# TOXICOKINETIC STUDIES OF CHLORINATED PHENOLS AND POLYCYCLIC AROMATIC HYDROCARBONS IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

by

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THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

in the

Department of

Biological Sciences



Simon Fraser University

February 1990

ISBN 0-315-66166-6

Canada

APPROVAL

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Degree: Doctor of Philosophy

Title of Thesis:

Toxicokinetic studies of chlorinated phenols and polycyclic aromatic hydrocarbons in rainbow trout (Oncorhynchus mykiss)

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Toxicokinetics of Chlorinated Phenols and Polycyclic Aromatic Hydrocarbons in Rainbow Trout (Onchorhynchus mykiss)

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# Abstract

Chlorinated phenols (CPs) and polycyclic aromatic hydrocarbons (PAHs) are widely distributed environmental pollutants. CPs are used both domestically and industrially for a wide range of purposes from the preservation of wood products to disinfectants in cooling systems due to their broad-spectrum biocidal properties. PAHs are released into the environment mainly from the pyrolysis of organic matter, from both natural and anthropogenic sources. Both CPs and PAHs enter the aquatic environment in surface runoff from urban and agricultural lands, industrial and domestic sewage and from direct deposition. The toxicity and fate of four CPs (2,4-dichlorophenol, 2,4,6-trichlorophenol, 2,3,4,6-tetrachlorophenol and pentachlorophenol) and three PAHs (2-methylnapthalene, fluorene and pyrene) were investigated in rainbow trout (*Oncorhynchus mykiss* ) following an intrarterial (i.a.) administration or exposure to one of the chemicals in water.

CPs were acutely toxic to rainbow trout and toxicity increased as the chlorination of the molecule increased. 2-Methylnapthalene was as acutely toxic to trout as the lower chlorinated phenols, however, fluorene and pyrene were virtually nontoxic at the concentrations studied.

The lower chlorinated phenols are eliminated from trout faster than the more lipophilic pentachlorophenol. The disposition of CPs and PAHs in trout following an i.a. administration was best described by a two-compartment and a three-compartment toxicokinetic model, respectively. CPs and PAHs were found in all tissues of trout and the highest levels found were in the liver and fat. CPs were eliminated in the urine and bile

of trout as conjugated metabolites and by the gill as unchanged chemical. PAHs were eliminated mainly in the bile as conjugated metabolites and to a lesser extent in the urine in a conjugated form. The half-lives of PAH elimination in trout were longer than those of CPs.

PAHs were taken up and rapidly metabolized by the isolated hepatocytes of trout. The isolated hepatocytes metabolized PAHs mainly to conjugated metabolites. The hepatocytes produced similar Phase I metabolites of PAHs as those produced by trout *in vivo*. Results indicate that the hepatocyte preparation may be useful in the study of xenobiotic metabolism and toxicity apart from whole animal influences.

# Dedication

This thesis is dedicated solely to my mother, Arlene.

# Acknowledgements

A thesis is not researched or written alone. The contributions of many people are involved in one way or another, be it in knowledge, experience, the lending of their hands, or in emotional support. There are many people who have been involved in this project from conception to delivery and I'd like to make mention of those foremost in my mind.

Firstly, I'd like to thank the late Dr. Peter C. Oloffs and Dr. Glen H. Geen for introducing me to the scientific discipline, environmental toxicology, which has become such a large part of my life. Also, thanks are given to my senior supervisor, Dr. Francis C.P. Law for his guidance and experience in all stages of this research and for his patience in certain 'atypical' work habits. Dr. B.A. McKeown is thanked for suggestions and encouragements. I am especially grateful to Dr. Tony P. Farrell for not only for his scientific suggestions and his critical reading of the thesis, but also for his humor, friendship and for imparting to me on many occasions, a glimpse of the 'big picture'.

I must thank Dick Joel at the animal care facility, everyone involved in the radioisotope lab and Carole Conlin for the use of her Macintosh in times of critical need. Thanks also goes to Dr. Francios Lallier, who instructed me on the wonders of Apple and in undoubtedly shaving months off my indenture.

Special thanks to Donna McKinnon for her technical assistance as well as for lending me that talented hand of hers in the illustrations in both my thesis and slides.

I am grateful for the support of many friends during all of this work, and in particular the L. Ledge Group, Dr. Rusty Sweeting, Jeff Johansen, John Keen and Adam

Lewis. Paul Bussanich, Lloyd Schulman and Jeff Silberman are acknowledged for continually reminding me that productivity always increases the day after a night out.

I have to thank my family for their support, especially my mother, who knew it would be done and had faith all along. And, last but certainly not least, I am grateful to Lara, for seeing it all through and bearing with me while this work was being completed, always encouraging and endlessly patient.

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# List of Abbreviations

| | |
|---|---|
| 2-MN | 2-methylnapthalene |
| AIC | Akaike's information criterion |
| BaP | benzo(a)pyrene |
| CDE | chlorinated diphenyl ether |
| Ci | curie |
| $Q_b$ | total body clearance |
| CP | chlorinated phenol |
| DCP | dichlorophenol |
| dpms | disintegrations per minute |
| EDTA | ethylenediamine-tetraacetic acid |
| g | gram |
| GC | gas chromatography |
| h | hour |
| HEPES | N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid |
| HPLC | high-performance liquid chromatography |
| i.a. | intraarterial |
| i.d. | inside diameter |
| i.p. | intraperitoneal |
| L | liter |
| $LC_{50}$ | median lethal concentration |
| LSC | liquid scintillation counting |

| | |
|---|---|
| M | molar |
| MFO | mixed-function oxidase |
| min | minute |
| MS 222 | 3-aminobenzoic acid ethyl ester |
| o.d. | outside diameter |
| PAH | polycyclic aromatic hydrocarbon |
| PCDD | polychlorinated dibenzo-dioxin |
| PCDF | polychlorinated dibenzo-furan |
| PCP | pentachlorophenol |
| ppm | parts per million |
| S.D. | standard deviation |
| TCP | trichlorophenol |
| TTCP | tetrachlorophenol |
| UV | ultraviolet |
| v/v | volume to volume |
| Vc | volume of central compartment |
| Vd | volume of distribution |
| w/v | weight to volume |
| WSSR | weighted sum of squares residuals |
| *g* | acceleration of gravity |

prefixes for units of measurement:

| | |
|---|---|
| p | pico $(10^{-12})$ |
| n | nano $(10^{-9})$ |
| μ | micro $(10^{-6})$ |
| m | milli $(10^{-3})$ |
| k | kilo $(10^{3})$ |

# Part I General Introduction

There is an ever increasing concern regarding the possible harmful effects to man and to other organisms of both synthetic and naturally occurring pollutants which enter aquatic ecosystems. Two groups of chemicals, namely chlorinated phenols (CPs) and the polycyclic aromatic hydrocarbons (PAHs) are present in the aquatic environment and are recognized as being harmful to many aquatic organisms. Concern regarding CPs is largely due to their high acute toxicity to various organisms. Concern over PAHs as environmental contaminants is primarily due to the fact that some are carcinogenic as well as mutagenic to the aquatic organisms exposed to them.

CPs are a major group of chemicals used for a variety of biocidal purposes and as precursors for other pesticides. CPs are usually marketed as complex mixtures and not as individual chemicals. Of the 19 possible CP isomers available to industry, only 8 have commercial utility. All are produced by the catalytic chlorination of phenol or the alkaline hydroxylation of hexachlorobenzene. CPs consist of a hydroxylated benzene ring which is substituted with one or more chlorine atoms. The structures of the CPs used in these studies are shown in Figure 1.1.

The sources of CPs to the aquatic environment include direct discharge, surface runoff from urban and agricultural lands, industrial and municipal effluent, aerial discharge and deposition as aerosols or in rain, or by indirect discharge such as that occuring from drift after aerial spraying.

CPs have biological activity as bactericides, slimicides, fungicides, herbicides and insecticides. The uses of CPs range from the preservation of wood to the treatment of water in industrial cooling systems. CPs are used domestically in wood treatment as a disinfectant and have been used in health care products. CPs are also used in the manufacture of other pesticides. For example 2,4-DCP is used in the production of 2,4-dichlorophenoxyacetic acid.

In the course of CP manufacture, at elevated temperatures in the latter stages of chlorination, toxic impurities such as polychlorinated-dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzo-furans (PCDFs) are formed. It is now believed that short-term toxicity, such as acute lethality, are mediated *via* CPs and that effects seen after 2-3 week exposures to sublethal concentrations are due to contaminants such as the PCDDs or PCDFs (NRCC, 1982).

The toxic effects of CPs are limited and are similar in a wide variety of organisms. The toxic mode of action of CPs in all eukaryotic cells is the uncoupling of oxidative phosphorylation in the mitochondria. The net effect of CP exposure is a dissociation of oxygen uptake from the synthesis of high energy phosphate bonds, resulting in an energy shortage in the cell.

The toxicity of various CPs, particularly PCP, has been reviewed extensively (U.S. E.P.A., 1979; Jones, 1981; NRCC, 1982). CPs are acutely toxic to many aquatic organisms and the toxicity tends to increase with increasing chlorination of the molecule. There have been few studies on the effects of chronic CP exposure to aquatic organisms, however, such studies with mammals have indicated that chronic exposure to CPs may be fetotoxic (Schwetz et al., 1974; Schwetz et al., 1978). There have been several studies describing the fate of PCP in fish (Kobayashi, 1979; Hodson and Blunt, 1981; Holcombe

et al., 1982; Spehar et al., 1985; McKim et al., 1986), however, this work has not been extended to include the lower chlorinated phenols.

PAHs are chemicals which consist solely of carbon and hydrogen arranged in the form of two or more fused benzene rings. There are thousands of PAHs ranging in molecular weight from 128.16 (napthalene) to 300.36 (coronene), each distinguished by the number of fused rings and the number and placement of substitutions on the aromatic rings. The structures of the three PAHs used in this study are shown in Figure 1.1.

The sources of PAHs in the environment are both natural and anthropogenic. Natural sources of PAHs include the synthesis by microorganisms (Mallet et al , 1967), algae and macrophytes (Graf and Diehl, 1966), as well as volcanic activity. However, the amounts of PAHs released to the environment from man-made sources and open burning such as forest fires (Youngblood and Blumer, 1975), are far greater than release by natural sources (Neff, 1979, 1985; Grimmer, 1983). Anthropogenic sources are those activities associated with the pyrolysis of organic materials, such as the burning of fossil fuels.

PAHs enter the aquatic environment from domestic and industrial sewage, surface runoff, deposition from the air and the direct deposition of petroleum products (Jackim and Lake, 1978; Lake et al., 1979; Neff, 1979; EPA, 1982; Prahl et al., 1984). PAHs are widely distributed in the aquatic environment and are found in water, sediment and biota (EPA, 1980).Trace amounts of PAHs have been detected in solution and associated with sediments in polluted and seemingly unpolluted areas (Barbier et al., 1973; Gordon et al., 1974). While PAH contamination of water, sediments and biota are directly related to the level of pollution in an area, the presence of PAHs in a particular habitat is not by itself an indication of pollution since the PAH contamination may have a natural source (Neff et al., 1976).

The biological fate of many PAHs in aquatic organisms has been thoroughly reviewed by Neff (1979), NRCC (1983) and Eisler (1987). The acute toxicity of PAHs appears to vary greatly with the molecule and species tested, but generally acute toxicity is due to low molecular weight compounds, with toxicity increasing with increasing substitution on the molecule. Interest in PAHs as environmental contaminants centres mainly on the relationship between the presence of PAHs or their metabolites in the tissues of organisms and the occurence of neoplasia and other cellular abberations (Wellings, 1969; Hodgins et al.,1977; Varanasi, 1989). Malins et al. (1984) have shown a positive correlation between idiopathic liver lesions and the presence of sediment-associated PAHs in fish. Krahn et al. (1984) have shown that fish with liver lesions contain high levels of metabolized xenobiotics with similar fluorescence characteristics as the metabolites of benzopyrene (BaP). Carcinogenic potential is usually associated with high molecular weight PAHs such as 3-methylcholanthrene and BaP.

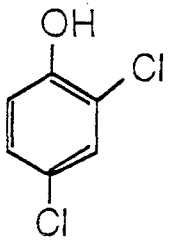
Fish were used in these studies as an experimental animal for a number of reasons. In the aquatic environment fish may be exposed to both water-borne and sediment - associated xenobiotics such as CPs and PAHs as well as eating contaminated food sources. The Environmental Protection Agency has published guidelines for the formulation of water quality criteria for the protection of aquatic species such as fish (U.S. EPA, 1983). Fish may also serve as alternative animal model systems in toxicological testing procedures. For example, there is increased interest in the use of fish species as alternative model systems for the study of the mechanisms of carcinogenesis and in carcinogenicity testing (Hoover, 1984). Powers (1989) stated that fish models may offer inexpensive and more acceptable alternatives in chemical carcinogenicity testing as well as being useful for the assessment of water-borne and sediment-deposited toxins. The use of standard species of fish in toxicological studies is important in order to extrapolate results between laboratories and there is a trend towards the use of such species, however, it

should be noted that no one species is suitable for all purposes. The rainbow trout (*Oncorhynchus mykiss*) was chosen as the model fish species in our experiments as it has attained world-wide use in representing the cool-water salmonid family (Sprague, 1973).

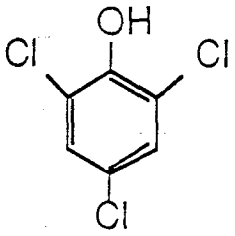
Isolated hepatocytes were used in the study of PAH metabolism in order to determine if this approach is suitable for the study of xenobiotic metabolism. Isolated cell preparations may reduce the number of experimental animals needed in xenobiotic testing by allowing for multiple and simultaneous testing of a number of chemicals on cells from one animal.

The first objective of the present study was to determine the acute toxicity of CPs and PAHs to trout in a short-term bioassay under standard test conditions. Acute toxicity data is not only important in classifying harmful substances and in the regulation and management of such substances, but is also important in the design and direction of further toxicological research. The second objective of the present study was to examine the toxicokinetics, especially the bioavailability, of both CPs and PAHs in trout following several routes of chemical administration. The absorption, distribution, metabolism and excretion of several CPs and PAH congeners in trout were studied. The third objective of this study was to examine the uptake and metabolism of PAHs by isolated trout hepatocytes and to compare the results obtained with those obtained *in vivo*. The use of isolated fish hepatocytes in toxicology may be a useful tool in the study of xenobiotic metabolism.

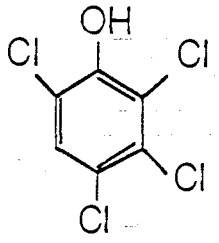
Figure 1.1. The chemical structures of CPs and the PAHs used in this study.



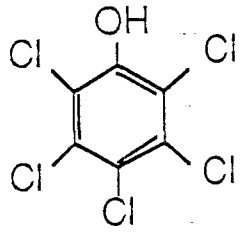

2,4-Dichlorophenol



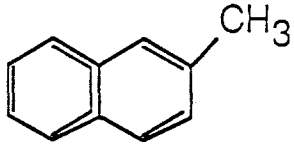

2,4,6-Trichlorophenol



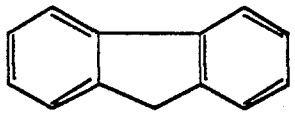

2,3,4,6-Tetrachlorophenol



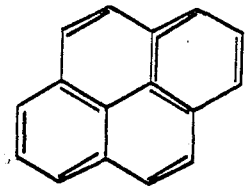

Pentachlorophenol




2-Methylnapthalene



Fluorene



Pyrene

# Part II Acute Toxicity of CPs and PAHs to Trout

## INTRODUCTION

Toxicological testing procedures, depending upon the number of chemical exposures and the duration of the study, are usually divided into three general classifications: 1) acute studies, 2) subacute or subchronic studies and 3) long term or chronic studies. Acute toxicity studies usually involve a single administration or several administrations of a test chemical within a 24- hour period in order to determine the short-term lethality of a compound to an organism. The $LC_{50}$ (median lethal concentration) is an estimate of acute toxicity to fish. The $LC_{50}$ can be described as a statistically derived expression of a single concentration of a chemical that can be expected to cause a 50% mortality of the test organisms.

Estimates of acute toxicity, such as the $LC_{50}$, are useful in classifying chemicals according to relative toxicity as well as in aiding in the evaluation of environmental risk from accidental spills into the aquatic environment. $LC_{50}$ determinations are also useful in the planning of subacute and chronic toxicity studies. Acute toxicity data are also useful in providing information about the mechanisms of toxicity, the probable target organ of the chemical and its specific toxic effect.

Two types of assays for determining $LC_{50}$ values of a chemical to aquatic organisms are the static and the continuous-flow or flow-through bioassay. Guidelines and references for standardized bioassay methods can be found in Sprague (1969, 1973), the American Public Health Association (1971) and Davis and Mason (1973). The continuous-

flow bioassay has a number of advantages over the static bioassay. In a flow-through system the concentration of the test compound remains constant, the fish remain undisturbed during changes of test water, and wastes and other metabolic products are continuously removed. However, well-designed and performed static bioassays can yield equally valid test results and in some cases results may be superior to those of a continuous-flow nature (Sprague, 1973). Several advantages of the static bioassay over the continuous-flow bioassay are that they do not require dosing devices which may be expensive as well as inaccurate if the test compound tends to settle out. Continuous-flow systems often require much larger amounts of test compound which may not be economically feasable, or less desirable from a safety point of view.

Acute toxicity data are usually analyzed by probit analysis (Bliss, 1935) or by methods which modify the probit method to utilize both 0 and 100% effect levels (Litchfield and Wilcoxin, 1949). Computer methods for probit analysis are now in widespread use and are often based on Finney's (1952) method for probit analysis which fits the line by iteration and is generally more accurate than the nomographic analysis described by Litchfield and Wilcoxin (1949).

It is now generally believed that the short-term effects, such as acute lethality, of technical CP formulations are mediated by CPs and that the long-term effects observed are mediated by various contaminants such as the PCDDs (Johnson *et al.*, 1973; Schwetz *et al.*, 1978). CPs act similarly in all eukaryotic cells by uncoupling oxidative phosphorylation in the mitochondria. It has been suggested, however, that the toxicity of higher chlorinated phenols may be attributed to the chlorophenate ion and that the toxic effect of lower chlorinated phenols is due to the undissociated molecule (Farquharson et al., 1958). The toxicity of PCP to a number of crustacean species has been reviewed by Rao (1978) and the acute toxicity of various CPs to fish have been studied previously by Goodnight (1942), Kobayashi *et al.* (1978) and Hattula *et al.* (1981).

Much of the toxicity of petroleum to aquatic species is attributable to aromatic hydrocarbons (Boylan and Tripp, 1971; Moore *et al.*, 1973; Moore and Dwyer, 1974). It is now generally believed that PAHs exert toxic effects by their ability to perturb cell membrane surface organization, and interfering with one or several cellular processes. Acute toxicity of PAHs may also be mediated by PAH metabolites due to their more hydrophilic, electrophilic and reactive nature, which may bind covalently to many cellular structures. Results from acute toxicity studies with PAHs have been carried out mainly with the lower molecular weight compounds such as napthalene (Anderson *et al.*, 1974b; Caldwell *et al.*, 1977). Studies with higher molecular weight PAHs have been few but have all indicated that the acute toxicity of petroleum constituents is not due to the higher-molecular-weight PAHs such as chrysene, BaP and dibenzanthracene (Neff, 1979) but due to napthalenes and phenols, perhaps due to the low water solubility of the larger molecules (Anderson *et al.*, 1974b; Rossi *et al.*, 1976; Eisler, 1987). Acute toxicity generally increases with increasing molecular weight, but is low with the higher molecular weight compounds as mentioned above. Within an aromatic series, acute toxicity also increases with increasing alkyl substitution on the aromatic ring. To various aquatic organisms, PAHs are most toxic to crustaceans and least toxic to teleosts (Neff, 1979). In general, concentrations of PAHs which are acutely toxic to aquatic organisms are not encountered in polluted environments, however, contaminated sediments may contain PAH concentrations which are acutely toxic to some species (Neff, 1979). The bioavailability of PAHs to fish from contaminated sediments can be substantial (Stein et al., 1974). The acute toxicity of many PAHs to various aquatic organisms has been reviewed by Neff (1979) and Eisler (1987).

Many factors such as fish loading density, acclimation and test temperature, frequency of observation, water pH, volume of test solution and test tank size may alter the results of acute toxicity tests. Standard guidelines and criteria as well as the use of reference

toxicants such as sodium pentachlorophenate or dehydroabietic acid may aid in obtaining consistent results between laboratories (Davis and Hoos, 1975). However, results of $LC_{50}$ tests vary greatly among species and studies due to biological and environmental variation and care must be taken in interpreting and extrapolating results.

## MATERIALS AND METHODS

### A) Fish and Chemicals

Rainbow trout (*Oncorhynchus mykiss*), weighing 2.5 to 4.0 g were obtained from the Fraser Valley Trout Hatchery, Abbotsford, B.C.. Fish were acclimated for 2 weeks in flowing fresh dechlorinated water at 10°C under an artificial photoperiod of 12-h light:12-h dark. Fish were fed daily with New Age Fish Feed obtained from Moore-Clarke Co., Vancouver, B.C., until 3 days before an experiment.

2,4-DCP, 2,4,6-TCP, PCP, 2-MN, fluorene and pyrene were purchased from Aldrich Chemical Co., Milwaukee, WS. Purified 2,3,4,6-TTCP was a gift from the MacMillan Bloedel Research Laboratories, Vancouver, B.C. Polyoxyethylene sorbitan monooleate (Tween 80) was purchased from Sigma Chemical Co., St. Louis, MO.

### B) Bioassays

Bioassay tanks were glass aquaria (45 L) placed into a refrigerated room which maintained the water temperature in the tanks at approximately 10°C. The sides of the aquaria were covered with black plastic and the tops covered with black plexiglass to minimize disturbance of the fish. Eleven fish were placed into each tank containing 40 L of dechlorinated water one day before the experiment. CPs or PAHs were dissolved in 2 drops of Tween 80 and then dissolved in water. Appropriate concentrations were added to

each aquarium at the start of the experiment. A tank containing only Tween 80 (0.1ml) was used as a control. Seventy-five percent of the water in the tank was changed every 24 hours to maintain constant chemical concentrations. Observations for mortalites were made daily for 96 h. Death was recorded when opercular movement ceased.

### C) Mathematical Analysis

The computer program package PROBIT (SAS, 1985) based on the procedures of Finney (1952) was used to calculate the $LC_{50}$, its 95% confidence limits and the slope of the line.

## RESULTS

No fish in control tanks died during the experiment. Pertinent information regarding test conditions (mean fish weight and length, water pH, temperature and fish loading density) and the calculated $LC_{50}$ values with associated 95% confidence limits and slope of the fitted line are reported in Table 2.1. Individual fish mortality data are shown in Tables A.1 to A.7.

