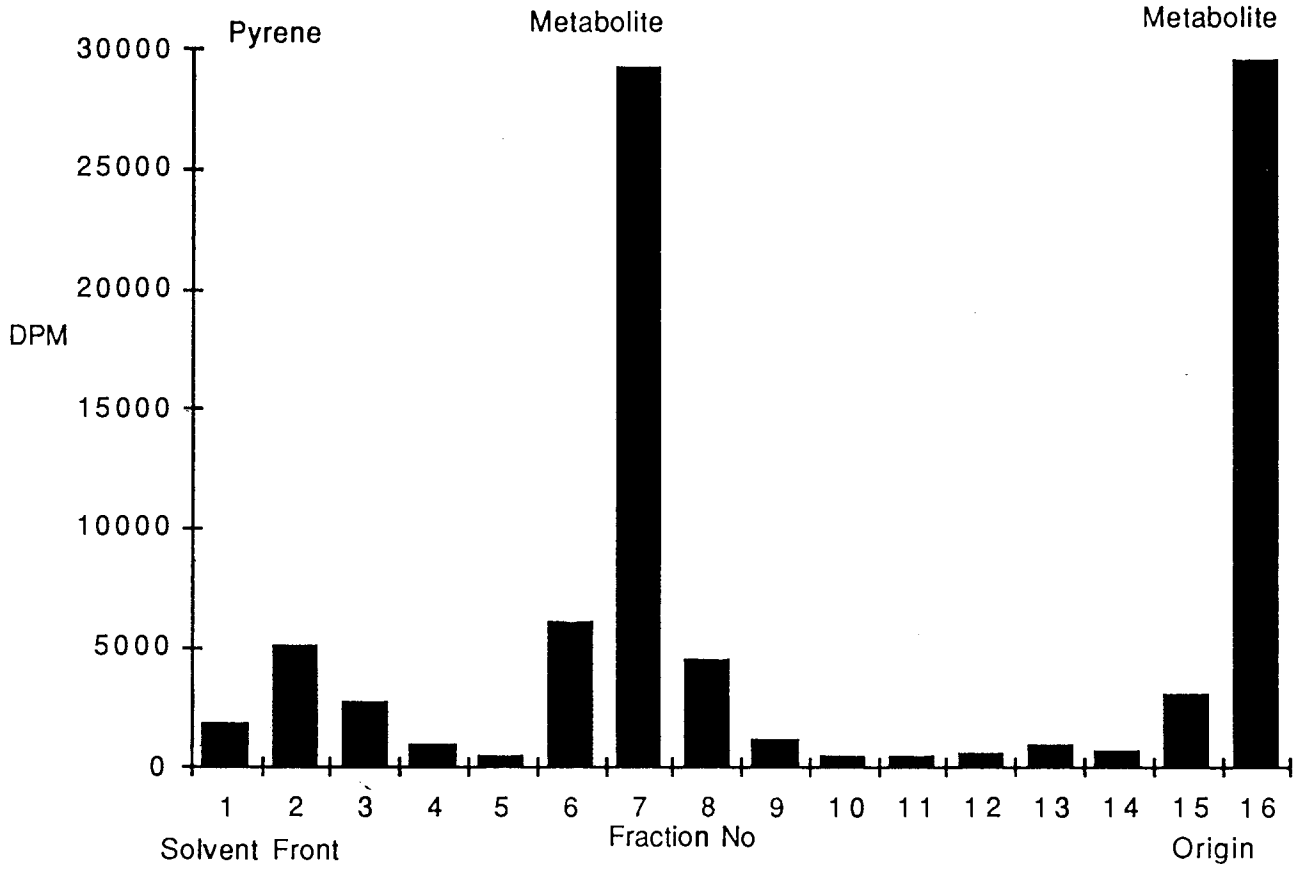

Fig. 8. TLC analysis of metabolites in fish after 16 hours of exposure

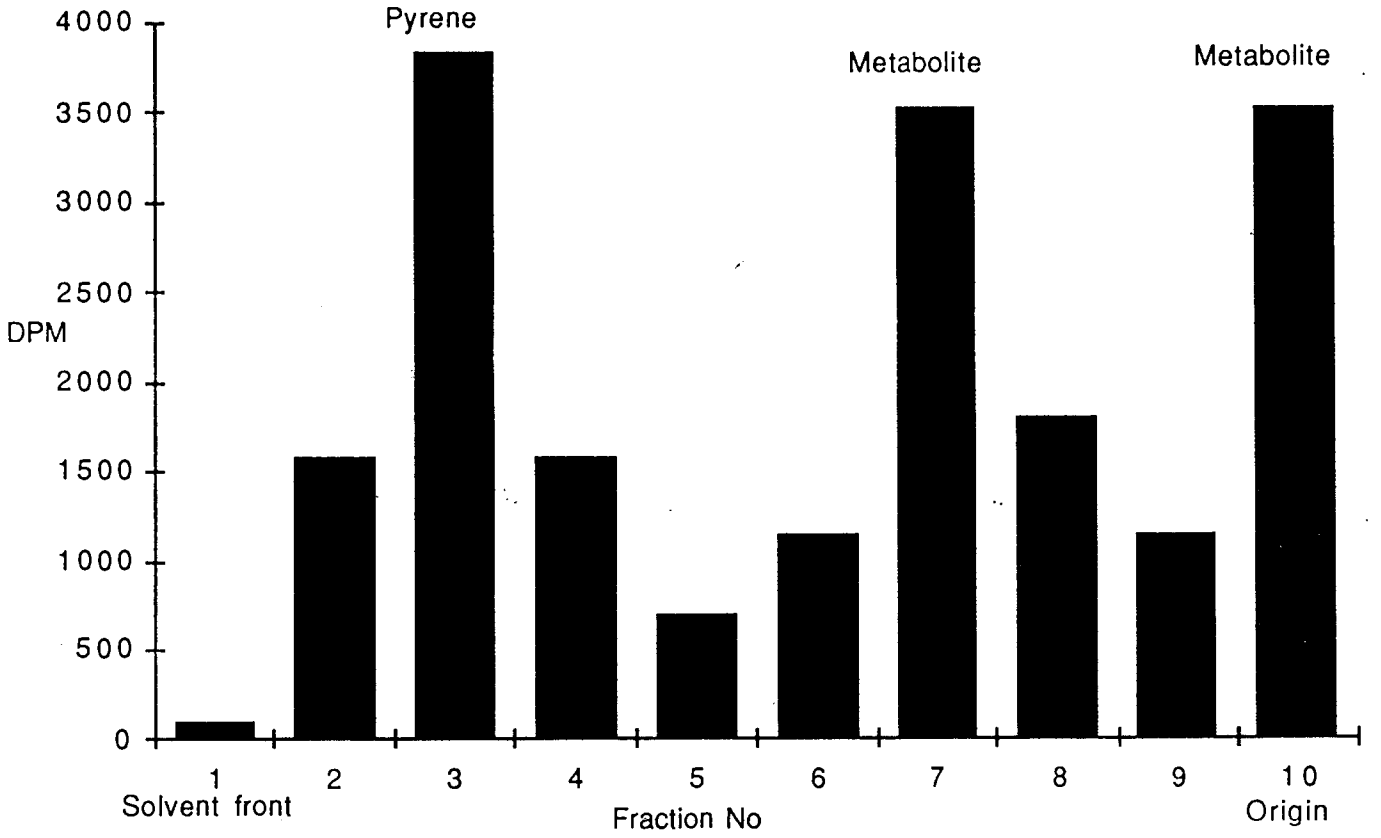

Fig. 9. TLC analysis of metabolites in water after 16 hours of exposure

5. Depuration of 14C-pyrene

Figure 10 and table 7 shows the time course of depuration of accumulated radioactivity from the fish after a 24 hrs exposure. The rate of 14C-pyrene depuration from fish was rapid in the initial 24 hrs. It levelled off after about 72 hrs in clean water. About 15.2 % of accumulated 14C-pyrene was eliminated in 6 days after the fish were transfered to clean water.