The 96-h $LC_{50}$'s for DCP and TCP were similar at 2.63 and 1.99, respectively. The 96-h $LC_{50}$ values for TTCP and PCP were also similar at 0.33 and 0.15, respectively. No $LC_{50}$ values for fluorene and pyrene were determined at the concentrations studied because of the few mortalites observed (18.2% of fish were dead 96 h in the tanks containing fluorene). The $LC_{50}$ of 2-MN was estimated as 1.46, which is similar in toxicity to DCP and TCP. In all cases, the $LC_{50}$ values, when calculated with mortality data at 24, 48, 72 and 96 h, declined with time (Tables A.1 to A.7) indicating that exposure to CPs or 2-MN for longer time periods may result in lower $LC_{50}$ values.

Results of 96-h static bioassays and test conditions for CPs and PAHs in trout.

| Chemical | $LC_{50}$ (ppm) | 95% Confidence limits | Slope | Loading density (g/L) | Mean initial temperature (°C) | Mean initial pH | Mean wt. (g) | Mean length (cm) |
|---|---|---|---|---|---|---|---|---|
| DCP | 2.630 | 2.182, 3.108 | 5.28 | 0.85 | 10.0 | 6.21 | 3.10 | 6.2 |
| TCP | 1.991 | 1.635, 2.381 | 5.14 | 0.86 | 10.1 | 6.20 | 3.14 | 6.1 |
| TTCP | 0.334 | 0.287, 0.377 | 7.71 | 0.88 | 9.7 | 6.24 | 3.21 | 6.2 |
| PCP | 0.153 | 0.126, 0.183 | 5.37 | 0.85 | 9.9 | 6.22 | 3.09 | 6.3 |
| 2-MN | 1.456 | 1.070, 1.841 | 3.80 | 0.80 | 11.4 | 6.20 | 2.92 | 6.0 |
| Fluorene | >2.00 | — | — | 0.88 | 10.9 | 6.21 | 3.21 | 6.4 |
| Pyrene | >2.00 | — | — | 0.87 | 11.0 | 6.19 | 3.17 | 6.2 |

## DISCUSSION

The results of the experiment revealed that all of the CPs studied were acutely toxic to trout. The overlap of the 95% confidence limits for DCP and TCP indicate that there is no significant difference in the $LC_{50}$ values (Sprague, 1969). The $LC_{50}$ calculated for PCP is similar to values determined in other studies (Davis and Hoos, 1974; Guo *et al.*, 1979). DCP and TCP can be classified as "toxic" having LC50 values between 1 and 100 ppm and both TTCP and PCP can be classified as "very toxic" having $LC_{50}$ values below 1ppm (Joint IMCO/FAO/UNESCO/WMO Group of Experts, 1969).

Spehar et al.(1985) and Hodson and Blunt (1981) found that water quality parameters such as pH and temperature influenced the toxicity of PCP. Koneman and Musch (1981) found that the toxicity of 11 CPs to the guppy (*Poecilia reticulata*) increased with decreasing pH and determined that at pH 3, the maximum toxicity of CPs would be reached. At this pH, most of the CP is in an unionized form and is more readily transported across cell membranes. They also reported that the toxicity of the phenol to the guppy increased with increasing chlorination of the molecule. Hattula *et al.* (1981) found that the 24-h $LC_{50}$ of chlorinated phenols decreased with increasing chlorination of the molecule in the trout (*Salmo trutta*) which is similar to these results. Kobayashi (1979) attributed the higher toxicity of PCP compared to lower chlorinated phenols to an increased accumulation of PCP to toxic levels. In studies using the rat, it was also noted that acute toxic effects of CPs administered intraperitoneally, decreased with a decreasing chlorination of the molecule (Farquaharson *et al.*, 1958). The results of our experiments are similar to those reported, as the acute toxicity of the CPs studied increased with an increased chlorine substitution of the molecule.

In a study with polychlorinated biphenyls (PCBs), the acute toxicities of less chlorinated PCBs were higher than higher chlorinated PCBs (Mayer *et al.*, 1977). Chui (1983) obtained lower $LC_{50}$ values in trout exposed to lower chlorinated diphenyl ethers (CDEs) than higher chlorinated ones and attributed this to a lower water solublity of the higher chlorinated CDEs. In our experiments, all of the chemicals were dissolved or available for uptake as suspended solutions.

Studies with petroleum indicate that acute toxicity is associated with napthalene and phenols and not PAHs with higher molecular weights (Anderson *et al.*, 1974; Rossi *et al.*, 1976). Gerhart and Carlson (1978) have injected rainbow trout with higher-numbered PAHs such as BaP with doses up to 30mg/kg with no apparent toxic effects. Water and sediments containing high levels of higher molecular weight PAHs appear not to be acutely toxic to several species of fish (Lee and Singer, 1979; Payne and May, 1979). The results of our experiments support this general hypothesis as 2-MN was found to be acutely toxic to trout and that pyrene and fluorene were virtually nontoxic at the concentrations studied. The 24-h $LC_{50}$ obtained for 2-MN was similar to the 24-h $LC_{50}$ (2.0 ppm) in the minnow, *Cyprinodon variegatus* , reported by Anderson *et al.* (1974c). 2-MN, with an $LC_{50}$ value between 1 and 100 ppm can be classified as "toxic" (Joint IMCO/FAO/UNESCO/WMO Group of Experts, 1969). An $LC_{50}$ value could not be estimated for fluorene due to low mortalities, however, Wofford and Neff (1978) obtained a 24-h $LC_{50}$ value of 1.68 for fluorene in the minnow. No data on the acute toxicity of pyrene to fish is available for comparison with results obtained in this study. It should be noted that although the acute toxicity of both fluorene and pyrene to trout were low in this study, sublethal effects of exposure to one of these chemicals may represent a potential hazard to aquatic organisms. For example, studies with bluegills (*Leopomis machrochirus*) have shown that sublethal levels of fluorene decreased growth of the fish and reduced the ability to capture prey and avoid predators (Finger et al., 1985).

Our results are similar to those reported in other studies. Zbinden and Flury-Roversi (1981) stated that the variability of acute toxicity tests can be kept at an acceptably low level if the tests are done under strictly controlled and standardized conditions in the laboratory and under such conditions, acute toxicity tests can yield useful information such as the classification of test compounds in official lists of toxic substances. Ours and other laboratories can increase the informational content of results with regard to toxicity if such studies are done in conjunction with other physiological, hematological, biochemical, pathological or histopathological investigations using reduced numbers of animals.

# Part III Toxicokinetics of CPs and PAHs in Trout

## INTRODUCTION

Biological responses to xenobiotics are usually governed by two categories of factors: 1)pharmacokinetic and 2)pharmacodynamic factors. Pharmacodynamic factors influence the sequence of events that result from the interaction of a chemical with a site of action, leading to the realization of a toxic response. Pharmacokinetic factors are those which affect the fate of a chemical in an organism, specifically the concentration of a chemical in tissues and at the site of action. Pharmacokinetics is a means of quantifying this fate, and is the study of the kinetics of absorption, distribution, metabolism or excretion of xenobiotics by an organism. Gilbaldi and Perrier (1975) describe pharmacokinetics as "the study of the time course of drug and metabolite levels in different fluids, tissues, and excreta of the body, and of the mathematical relationships required to develop models to interpret such data". Several mathematical tools have been developed to evaluate those factors which affect the time course of xenobiotics in an organism and include both compartmental analysis and physiologically-based models. Both models have tremendous potential for providing a mechanism for the screening of chemicals prior to costly in-depth testing in risk assessment (Bischoff, 1987) .

Toxicokinetics have been used extensively in the study of xenobiotics in mammals and has been adapted to piscine systems. The toxicokinetics of xenobiotics in many marine vertebrates has been reviewed by Guarino and Arnold (1979). The use of physiologically-based models may provide a new approach in the use of toxicokinetics as a

means of extrapolating toxicological data among different aquatic species and between lower and higher vertebrates (Dedrick and Bischoff, 1980).

McKim and Goeden (1982) have developed a method for empirically determining the necessary *in vivo* rate constants for the toxicokinetic modelling of xenobiotics. The direct measurement of uptake and elimination of tetrachlorobiphenyl and PCP (McKim *et al.*, 1986) by the gill of rainbow trout was coupled with urinary and fecal elimination rates from the same fish. Bradbury *et al.* (1986) employed similar toxicokinetic modelling to study the fate of the pyrethroid insecticide fenvalerate in fish. Barron *et al.* (1987) utilized plasma concentration data of rainbow trout following a single intravenous dose in the toxicokinetic modelling of di-2-ethylhexyl phthalate (DEHP). A similar study utilizing blood concentration data was employed in the study of the toxicokinetics of 4-chlorodiphenyl ether in the skate, *Raja ocellata*, following intravenous administration (Chui *et al.*, 1986).

The uptake, distribution, metabolism and excretion of PCP has been extensively studied in fish (Kobayashi and Akitake, 1975; Glickman *et al.*, 1977; Kobayashi, 1979; Kobayashi and Nakamura, 1979; Veith *et al.*, 1979; McKim *et al.*, 1985; Spehar *et al.*, 1985; McKim *et al.*, 1986). PCP is rapidly taken up from water by rainbow trout *via* the gill and is found in all tissues examined (McKim *et al.*, 1986). PCP has been found to be metabolized to conjugates by both the rainbow trout (McKim *et al.*, 1986) and goldfish (Kobayashi, 1979). However, little information exists on the fate of the lower chlorinated phenols in rainbow trout.

The biological fates of PAHs in aquatic organisms have been thoroughly reviewed (Neff, 1979; NRCC, 1983: Eisler; 1987). In general, waterborne PAHs and those associated with sediments are rapidly taken up by fish and are found in almost all tissues after exposure to the chemicals in water (Lee *et al.*, 1972; Roubal *et al.*, 1977; Jiminez *et*

*al.*, 1987; Kennedy *et al.*, 1989a). However, very few of these studies actually deal with the quantitative aspect of uptake and elimination. The uptake rates of PAHs by fish appear to vary with the exposure route, the lipophilicity and size of the PAHs, and the surrounding matrices. For example, equilibrium concentrations of PAHs in tissues are often reached within 24 h after fish are exposed to waterborne PAHs (Lee *et al.*, 1972). Fish appear to take up PAHs slowly from food and sediments; more than half of an orally administered chemical is unabsorbed but associated with the digestive tract or its contents (Roubal *et al.*, 1977; Whittle *et al.*, 1977; Stein *et al.*, 1984). Although fish accumulate PAHs from water and contaminated sediments rapidly, the metabolism of the PAHs may be rapid and fish sampled from contaminated areas often have concentrations lower than might be estimated from bioconcentration factors (Schnitz *et al.*, 1987). A growing literature exists on the ability of fish to metabolize PAHs (for review: see Varanasi, 1989). Fish are capable of readily metabolizing PAHs *via* the mixed function oxidase system (Law, 1982) or by conjugating enzymes ( Gmur *et al.*, 1982; Melcanon and Lech, 1984; Varanasi *et al.*, 1988; Foureman, 1989). The elimination of PAHs by fish appears to vary with the molecular weight of the molecule and between species but is generally faster in organisms possessing higher levels of detoxifying enzymes (Neff *et al.*, 1976). The major route of excretion of PAHs such as BaP, in fish, regardless of the route of exposure, is by the bile (Varanasi *et al.*, 1981; Thomas *et al.*, 1982; Stein *et al.*, 1984; Kennedy *et al.*, 1989b). The distribution, elimination and metabolism of 2-MN was examined extensively in rainbow trout (Melancon and Lech, 1978; Melancon and Lech,1984), however, little is known of the toxicokinetics of pyrene or fluorene in fish.

This study was undertaken to examine the toxicokinetics, especially the bioavailability, of CPs and PAHs in rainbow trout following i.a., oral or branchial exposure. The fate of both CPs and PAHs in rainbow trout following a single i.a. dose was investigated.

## MATERIALS AND METHODS

### A) Fish and Chemicals

Rainbow trout, weighing 400 to 500 g, were obtained from Spring Valley Trout Farms, Langley, B.C.. Fish were acclimated for three weeks in flowing fresh dechlorinated water at 10°C under an artificial photoperiod of 12-h light: 12-h dark. Fish were fed daily with New Age Fish Feed, Moore-Clarke Co., Vancouver, B.C.. Fish were not fed three days before an experiment.

Unlabeled CPs and PAHs were purchased as described in Part II.A. [4,5,9,10-$^{14}$C]pyrene (specific activity 56 mCi/mmol) and [8-$^{14}$C] 2-MN (specific activity 20 mCi/mmol) were purchased from Amersham Corporation, Arlington Heights, IL. [9-$^{14}$C] fluorene (specific activity 2.6 mCi/mmol) was purchased from California Bionuclear Corporation, Sun Valley, CA. The $^{14}$C-labeled PAHs were purified by thin-layer chromatography (TLC) on silica-gel plates using hexanes as the developing solvent. The radiochemical purity of the $^{14}$C-labeled PAHs, determined by TLC, exceeded 99%. Protosol and Biofluor were obtained from New England Nuclear, Lachine, Que.

### B) Toxicokinetics of CPs and PAHs After Different Routes of Exposure

#### a) Cannulation of the Dorsal Aorta of Trout

The dorsal aorta of trout were cannulated using the general surgical procedures of Smith and Bell (1964) one day before the experiment. Trout were anaesthetized with 0.2 g/l MS 222 (ethyl-N-aminobenzoate methane sulfonic acid, Sigma Chemical Co., St. Louis, MO.) and 0.2 g/l sodium bicarbonate as a buffer (Wedemeyer, 1970) and placed on

an operating table when all opercular movement had stopped. Recirculating water containing 0.1 g/l MS 222 and 0.1 g/l sodium bicarbonate was continuously passed over the gills of the trout to maintain anaesthesia during the operation. A catheter was inserted into the dorsal aorta of the trout at the first gill arch. When the catheter had entered the dorsal aorta and blood had filled the catheter, PE50 tubing (0.58 mm i.d., 0.965 mm o.d., Clay Adams, Parsippany, NJ.) was inserted into the dorsal aorta through the catheter. The catheter was removed and the cannula was sutured at two points along the roof of the mouth with silk sutures (size 3-0, Ethicon, Inc., Somerville, NJ.) to secure it in place. The cannula was passed through a hole in the nasal area of the trout and secured with another suture. The cannula was filled with heparinized saline (5 I.U./ml) and heat sealed. The entire procedure took approximately 20 min. Fish were placed into darkened plexiglass boxes supplied with fresh flowing dechlorinated water and allowed one day to recover before the experiment. The cannula was washed twice daily with heparinized saline to prevent clotting .

**b) Intraarterial Administration**

Unlabeled CPs or PAHs dissolved in 0.2 ml of a modified Hanks medium (see Medium A excluding EDTA and HEPES, Appendix, Table A.29) (Hanks and Wallace, 1949) containing 1% Tween 80 (w/v) and heparin (5 I.U./ml) were injected as a bolus through the cannula into the dorsal aorta. This was followed by an injection of 0.2 ml of the Hanks medium through the cannula to ensure that the entire dose of 10 mg/kg entered the circulatory system.

In separate experiments, trout were dosed with 1 mg/kg of CPs or PAHs to determine if the dose effected the kinetics of the chemicals after i.a. administration. Elementary superposition (or overlay technique) as described by Westlake (1971) involved the superimposition of the new plasma concentration-time curve over the previous curve.

c) **Branchial Exposure**

Trout implanted with an indwelling cannula in the dorsal aorta were placed individually in aerated (10 ml/min) glass aquaria containing 12 l of water maintained at 10°C by a water bath. The sides of the aquaria were covered with black plastic and the tops covered with black plexiglass to minimize disturbance of the fish. Unlabeled CPs or PAHs dissolved in 2 ml distilled water containing 0.1% Tween 80 (w/v) had been added to each tank to obtain a final concentration of 0.5 mg/l.

d) **Blood Sampling**

A control blood sample was withdrawn from all trout prior to chemical administration. At different times after chemical administration, a 0.2-ml blood sample was withdrawn through the cannula with a glass syringe. An equal volume of heparinized Hanks solution was injected to replace the volume of blood removed and to prevent the blood in the cannula from clotting.

e) **Analysis of Unchanged CPs**

A blood sample (0.2ml) was pipetted into a 12-ml glass centrifuge tube. Sulphuric acid (1N, 0.5 ml) and distilled water (0.5 ml) were added to the tube to deproteinize the blood. The tube was stoppered and the contents of the tube were vortexed for 5 min and extracted three times with 3-ml portions of hexanes. After each addition of hexanes the tubes were shaken on a reciprocating shaker for 30 min and centrifuged at 2000 *g* for 15 min on a low speed centrifuge to separate the aqueous and organic phases. The extracts were combined and placed into a 20-ml scintillation vial. The vial was placed into a water

bath at 0°C and the extracts concentrated to approximately 1 ml under a gentle stream of nitrogen.

To increase the detection sensitivity in the GC analysis of CPs, diazomethane, a reagent used in the preparation of methyl ester derivatives of a variety of hydroxy compounds, was prepared using a Diazald Kit (Aldrich Chem. Co., Milwaukee, WS.)as follows: 2-(2-ethoxyethoxy)-ethanol and ether (20 ml) were added to a solution of KOH (6 g) in water (10 ml). The solution was placed in a 100-ml long-necked distilling flask fitted with a dropping funnel and efficient condenser in a water bath at 70°C. As the distillation of ether began, a solution of 21.5 g Diazald (N-methly-N-nitroso-p-toluenesulfonamide) (Aldrich Chem. Co., Milwaukee, WS.) in 200 ml ether was added slowly through the dropping funnel over 20 min. When the dropping funnel was empty, another 40 ml of ether was added and the distillation continued until the distilling ether was colorless. The combined ethereal distillate in the ice-cooled collection-flask contained approximately 3 g diazomethane (P.C. Oloffs, personal communication).

Diazomethane in ether (0.1 ml) was added to the hexane extract of blood. The methylation reaction was allowed to proceed for 30 min after which the extract was evaporated to dryness under nitrogen. Unreacted diazomethane evaporated with the ether. The remaining residue was redissolved in an appropriate volume of hexanes containing an internal standard. 2,4,4'-trichloro-diphenyl-ether (2,4,4'-triCDE, 0.2 ng/ml) was used as an internal standard in the analysis of methylated TTCP and PCP. Methylated TTCP was used as an internal standard in the analysis of methylated DCP and TCP.

The CP derivatives were analyzed with a Model 5880A Hewlett-Packard gas chromatograph equipped with an OV-101 capillary column (12 m, 0.2 mm i.d.) and a $^{65}$Ni electron capture detector. The carrier gas was nitrogen at a flow rate of 30 ml/min. The injector and detector temperature were 250° and 300°C, respectively. The oven

temperature for the analysis of TTCP and PCP was 160°C and was 120°C in the analysis of DCP and TCP.

### f) Analysis of Unchanged PAHs

Distilled water (0.5 ml) and 0.1N sulphuric acid (0.5 ml) were added to the blood samples (0.2 ml) in 12-ml glass centrifuge tubes. The mixture was vortexed for 5 min and extracted three times with 4-ml portions of methylene chloride. After each addition of methylene chloride, the tubes were shaken for 30 min on a reciprocating shaker and centrifuged at 2000 *g* on a low speed centrifuge for 15 min to adequately separate the aqueous and organic layers. The extracts were combined and placed into a 20-ml scintillation vial. The vials were placed into a water bath at 0°C and the extracts evaporated under a gentle stream of nitrogen. The residues were redissolved in hexanes containing benzanthracene (1 μg/ml) as an internal standard. The sample in hexane was analyzed by a Hewlett-Packard Liquid Chromatograph (Model 1090) equipped with an ODS-Hypersil column (Hewlett-Packard Co., 5um, 100 X 4.6 mm i.d.) and a diode-array UV detector. The wavelength of the UV detector was fixed at 230 nm. The PAHs were eluted from the column isocratically with methanol:$H_2O$ (70%:30%).

### g) Mathematical Analysis

The blood concentration-time data of CPs and PAHs were analyzed by a nonlinear least-squares regression program (NONLIN) (Metzler *et al.*, 1974) in conjunction with a specific subroutine depending upon the toxicokinetic model chosen. The time course of the CPs or PAHs in the blood of trout after i.a. administration were described by the equation:

$$C_B = \sum_{i=1}^{n} A_i e^{-x_i t} \qquad (1)$$

where CB is blood concentration of CPs or PAHs at time t, and $A_i$ and $X_i$ are the coefficients and constants of the exponential components, respectively. The blood-concentration-time data from branchially exposed trout were described according to the equation:

$$C_B = \frac{FDk_a}{Vd(k_a - k_e)} (e^{-ket} - e^{-kat}) \qquad (2)$$

where F is the fraction of the dose (D) absorbed ( by estimating the area under the blood concentration-time curve (AUC) as described below), Vd is the volume of distribution estimated from i.a. administration, and $k_a$ and $k_e$ are the first-order rate constants describing the appearance and elimination of CPs or PAHs from the blood.

The pharmacokinetic parameters were estimated by the damping Gauss-Newton method and the statistical weighting factor in the least-squares procedure was the square of the inverse of the observed blood concentrations (Ottaway, 1973). The overall goodness of fit was determined by comparing the sum of the squared deviations and by the scatter of the data points around the fitted function. Akaike's information criterion (AIC) (Yamaoka *et al.*, 1978) was used to select the most appropriate model. The parameters estimated from the nonlinear regression analysis were used to calculate the model-dependent parameters and the volume of the central compartment according to Gibaldi and Perrier (1975). Total body clearance (Qb) was calculated as the ratio of dose divided by the AUC. The AUC was calculated using the trapezoidal rule (Gilbaldi and Perrier, 1985).

The apparent bioavailability (A) of CPs and PAHs administered branchially to trout was calculated by:

$$A(\%) = \frac{(AUC_b)\,(Dose_{i.a.})}{(AUC_{i.a.})(Dose_b)} \times 100 \qquad (3)$$

where $AUC_{i.a.}$ and $dose_{i.a.}$ are the area under the blood concentration-time curve and dose, respectively, following an i.a. administration. $AUC_b$ and $dose_b$ are the corresponding parameters of branchial exposure.

## C) Disposition of CPs and PAHs in Trout Tissues

### a) Chemical Administration

Trout were cannulated in the dorsal aorta as described in Part III.B. Unlabeled CPs or $^{14}C$-labeled PAHs dissolved in modified Hanks solution were injected through the cannula to achieve a dose of 10 mg/kg. Fish dosed with DCP, TCP, or TTCP were sacrificed at 2, 4, 6, 8 and 10 h following chemical administration. Fish dosed with PCP were sacrificed at 4, 8, 12, 16 and 24 h after chemical administration, and fish dosed with one of the PAHs were sacrificed at 16, 24, 48, 72 and 144 h.

In a separate experiment, trout were given a single dose of 50 mg/kg containing 10 μCi of $^{14}C$-DCP or $^{14}C$-pyrene by gavage. CPs or PAHs given by gavage were dissolved in 2 ml distilled water containing 0.1% Tween 80 (w/v). Fish were sacrificed 24 h following chemical administration.

### b) Analysis of Unchanged CPs and PAHs in Trout Tissues

The major organs and tissues (liver, kidney, fat, gill, intestine and stomach) and a subsample of muscle and skin taken near the lateral line above the anal fin of the fish were removed, blotted, weighed and homogenized separately in three volumes of 0.9% NaCl with a Polytron homogenizer (Brinkman Co., Rexdale, Ont.). Bile was collected by gall bladder puncture and was stored at -20°C in vials containing sodium citrate buffer (pH 5 for CPs and pH 7.0 for PAHs). Bile was analyzed as described in Parts III.e and III.f. The

tissue homogenates of CP-treated fish were extracted three times with 3-ml portions of hexanes. The tissue homogenates of PAH-treated fish were extracted similarly with 3-ml portions of methylene chloride. After each addition of organic solvent, the tubes were shaken on a reciprocating shaker for 30 min and centrifuged at 2000 *g* for 15 min. The extracts from each sample were combined and passed through a pasteur pipette containing 5 g florosil to remove any interfering biological compounds which may be coextracted with the CPs or PAHs from the tissues. Organic solvent (10 ml) was passed through the column to ensure all of the CPs or PAHs had been eluted. The tubes were placed in an ice-cooled water bath and the extracts containing CPs were evaporated to approximately 1.0 ml and 0.1 ml of the diazomethane solution added to derivatize the CPs. The methylating reaction was allowed to continue for 30 min after which the extracts were evaporated to dryness and redissolved in an appropriate volume of hexanes containing the appropriate internal standard (0.2 ng/ml). The extracts containing PAHs were evaporated to dryness and redissolved in hexanes containing benzanthracene (1 ug/ml) as an internal standard.

The derivatized CPs were analyzed by GC and the PAHs were analyzed by HPLC as described in Parts III.e and III.f. Percent recovery values were used in the calculation of CP or PAH concentrations in tissues.

After extraction, the radioactivity in the aqueous layer of tissue homogenates from $^{14}$C-PAH dosed trout (144 h sample) were determined after the tissues were digested by the addition of 1 ml of a Protosol:ethanol mixture (1:1 v/v) and heating in an oven at 80°C for 4 h. Samples were decolorized by the addition of 0.1 ml of 10% $H_2O_2$ and heating for 1 h at 60°C. Biofluor (15 ml) was added to the sample in a 20 ml scintillation vial and counted by LSC.

Radioactivity was determined by a Beckman LS-8000 liquid scintillation counter. A correction for quenching was made using the external standard technique.

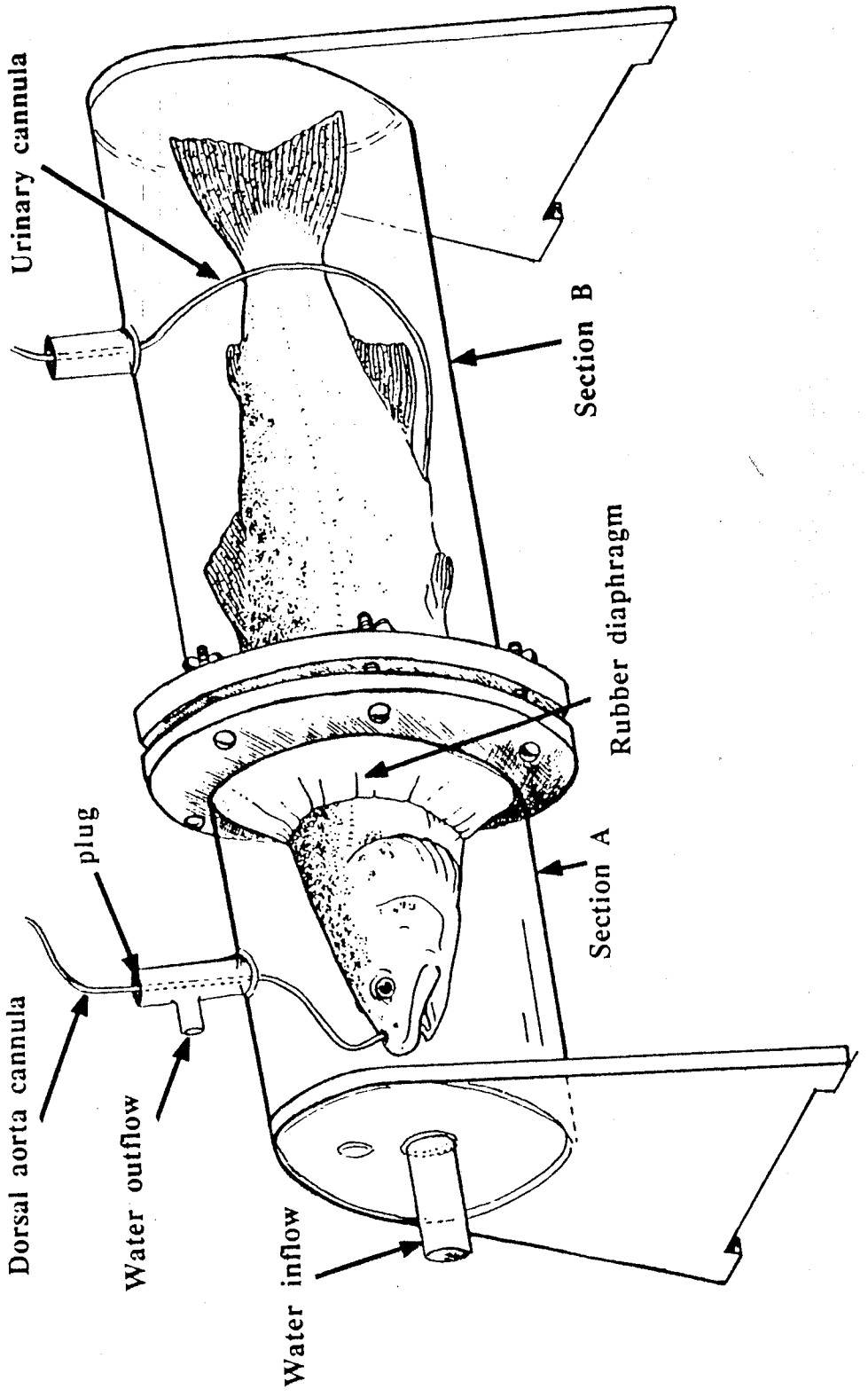
Tissues from trout dosed orally with 50 mg/kg $^{14}$C-DCP or $^{14}$C-pyrene were removed and prepared as described above and analyzed for total radioactivity. When the gastrointestinal tract was removed, it was rinsed with 10 ml saline and an aliquot removed and analyzed for total radioactivity.

## D) Excretion of CPs and PAHs by Trout

### a) Cannulation of Trout and Metabolic Chambers

The dorsal aorta of trout were cannulated as described in Part III.B. A cannula was also inserted into the urinary tract of the fish as follows: PE50 tubing with a slightly heat-flared end, filled with 0.9% saline, was inserted approximately 2 cm into the urinary tract of the fish and secured at both the urogenital flaps and on the anal fin with silk sutures. Fish were allowed to recover in darkened plexiglass boxes supplied with fresh flowing dechlorinated water for 24 h. Fish were then placed individually in metabolic chambers modified from Maren *et al.* (1968). A fitted rubber diaphragm divided the chamber into two chambers. A preliminary experiment indicated that there was no mixing of the water in the front section (chamber A) and the rear section (chamber B) of the chamber. Fresh dechlorinated water was supplied to chamber A at a flow rate of 10 ml/min and was aerated at 10ml/min. The entire chamber was immersed in a 10°C water bath. Water passing through chamber A was passed through an activated charcoal trap (50 g, Aldrich Chemical Co.) which was covered in black plastic and collected in an ice-cooled 12 l glass aquarium. Urine was collected *via* the urinary cannula into an ice-cooled volumetric flask which was covered in black plastic. Air coming out of chamber A was passed through a series of tubes containing toluene to collect any volatile compound. A typical chamber is shown in Figure 3.1.

Figure 3.1. Schematic diagram of the metabolism chamber containing a fish, indicating the position of the dorsal aorta and urinary cannulas. The metabolism chamber was divided into two chambers by a rubber diaphragm.


Urinary cannula
Section B
Rubber diaphragm
Section A
plug
Dorsal aorta cannula
Water outflow
Water inflow

b) Chemical Administration and Sampling

Unlabeled CPs and $^{14}C$-labeled PAHs dissolved in a modified Hanks solution containing 0.1% Tween 80 (w/v) were administered (10 mg/kg) to trout in the metabolic chambers through the cannula in the dorsal aorta.

Water passing through chamber A , water from chamber B (2 ml) and urine were sampled at 2,4,8,16,24 and 144 h for DCP, TCP and TTCP and every 24 h for PCP and PAHs.. The activated charcoal traps were rinsed with organic solvent at similar sampling times. An aliquot of toluene in traps was evaporatedto dryness and anlayzed for CPs or $^{14}C$-PAH derived radioactivity as described previously. Fish dosed with $^{14}C$-PAHs were sacrificed 144 h after chemical administration and analyzed for total radioacitivy as in Part III. C.

c) Analysis of Unchanged CPs and Metabolites in Urine, Bile and Water

Charcoal traps were rinsed twice with 50-ml portions of hexanes for the analysis of CPs. Bile, water from chamber B and an aliquot of gill water or urine were pipetted into 12-ml centrifuge tubes and extracted three times with 3-ml portions hexanes as described in Part III.e. The extracts from each sample were combined and evaporated under nitrogen to approximately 1.0 ml, derivatized with diazomethane and analyzed for unchanged CPs by GC as described in Part III.e. The remaining aqueous layers were adjusted to pH 2 with 1N sulphuric acid. The samples were hydrolyzed by heating at 80°C for 24 h. The cooled, hydrolyzed samples were extracted again three times with 3-ml portions of hexanes. Extracts were combined, evaporated to approximately 1.0 ml, derivatized with diazomethane and analyzed for unchanged CPs as described in Part III.e.

**d) Analysis of Unchanged PAHs and Metabolites in Urine, Bile and Water**

Bile, water from section B of the chamber and aliquots of gill water or urine were pipetted separately into centrifuge tubes and extracted three times with 3-ml portions of methylene chloride. Charcoal traps were rinsed twice with 50 ml methylene chloride. Sample extracts were combined and an aliquot of the extracts was pipetted into 20-ml scintillation vials. The radioactivity in the extracts was determined by LSC after the addition of Biofluor.

The remaining extracts were evaporated under nitrogen to dryness. The residues were redissolved in an appropriate volume of hexanes containing an internal standard and analyzed for unchanged PAHs as described in Part III.f.

The remaining aqueous layers were acidified to pH 2 with 1N sulfuric acid. The samples were hydrolyzed and extracted with methylene chloride as described above. The radioactivity in the remaining aqueous layer of the sample were determined directly by LSC after the addition of 15 ml Biofluor to an aliquot of the sample in a 20 ml scintillation vial.

**E) Metabolites of PAHs in Trout Bile**

$^{14}$C-labeled PAHs or $^{14}$C-DCP were administered to cannulated trout as described in Part III.B. Fish were sacrificed 144 h after chemical administration and the bile removed by gall bladder puncture. Sodium citrate buffer (pH 7.0, 1 ml) was added to the bile. Bile from PAH-dosed trout was extracted three times with 3-ml portions of methylene chloride. Bile from DCP dosed trout was acidified to pH 2 with 1N $H_2SO_4$ and extracted with hexanes. Extracts were combined and evaporated to dryness under nitrogen. The aqueous layers were adjusted to pH 2 with 1N sulfuric acid. The samples were hydrolyzed at 80°C for 24 h. The cooled, hydrolyzed bile was reextracted with 3-ml portions of the

appropriate organic solvent. The extracts were combined and evaporated under nitrogen. All residues were redissolved in hexanes (0.5 ml) and chromatographed on thin layer silica-gel plates. Developing solvent for 2-MN, fluorene and pyrene was benzene:ethanol (19:1) (Boyland and Sims, 1964) and hexanes for DCP. Plates were scraped into 0.5 cm strips and the silica gel in each zone was added to scintillation vials containing 10 ml Biofluor. The radioactivity in each vial was determined by LSC.

## RESULTS

### A) Chromatographic analysis of CPs and PAHs

Figure 3.2 shows a typical gas chromatograph of derivatized CPs. Recovery of CPs from spiked blood samples or water using hexanes as organic phase yielded recoveries of greater than 94% and recoveries from charcoal traps treated with spiked water was greater than 85%. The detection limit for CPs by GC analysis ranged from 10-20 pg/ml. Figure 3.3 shows a typical high-performance liquid chromatograph of PAHs. Values in excess of 96% were obtained for the recovery of PAHs from spiked blood and water samples using methylene chloride as organic phase, and was greater than 90% from charcoal traps treated with spiked water. Recoveries of both CPs and PAHs from spiked tissue samples with known quantities of the chemicals, exceeded 85% in all tissues. The detection limit for PAHs by HPLC analysis ranged from 0.05- 0.1 µg/ml.

### B) Toxicokinetics of CPs in Trout

The cannulation technique employed in these studies was suitable for the continuous blood sampling for the duration of the experiment.

The time courses of unchanged DCP and TCP in the blood of trout following an i.a. administration of 10 mg/kg are shown in Figure 3.4. The time courses of TTCP and PCP following an i.a. administration are shown in Figure 3.5. The concentration of the CPs in the blood appeared to decline biphasically with time.

The mean data from six trout were fitted to a two-compartment and a three-compartment model using a nonlinear least-squares regression analysis program. The low WSSR values obtained (data not shown) when fitting to the two- compartment model indicated the goodness of fit. The WSSR measures the deviation between the observed concentrations and the concentrations predicted by the fitted curve (Balant and Garrett, 1983). In addition to low WSSR values, the scatter of observed values about the predicted values was randomly distributed (data not shown) and the value for AIC was lower for the two-compartment model indicating a better interpretation of the experimental data than the three compartment model. Figure 3.6 shows the toxicokinetic model which was used to describe the disposition of CPs in trout.

The derived pharmacokinetic parameters for CPs in trout following an i.a. dose are shown in Table 3.1. The estimated half-lives for DCP, TCP, TTCP and PCP were 77.0, 69.3, 86.6 min and 7.6 h, respectively. Total body clearance of DCP, TCP, TTCP and PCP from trout were 6.4, 8.7, 6.0, and 2.7 ml/min, respectively.

The time course of unchanged DCP and TCP in the blood of trout exposed to 0.5 mg/l in water are shown in Figure 3.7. Similarily, the time course of TTCP and PCP

Figure 3.2. A typical gas chromatogram of derivatized CPs and internal standard. The peak identities are for a: I) solvent, II) 2,4-DCP, III) 2,4,6-TCP, IV) 2,3,4,6-TTCP and for b: I) solvent , II) 2,3,4,6-TTCP, III) PCP, and IV) 2,4,4'-tetraCDE.

b)

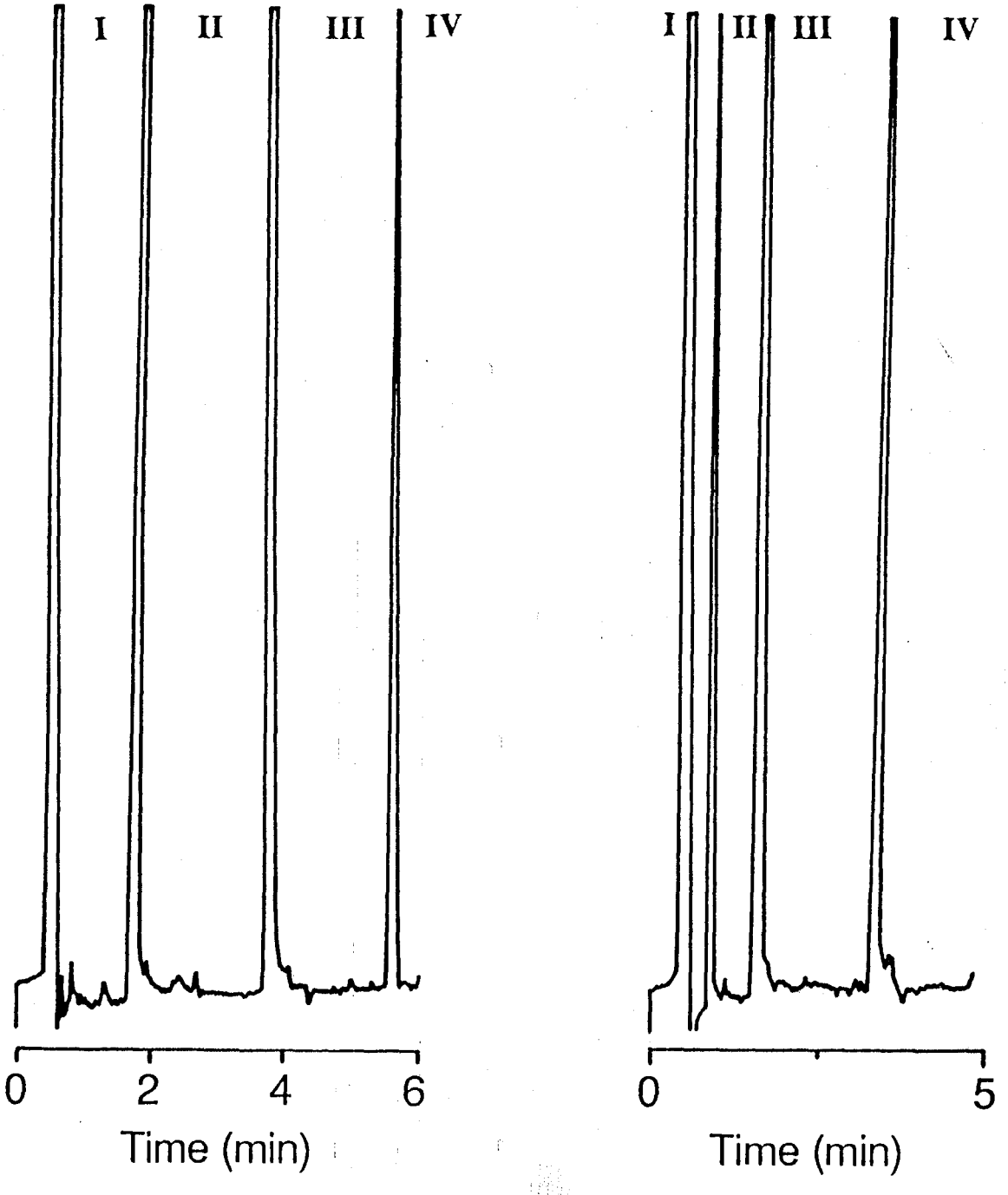


Figure 3.3. A typical high-performance liquid chromatogram of PAHs and internal standard. The peak identities are: I) 2-MN, II) fluorene, III) pyrene and IV) benzanthracene.

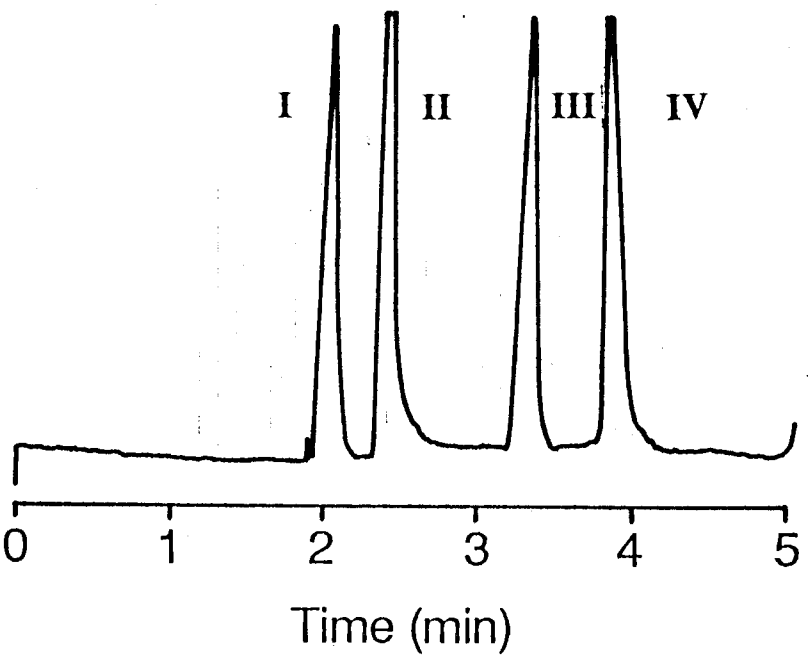

I
II
III
IV
0
1
2
3
4
5
Time (min)

Figure 3.4. Time course of unchanged a) 2,4-DCP and b) 2,4,6-TCP in trout blood following a single intraarterial adminstration of 10 mg/kg. The curve represents the two-compartment model prediction of the data. The values are the means ± S.D. of six fish.