II. Plant

1. Uptake of 14C-pyrene

Figure 11, 12 and table 8, 9 show the time course of 14C-pyrene uptake by water-hyacinth at different exposure conditions. Uptake of pyrene by the plant was very rapid in the first 8 hrs of exposure. But the uptake rate began to level off after the plant was exposed to pyrene for more than 8 hrs. The uptake rates were affected by exposure temperature and pH. At pH 6, a higher uptake rate of was found in water-hyacinth exposed to pyrene at 30 °C than those exposed at 20 °C.

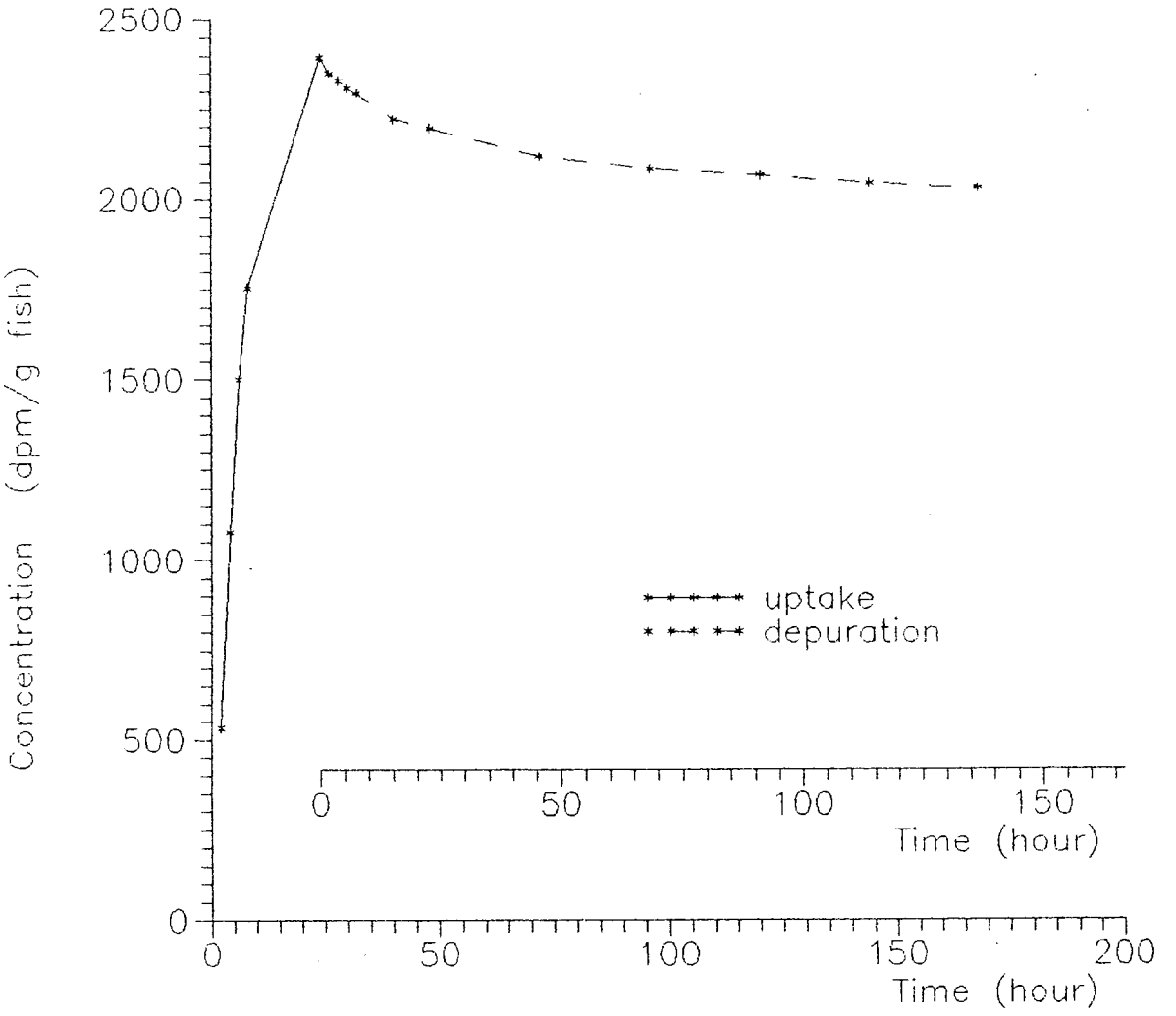

Fig. 10. Time course of radioactivity uptake and depuration by rainbow trout.

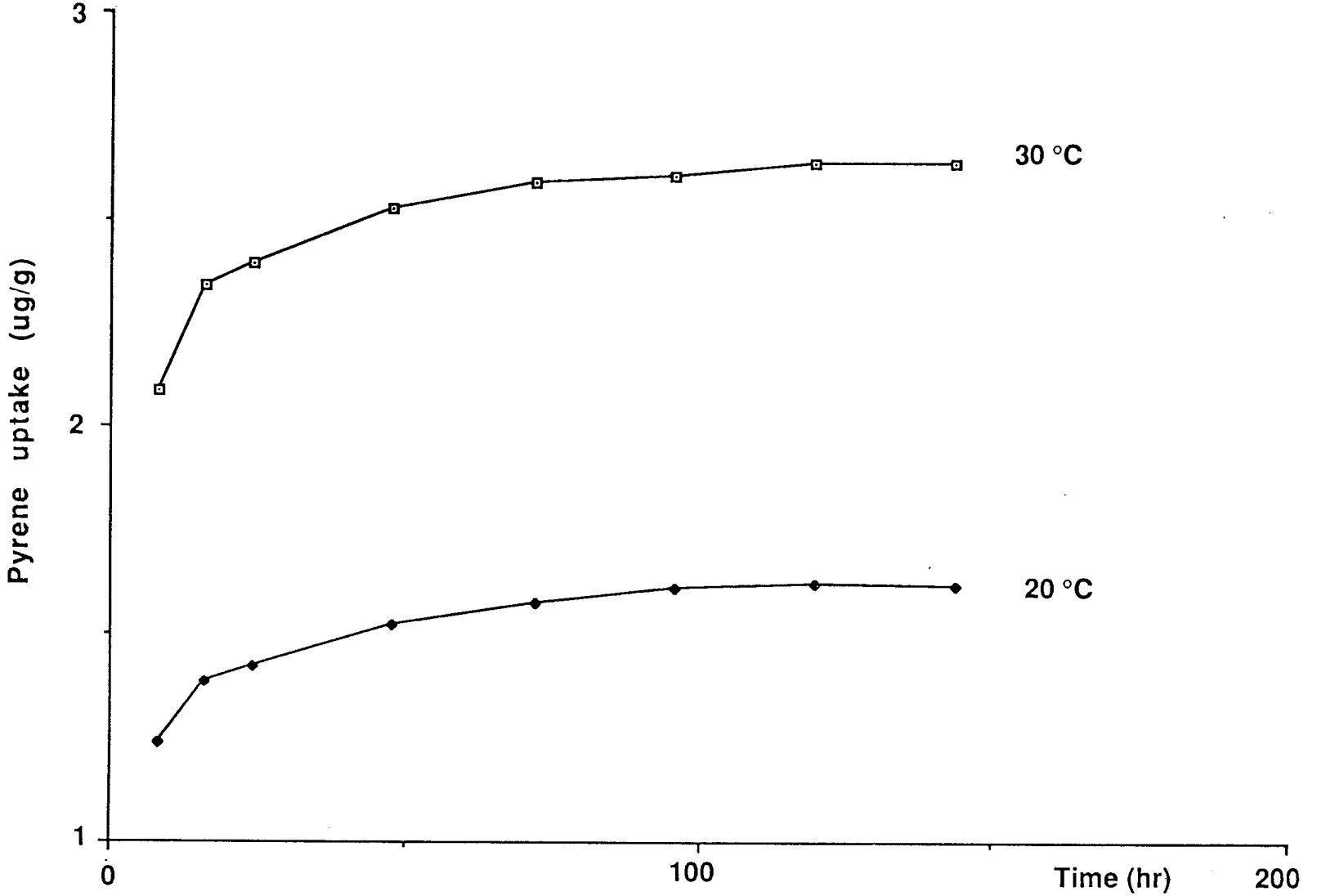

Fig. 11. Time course of pyrene uptake by water-hyacinth exposed to pH 6, at temperature 20 °C and 30 °C