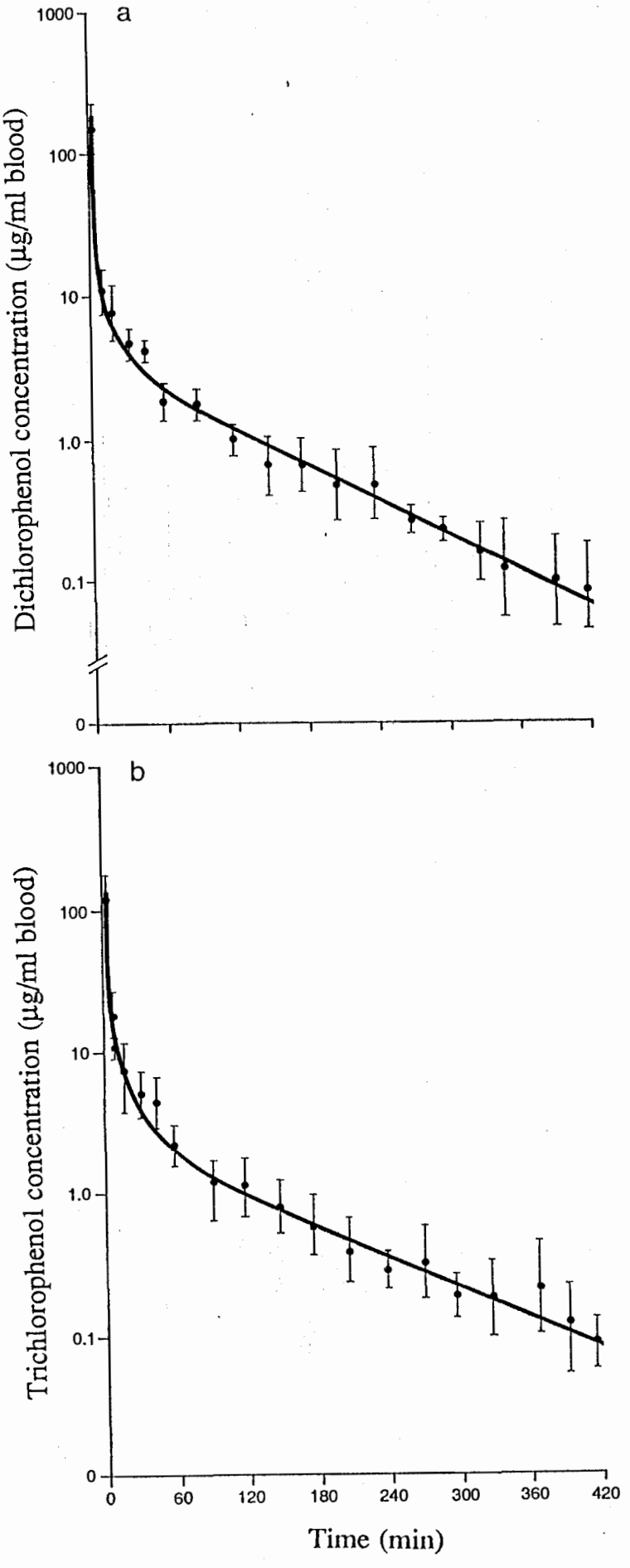
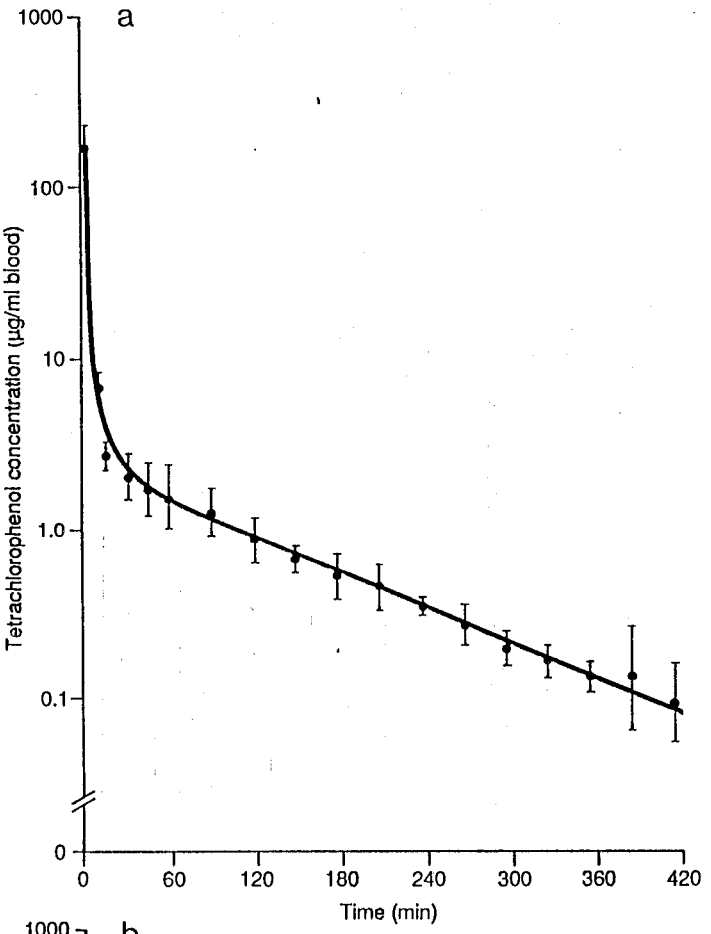
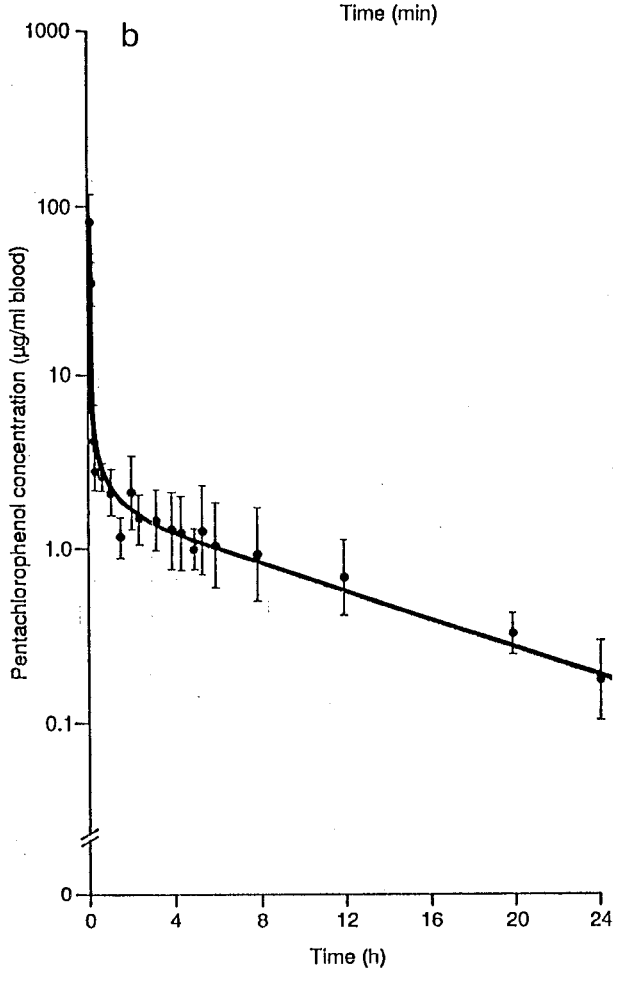

a
1000
100
10
1.0
0.1
0
Dichlorophenol concentration (µg/ml blood)
b
1000
100
10
1.0
0.1
0
Trichlorophenol concentration (µg/ml blood)
0
60
120
180
240
300
360
420
Time (min)

Figure 3.5. Time course of unchanged a) 2,3,4,6-TTCP and b) PCP in trout blood following a single intraarterial adminstration of 10 mg/kg. The curve represents the two-compartment model prediction of the data. The values are the means ± S.D. of six fish


a
1000
100
10
1.0
0.1
0
Tetrachlorophenol concentration (µg/ml blood)
0
60
120
180
240
300
360
420
Time (min)


b
1000
100
10
1.0
0.1
0
Pentachlorophenol concentration (µg/ml blood)
0
4
8
12
16
20
24
Time (h)

during exposure to 0.5 mg/l in water are shown in Figure 3.8. CPs were absorbed rapidly by trout since they could be detected in the blood 5 minutes after exposure to CPs in water.

The mean data from three trout were fitted to a one-compartment model using a nonlinear least-squares regression analysis program. Low WSSR values and a random distribution of observed values about the predicted values indicated the goodness of fit of the predicted line. The derived toxicokinetic parameters for CPs in trout during an exposure to CPs in water are shown in Table 3.2. The bioavailabilities calculated by equation (3) for DCP, TCP, TTCP and PCP were 11, 9, 16 and 36%, respectively.

## C) Toxicokinetics of PAHs in trout

Figure 3.9 shows the time course of unchanged 2-MN, fluorene and pyrene in the blood of trout following an i.a. administration. The concentration of the PAHs in blood appeared to decline triexponentially with time. The mean data from six trout were fitted to a two-compartment and a three-compartment model using a nonlinear least-squares regression analysis program. The WSSR and AIC values for the three-compartment model were in all cases lower than those of the two-compartment model. The scatter of observed values about the predicted values in the three compartment model were random. The three-compartment model was considered the best interpretation of the data. The three-compartment model chosen to describe the disposition of PAHs in trout is shown in Figure 3.10.

The parameter estimates of the fitted equation, along with the derived pharmacokinetic parameters are shown in Table 3.3. The terminal half-lives of decay in blood for 2-MN, fluorene and pyrene were 9.6, 10.5 and 12.8 h, respectively. Total body clearance of 2-MN, fluorene and pyrene from trout were 0.8, 1.2 and 1.1 ml/min, respectively.

Figure 3.6. Schematic representation of a two-compartment model consisting of a central compartment and a peripheral compartment used to describe the disposition of CPs in trout following intraarterial administration.

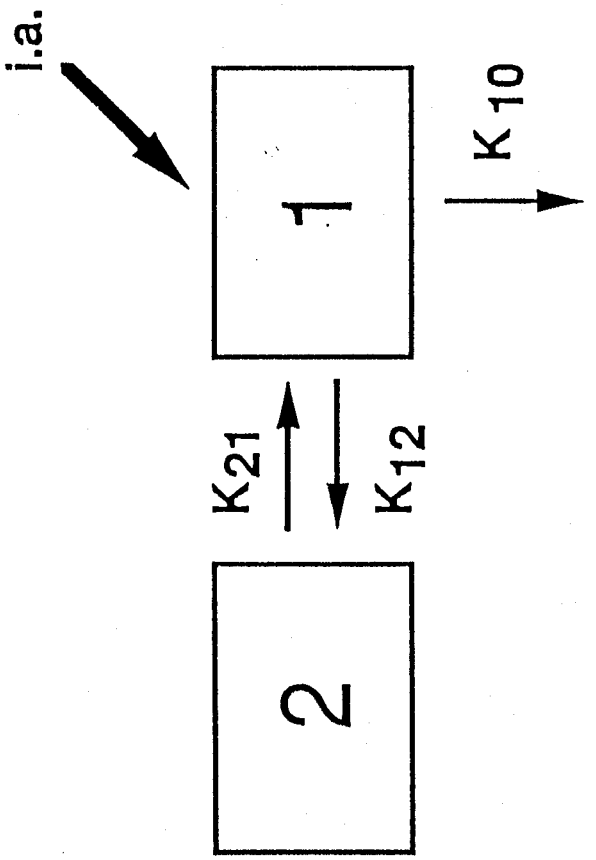

i.a.
1
K 10
K21
K12
2

Table 3.1. Model parameters describing blood concentrations of 2,4-DCP, 2,4,6-TCP, 2,3,4,6-TTCP and PCP in trout following a single inraarterial administration of 10 mg/kg.

| Parameters | 2,4-DCP | 2,4,6-TCP | 2,3,4,6-TTCP | PCP |
|---|---|---|---|---|
| $A_1$ (ug/ml) | 169 | 155 | 133 | 150 |
| $A_2$ (ug/ml) | 3.3 | 2.1 | 2.2 | 1.6 |
| $X_1$ ($min^{-1}$) | 0.565 | 0.501 | 0.348 | 0.198 |
| $X_2$ ($min^{-1}$) | 0.009 | 0.010 | 0.008 | 0.002 |
| $X_{1Hl}$ (min) | 1.2 | 1.4 | 2.0 | 3.5 |
| $X_{2HL}$ (min) | 77.0 | 69.3 | 86.6 | 346.5 |
| $k_{12}$ ($min^{-1}$) | 0.300 | 0.199 | 0.143 | 0.097 |
| $k_{21}$ ($min^{-1}$) | 0.020 | 0.017 | 0.014 | 0.004 |
| $k_{10}$ ($min^{-1}$) | 0.254 | 0.295 | 0.199 | 0.099 |
| AUC(ug•min/ml) | 1256 | 1377 | 1294 | 1949 |
| $V_c$ (ml) | 25.0 | 29.6 | 29.9 | 27.4 |
| $Q_b$ (ml/min) | 6.4 | 8.7 | 6.0 | 2.7 |

Figure 3.7. Time course of unchanged a) 2,4-DCP and b)2,4,6-TCP in the blood of trout exposed to 0.5 mg/l of one of the chemicals in water .The curve represents the one-compartment model prediction of the data. The values are the means ± S.D. of three fish.

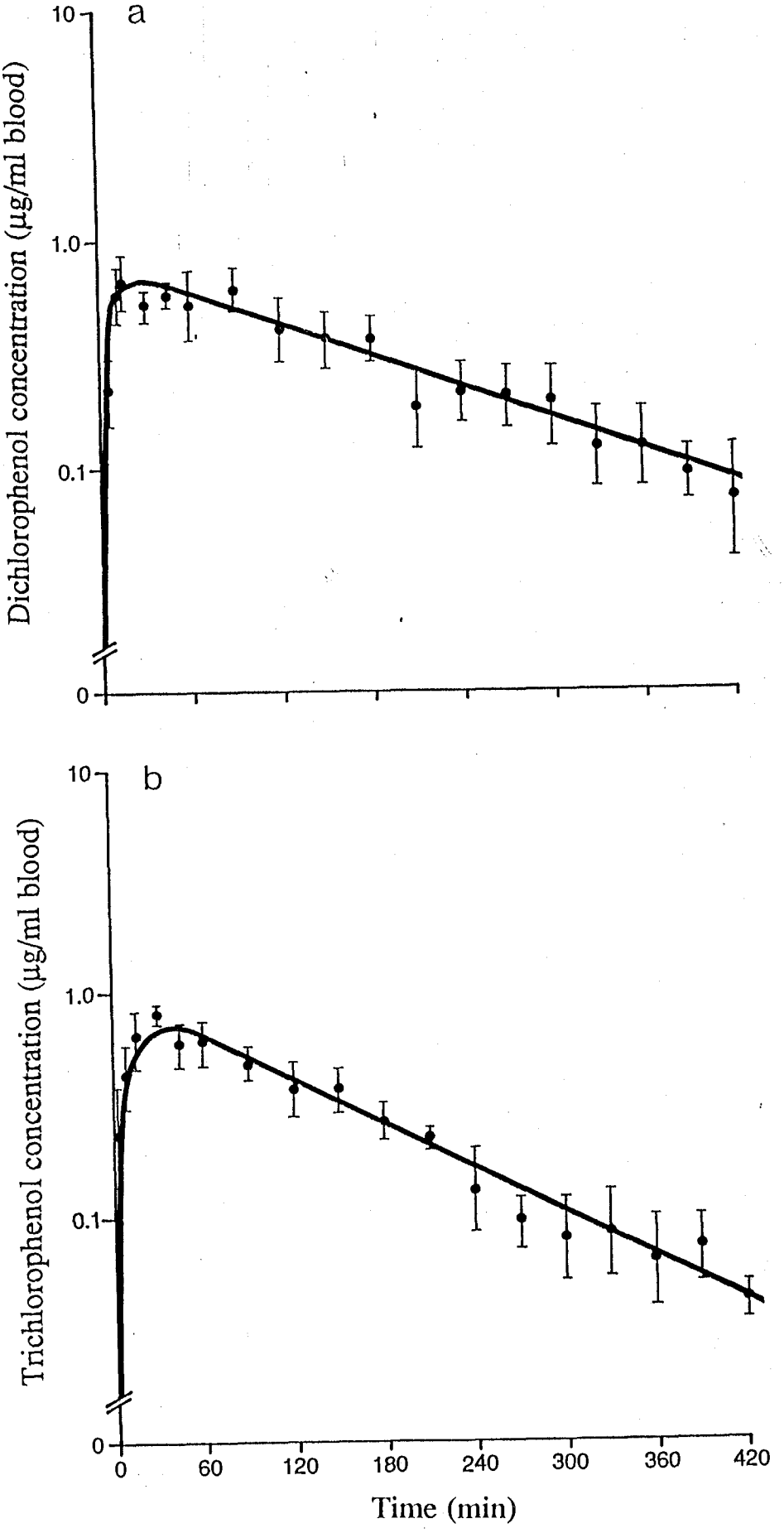
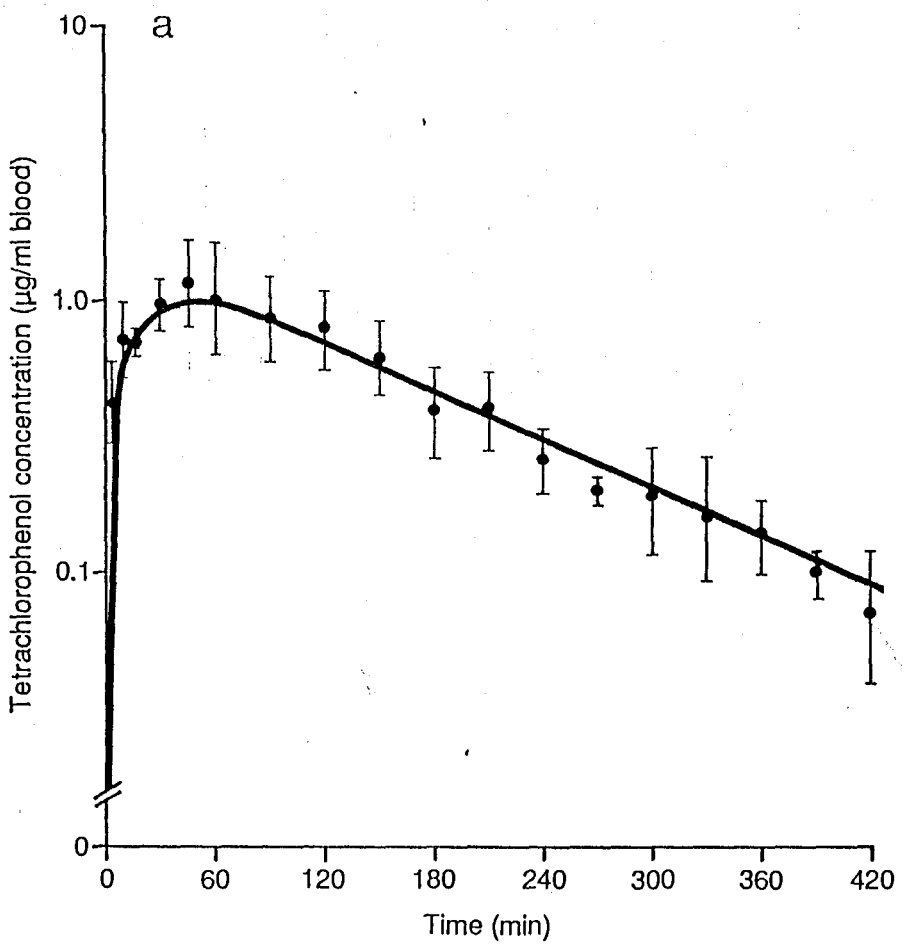

a
10
1.0
0.1
0
Dichlorophenol concentration (µg/ml blood)
b
10
1.0
0.1
0
Trichlorophenol concentration (µg/ml blood)
0
60
120
180
240
300
360
420
Time (min)

Figure 3.8. Time course of unchanged a) 2,3,4,6-TTCP and b) PCP in the blood of trout exposed to 0.5 mg/l of one of the chemicals in water. The curve represents the one-compartment model prediction of the data. The values are the means ± S.D. of three fish.


a
10
1.0
0.1
0
Tetrachlorophenol concentration (µg/ml blood)
0
60
120
180
240
300
360
420
Time (min)

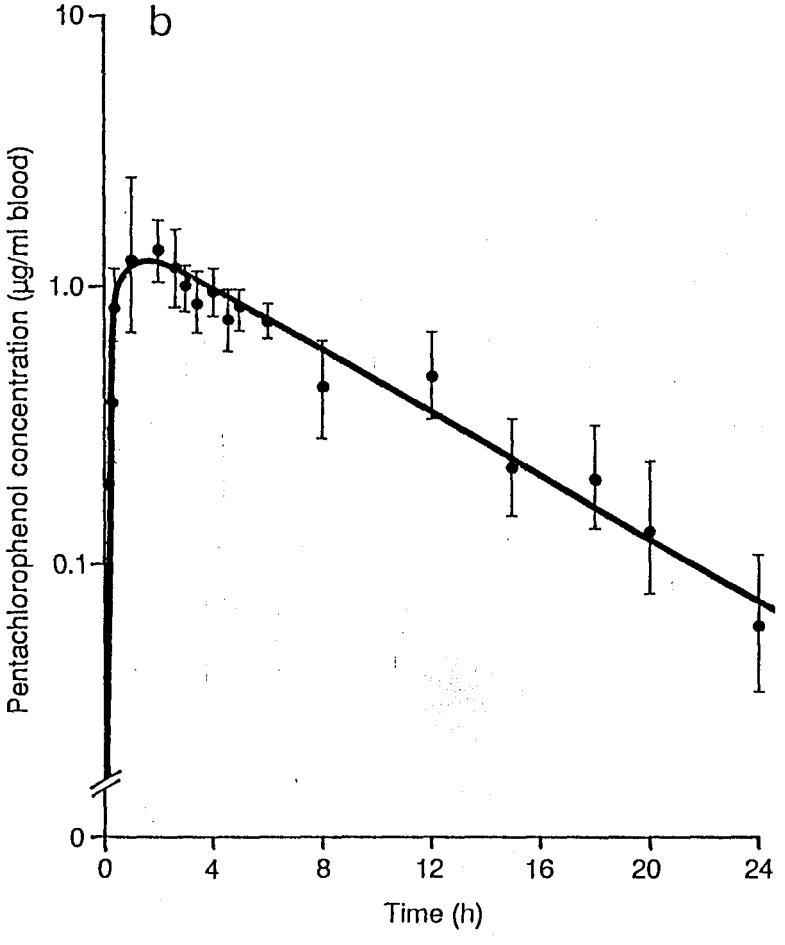

b
10
1.0
0.1
0
Pentachlorophenol concentration (µg/ml blood)
0
4
8
12
16
20
24
Time (h)

Table 3.2. Model parameters describing blood concentrations of 2,4-DCP, 2,4,6-TCP, 2,3,4,6-TTCP and PCP in trout exposed to a concentration of 0.5 mg/l of one of the chemicals in water.

| Parameters | 2,4-DCP | 2,4,6-TCP | 2,3,4,6-TTCP | PCP |
|---|---|---|---|---|
| $k_a$ ($min^{-1}$) | 0.573 | 0.229 | 0.385 | 0.096 |
| $k_e$ ($min^{-1}$) | 0.005 | 0.008 | 0.007 | 0.002 |
| $k_{aHL}$ (min) | 1.21 | 3.02 | 1.81 | 7.2 |
| $k_{eHL}$ (min) | 132 | 91.8 | 99.6 | 312 |
| $T_{max}$ (min) | 30.2 | 42.0 | 51.0 | 88.8 |
| $C_{max}$ (ug/ml) | .69 | 0.72 | 1.00 | 1.28 |
| AUC(ug•min/ml) | 142 | 117 | 201 | 702 |
| A (%) | 11 | 9 | 16 | 36 |

Figure 3.9. Time course of unchanged a) 2-MN, b) fluorene and c)pyrene in trout blood following a single intraarterial adminstration of 10 mg/kg. The curve represents the three-compartment model prediction of the data. The values are the means ± S.D. of six fish

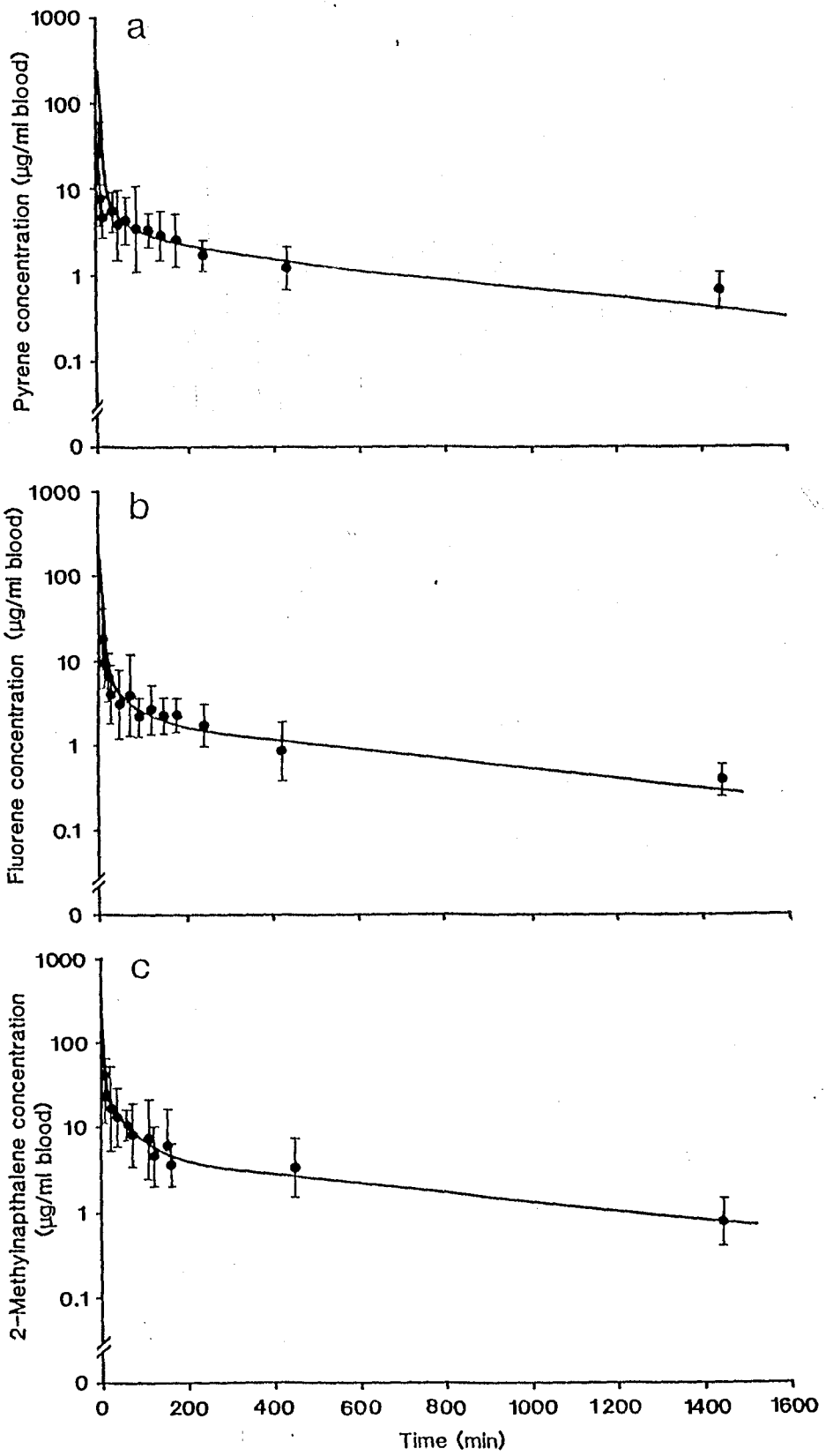

a
Pyrene concentration (µg/ml blood)
1000
100
10
1
0.1
0
b
Fluorene concentration (µg/ml blood)
1000
100
10
1
0.1
0
c
2-Methylnapthalene concentration (µg/ml blood)
1000
100
10
1
0.1
0
0
200
400
600
800
1000
1200
1400
1600
Time (min)

Figure 3.10. Schematic representation of a three-compartment model consisting of a central compartment, a "shallow" peripheral compartment, and a "deep" peripheral compartment, used to describe the disposition of CPs in trout following intraarterial administration.

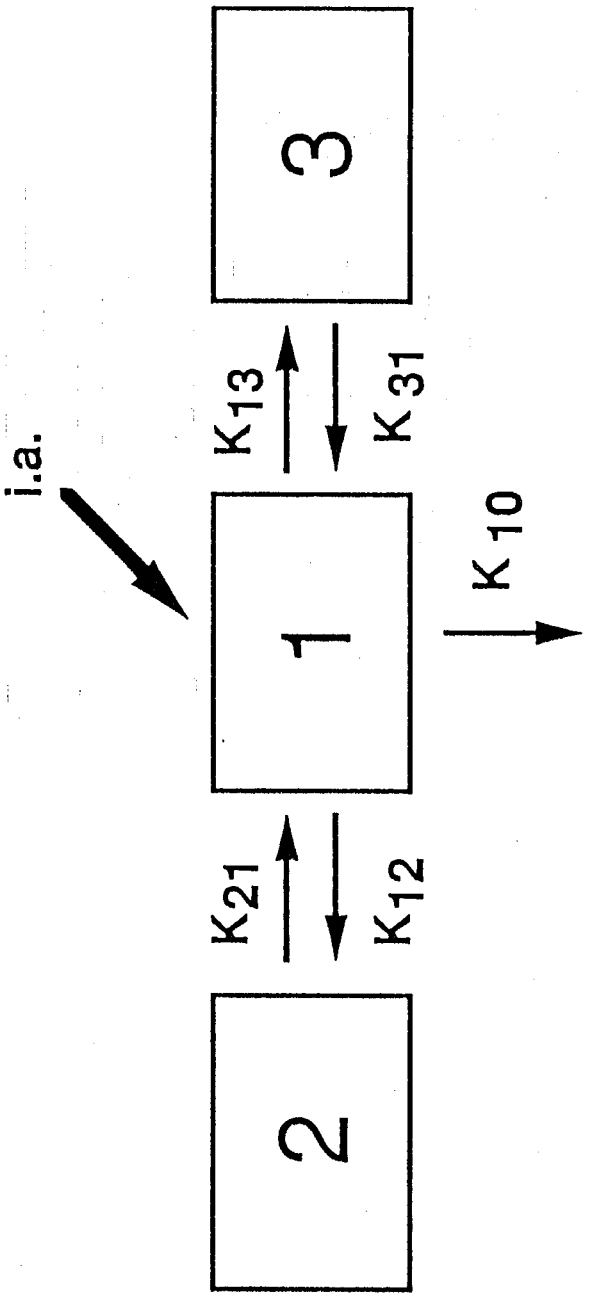

3
i.a.
K13
K31
1
K10
K21
K12
2

Table 3.3. Model parameters describing blood concentrations of 2-MN, fluorene and pyrene in trout following a single intraarterial administration of 10 mg/kg.

| Parameters | 2-MN | Fluorene | Pyrene |
|---|---|---|---|
| $A_1$ (ug/ml) | 141 | 140 | 194 |
| $A_2$ (ug/ml) | 21.9 | 14.2 | 4.7 |
| $A_3$ (ug/ml) | 6.6 | 3.8 | 2.8 |
| $X_1$ ($min^{-1}$) | .500 | 0.589 | .0443 |
| $X_2$ ($min^{-1}$) | 0.025 | 0.057 | 0.011 |
| $X_3$ ($min^{-1}$) | 0.001 | 0.001 | 0.001 |
| $X_{1HL}$ (min) | 1.4 | 1.2 | 1.6 |
| $X_{2HL}$ (min) | 27.7 | 12.1 | 63.0 |
| $X_{3HL}$ (min) | 577 | 630 | 770 |
| $k_{12}$ ($min^{-1}$) | 0.320 | 0.250 | 0.260 |
| $k_{21}$ ($min^{-1}$) | 0.100 | 0.112 | 0.025 |
| $k_{13}$ ($min^{-1}$) | 0.074 | 0.236 | 0.118 |
| $k_{31}$ ($min^{-1}$) | 0.006 | 0.008 | 0.004 |
| $k_{10}$ ($min^{-1}$) | 0.026 | 0.041 | 0.049 |
| AUC(ug•min/ml) | 5405 | 3556 | 3888 |
| $V_c$ (ml) | 25.4 | 28.1 | 22.4 |
| $Q_b$ (ml/min) | 0.8 | 1.2 | 1.1 |

Figure 3.11 shows the time course of 2-MN, fluorene and pyrene in the blood of trout after exposure to one of the PAHs in water. The PAHs were absorbed rapidly by trout since they were detected in the blood within 5 minutes of exposure. The mean data from three trout were fit to a one-compartment model using a nonlinear least-squares regression analysis program. Low WSSR values and a random distribution of observed values about the predicted values indicated the goodness of fit of the predicted line.

The derived pharmacokinetic parameters are shown in Table 3.4. The apparent bioavailabilities of 2-MN, fluorene and pyrene following branchial exposure are 20, 36 and 35%, respectively.

Individual fish blood-concentration data for both CPs and PAHs are shown in Tables A.8 to A.21.The pharmacokinetic parameters describing the i.a. administration of CPs and PAHs did not change when the chemicals were administered at a lower dose (1mg/kg) as determined by the method of superposition (Gibaldi and Perrier, 1975).

Figure 3.11. Time course of unchanged a) 2-MN, b) fluorene and c) pyrene in the blood of trout exposed to 0.5 mg/l of one of the chemicals in water . The curve represents the one-compartment model prediction of the data. The values are the means ± S.D. of three fish.

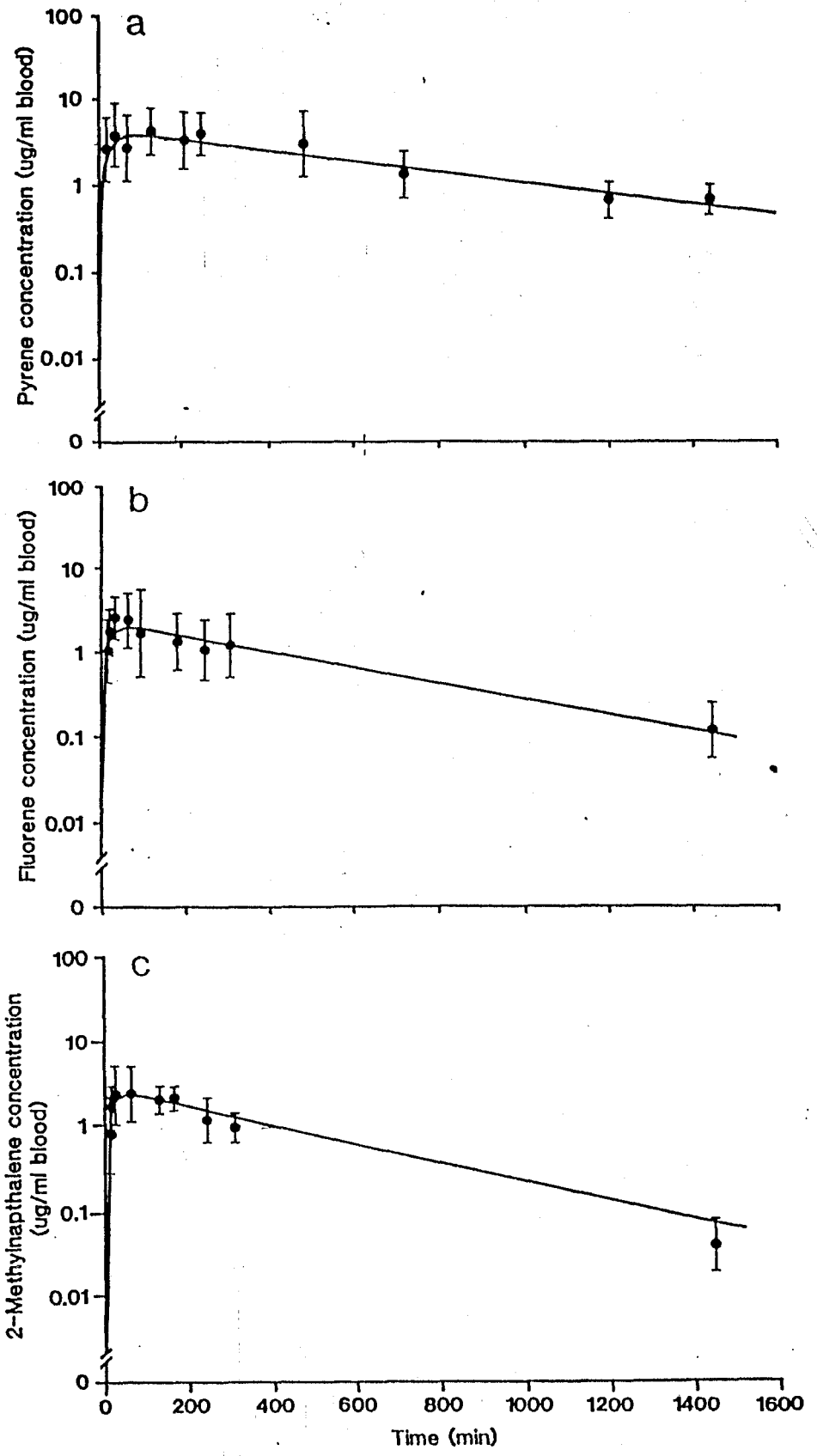

a
Pyrene concentration (ug/ml blood)
100
10
1
0.1
0.01
0
b
Fluorene concentration (ug/ml blood)
100
10
1
0.1
0.01
0
c
2-Methylnapthalene concentration (ug/ml blood)
100
10
1
0.1
0.01
0
0
200
400
600
800
1000
1200
1400
1600
Time (min)

Table 3.4. Model parameters describing blood concentrations of 2-MN, fluorene and pyrene in trout exposed to a concentration of 0.5mg/l of one of the chemicals in water.

| Parameters | 2-MN | Fluorene | Pyrene |
|---|---|---|---|
| $k_a$ ($min^{-1}$) | 0.097 | 0.115 | 0.177 |
| $k_e$ ($min^{-1}$) | 0.003 | 0.003 | 0.004 |
| $k_{aHL}$ (min) | 7.1 | 6.0 | 3.9 |
| $k_{eHL}$ (min) | 265 | 247 | 173 |
| $T_{max}$ (min) | 38.2 | 33.1 | 21.8 |
| $C_{max}$ (ug/ml) | 2.4 | 3.0 | 3.3 |
| AUC(ug•min/ml) | 1275 | 1524 | 1347 |
| A (%) | 20 | 36 | 35 |

## D) Disposition of Unchanged CPs and PAHs in Trout Tissues

CPs and PAHs were found in all of the tissues examined after a bolus injection of 10 mg/kg of one of the chemicals *via* the dorsal aorta of trout. The highest levels of unchanged CPs or PAHs in all tissues seemed to have reached a maximum level by the first sampling period (2 h for 2,4-DCP, 2,4,6-TCP and 2,3,4,6-TTCP; 4 h for PCP; 16 h for 2-MN, fluorene and pyrene). The highest levels of unchanged CPs were found in the liver, fat and the kidney and the lowest levels were usually found in the muscle and skin. The highest levels of unchanged PAHs were found in the liver and fat and the lowest levels in the muscle and skin. PAHs remained in the tissues of trout longer than CPs which were eliminated from trout tissues at a much faster rate. The time course of CPs and PAHs in the tissues are shown in Tables A.22 to A.28.

Fat was the tissue with the longest half-life of elimination for CPs and PAHs in trout. Table 3.5 shows the tissue distribution of radioactivity in trout 144 h following an i.a. administration of $^{14}$C-PAHs. In all tissues, the radioactivities (expressed as ug equivalents of $^{14}$C/g tissue) were higher than the amounts of unchanged PAHs/g tissue as determined by HPLC indicating that the radioactivity is attributable to both unchanged $^{14}$C-PAH and its metabolites. Radioactivity was highest in fat followed by liver, skin and kidney. The amount of radioactivity in most tissues, especially in the fat was much higher in trout exposed to $^{14}$C-pyrene than fish exposed to $^{14}$C-fluorene or $^{14}$C-2-MN.

When trout were given a single dose of 50 mg/kg containing 10 μCi of $^{14}$C-DCP or $^{14}$C-pyrene by gavage, CPs or PAHs were found in low levels in all tissues. In both cases, most of the absorbed radioactivity was associated with the gastrointestinal tract. The amount of DCP or pyrene absorbed was approximately 20 and 37%, respectively. Other than the gut, the bile, fat and liver also contained high levels of radioactivity. The results are shown in Table 3.6.

Table 3.5. Tissue distribution of PAH-derived radioactivity in trout 144 h following an intraarterial administration of 10 mg/kg. Values are the means ± S.D. of 3 trout.

| Organs and Tissues | Radioactivity (ug equivalent of $^{14}C$/g) | | |
|---|---|---|---|
| | 2-MN | Fluorene | Pyrene |
| Visceral fat | 190.7±21.4 | 192.7±35.6 | 345.3±63.3 |
| Liver | 33.5±4.9 | 27.0±3.9 | 48.9±6.7 |
| Stomach | 3.1±0.7 | 1.7±0.1 | 2.7±1.2 |
| Intestine | 3.9±1.2 | 1.4±0.9 | 2.5±0.9 |
| Kidney | 5.3±2.4 | 5.6±3.2 | 5.8±2.1 |
| Brain | 3.6±0.9 | 3.8±1.7 | 1.7±0.7 |
| Gill | 1.1±0.2 | 0.6±0.1 | 3.0±1.0 |
| Muscle | 0.2±0.0 | 0.1±0.1 | 0.4±0.3 |
| Skin | 6.8±2.7 | 2.7±1.3 | 6.0±1.4 |
| Swim bladder | 4.6±3.7 | N.D. | N.D. |

Table 3.6. Percent of total dose and percent body burden of $^{14}$C-DCP and $^{14}$C-pyrene-derived radioactivity in the tissues of trout 24 h following a single oral administration of 50 mg/kg. Values are from one fish.

| Tissue | % Total administered dose | | % Body burden | |
|---|---|---|---|---|
| | Pyrene | DCP | Pyrene | DCP |
| Liver | 2.0 | 0.9 | 5.4 | 4.5 |
| Gall bladder | 5.1 | 3.2 | 13.8 | 15.8 |
| Kidney | 0.7 | 1.1 | 1.9 | 5.5 |
| Muscle | 1.0 | 0.2 | 3.7 | 1.0 |
| Skin | 0.0 | 0.0 | 0.0 | 0.0 |
| Gill | 0.2 | 0.7 | 0.5 | 3.5 |
| Fat | 2.9 | 0.8 | 7.8 | 4.0 |
| Stomach | 19.8 | 11.2 | 53.4 | 55.5 |
| Intestine | 5.3 | 2.1 | 14.3 | 10.4 |
| Total in tissues | 37.0 | 20.2 | | |
| Total in wash | 52.3 | 57.9 | | |

## E) Excretion of CPs and PAHs by Trout

Fish housed in metabolic chambers seemed to remain healthy for at least six days.

Figure 3.12 shows the cumulative excretion of CPs in the urine, bile and gill water following an i.a. dose of 10 mg/kg. Unchanged CPs or their conjugates were not detected in any water sample from chamber B. Total percent dose of DCP, TCP, TTCP and PCP excreted in 144 h following an i.a. administration were 84, 83, 75 and 78%, respectively.

The percentage of total amount excreted in the urine, bile and gill water are shown in Table 3.7. CPs are excreted mainly by the gill of trout; greater than 46% of the total amount excreted was *via* the gill.

The percentages of CPs excreted as unchanged chemical and as conjugated CPs in the urine, bile and gill water following an i.a. administration of 10 mg/kg are shown in Table 3.8. Only unchanged CPs were detected in water which had passed over the gills. The urine and the bile contained mainly conjugated CPs (>90%). No CPs were found in the toluene traps indicating that all of the CPs excreted by the gill were in the water.

The cumulative excretion of $^{14}$C-PAHs in the urine, bile and gill water 144 h following an i.a. administration of 10 mg/kg are shown in Figure 3.13. Total percent dose of 2-MN, fluorene and pyrene excreted by 144 h were 74, 77 and 68%, respectively. The percentage of total amount excreted in the urine, bile and gill water is shown in Table 3.7. Little or no PAHs were excreted by the gill. It was possible that the metabolites of the chemicals were volatile, however, no PAHs were found in the toluene traps from chamber A. In all cases, greater than 53% of the radioactivity excreted was found in the bile. No radioactivity was detected in the water samples from chamber B.

Figure 3.12. Cumulative excretion of a) 2,4-DCP, b)2,4,6-TCP, c) 2,3,4,6-TTCP and 4) PCP by the gill (▨) and in the urine (■) and bile (□) of trout and the total percent dose excreted (▩). The values represent the means ± S.D. of three fish.trout following a single intraarterial administration of 10 mg/kg. The values represent the means ± S.D. of three fish.

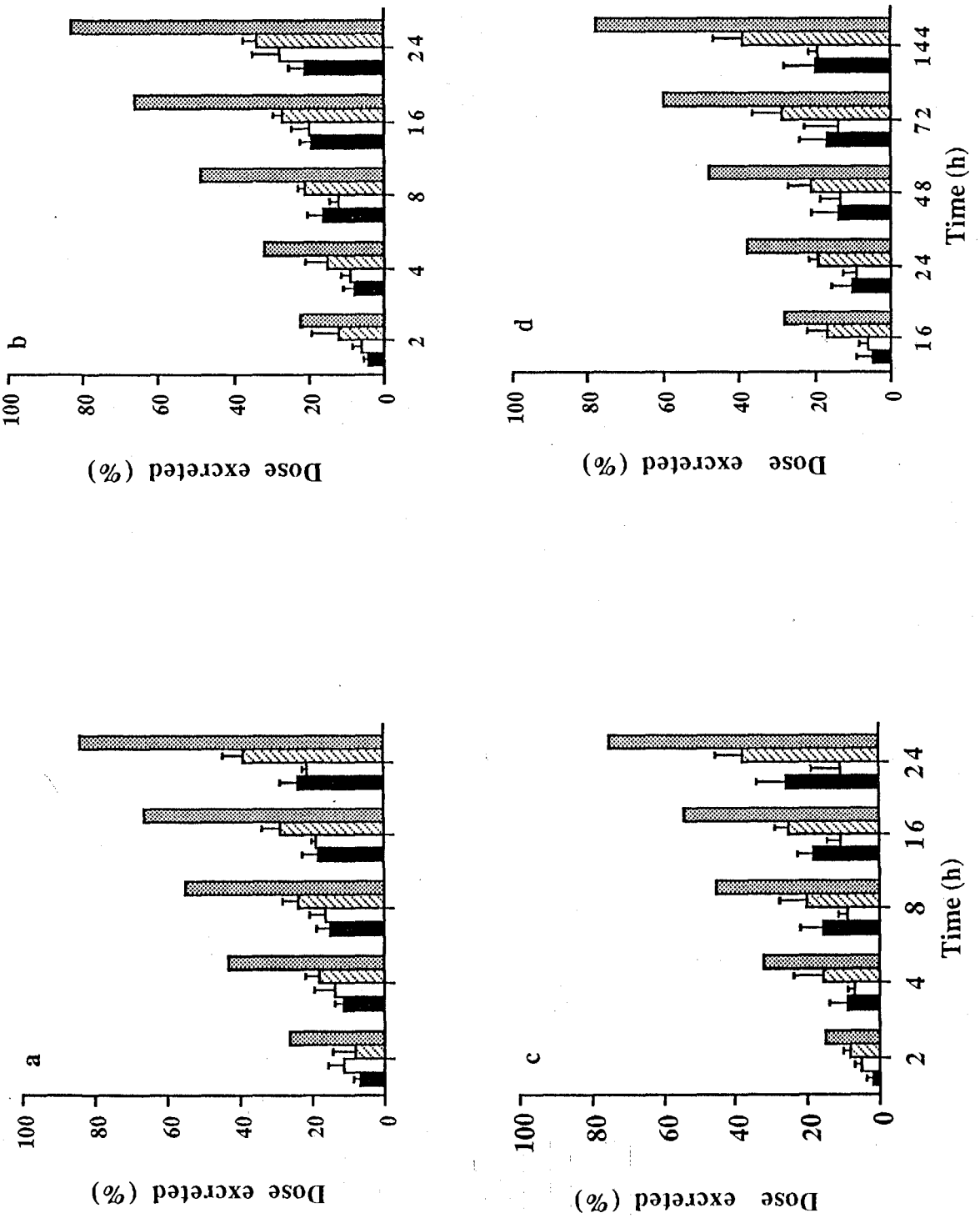

a
b
c
d
Dose excreted (%)
Time (h)
0
20
40
60
80
100
2
4
8
16
24
48
72
144

Table 3.7. The percent dose of CPs and $^{14}$C-PAHs excreted in the urine, bile and gill water following an intraarterial dose of 10 mg/kg. Values in parentheses are expressed as a percentage of total amount excreted dose. Values are the means ± S.D. for 3 trout.

| | % Dose excreted by 144 h. | | |
|---|---|---|---|
| Compound | Bile | Urine | Gill water |
| DCP | 21±2 (25±2) | 24±5 (29±6) | 39±5 (46±6) |
| TCP | 28±7 (34±8) | 21±4 (25±5) | 34±4 (41±5) |
| TTCP | 11±8 (15±11) | 26±8 (35±11) | 38±7 (51±9) |
| PCP | 19±3 (24±4) | 20±8 (26±10) | 39±8 (50±10) |
| 2-MN | 54±3 (73±4) | 20±4 (27±5) | <1 |
| Fluorene | 56±6 (73±8) | 21±4 (27±5) | <1 |
| Pyrene | 53±4 (78±6) | 15±3 (22±4) | <1 |

Table 3.8. The percentages of CPs excreted as parent compound and conjugated CPs in the urine, bile and gill water 144 h following an intraarterial administration of 10 mg/kg. Values are the means ± S.D. of three trout.

| Excretion route | Coumpound DCP | TCP | TTCP | PCP |
|---|---|---|---|---|
| | | %form excreted | | |
| Urine | | | | |
| Parent | 9.3±1.7 | 3.3±0.9 | 5.7±1.2 | 9.1±2.5 |
| Conjugates | 90.7±1.7 | 96.7±0.9 | 94.7±1.2 | 90.9±2.5 |
| Bile | | | | |
| Parent | 3.0±0.8 | 6.3±2.5 | 2.0±0.8 | 4.3±1.2 |
| Conjugates | 97.0±0.8 | 93.7±2.5 | 98.0±0.8 | 95.7±1.2 |
| Gill water | | | | |
| Parent | 100.0 | 100.0 | 100.0 | 100.0 |
| Conjugates | 0.00 | 0.00 | 0.00 | 0.00 |

Figure 3.13. Cumulative excretion of a) 2-MN, b) fluorene and c) pyrene in the urine(■), bile (□) and total percent dose excreted (▨), following a single intrarterial administration of 10 mg/kg. The values represent the means ± S.D. of three fish.