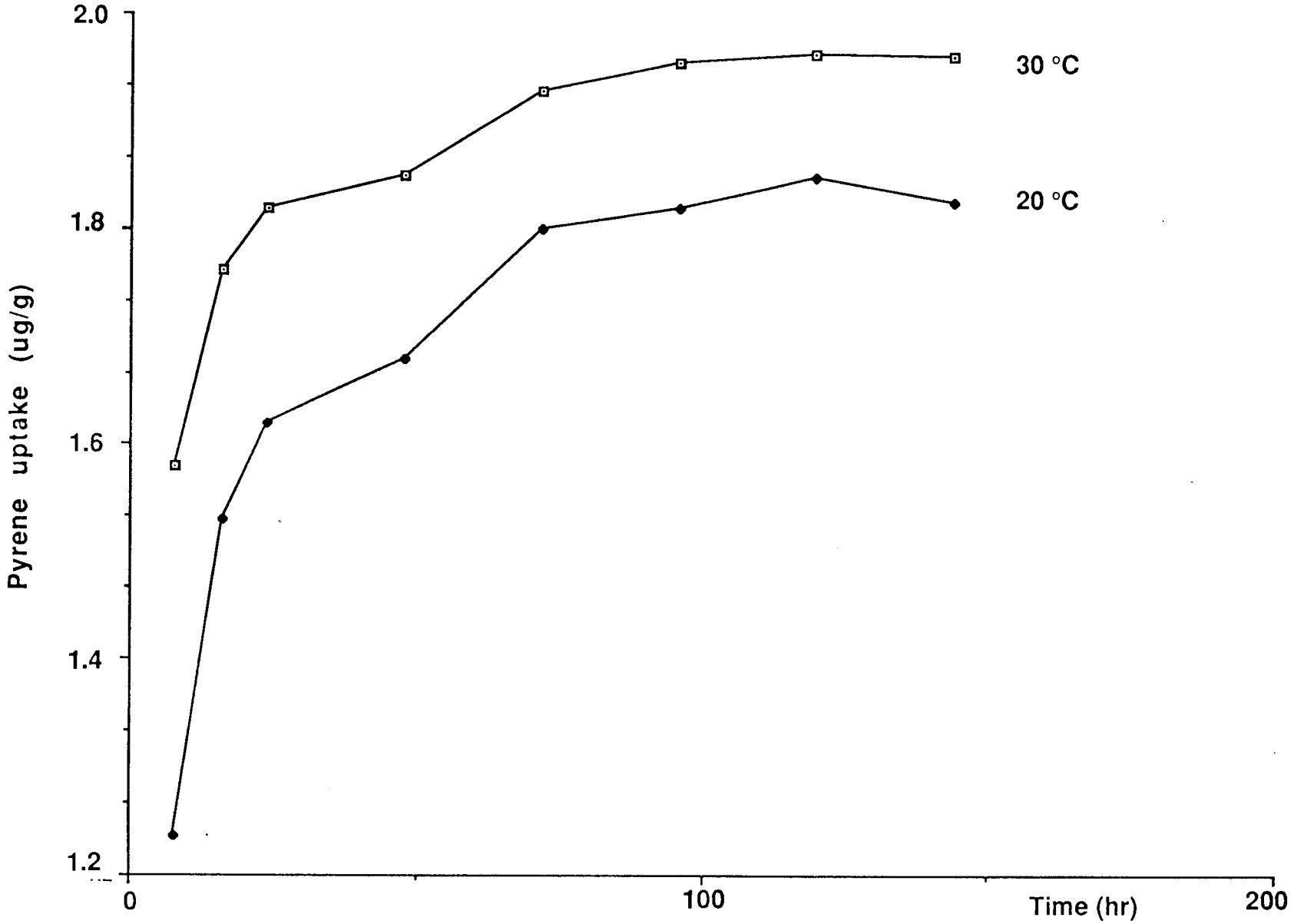

Fig. 12. Time course of pyrene uptake by water-hyacinth exposed to pH 8, at temperature 20 °C and 30 °C

2. Tissues distribution of 14C-pyrene in water-hyacinth

Distribution of radioactivity in water-hyacinth exposed to 14C-pyrene at 30 °C is presented in figure 13,14 and table10,11. More than 95 % of the absorbed radioactivity was found in the root, although a small amount of 14C was found in the stem and leaf. A similar pattern of radioactivity distribution was found in water-hyacinth tissues following exposure at pH 6 or pH 8.

Figure 13 and table 10 shows the radioactivity distribution in water-hyacinth tissues exposed to 14C-pyrene at 20 °C. 14C-pyrene distribution in water-hyacinth is very similar to that found in figure 14 and table 11.

3. Depuration of 14C-pyrene

Figure15 and table12 shows the depuration of accumulated radioactivity by water-hyacinth after a120 hrs exposure. Water-hyacinth eliminated about 2.9 % of the absorbed radioactivity after being transferred to pyrene-free water for 4 hrs. At the end of a144 hrs depuration period, only 4.28 % of the accumulated 14C-pyrene was eliminated. The plant did not show any sign of deterioration at the conclusion the experiment.