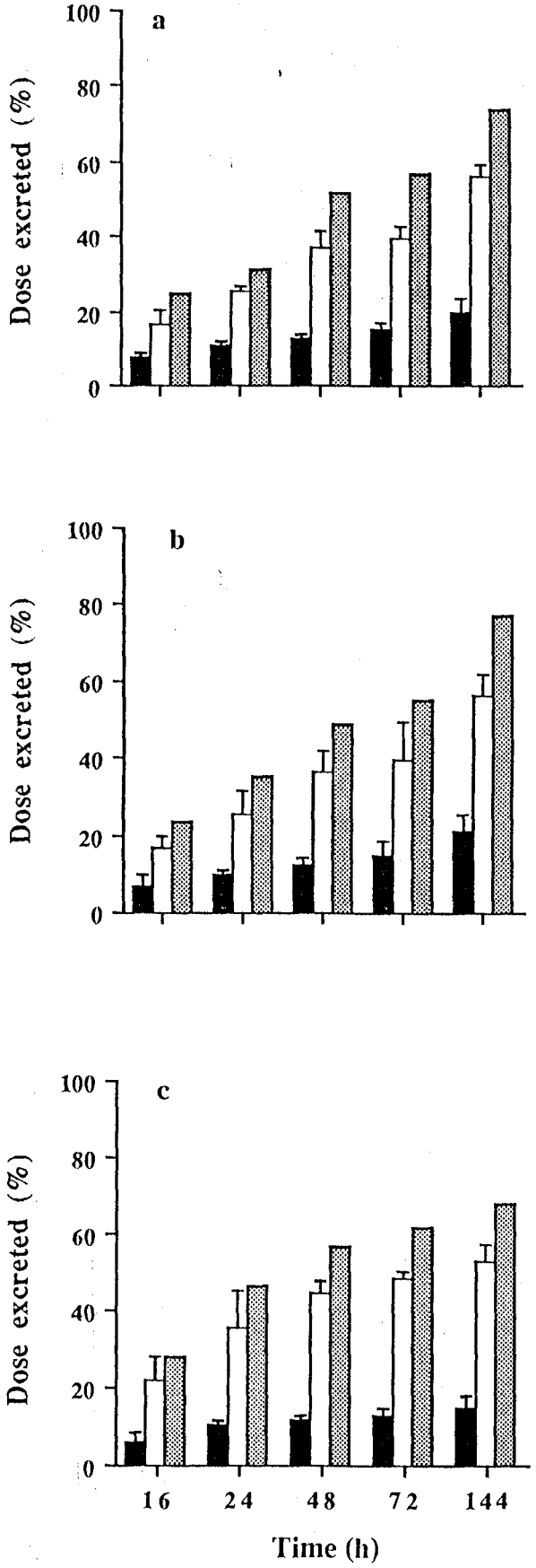

a
b
c
Dose excreted (%)
100
80
60
40
20
0
16
24
48
72
144
Time (h)

Table 3.9. The percentages of PAHs excreted as parent compound, organic soluble metabolites and conjugated metabolites in the urine and bile 144 h following an intraarterial administration of 10 mg/kg. Values are the means ± S.D. of three trout.

| Excretion route | Coumpound 2-MN | Fluorene | Pyrene |
|---|---|---|---|
| | | %form excreted | |
| Urine | | | |
| Parent | N.D. | N.D. | N.D. |
| Organic-soluble | 2.0±1.7 | 3.0±2.4 | 2.5±1.4 |
| Conjugates | 83.8±3.0 | 86.3±4.5 | 81.0±4.6 |
| Bile | | | |
| Parent | 0.4±0.1 | 0.1±0.1 | 0.5±0.4 |
| Organic-soluble | 21.6±6.0 | 9.8±2.6 | 5.1±1.9 |
| Conjugates | 68.6±5.5 | 81.6±0.9 | 86.7±4.1 |

The percentages of $^{14}$C-PAHs excreted as parent compound, organic soluble metabolites and as conjugated metabolites in the urine and bile 144 h following an i.a. dose of one of the PAHs are shown in Table 3.9. None of the radioactivity present in the urine was attributed to unchanged PAHs. The organic soluble metabolites constituted approximately 2-3% of the radioactivity excreted in the urine. The majority of the radioactivity in the urine was represented by conjugated metabolites which contributed to 84, 86 and 81% of the total radioactivity in the urine for 2-MN, fluorene and pyrene, respectively. Unchanged $^{14}$C-PAHs were detected in the bile of trout, however, the percent of total bile radioactivity as unchanged chemical was low. A small percentage of biliary radioactivity was as organic-soluble metabolites and the majority of bile radioactivity was attributed to conjugated metabolites.

## F) Metabolism of PAHs and CPs in Trout

The TLC profiles of organic extracts of the untreated and hydrolyzed bile from trout dosed with $^{14}$C-DCP and $^{14}$C-PAHs are shown in Figures 3.14 and 3.15, respectively. The peak which migrated the furthest was the parent compound (as determined by co-development with unlabeled standards). The radioactivity at the origin of the TLC profiles of unhydrolyzed bile may represent the more polar metabolites of the organic-soluble fraction of bile.

In the bile of trout treated with $^{14}$C-DCP, very little radioactivity was present in the organic-soluble fraction. The TLC profile of the organic extract of the hydrolyzed bile revealed a large peak that migrated the same distance as the DCP standard. The TLC profiles of bile of trout treated with $^{14}$C-pyrene revealed at least two metabolite peaks. The TLC profile of bile of trout treated with $^{14}$C-fluorene or $^{14}$C-2-MN revealed at least one metabolite peak. Little radioactivity was present in peaks corresponding to the parent PAHs

Figure 3.14. Radiochromatogram of organic solvent extract of the bile of trout injected with 10 mg/kg of a) $^{14}$C-DCP, b) $^{14}$C-2-MN, c) $^{14}$C-fluorene or d) $^{14}$C-pyrene.

Radioactivity (dpm x 10 3 )
0
1
2
3
4
a
0
13


Radioactivity (dpm x 10 3 )
0
1
2
3
4
5
c
0
8
16
Distance from origin (cm)


Radioactivity (dpm x 10 3 )
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
b


Radioactivity (dpms x 10 3 )
0
2
4
6
8
d
0
8
16
Distance from origin (cm)

Figure 3.15. Radiochromatogram of organic solvent extract of acid hydrolyzed bile of trout injected with 10 mg/kg of a) $^{14}$C-DCP, b) $^{14}$C-2-MN, c) $^{14}$C-fluorene or d) $^{14}$C-pyrene.

a
b
c
d
Radioactivity (dpms x 10 5 )
Distance from origin (cm)
0
8
13
16
1.0
0.8
0.6
0.4
0.2
0.0
1
2
3

in the profiles of bile from trout treated with fluorene and pyrene. The peak corresponding to 2-MN in the profile of the organic-soluble fraction of bile was large, indicating that 2-MN may be excreted unchanged into the bile or a metabolite was co-developed with 2-MN. Hydrolysis and subsequent extraction of the bile of trout treated with labeled PAHs resulted in TLC profiles which were similar to those of the organic extracts of unhydrolyzed bile, however, the peaks which chromatographed with the parent compounds were smaller in relation to the other peaks.

## DISCUSSION

The results of these experiments indicate that the toxicokinetics of i.a. administered CPs can be described adequately by a two-compartment open toxicokinetic model which consists of a central compartment and a peripheral compartment. The central compartment represents the vascular system of trout. The calculated volume of the central compartment (Vc) (Tables 3.1) is similar to the blood volume of trout (5.58% of body weight) reported by Milligan and Wood (1982).

The terminal half-lives of elimination of CPs in trout are similar for DCP, TCP and TTCP, however, the terminal half-life of elimination of PCP is considerably longer. The estimated half-life of PCP in trout blood after an i.a. administration was similar to that obtained by Glickman *et al.* (1977) in the blood of trout exposed to PCP in water at a concentration of 0.05 mg/l for 24 h. McKim *et al.* (1986) estimated the whole body half-life of PCP in trout to be approximately 65 h but stated that the loss of PCP in gill tissue and blood may be more rapid than that from the fish as a whole. PCP has a longer terminal half-life of elimination and a smaller body clearance (Qb) than the lower chlorinated phenols and is consistent with the fact that PCP is more lipophilic than the lower chlorinated phenols.

The data indicated that the toxicokinetics of i.a administered PAHs in trout can be described adequately by a three-compartment open toxicokinetic model which consists of a central compartment, a shallow peripheral compartment and a deep peripheral compartment. As with CPs, the central compartment represents the vascular system of trout since the calculated volume of the central compartment is similar to the blood volume of the trout used in the experiments. Since high levels of unchanged PAHs are found in fat and liver, these tissues may represent the deep peripheral compartment.

The PAHs have long terminal half-lives of elimination and small body clearances. The half-lives of the PAHs range from 9.6 h to 12.8 h and the Qb values range between 0.65-1.09 ml/min/kg. These results indicate that the PAHs are eliminated slowly by trout, which are in general agreement with the values of the calculated rate constants (Table 3.3). The large k12 and k13 (the rate constants describing the transfer of the PAHs into the peripheral compartments) and the small k21 and k31 ( the rate constants describing the transfer of the PAHs out of the same compartments) values indicated that the PAHs tend to remain in the peripheral compartments and return slowly to the central compartment for elimination. The half-life of napthalene in the blood of fingerling trout exposed to $^{14}$C-napthalene in water (Melancon and Lech, 1978) was similar to the half-life of 2-MN in the present study. Our results indicate that pyrene had a longer half-life in trout than did 2-MN. Lee et al. (1972b) reported similar findings in that the more lipophilic BaP was more persistent in the tissues of fish than naphthalene.

Bioavailability describes the percentage of a given dose of a chemical which reaches the general circulation. Both $^{14}$C-DCP and $^{14}$C-pyrene were found in all major tissues of trout 24 h following an oral administration of 10 mg/kg. At 24 h, 20 and 37% of the administered dose of DCP and pyrene was found in tissues of the trout. It should be noted that the more lipophilic pyrene was absorbed to a greater extent than DCP. In the environment, however, PAHs may not be absorbed to a greater extent than less lipophillic compounds, due to such factors as a lower water solubility or greater affinity to sediment particles. In each case over 65% of the body burden of DCP or pyrene was associated with the stomach and intestine. Whittle *et al.* (1977) have reported that more than 98% of orally administered BaP to fish was associated with the stomach and gut. Schnitz *et al.* (1987) have shown that no more than 12.4% of an orally administered dose of 7,12-dimethylbenzanthracene to trout was associated with the non-alimentary tissues. Jiminez *et*

*al.* (1987) have reported that dietary consumption of food contaminated with BaP does not contribute significantly to the total BaP body burden of fish.

The rapid appearance of CPs and PAHs in the blood of trout following whole body exposure indicate that the chemicals are removed rapidly from water. The estimated bioavailabilities are similar for DCP and TCP but are higher for TTCP and PCP. McKim *et al.*, (1986) calculated the uptake efficiency value of 33% for PCP in rainbow trout, which is similar to the bioavailability value calculated in our experiments (36%). PAHs are also rapidly taken up from water by fish (Lee *et al.*, 1972; Neff *et al.*, 1976). Kennedy *et al.* (1989) found that the gulf toadfish (*Opsanus beta* ) removed approximately 65% of the added BaP in a 1 L flask within 24 h. The results of our experiments indicated that the higher-ring numbered PAHs were more bioavailable by the branchial route than was 2-MN. This is probably due in part to an increase in lipophilicity with increasing molecular weight, however with very large PAHs, molecular size may actually inhibit accumulation (Varanasi et al., 1985). Our findings were contrary to the results of Lee *et al.* (1972) who reported that the uptake of $^{14}$C-napthalene from water *via* the gills of 3 species of marine fish (*Gillichthys mirabilis*, *Oligocoltus maculosus* and *Citharichthys stigmaeus*) was greater than $^{3}$H-BaP and could be detected in the tissues of the fish within a few minutes after the introduction of the hydrocarbons to the water. Jiminez *et al.* (1987) found that the uptake of $^{14}$C-BaP by the Bluegill sunfish, *Lepomis macrochirus*, was rapid during exposure to BaP in water. The bioavailability values estimated for both CPs and PAHs are constant, however, the absolute amount of a chemical taken up into a fish depends upon the concentration in the water and the amount of water passing over the gills. Kennedy *et al.* (1989) found that the uptake of BaP by the gulf toadfish was directly proportional to the chemical concentration in water and that calculated $Q_{10}$ values ( 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 (Hochachka and Somero, 1984)) suggest that uptake is modulated

by temperatue-induced changes in respiration rate or convection volume. Bioavailability data can aid in the determination of the actual dose of a waterborne chemical which enters the fish *via* the gills. Such data may be useful in comparing toxicity data on fish exposed to chemicals in water with that of mammalian data (McKim and Heath, 1983).

CPs and PAHs were found in all tissues of trout following i.a. administration. The levels of unchanged parent compound had appeared to have reached a maximum concentration in all tissues by the first sampling period. The highest levels of unchanged CPs and PAHs were found in the liver and fat of the fish. These results are consistent with those of other studies (Glickman *et al.*, 1977; Melcanon and Lech, 1977; Roubal *et al.*, 1977). Glickman *et al.*, (1977) reported that higher levels of PCP were found in fat and liver than in the blood or muscle of trout exposed to PCP in water, but declined rapidly when fish were transferred to fresh uncontaminated water. The decline of unchanged CPs in the fat of trout was slower than in other tissues; the half-lives of PCP in the fat of trout after exposure to PCP in water was approximately 23.7 h compared to blood (6.2 h), muscle (6.9 h) and liver (9.8 h). Previous studies have also shown that the fat and liver contained higher levels of unchanged chemicals than other tissues after exposing fish to PAHs in water (Roubal *et al.*, 1977; Melcanon and Lech, 1978; Kennedy *et al.*, 1989). Melcanon and Lech (1978) found that the amount of unchanged $^{14}$C-napthalene in fat and liver of trout declined more slowly than in blood and muscle. These results are consistent with the data obtained in the present study with 2-MN, fluorene and pyrene. Varanasi *et al.* (1978), however, found that within a few hours of exposing rainbow trout to napthalene, detectable amounts of napthalene and its metabolites were found in the skin of rainbow trout regardless of route of chemical exposure. When trout were exposed to labelled PAHs in our experiment, only low levels of radioactivity were found in the skin or associated mucous.

Forster and Goldstein (1969) indicated that the gills of fish are not very efficient in eliminating xenobiotics. Adamson and Serber (1974) showed that only a limited amount of a foreign compound is excreted across the gill of the dogfish. In contrast, Maren *et al.* (1968) reported that lipid soluble drugs such as MS 222 were rapidly excreted by diffusion across the gill. Our results show that CPs were eliminated mainly across the gills of the fish. Other studies have shown that the majority of PCP taken up by fish is eliminated mainly by the gill (Kobayashi, 1979; McKim *et al.*, 1986). Branchial elimination was the major route of phenol, 3-nitrophenol and 3,5-diethylphenol in the goldfish (Nagel and Urich, 1980). Braun *et al.* (1977) found that about 80% and 19% of administered PCP was excreted in the urine and feces of rats, respectively. Similar results were found in the mouse (Jakobson and Yllner, 1971) and the monkey (Braun and Sauerhoff, 1976)). In the present study, trout excreted approximately 50% , 26% and 24% of the administered dose by the gills, urine and bile, respectively. These results are similar to those found in mammals since the gill of fish is in many ways analagous to the mammalian kidney.

Trout excreted CPs *via* the gill mainly as unchanged CPs, which is consistent with Thomas and Rice (1982) who reported that the polar metabolites of xenobiotics such as conjugates are not easily released by the gills of fish. In contrast, the urine contained approximately 10% unchanged CPs and 90% conjugated CPs, whereas the bile consisted of approximately 97% conjugated CPs and 3% unchanged compound. The values are consistent with those reported in rainbow trout exposed to PCP in water (McKim *et al.* ,1986). Glickman *et al.*(1977) reported that PCP was excreted into the bile of trout exclusively as a glucuronide conjugate. However, Stehly *et al.* (1989) reported that a significant amount of PCP-sulfate was excreted in the bile of fish exposed to PCP in water. Kobayashi *et al* (1976) found that among four species of fish studied, trout liver had the lowest activity of sulfate conjugation to PCP. It has also been reported that goldfish excreted PCP as a sulfate conjugate in the urine and branchial water.

The present study indicates that rainbow trout excreted the PAHs *via* the urine and the bile. These results are similar to those obtained by other investigators; Stein *et al.* (1984) suggested that gills may be a significant non-dietary route of uptake of BaP but not a major route of elimination.Thomas *et al.* (1982) found that only small amounts of BaP-derived radioactivity were found to be excreted by the gill of dolly varden char (*Salvelinus malma* ) force-fed BaP. The major route of PAH excretion in fish, regardless of the route of exposure, appears to be *via* the bile (Varanasi *et al.*, 1981; Stein *et al.*, 1984; Kennedy *et al.*, 1989). However, low molecular weight compounds such as benzene and napthalene are known to be excreted by the gill and skin(Thomas and Rice, 1982; Varanasi et al., 1978). 2-MN was not excreted via the gill in trout and may be due to an increased lipophilicity or size associated with the substitution of the molecule. The additional methyl group was shown to increase the bioaccumulation 5- fold compared to napthalene in coho salmon (*Onchorhychus kisutch*) exposed to the PAHs in water (Korn and Rice, 1981).

The large proportion of aqueous soluble metabolites found in the bile of PAH-treated fish indicate that many Phase I metabolites were formed by the hepatic microsomal monooxygenase system. Many studies have shown that fish can metabolize xenobiotics to Phase I metabolites (for review see: Buhler and Williams, 1989). For example, Kennedy *et al.* (1989) identified 13 Phase I metabolites of BaP, including phenols, diols, triols, tetrols and quinones in the bile of the gulf toadfish exposed to BaP in water. Melancon and Lech (1982) showed that rainbow trout metabolized 2-MN to three dihydrodiols and a hydroxymethyl metabolite. The levels of unchanged PAH and organic-soluble metabolites in the bile are similar to the results from other studies ( Gmur *et al.*, 1982; Stein *et al.*, 1987; Kennedy *et al.*, 1989).

The prescence of aqueous soluble radioactivity in the bile of trout after extraction with organic solvent indicates that trout can readily detoxify environmental pollutants such

as 2-MN, fluorene and pyrene. Acid hydrolysis and subsequent solvent extraction indicated that the majority of the aqueous soluble radioactivity in bile was as conjugated metabolites. The levels of conjugated PAHs found in this study are similar to those found in English sole (Gmur *et al.*, 1982), rainbow trout (Melancon and Lech, 1984) and the gulf toadfish (Kennedy *et al.*, 1989). It was demonstrated by B-glucuronidase hydrolysis that approximately 50% of the biliary metabolites of 2-MN were glucuronide conjugates in rainbow trout (Melancon and Lech, 1984). The conjugation of many xenobiotics with glucuronic acid in rainbow trout (Lech, 1973; Statham *et al.*, 1975; Statham and Lech, 1975; Melancon and Lech 1976a; Melancon and Lech, 1976b; Glickman *et al.*, 1977; Glickman *et al.*, 1981) and in the bile of coho salmon (Roubal *et al.*, 1977; Krahn *et al.*, 1980) has also been described.

# Part IV Uptake and Metabolism of PAHs by Trout Hepatocytes

## INTRODUCTION

Xenobiotics which are absorbed by an aquatic organism are usually lipophillic in nature and must be converted to more polar derivatives in order to facilitate or enhance the excretion of the chemical. Such reactions allow an organism to excrete xenobiotics which may otherwise accumulate to harmful levels. The metabolism of xenobiotics has been called detoxification or deactivation since metabolism usually results in the production of more polar and less toxic metabolites. It is now widely recognized that the products of xenobiotic metabolism may result in the "bioactivation" or "toxication" of the parent compound since metabolites which are as toxic, or even more toxic than the parent compound are often formed (Guengerich and Liebler, 1984; Mitchell and Horning, 1984; Nelson, 1982).

As in mammals, the liver of fish is the site of the highest specific and total xenobiotic metabolizing enzymes of any tissue, although biotransformation activities have been noted in extrahepatic tissues. For example, high levels of aniline hydroxylase activity have been found in the 10,000 *g* supernatant fraction of the anterior and posterior kidney (approximately 40% of hepatic values), heart, muscle and blood of rainbow trout (Buhler and Rasmusson, 1968). This is not the case with all enzymes or in all species, as Pederson *et al.* (1974), using a sensitive assay for BaP hydroxylation, did not detect any activity in the blood, muscle or gill of the steelhead trout, *O. mykiss*.

Reactions of xenobiotic metabolism are usually classified into two categories: Phase I and Phase II reactions. Phase I reactions include reduction, hydrolysis and oxidation reactions and usually introduce a polar functional group into the molecule ( for review see: Buhler and Williams, 1989). Oxidation reactions are the most important Phase I reactions and occur in both the microsomal and cytosolic fractions of the cell. These reactions include the cytochrome P-450-dependent mixed function oxidases (MFOs or monooxygenases), flavin-containing monooxygenases (FMO) and the epoxide hydrolases. The cytochrome P-450 system is the most important of the aforementioned, and is largely located in the membranes of the endoplasmic reticulum. Like many of the other xenobiotic enzymes, upon disruption of the cell, the cytochrome P-450 monooxygenases are associated with the microsomal fraction. The cytochrome P-450 system is associated with the phospholipid of the endoplasmic reticulum and consists of a two-enzyme system containing cytochrome P-450 and a flavoprotein which inserts an atom of oxygen into the substrate and reduces another to form water. Although significant amounts of cytochrome P-450 occur in hepatic microsomes of many teleosts, it is generally lower than that found in rat microsomes, (Malins and Sargen, 1974) although the substrate specificities appear to be similar to those seen in mammalian systems (Vodicnik *et al.*, 1981).

Xenobiotics with polar functional groups may undergo Phase II reactions which involve the conjugation of the foreign chemical with an endogenous molecule such as glucuronic acid, sulfate or glutathione. Xenobiotics without a polar functional group must first be metabolized by Phase I reactions before being metabolized by Phase II reactions. Conjugated products are highly water-soluble and are more easily excreted than either the parent compound or Phase I metabolite (for review see: Foureman, 1989).

As mentioned earlier, metabolism of xenobiotics may bioactivate certain compounds. Most bioactivations occur *via* Phase I reactions. For example, BaP is

converted by the cytochrome P-450 system to BaP-7,8-epoxide which is subsequently hydrolyzed by epoxide hydrolase to from BaP-7,8-dihydrodiol. BaP 7,8-dihydrodiol is again oxidized by the cytochrome P-450 system to yield BaP-7,8-dihydrodiol-9,10-epoxide which is the ultimate carcinogen of BaP. Phase II metabolites are almost invariably less toxic than the parent compound or Phase I metabolites, however, Phase II reactions may bioactivate a chemical. The formation of certain glucuronide or sulfate conjugates have been shown to bind to cell macromolecules. For example, the sulfate conjugate of N-hydroxy-2-N-acetyl-N-aminofluorene (a Phase I metabolite) is thought to be the ultimate carcinogen of the procarcinogen 2-acetylaminofluorene( Malins and Sargent, 1978).

The biotransformation of xenobiotics has been studied intensively in mammalian species and has generally been restricted to the use of whole animals, perfused organs or tissue homogenates. These studies have several limitations, although they have provided much information regarding biochemical, pharmacological and physiological mechanisms which regulate xenobiotic metabolism. The use of whole animals or isolated perfused organs are often subject to effects which are both uncontrollable and unidentifiable. The drawback in studies utilizing tissue homogenates are that they do not retain cellular organization which may play an important role in the metabolism of xenobiotics by the intact animal.

Various techniques including mechanical (Lipson *et al.*, 1967), chemical (Rappaport and Howgi, 1972) and enzymatic (Howard and Christensen, 1967) methods have been employed to prepare isolated hepatocytes. Modified versions of the isolation technique involving the perfusion of the liver by collagenase (Berry and Friend, 1969) is still in use. The isolated hepatocyte system has been suggested as an ideal system for the study of hepatic metabolism by fish ( Moon *et al.*, 1985).

There are several advantages of using isolated hepatocytes over other methods in studying xenobiotic metabolism. Isolated hepatocytes retain cellular organization which may be necessary in the regulation of metabolism in the intact animal. Another advantage is the ease in which this system can be manipulated, as in the alteration of the extracellular milieu, independent of other factors. Isolated cell preparations may reduce the number of experimental animals needed in xenobiotic testing by allowing for multiple and simultaneous testing of a number of chemicals on cells from one animal. Another advantage of isolated cell preparations is that the mechanism of xenobiotic metabolism can be studied apart from whole animal influences. However, there are several disadvantages using isolated cell preparations. A number of criteria are used to judge cell viability, which may not reveal information about cell integrity on different organizational levels; for example, the intactness of hormone receptors (Moon *et al.*, 1985). As well, the isolation procedure may adversely affect cells. For example, Schwarze and Seglen (1980) reported that rat hepatocytes are in negative nitrogen balance following isolation, and the procedure may affect cell membrane potential.

The adaptation of hepatocyte isolation techniques for the preparation of fish hepatocytes has been described by several laboratories (Birnbaum *et al.*, 1976; Hayashi and Ooshiro, 1978; Bouche *et al.*, 1979; Walton and Comey, 1979; Renaud and Moon, 1980; French *et al.*, 1981). There are a number of important differences in the isolation of hepatocytes between mammalian and piscine systems. Therefore, balanced salt solutions (Hanks and Wallace, 1949; Lockwood, 1961; Wolf, 1963; King *et al.*, 1980) used as the perfusion and incubation media should be similar to the plasma of the species of fish used in the study. The perfusion media employed are buffered and adjusted to hydrogen ion concentrations that are similar to those found *in vivo*, which are typically lower than those used in mammalian cell preparations. Related to this, the thermal history of the fish must be taken into consideration as plasma pH changes with changing temperature (Moon *et al.*,

1985). It should also be noted that fish blood is low in bicarbonate concentration as well as concomitant low $P_{CO2}$ values (Albers, 1970) and thus, low concentrations of bicarbonate and $CO_2$-poor gas phases are used to approach physiological conditions. Moon *et al.* (1985) noted that although the importance of exogenous glucose to liver metabolism is still unknown, it may be of an advantage to include a carbon source such as glucose, lactate or amino acids in the perfusion medium.

Viable isolated hepatocytes remain metabolically active for a number of hours and have been used extensively in the study of fish liver metabolism. Hepatocytes have been used in the study of the regulation of fish liver metabolism ( for review see: Moon *et al.*, 1985) as well as the pathways of gluconeogenesis (Birnbaum *et al.*, 1976), lipid metabolism (Moerland and Sidell, 1981; Walsh *et al.*, 1985) and protein and amino acid metabolism (Bouche *et al.*, 1979; Bhattacharyn *et al.*, 1985) in the liver. Studies with hepatocytes are providing information on the effects of external factors such as pH, temperature and hormones on these biochemical pathways (Moon *et al.*, 1985). For example, the control of the metabolic processes in the liver cell appear to reside within the cell itself and are not dependent upon complex systemic changes (Kent *et al.*, 1983; Kent and Prosser, 1984; Koban *et al.*, 1984).

Biotransformation reactions can significantly influence the biological properties of xenobiotics depending on the nature of the reaction and the rate at which it occurs *in vivo* . It also may influence blood and tissue chemical concentrations, the control of bioaccumulation and persistence and the conversion of a toxic compound to a less toxic one and *vice versa* (Lech and Bend, 1980).

Isolated hepatocytes of rainbow trout are capable of producing both Phase I and Phase II metabolites of xenobiotics (Parker *et al.*, 1975; Bailey *et al.*, 1984; Andersson, 1986; Andersson and Koivussari, 1986). Gill and Walsh (in press) found that isolated

hepatocytes of the gulf toadfish, are capable of converting BaP to Phase I metabolites (both carcinogenic and noncarcinogenic) as well as to conjugated metabolites.

The purpose of these experiments was to study PAH uptake and metabolism by hepatocytes. Specifically, the rates of uptake and metabolism of 2-MN, fluorene and pyrene by isolated hepatocytes from rainbow trout was examined.

## MATERIALS AND METHODS

### A) Fish and Chemicals

Rainbow trout, weighing 300 to 500 g, were obtained from Spring Valley Trout Farms, B.C., and were held and fed as described in Part III.A (except that the water temperature was 12°C) until one day before the experiment. $^{14}$C-labeled PAHs and chemical reagents were purchased as described in Part III.A.

### B) Isolation of Hepatocytes

Hepatocytes were isolated by a modified procedure of French *et al.* (1981) and Walsh (1986). Fish were anaesthetized with 0.2 g/l MS222 and 0.2 g/l sodium bicarbonate until opercular movement ceased. A ventral incision was made in the abdomen and the hepatic portal veins cut. The intestinal vein was cannulated with a 23 gauge stainless steel needle and sutured in place with silk sutures. The liver was perfused with a well-oxygenated Medium A (see appendix Table A.29) supplied by a peristaltic pump (Cole-Parmer Instrument Co., Chicago, IL) for 10 minutes to clear the liver of blood. The flow rate was adjusted to approximately 2 ml/min/g liver to guarantee fully aerobic conditions. The liver was massaged gently by hand during perfusion with Medium A in order to enhance blood clearance and decrease red blood cell contamination. After

perfusion with Medium A, the liver was perfused for an additional 30-45 min with Medium B (see Appendix, Table A.29) which contained collagenase and hyaluronidase. After perfusion with Medium B, the liver was removed from the fish and placed on an ice-cold watchglass and doused with ice-cold Medium A. The gall bladder was gently removed. Care was taken to ensure that bile did not come in contact with the liver. The liver was then chopped with a razor blade. Unperfused parts of liver or connective tissue were removed. The liver was then disaggregated by filtration through a 253 μm and then a 73 μm nylon mesh. The cells were collected by centrifugation (50 *g*) for 2 min in a Sorval RC-5B superspeed centrifuge (DuPont, Wilmington, DW). The supernatant was decanted and the cell pellet resuspended in ice-cold Medium C (Table A.29). The cells were washed twice more with Medium C and collected each time by centrifugation. The final cell pellet was resuspended in Medium C and maintained at 12°C with a very gentle agitation under a constant atmosphere of 0.25% $CO_2$, balance air.

An aliquot of cell suspension (0.1 ml) was diluted by a factor of 100 with ice-cold Medium C. Equal volumes (1 ml) of diluted cell suspension and 0.8% (w/v) trypan blue in Medium C were mixed. This cell suspension was counted by light microscopy using a hemocytometer. For the purpose of this study, those cells excluding trypan blue were determined as viable, although exclusion of the dye determines membrane integrity and may or may not be related to metabolic activity. Cell preparations with <90% of cells excluding trypan blue or preparations containing clumped cells or debris were discarded (Moldeus *et al.*, 1978; Walsh, 1986). All experiments were performed within 3 h following cell preparation.

## C) Hepatocyte Incubation

Separate incubations were performed for PAH uptake and PAH metabolism by isolated hepatocytes.

An aliquot of the cell suspension was diluted with Medium C to obtain a final cell concentration of 3 x $10^6$ cells/ml in scintillation vials which served as reaction flasks. Final incubate volumes after the addition of chemicals was 2.0 ml. The reaction flasks were equipped with serum stoppers and maintained at 12°C by water bath. The cells were gassed with 0.25% $CO_2$, balance air for 10 min before the addition of $^{14}$C-PAHs.

$^{14}$C-labeled PAHs were dissolved in Medium C containing 1% Tween 80 (w/v). Upon the addition of $^{14}$C-2-MN, $^{14}$C-fluorene or $^{14}$C-pyrene to the reaction flasks, the final concentrations of $^{14}$C-PAHs obtained were 0.5, 2.5, 5.0, 25, 50 and 200 µM. To initiate exposure, 0.1 ml of a $^{14}$C-PAH solution was added to each reaction flask containing 1.9 ml of the cell suspension. The reaction flasks were covered with serum stoppers and a gentle stream of the gas mixture was delivered to the flasks *via* a needle through the serum stopper for the duration of the incubation. Reaction flasks were gently shaken on a reciprocating shaker during incubation. Care was taken to ensure that the gas mixture was not bubbled directly into the cell suspension. Reactions were terminated at 1, 5, 10, 15, 30 min for experiments with $^{14}$C-2-MN and $^{14}$C-fluorene and additionally for 60 and 90 min for preliminary experiments with $^{14}$C-pyrene.

## D) Analysis of Hepatocytes and Incubation Medium for PAHs and Metabolites

In uptake experiments, cells were separated from the incubation medium by centrifugation at 0°C for 1 min at 50 *g* on a Sorval SS-34 centrifuge and the supernatant discarded. The cell pellet was suspended in 2 ml sodium citrate buffer (pH 7.0) and homogenized using a Polytron homogenizer.

In metabolism experiments, both the cells and supernatant were homogenized. Homogenates from uptake experiments (containing only cells) and from metabolism

experiments (containing cells and incubation medium) were extracted with methylene chloride as described for fish tissues in Part III.C. Aliquots of the extracts from the metabolism experiments were taken and analyzed for total $^{14}C$-radioactivity by LSC after the addition of 15 ml Biofluor for the determination of unchanged PAH and Phase I metabolites. The combined extracts from the homogenates were analyzed for unchanged PAHs by HPLC as described in Part III.f. An aliquot of the remaining aqueous phases of homogenates from the metabolism experiments was removed and counted for $^{14}C$-radioactivity by LSC for the determination of aqueous-soluble metabolites. The aqueous phases was then adjusted to pH 2 with $H_2SO_4$ and hydrolyzed and extracted with methylene chloride as described in Part III.E. The extracts were combined, an aliquot taken and counted for radioactivity by LSC to determine the amount of conjugated metabolites produced. The extracts were dried down to approximatedly 0.1 ml and developed by TLC as described in Part III.E.

## RESULTS

### A) Viability of Isolated Cells

Cells in the suspension medium were essentially separated, although a few adhering cells were observed. Examination by light microscopy also revealed little contaminating debris in any hepatocyte preparation used in an experiment. Cells were used in an experiment only when viability, as judged by trypan blue exclusion, exceeded 95%. Cell preparations typically exceeded 99% viability. At the end of a 2-h incubation, the proportion of cells excluding trypan blue was always greater than 91%. Cells which served as controls were incubated with Tween 80 only. Incubations with the surfactant had no apparent effect on hepatocyte viability as judged by our criterion.

## B) PAH Uptake by Isolated Hepatocytes

The time course of $^{14}$C-2-MN, $^{14}$C-fluorene and $^{14}$C-pyrene uptake by hepatocytes is shown in Figure 4.1. The uptake of the three PAHs is linear with time for about 5 to 10 min after which the amount of unchanged PAH in the cells remained constant. In preliminary experiments the amount of unchanged pyrene in the cells remained constant up to 90 min. Maximum levels of unchanged PAHs in the cells were concentration-dependent. Initial uptake rates for 2-MN, fluorene and pyrene were calculated from the linear portions of the time-course curves. Initial PAH uptake rates were linearly related to the initial PAH concentrations in the medium up to 50 μM(Figure 4.2).

## C) PAH Metabolism by Isolated Hepatocytes

Isolated hepatocytes of trout were found to metabolize 2-MN, fluorene and pyrene rapidly. Hepatocytes metabolized the PAHs at a linear rate for at least 30 min. The cumulative metabolism of the PAHs are shown in Figure 4.3. The rate of 2-MN, fluorene and pyrene metabolism was linearly correlated with the concentration in the medium up to 50 μM (Figure 4.4).

The double-reciprocal plots of PAH metabolism are shown in Figure 4.5. The estimated ranges of maximal velocity ($V_{max}$) for the metabolism of 2-MN, fluorene and pyrene were0.0020 to 0.0028, 0.0008 to 0.0012 and 0.0012 to 0.0015, nmol/min/$10^6$ cells, respectively. The estimated ranges for the Michaelis constant s($K_m$) for 2-MN, fluorene and pyrene metabolism were 13.3 to 17.4, 8.2 to 11.7 and 12.6 to 14.5 μM, respectively.

Isolated hepatocytes metabolize 2-MN, fluorene and pyrene mainly to water soluble metabolites since organic soluble metabolites constitute less than 5% of the organic-soluble

Figure 4.1. Time course of a)$^{14}$C-2-MN, 1b)$^{14}$C-fluorene and c)$^{14}$C-pyrene accumulation in isolated hepatocytes of trout. Initial PAH concentrations were 0.5 μM (□), 2.5 μM (■), 5.0 (○), 25 μM (●), 50 μM (△) and 200 μM (▲). Values are the means ± S.D. of three different hepatocyte preparations.

2-MN accumulation
(nmol/10 6 cells)
Fluorene accumulation
(nmol/10 6 cells)
Pyrene accumulation
(nmol/10 6 cells)
a
b
c
Time (min)
Time (min)
Time (min)

Figure 4.2. Initial uptake rates of a) $^{14}$C-2-MN, b) $^{14}$C-fluorene and c) $^{14}$C-pyrene in isolated hepatocytes of trout. Values are the means ± S.D. of three different hepatocyte preparations.

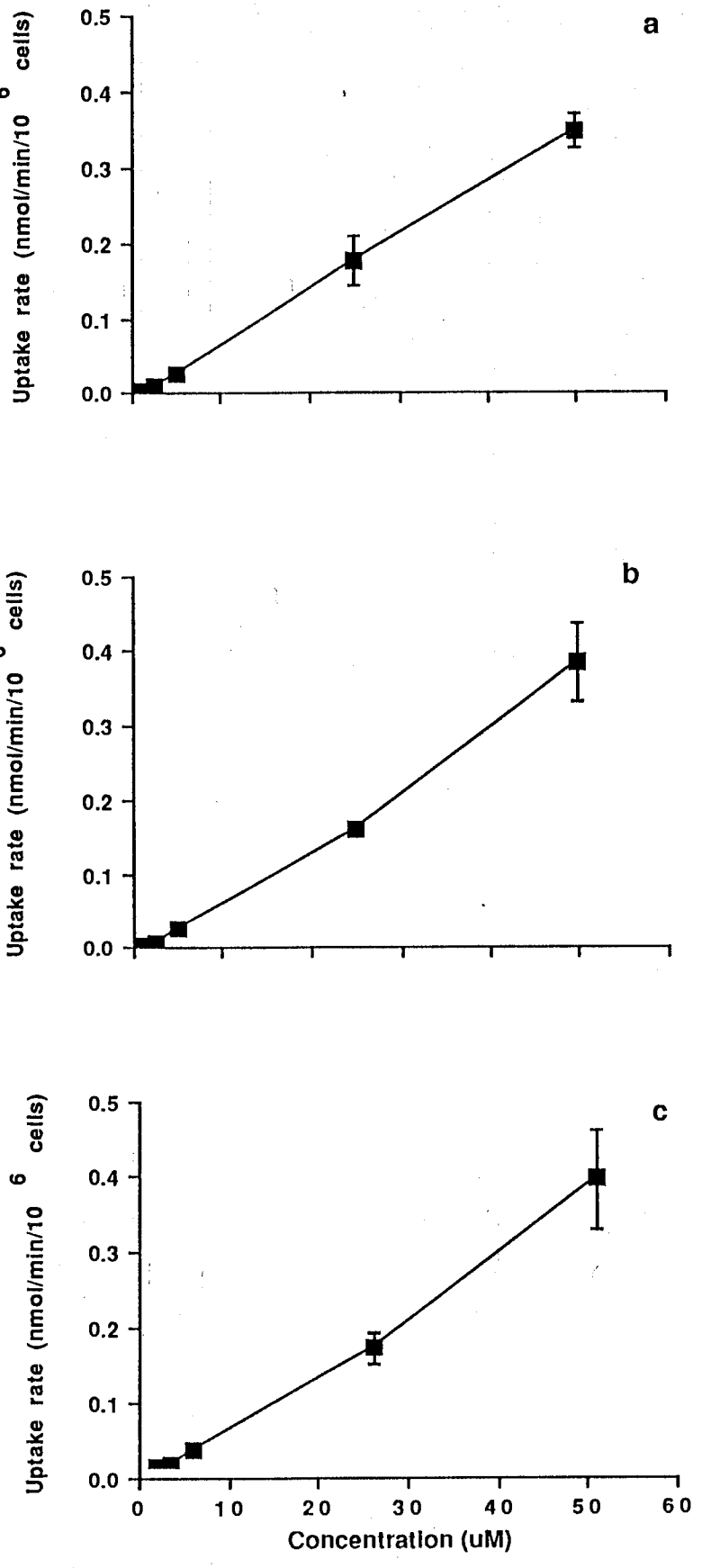

a
b
c
Uptake rate (nmol/min/10 6 cells)
0.0
0.1
0.2
0.3
0.4
0.5
0
10
20
30
40
50
60
Concentration (uM)

Figure 4.3. Cumulative metabolism of a) $^{14}$C-2-MN, b) $^{14}$C-fluorene and c) $^{14}$C-pyrene in isolated hepatocytes of trout. Initial PAH concentration was 50 μM. Values are the means ± S.D. of three different hepatocyte preparations.

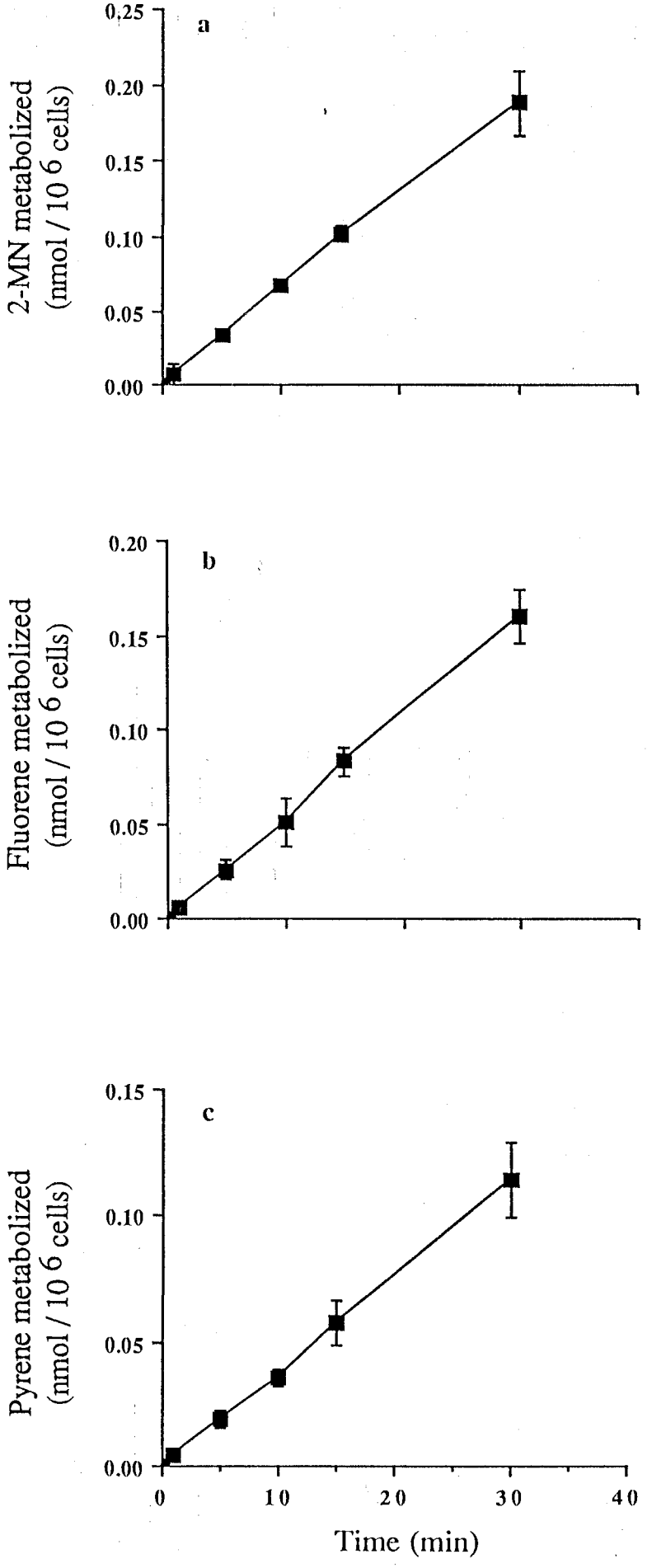

a
2-MN metabolized
(nmol / 10 6 cells)
0.25
0.20
0.15
0.10
0.05
0.00
b
Fluorene metabolized
(nmol / 10 6 cells)
0.20
0.15
0.10
0.05
0.00
c
Pyrene metabolized
(nmol / 10 6 cells)
0.15
0.10
0.05
0.00
0
10
20
30
40
Time (min)

Figure 4.4. Metabolism rate of a) $^{14}$C-2-MN, b) $^{14}$C-fluorene and c) $^{14}$C-pyrene in isolated hepatocytes of trout. Values are the means ± S.D. of three different hepatocyte preparations.