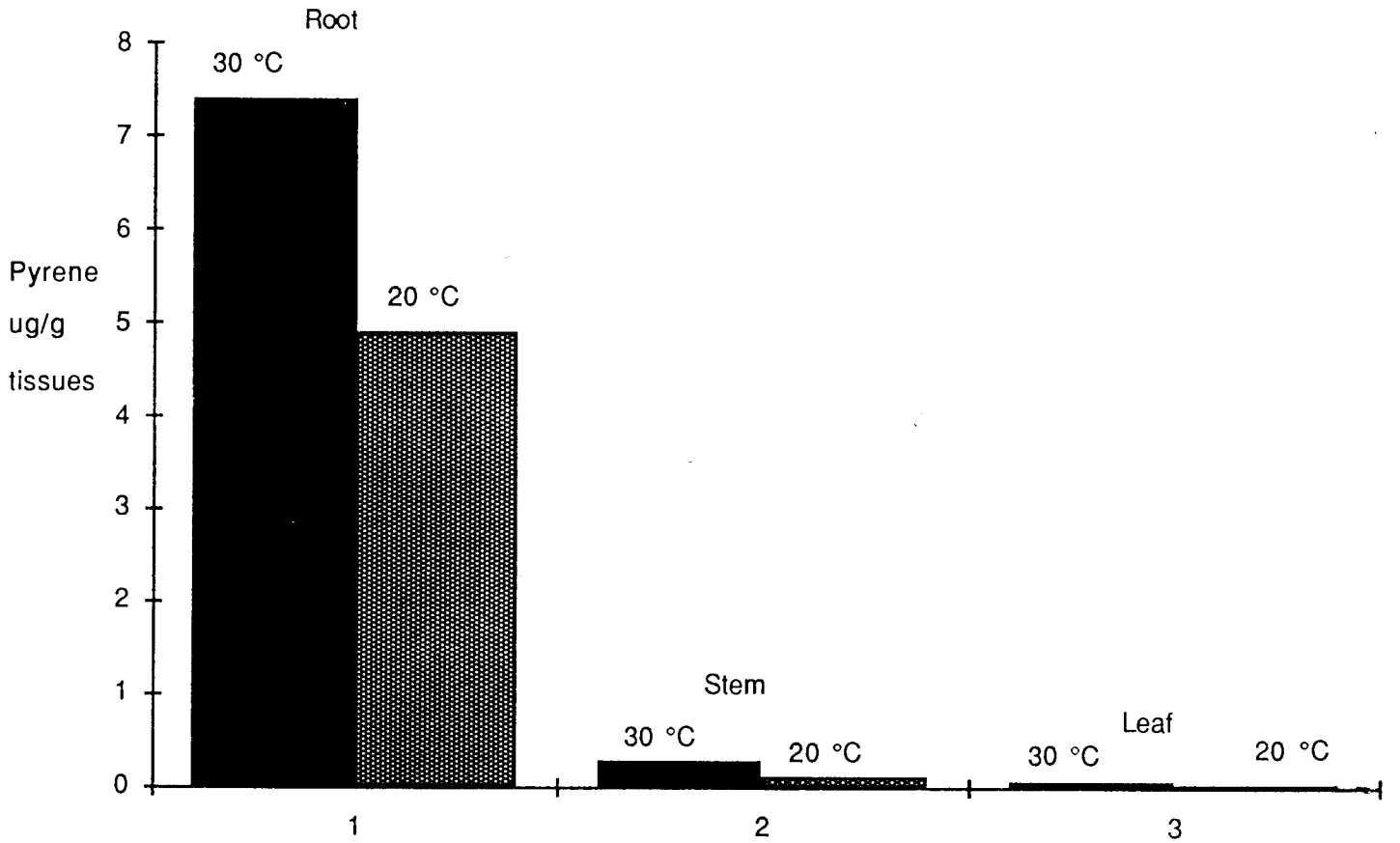

Fig. 13. Distribution of 14C-pyrene in the tissues of water-hyacinth exposed to pH 6, at temperature 20 °C and 30 °C

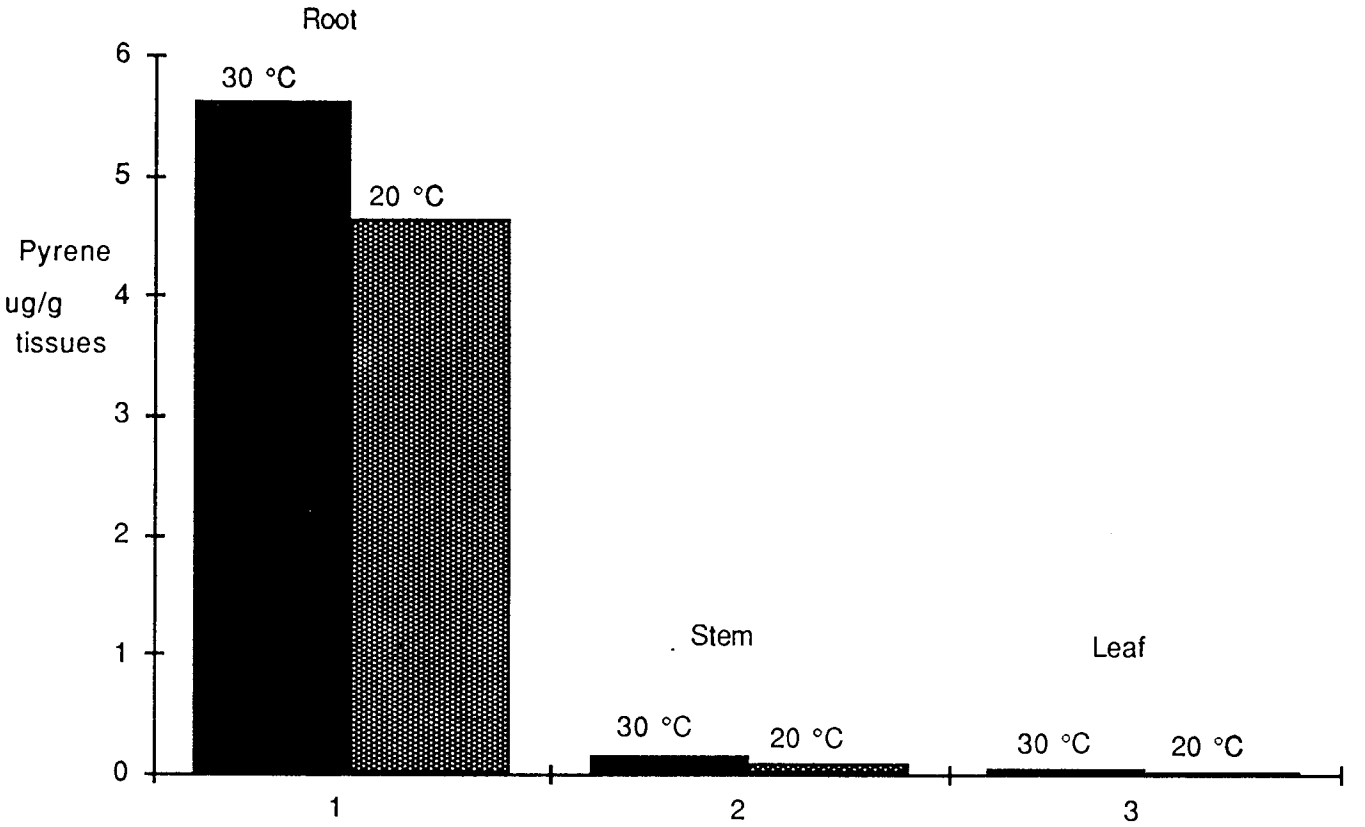

Fig. 14. Distribution of 14C-pyrene in the tissues of water-hyacinth exposed to pH 8, at temperature 20 °C and 30 °C

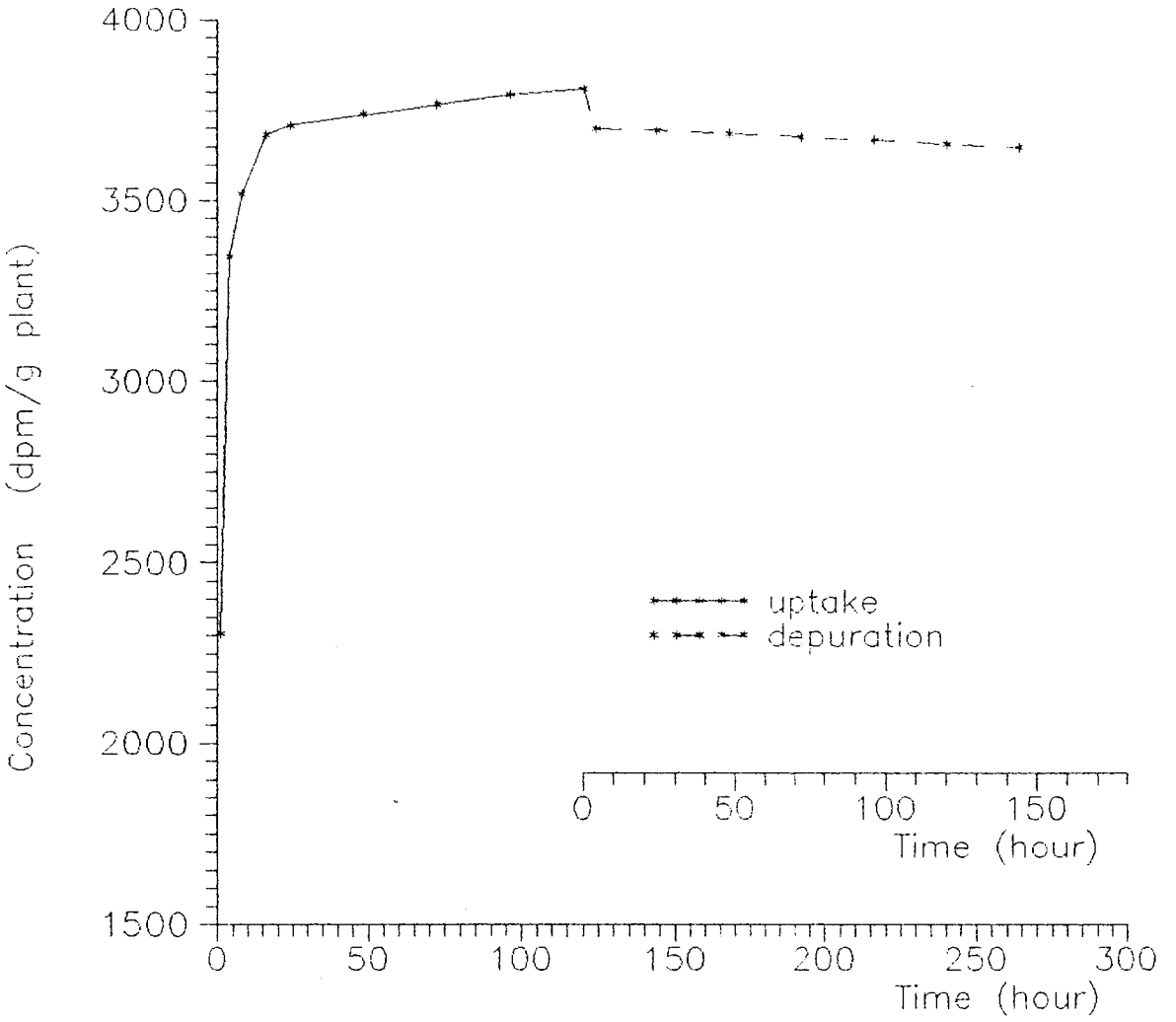

Fig. 15. Time course of radioactivity uptake and depuration by water-hyacinth.

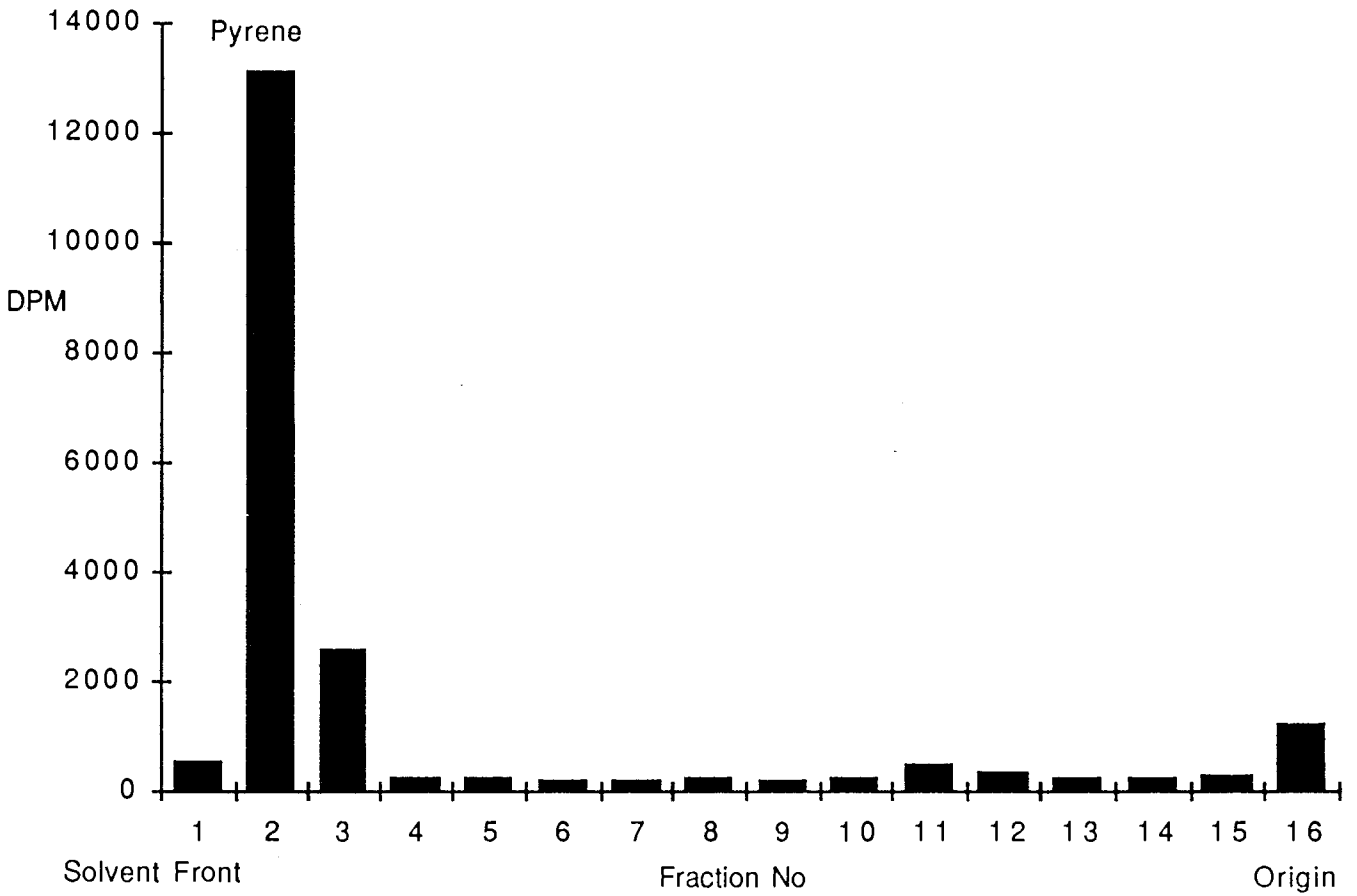

Fig. 16. TLC analysis of metabolites in root of water-hyacinth after 144 hrs of exposure

PART D

DISCUSSION

D. Discussion

Results of the present study show that pyrene is taken up rapidly by rainbow trout from water. Losses of 14C-pyrene due to adsorption onto the wall of the Erlenmeyer flask and evaporation were small (<5 %) and should not affect significantly the dynamics of pyrene in the system.

The rate of 14C-pyrene uptake by trout increases with increasing exposure temperature (Fig. 1 and 2). Dejours (1981) has reported that as water temperature increases, the oxygen demand of aquatic organisms increases, since aerobic metabolism increases and oxygen solubility in water decreases with increasing temperature. Consequently, the ventilation rate and volume of the water passing through the gill of fish are increased, resulting in a higher uptake rate of xenobiotics by the gill. Feuga (1978) also has demonstrated that oxygen consumption by small rainbow trout at 6 °C and at16 °C are 100 and 450 mg O_2. per kg of fish per hour, respectively. He concluded that the uptake of organic pollutants by smaller fish increases with exposure temperature.

In the present study, the Q10 values of 14C-pyrene uptake by trout from 6 °C to 16 °C was high at the beginning of the exposure period (up to 2 - 3 fold for a 10 °C increase in temperature), but was decreased to 1 - 2 fold in 24 hrs. A Q10 value refers to the ratio of a physiological or biochemical rate at T + 10 °C to the rate at T, or the fold-increase in rate for a 10 °C increase in

temperature. A possible explanation for a higher rate of pyrene absorption in the first 2 hrs of exposure may be due to an initially higher level of metabolism, the "overshoot" (Cairn, J. Jr., 1975), which is caused by an acute temperature change. Since fish tend to swim more rapidly after transferring and handling, both gill blood flow and gill ventilation rate may increase, producing a temporary increase of Q10 value. The ventilation rate gradually decreases with time following acclimation to the new temperature.

At pH 6, the Q10 value of 14C-pyrene uptake by trout (1.5) is very close to the reported value of 1.7 for a temperature change of 12 °C (Feuga, 1978). However, at pH 8, the Q10 was 1.1 which is lower than that at pH 6. Probably due to the physiological variability of fish since gill blood flow has been shown to range from 0.3 to 6 ml. h^{-1}. g. fish (Goldstein, et. al.,1964.).

Pyrene uptake by rainbow trout at 16 °C water temperature is not affected significantly by pH change from 6 to 8 although the steady-state level of pyrene in trout is a higher at pH 6. A water pH range between 5 - 9 is considered safe for fish (EIFAC, 1969), although differences in the chemical constituents of the water and sensitive fish species may modify the potential hazard of toxicants within this range of pH values. For example nickelocyanide complex is 500 times more toxic at pH 7 than at 8, and ammonia is one tenth as toxic at pH 7 as at 8. Daye et. al. (1976) and Alabaster (1980) reported that toxic action of hydrogen-ions and hydroxyl-ions could

destruct the gill mucus and gill epithelium causing death by suffocation, particularly at extreme pH values below 5 and above 9.

The distribution data show that fish exposed at 6 °C has a higher concentration of pyrene in the tissues than those exposed at 16 °C. This confirm the finding Jiminez (1987) that a decrease in exposure temperature decreases metabolic rates and increases the retention of organic pollutants in fish.

The pyrene concentration was decreased in liver and increased in intestine at a higher exposure temperature. This may be explanable by an increase in the hepatic rate of pyrene detoxification and release of pyrene metabolites into the intestine (Niimi, et. al., 1985). In the present study low concentrations of pyrene were found in the brains of trout. Similar observations were reported for benzo(a)pyrene (Spacie, et. al., 1983) and for toluene and naphtalene (Thomas, et. al. , 1981).

At least two major pyrene metabolites were identified in fish and water. Although the metabolites were not characterized chemically, they probably were similar to those reported in the rat (Boyland, et. al., 1964, Simm, 1970 and Yang, et. al., 1988). These authors reported that pyrene is converted by rats to 1-hydroxypyrene, 4,5-epoxy-4,5-dihydroxypyrene, 1,6 and trans-4,5-dihydro-4,5-dihydroxypyrene and 4,5-dihydro-4hydroxy-5-pyrenil (Appendix 4).

My results show that about 15.2 % of the accumulated 14C-pyrene was eliminated by the trout in 6 days after they were transferred to clean water. 14C-pyrene is eliminated slowly by trout since low exposure temperature causes a decrease in metabolism and an increase in half-life of pyrene in fish tissues. Other PAHs such as benz(a)pyrene, anthracene, and azarane are also eliminated slowly (< 4 %) from trout (Thomas, et. al. , 1982 ; Varanasi, et. al., 1981 and Southworth, et. al., 1981). Our results confirm these findings.

My results show that 14C-pyrene is taken up by the root of water-hyacinth rapidly during the first 8 hrs of exposure. This may be explanable by the morphological and physiological characteristics of the root system. Water-hyacinth roots are hairy, adventitious, fibrous and unbranched. Thereforelarge and capable of absorbing chemicals extensively. A similar finding has been reported by Reiners, et. al. (1988) on the absorption of 14C-thiobencarb from solution by lettuce. Water-hyacinth has a high transpiration rate; it loses water rapidly. The high transpiration rate may be explained by the number and size of its stomata, which are responsible for regulating the transpiration process of the plant. Penfound and Earle (1946) reported that the stomata are occur on both surfaces of water-hyacinth leaves with a frequency of 120/mm2. They also occur with frequency of 20/mm2 on the isthmus and 1/mm2 on the petiole. The size of the stomatal aperture is 12 x 27 u which is about twice as large as those of most other plants. The interstomatal distance is about eight time the pore aperture, which

would not allow overlapping of water vapour diffusion shells which would prevent further water loss to the atmosphere. In a water-hyacinth uptake experiment, Bingham (1977) showed that water-hyacinth could remove 88 % of diphenamid in 4 days. In contrast, waterthread pondweed was less effective in removing this compound from water. He explained this difference by the fact that waterthread pondweed have small floating leaves and low rates of transpiration, and therefore a low uptake rate of toxicant from water. Wolverton, et. al. (1976) studied the ability of water-hyacinth to remove phenol from water at different exposure concentrations and found that the higher the concentration of phenol in water, the higher the uptake rate of water-hyacinth.

My results demonstrate that water-hyacinth had absorbed between 60 to 77 % of14C-pyrene from water at the conclusion of an 8 hrs exposure. The highest uptake rate was found in water-hyacinth exposed to 30 °C at pH 6 (Q10 value 1.63). A similar finding has been described by Hoagland et. al. (1936), Jacobson et. al. (1957) and Overstreet et. al. (1957). They reported a Q10 value of 1.6 for ion uptake over the temperature range 6 - 30 °C. Lange et. al. (1975) also reported the relationship between water transpiration and ion absorption; ion uptake increases with increase in transpiration. This agrees with the observation that temperature affects transpiration and therefore chemical absorption in most plants.

The present study shows that high water pH reduces 14C-pyrene uptake by as much as 26 % in water-hyacinth. This is

consistent with the 50 % reduction in the uptake of phosphate and nitrate at a higher pH reported by Honert and Hooyman (1955).

In the present study, more than 96 % of 14C-pyrene absorbed by water-hyacinth was in the root. Less than 4 % of the absorbed 14C-pyrene was in the shoot system. My results are consistent with the findings of Singh, et. al. (1979) that 2, 4-D was concentrated from culture medium by water-hyacinth root. At the lower exposure temperature, water-hyacinth had a lower amount but higher proportion of 14C-pyrene in shoot system. This indicates that temperature has an important role not only in the uptake process , but also in the transportation of the compound from root to shoot. The effect of temperature on transportation of 14C-pyrene probably is be due to the dilution of 14C-pyrene as a result of an increase in water flow. It may also be due to a high transpiration rate. Similar findings were reported by Cooley, et al. (1979) on the distribution of metals in various parts of water-hyacinth; about 99 %, 77 %, 82 % and 60 % of Fe, Cd, Cu, and Co, respectively are localized in root with the remainder in the stem and the leaf. Pitman (1963) and Hoagland et. al. (1936), proposed that plant cells store ions mainly in the vacuolated tissues of the root cortex which has the capacity to accumulate large amounts of metals in a short period. These vacuolated tissues occupy about 70 % of the tissue volume.

The results show that only 4.28 % of the 14C-pyrene absorbed by water-hyacinth is released back to the culture medium in 6 days. Absorbed radioactivity may be released via root hairs the epidermal

structure andsurounding intercellular space. The excretion product is usually discharged by the disintegration of internal cells. According to Lutge et. al. (1982), excretion products in many plants are eliminated by glands as liphophilic materials such as slime or alkaloids, flavonoids, glycones, fatty oils, and qiunones. Water-hyacinth could produce some amino acids and carbohydrates which it released from the roots in to the water (Sioli, 1968). Secondly, 14C-pyrene might also be released into water due to leaching from the very large outer surface of the root. Also, the absorbed radioactivity could be released from dead root hairs left in the medium.

In contrast to trout studies, no metabolites was found in the water-hyacinth (fig.16 and table 13). Perhaps, this is due to low or a lack of mixed function oxidases activities in water-hyacinth.

CONCLUSION

The present studies show that water temperature and pH affect the uptake and disposition of 14C-pyrene by rainbow trout and water-hyacinth. 14C-pyrene uptake is high in fish and water-hyacinth when they are exposed to high exposure temperature (30 °C) and low pH (pH 6). 14C-pyrene is distributed rapidly in the tissues of trout since high levels of radioactivity in the bile, liver and intestine and low levels of radioactivity are found in gill, muscle and brain. In the water-hyacinth radioactivity is found mainly mostly in root (> 95 %); less than 1 % of the radioactivity is in the leaf.

At least two major pyrene metabolites are formed by trout. The metabolite profile is similar to that of the rat. No pyrene metabolites are found in the water-hyacinth. Elimination of 14C-pyrene by rainbow trout is slow but is 6 fold faster than water-hyacinth.

The mechanism of 14C-pyrene elimination by water-hyacinth remains to be determined since it may be actively eliminated from the inside of the root or simply leach from the root surface.

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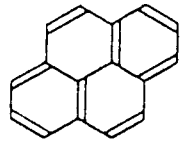
Appendix 1 . Relative Carcinogenicity of PAH to Laboratory Mammals (NAS,1972)

Compound	Carcinogenicity	Compound	Carcinogenicity
Anthracene	-	Aceanthrylene	-
Phenenthrene	-	Cholanthrene	++
7,12-Dimethylbenz(a) anthracene	++++	3-Methylcho-lanthrene	++++
Dibenz(aj)anthracene	+	Naphthacene	-
Dibenz(ah)anthracene	+++	Pyrene	-
Dibenz(ac)anthracene	+	Benzo(a)pyrene	+++
Benzo(a)phenanthrene	+++	Benzo(e)pyrene	-
Flourene	-	Dibenzo(al)pyrene	±
Benzo(a)flourene	-	Dibenzo(ah)pyrene	+++
Benzo(b)flourene	-	Dibenzo(ai)pyrene	+++
Benzo(c)flourene	-	Dibenzo(cd,jk)pyrene	-
Dibenzo(ag)flourene	+	Indeno(1,2,3-cd)pyrene	+
Dibenzo(ah)flourene	±	Chrysene	±
Dibenzo(ac)flourene	±	Dibenzo(b,def)chrysene	++
Flouranthene	-	Dibenzo(def,p)chrysene	+
Benzo(b)flouranthene	++	Anthanthrene	-
Benzo(j)flouranthene	++	Perylene	-
Benzo(k)flouranthene	-	Benzo(ghi)perylene	-
Benzo(mno)flouranthene	-	Coronene	-

Notes: a_,not carcinogenic; ± uncertain or weakly carcinogenic; +, carcinogenic;++,+++, ++++, strongly carcinogenic.

Appendix 2. Physical and Chemical Properties of Pyrene (Py)

Molecular Structure	
Molecular Formula	C16 H10
Molecular Weight	202.2
Solubility in water (25 °C)	129 - 175 ug/l
Melting Point (°C)	156
Boiling Point (°C)	404
Density g.ml-1	1.271
Vapor Pressure (25 °C) k Pa	9.1 x 10 -8
Saturation Vapor concentration ng.m-3 (25 °C)	7.4 x 10 -4

Appendix 3. Concentration of Pyrene from Different Aquatic Sources

S o u r c e	Concentration (ug/l)	References
Domestic effluent	1763	(After Borneff and Kunte, 1965)
Common sewage	254 - 16050	(,,)
Industrial sewage	2560 - 3120	(,,)
Crude oils	3500 - 41000	(Pancirof and Brown, 1975)
Refinery	106	(After Searl et al., 1979)

Appendix 4 . Metabolic pathways of pyrene in rats

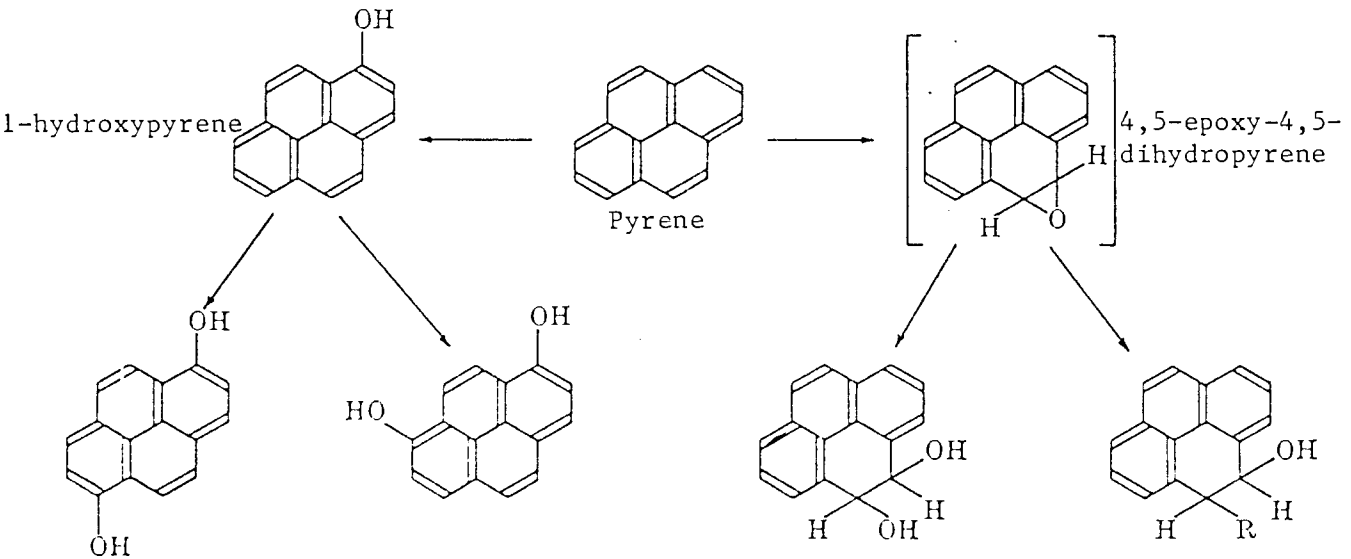


Table 1. Time course of radioactivity uptake (dpm) by rainbow trout exposed to 6 °C or 16 °C water temperature at pH 6 or pH 8

Time (hr)	pH 6		pH 8	
	6 °C	16 °C	6 °C	16 °C
2	470 ± 156*	1535 ± 160*	723 ± 92*	1294 ± 94*
4	738 ± 95*	2252 ± 229*	1044 ± 92*	2304 ± 172*
6	924 ± 106*	2500 ± 316*	1351 ± 84*	2514 ± 218*
8	1183 ± 54*	2874 ± 302*	1531 ± 89*	2605 ± 210*
24	2104 ± 126*	3129 ± 318*	2533 ± 28	2663 ± 268

- Values are the mean ± S.E of 3 fish (dpm)
- * Significantly different between 6 °C and 16 °C ($p<0.05$)

Table 2. Distribution of radioactivity in the tissues of rainbow trout exposed to 6 °C water temperature at pH 6 or pH 8

Tissues	pH 6		pH 8	
	% body burden	ug/g tissue	% body burden	ug/g tissue
Gill	2.86 ± 0.69	0.656 ± 0.069	2.33 ± 0.22	0.610 ± 0.116
Muscle	2.56 ± 2.36	0.587 ± 0.314	0.94 ± 0.14	0.257 ± 0.088
Liver	32.11 ± 10.49	7.364 ± 2.099	29.23 ± 8.47	8.293 ± 4.050
Stomach	4.29 ± 0.75	0.983 ± 0.101	5.45 ± 1.65	1.427 ± 0.479
Intestine	13.75 ± 4.25*	3.152 ± 0.394	24.18 ± 9.10*	6.266 ± 2.613
Skin	12.46 ± 3.02	2.857 ± 0.738	10.50 ± 5.31	2.479 ± 0.640
Kidney	14.12 ± 1.42	3.238 ± 0.205	12.17 ± 0.70	3.186 ± 0.584
Swimbladder	15.71 ± 1.37	3.600 ± 0.183	14.13 ± 7.90	5.670 ± 2.039
Brain	2.14 ± 0.89	0.491 ± 0.137	1.07 ± 0.10	0.276 ± 0.044

- Values are the mean ± S.E of 3 fish
- * Significantly different between pH 6 and pH 8 ($p<0.05$)

Table 3. Distribution of radioactivity in the tissues of rainbow trout exposed to 16 °C water temperature at pH 6 or pH 8

Tissues	pH 6		pH 8	
	% body burden	ug/g tissue	% body burden	ug/g tissue
Gill	1.69 ± 0.50	0.435 ± 0.080	2.19 ± 1.88	0.365 ± 0.267
Muscle	1.32 ± 0.21	0.340 ± 0.036	1.37 ± 0.71	0.221 ± 0.067
Liver	20.25 ± 5.53	5.195 ± 0.802	25.80 ± 14.84	4.619 ± 2.356
Stomach	7.19 ± 3.25	1.824 ± 0.545	2.35 ± 1.57	0.365 ± 0.136
Intestine	37.69 ± 12.00	9.961 ± 3.045	39.50 ± 10.90	6.891 ± 2.561
Skin	7.74 ± 0.66	2.006 ± 0.170	5.66 ± 1.24	0.949 ± 0.170
Kidney	7.88 ± 0.65	2.049 ± 0.188	6.85 ± 4.48	1.068 ± 0.380
Swimbladder	12.18 ± 1.91	3.150 ± 0.279	14.05 ± 7.18	2.222 ± 0.602
Brain	4.06 ± 0.37	1.064 ± 0.147	2.13 ± 1.42	0.338 ± 0.121

- Values are the mean ± S.E of 3 fish

Table 4. Concentration of unchanged pyrene in water and fish and total metabolites formed after exposure

Time (hr)	Water (ug/l)	Fish (ug/gr)	Metabolites (ug/gr)
2	4.748 ± 0.188	0.087 ± 0.036	0.088 ± 0.015
4	3.792 ± 0.183	0.081 ± 0.007	0.117 ± 0.022
8	3.630 ± 0.189	0.110 ± 0.013	0.218 ± 0.073
16	3.127 ± 0.494	0.166 ± 0.054	0.312 ± 0.045
32	1.876 ± 0.282	0.275 ± 0.071	0.395 ± 0.076
48	1.333 ± 0.123	0.272 ± 0.121	0.488 ± 0.123
72	0.841 ± 0.208	0.250 ± 0.042	0.565 ± 0.072
96	0.831 ± 0.147	0.216 ± 0.065	0.562 ± 0.084

- Values are the mean ± S.E of 4 fish

Table 5. Metabolites distribution in fish and water after exposure

Time (hr)	Metabolite in fish (ug/g)	Metabolite in water (ug/l)
2	0.063 ± 0.013	0.025 ± 0.007
4	0.080 ± 0.015	0.039 ± 0.014
8	0.148 ± 0.055	0.070 ± 0.020
16	0.210 ± 0.049	0.102 ± 0.040
32	0.204 ± 0.059	0.191 ± 0.030
48	0.199 ± 0.041	0.289 ± 0.079
72	0.208 ± 0.052	0.357 ± 0.056
96	0.181 ± 0.046	0.381 ± 0.025

- Values are the mean ± S.E of 4 fish

Table 6. TLC analysis of metabolites in fish and water after 16 hrs of exposure (dpm/spot)

Fraction No.	Fish	Water
1	1796	102
2	5034	1574
3	2709	3829
4	961	1572
5	472	700
6	6048	1135
7	29216	3520
8	4546	1797
9	1114	1143
10	439	3515
11	423	____
12	595	
13	898	
14	645	
15	3099	
16	29555	

Table 7. Time course of 14C-pyrene uptake and depuration for rainbow trout

Time (hr)	Uptake (ug/g fish)	Time (hr)	Depuration (ug/g fish)
2	0.176 ± 0.037	2	0.015 ± 0.003
4	0.356 ± 0.003	4	0.021 ± 0.004
6	0.495 ± 0.054	6	0.028 ± 0.007
8	0.579 ± 0.029	8	0.033 ± 0.008
24	0.790 ± 0.105	16	0.057 ± 0.012
		24	0.066 ± 0.015
		48	0.091 ± 0.012
		72	0.103 ± 0.014
		96	0.108 ± 0.014
		120	0.116 ± 0.015
		144	0.120 ± 0.016

- Values are the mean ± S.E of 3 fish

Table 8. Time course of 14C-pyrene uptake for water-hyacinth exposed to 30 °C, at pH 6 and pH 8

Time (hr)	pH 6 (ug/ g tissue)	pH 8 (ug/g tissue)
8	2.088 ± 0.170*	1.580 ± 0.306*
16	2.340 ± 0.183*	1.762 ± 0.310*
24	2.392 ± 0.187*	1.818 ± 0.299*
72	2.590 ± 0.205*	1.928 ± 0.322*
96	2.606 ± 0.205*	1.954 ± 0.333*
120	2.635 ± 0.212*	1.962 ± 0.330*
144	2.638 ± 0.211*	1.960 ± 0.339*

- Values are the mean ± S.E. of 4 plants

- *Significantly different between pH 6 and pH 8 ($p<0.05$)

Table 9. Time course of 14C-pyrene uptake for water-hyacinth exposed to 20 °C at pH 6 and pH 8

Time (hr)	pH 6 (ug/g tissue)	pH 8 (ug/g tissue)
8	1.236 ± 0.262	1.236 ± 0.158
16	1.383 ± 0.251	1.530 ± 0.192
24	1.420 ± 0.257	1.620 ± 0.212
48	1.520 ± 0.269	1.680 ± 0.209
72	1.576 ± 0.256	1.800 ± 0.213
96	1.613 ± 0.269	1.818 ± 0.218
120	1.622 ± 0.276	1.847 ± 0.236
144	1.616 ± 0.268	1.825 ± 0.218

- Values are the mean ± S.E. of 4 plants

Table 10. Distribution of radioactivity in the tissues of water-hyacinth exposed to 30 °C, at pH 6 and pH 8

Tissues	pH6	pH 8
Leaf (ug eq. of py./g tissue)	0.053 ± 0.023	0.039 ± 0.033
Leaf (g of dry weight)	0.537 ± 0.040	0.678 ± 0.040
Leaf (% of whole plant)	0.69 ± 0.29	0.67 ± 0.57
Stem (ug eq. of py./g tissue)	0.253 ± 0.069	0.164. ± 0.078
Stem (g of dry weight)	0.879 ± 0.045	1.202 ± 0.186
Stem (% of whole plant)	3.28 ± 0.89 *	2.82 ± 1.34 *
Root (ug eq. of py./g tissue)	7.402 ± 1.744	5.609 ± 0.315
Root (g of dry weight)	0.403 ± 0.076	0.509 ± 0.075
Root (% of whole plant)	96.03 ± 22.62	96.51 ± 5.42

- Values are the mean ± S.E. of 4 plants

- *Significantly different between pH 6 and pH 8 ($p<0.05$)

Table 11. Distribution of radioactivity in the tissues of water-hyacinth exposed to 20°C, at pH 6 and pH 8

Tissues	pH 6	pH 8
Leaf (ug eq. of py./g tissue)	0.022 ± 0.004	0.026 ± 0.007
Leaf (g of dryweight)	0.855 ± 0.147	0.711 ± 0.084
Leaf (% of whole plant)	0.44 ± 0.08	0.59 ± 0.010
Stem (ug eq. of py./g tissue)	0.090 ± 0.080	0.082 ± 0.024
Stem (g of dryweight)	1.351 ± 0.246	1.014 ± 0.130
Stem (% of whole plant)	1.79 ± 1.59	1.88 ± 0.05
Root (ug eq. of py./g tissue)	4.902 ± 0.868	4.621 ± 0.518
Root (g of dry weight)	0.743 ± 0.123	0.682 ± 0.102
Root (% of whole plant)	97.77 ± 17.31	97.53 ± 11.86

- Value are the mean ± S.E. of 4 plants

Table 12. Time course of 14C-pyrene uptake and depuration for water-hyacinth

Time (hr)	Uptake (ug/g plant)	Time (hr)	Depuration (ug/g plant)
1	0.922 ± 0.108	4	0.044 ± 0.008
4	1.338 ± 0.147	24	0.046 ± 0.007
8	1.408 ± 0.152	48	0.050 ± 0.004
16	1.473 ± 0.167	72	0.054 ± 0.006
24	1.483 ± 0.167	96	0.057 ± 0.005
48	1.496 ± 0.173	120	0.062 ± 0.004
72	1.507 ± 0.176	144	0.065 ± 0.007
96	1.517 ± 0.178		
120	1.524 ± 0.178		

- Values are the mean ± S.E. of 4 plants

Table13. TLC analysis of metabolites in root of water-hyacinth after 144 hrs of exposure

Fraction No.	dpm/spot
1	504
2	13095
3	2555
4	218
5	236
6	185
7	164
8	219
9	189
10	240
11	476
12	327
13	243
14	214
15	288
16	1187