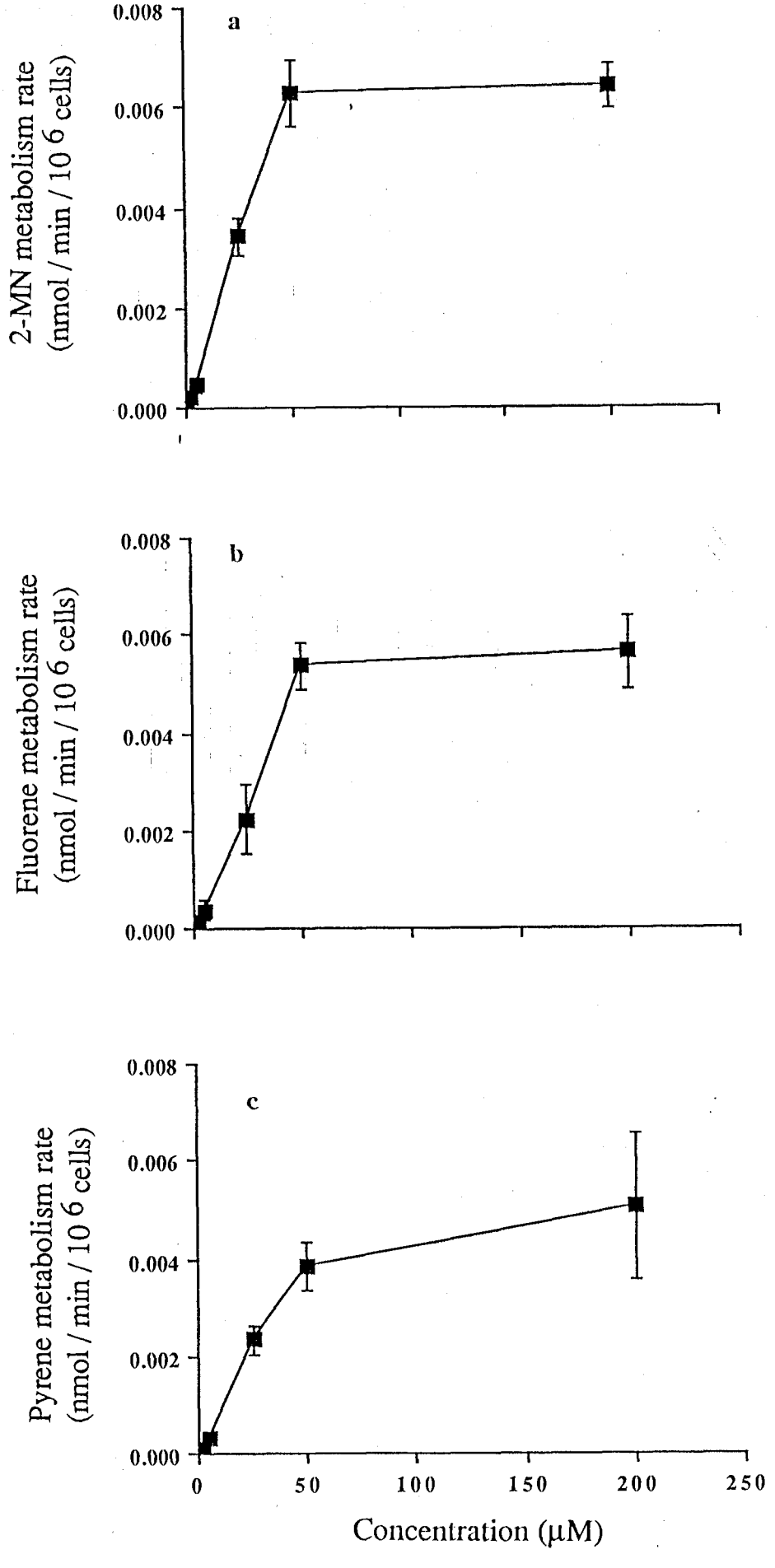
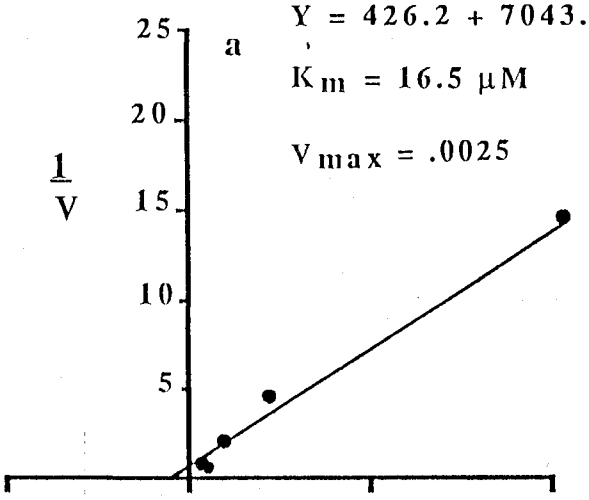
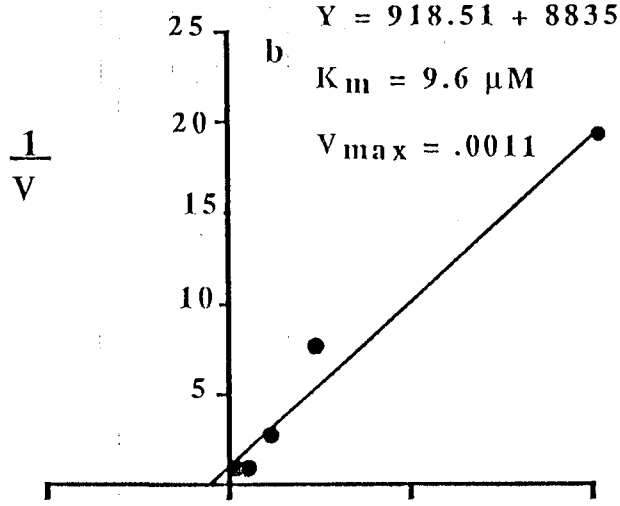

a
2-MN metabolism rate
(nmol / min / 10 6 cells)
0.008
0.006
0.004
0.002
0.000
b
Fluorene metabolism rate
(nmol / min / 10 6 cells)
0.008
0.006
0.004
0.002
0.000
c
Pyrene metabolism rate
(nmol / min / 10 6 cells)
0.008
0.006
0.004
0.002
0.000
0
50
100
150
200
250
Concentration (μM)

Figure 4.5. Double-reciprocal plot of rate of metabolism (nmol/min/$10^6$ cells) and substrate concentration (μM) of a) 2-MN, b) fluorene and c) pyrene by isolated trout hepatocytes. Values are the means of three hepatocyte preparations.


a
Y = 426.2 + 7043.1X
r2 = 0.989
Km = 16.5 µM
Vmax = .0025
1/V
25
20
15
10
5


b
Y = 918.51 + 8835.7X
r2 = 0.952
Km = 9.6 µM
Vmax = .0011
1/V
25
20
15
10
5

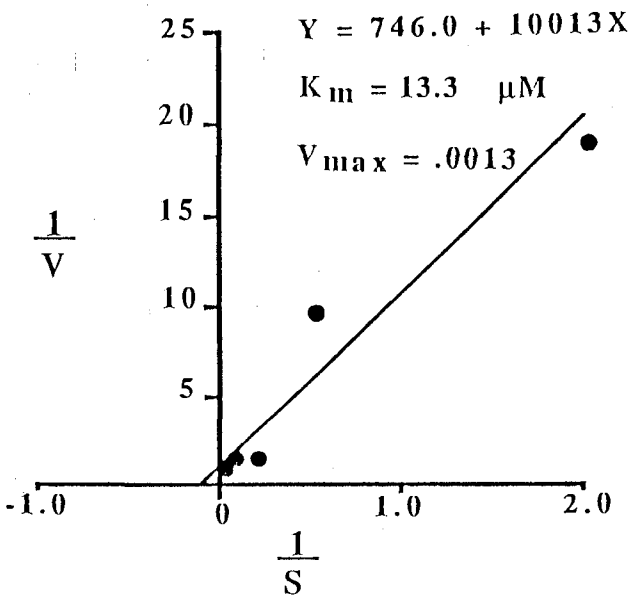
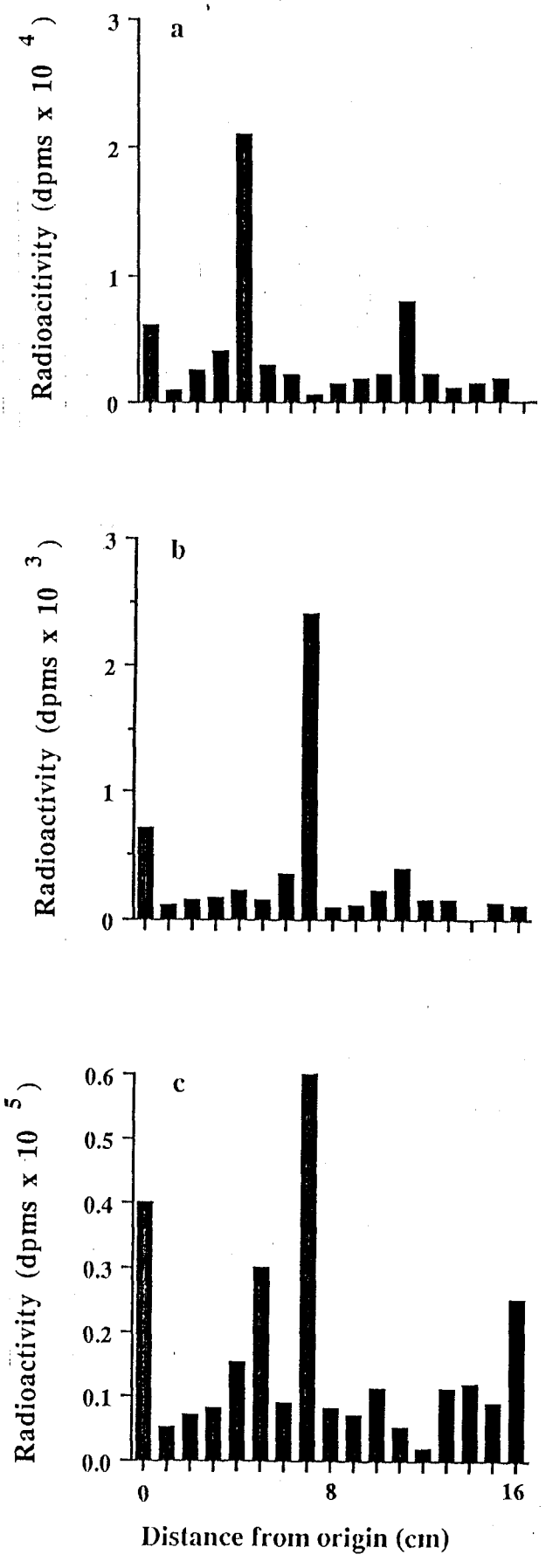

Y = 746.0 + 10013X
r2 = 0.895
Km = 13.3 µM
Vmax = .0013
1/V
25
20
15
10
5
-1.0
0
1.0
2.0
1/S

Figure 4.6. Radiochromatogram of methylene chloride extract of acid hydrolyzed trout hepatocytes exposed to a) $^{14}$C-2-MN, b) $^{14}$C-fluorene and c) $^{14}$C-pyrene.


a
Radioacitivity (dpms x 10 4 )
3
2
1
0
b
Radioactivity (dpms x 10 3 )
3
2
1
0
c
Radioactivity (dpms x 10 5 )
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
8
16
Distance from origin (cm)

radioactivity and more than 80% of the aqueous-soluble radioactivity were conjugated metabolites.

TLC analysis revealed that isolated hepatocytes produced a similar migration pattern to those produced by the liver *in vivo*. Figure 4.6 shows the TLC profiles of organic solvent extracts of acid hydrolyzed samples.

## DISCUSSION

The results of these studies indicate that the modified procedure of French *et al.*, (1981) and Walsh (1986) gives an abundant yield of metabolically active and viable cells. Isolated trout hepatocytes rapidly take up 2-MN, fluorene and pyrene from the incubation medium. Vadi *et al.* (1975) found that isolated rat hepatocytes took up BaP quickly and required approximately 15 min to complete the binding of BaP. The amount of bound substrate in cells remained constant if the uptake of BaP from the medium compensated the metabolism of BaP. Vadi *et al.* (1975) also found that after a 30 min incubation, the medium was almost depleted of BaP and that the levels of BaP in the cells began to decline. In our experiments with trout hepatocytes, no decline in the amount of unchanged PAHs in the cells was observed after a 30 min incubation indicating that the amount of PAHs metabolized was compensated for by PAH uptake from the medium for the entire incubation period.

The metabolism of many xenobiotics proceeds through a series of reactions which include the oxidation of the molecule by the cytochrome P-450 system and the subsequent conjugation of the oxidized xenobiotic with endogenous molecules such as glucuronic acid, glutathione, glycine, sulfate, taurine and acetate (Buhler and Williams, 1988). The

metabolism of PAHs by fish has been reported to proceed through metabolism by the cytochrome P-450 system and further conjugation. The metabolism of 2-MN, fluorene and pyrene to both organic and aqueous soluble metabolites by the isolated hepatocytes of trout was linear with time at all concentrations for incubations up to 90 min. The linearity of metabolite production indicates that the isolated cells can maintain a substantial level of reduced NADPH which is necessary for Phase I reactions. The linearity of metabolite production also indicates that product inhibition does not occur in the cells. It is possible that the metabolic products of Phase I reactions are not accumulated because they are immediately conjugated *via* Phase II reactions. Vadi *et al.* (1975) found that the metabolism of BaP by isolated rat hepatocytes was linear with time for 60 min, whereas metabolite production by rat liver microsomes was linear with time only for the first few minutes of incubation probably due to inhibition by accumulated hydroxylated products. In the present study, PAH metabolism became saturated between 50 and 200 μM of initial concentrations. Andersson and Kiovusarri (1986) showed that the metabolism of BaP by isolated trout hepatocyes became saturated at 80 μM and demonstrated that the cells formed polar metabolites for at least 90 min. The calculated rates of 2-MN, fluorene and pyrene metabolism by isolated hepatocytes were within the range of BaP metabolism reported by Andersson and Koivussari (1986) for isolated trout hepatocytes and are similar to the rates of BaP metabolism by isolated hepatocytes of the gulf toadfish (Gill and Walsh, in press). These rates are also similar to those reported for BaP using rainbow trout liver microsomes (Forlin, 1980; Koivussari *et al.*, 1981; Koivussari, 1983). Many studies have indicated that cytochrome P-450 levels are lower in fish than mammalian species (Parker *et al.*, 1981; Ahokas *et al.*, 1975; Ahokas *et al.*, 1976). However, Pederson *et al.*, (1974) found that rainbow trout liver homogenates metabolized BaP at a faster rate than the rat based on per g protein. Ahokas *et al.* (1975) reported that lake trout (*S. trutta lacustris* ) had a higher specific activity of BaP metabolism than the rat based on mg microsomal protein. Other studies have indicated that isolated rat hepatocytes metabolize BaP

approximately ten times faster (Vadi *et al.*, 1975) than rainbow trout hepatocytes (Andersson and Koivassari, 1986). However, Koivassari (1983) reported that BaP hydroxylase activity in the liver microsomes of rainbow trout in different seasons varied from 0.02nmol/nmol cytochrome P-450/min to 0.64nmol/mg cytochrome P-450/min, which is similar to the activity in isolated rat hepatocytes.

The kinetic constants of 2-MN, fluorene and pyrene metabolism in isolated trout hepatocytes were very similar. The Michaelis constant ($K_m$) of the PAHs were similar to those reported for BaP with rat liver homogenates (13.3 μM) (Alvarez *et al.*, 1968) or lake trout (8 μM) (Ahokas *et al.*, 1975). These $K_m$ values may be similar since PAHs are known to be metabolized similarly by the MFO system in fish. The calculated maximum velocities of 2-MN, fluorene and pyrene by isolated hepatocytes on a per gram liver basis are greater than those by the intact liver as calculated from the amount of PAH metabolites in the urine and bile of trout. Gill and Walsh (in press) also found that gulf toadfish hepatocytes metabolized BaP at rates approaching or exceeding *in vivo* rates. However, care must be used when extrapolating these *in vitro* data to the *in vivo* situation. *In vitro* experiments are often carried out under optimal biochemical conditions and substrate concentrations, as well, the purification process itself may alter the biochemical parameters such that great differences between *in vitro* and *in vivo* results occur (Wilkinson, 1987). The use of isolated hepatocytes eliminates many of these differences, however, it should be noted that in hepatocyte preparations substrate is readily available and often saturating. In contrast, some cells in the intact liver of the fish may be perfusion-limited and involved in intraorgan shunting of xenobiotics. Pang et al. (1985) suggested that isolated hepatocyte preparations may not reflect the *in vivo* situation better than other *in vitro* preparations.

Many studies have shown that fish metabolize PAHs extensively. For example, BaP is extensively metabolized by jeuvenile English sole (*Parophyrus vetulus)* (Gmur *et al.*, 1982) and the gulf toadfish,(Kennedy *et al.*, 1989b). More specifically, trout can

metabolize 2-MN to dihydrodiols and their glucuronide conjugates (Melcanon and Lech, 1984). The presence of aqueous-soluble radioactivity in both the cells and incubation medium indicate that isolated trout hepatocytes readily detoxify PAHs such as those studied in our experiments. Other studies have shown that isolated fish hepatocytes metabolize BaP to aqueous-soluble metabolites (Andersson and Koivussari, 1986: Gill and Walsh, in press). Vadi *et al.* (1975) found that isolated rat hepatocytes also metabolized BaP to water-soluble conjugates.

The analysis of organic-soluble extracts of acid hydrolyzed cell suspension indicate that the isolated hepatocytes of trout metabolized 2-MN, fluorene and pyrene to similar metabolites found in the bile of trout exposed to one of the PAHs *in vivo*. However, Gill and Walsh (in press) found that the pattern of BaP metabolites produced by gulf toadfish hepatocyte preparations was different from those produced *in vivo*: a 24-h exposure of gulf toadfish to BaP resulted in the accumulation of at least thirteen metabolites in the bile (Kennedy et al., in press) whereas only three metabolites were identified in hepatocyte preparations. This may be explained by the shorter incubation time compared to the in vivo studies, or to an inability to detect lower concentrations of other Phase I metabolites.

In summary, isolated hepatocytes from rainbow trout were found to take up and metabolize PAHs rapidly . The rates of PAH metabolism in this study were similar to those obtained for BaP in both isolated trout hepatocytes and isolated microsomes. Isolated hepatocytes metabolized 2-MN, fluorene and pyrene at a faster rate than is seen *in vivo*, possibly due to a greater availability of substrate in the hepatocyte suspension. Isolated hepatocytes convert 2-MN, fluorene and pyrene to metabolites which are similar to those found in the bile of fish after PAH-exposure.

# Part V Summary and Conclusions

The results of the acute toxicity test show that all of the CPs studied are toxic to rainbow trout fingerlings. The acute toxicity of CPs increases with increasing chlorination of the molecule. The results with PAHs indicate that although pyrene and fluorene are virtually nontoxic to fish, 2-MN was as toxic as the lower chlorinated phenols.

The disposition of CPs in trout following an i.a. administration could be described by a two compartment open toxicokinetic model. The uptake of CPs from water was rapid and the bioavailability of the chemicals by this exposure route seemed to increase with increasing chlorination of the molecule. The bioavailability of CPs by the oral route could not be determined by classical or compartmental toxicokinetic models; although DCP was found to be absorbed rapidly by this route, most of the administered dose was associated with the gastrointestinal tract. The time course of PAH concentration in blood following an i.a. injection to trout could be described by a three-compartment open toxicokinetic model. Uptake of the PAHs was rapid and the bioavailability of the PAHs was similar to that determined for PCP. As with CPs, the bioavailability of PAHs by the oral route could not be determined, however, pyrene was absorbed by the gastrointestinal tract and remained associated with these tissues.

Trout detoxify CPs and PAHs and therefore, at low environmental concentrations, the accumulation of these xenobiotics may not reach levels necessary to illicit a toxic response. The induction of both Phase I and Phase II metabolizing enzymes by other xenobiotics may further enhance this detoxification potential, enabling fish to survive in heavily contaminated environments.

After i.a. administration of CPs or PAHs, most of the unchanged chemical was found in the fat and liver of the fish. PAHs in the tissues were attributed to unchanged chemical and organic-soluble metabolites. Trout appeared to conjugate unchanged CPs directly with little or no metabolism by Phase I enzymes. PAHs were extensively metabolized by Phase I enzymes and subsequently conjugated to more water-soluble metabolites. All CPs were excreted in the urine, bile and by the gill. CPs were excreted by the gill exclusively as unchanged compound. Only conjugated metabolites were excreted into the urine and bile. PAHs were excreted in the urine and bile only. PAHs were excreted into the urine mainly as conjugated metabolites. The majority of PAHs excreted into the bile were as conjugated metabolites, small amounts of organic-soluble metabolites and the least amount as unchanged PAHs.

Isolated trout hepatocytes appeared to be an ideal system in the study of PAH metabolism; the hepatocytes remained metabolically active for the entire incubation period and the metabolic rates of the PAHs were similar to that reported for BaP. Calculated Vmax values indicated that trout *in vivo* might be exposed to doses which saturated the metabolic clearance process.

Since the metabolites of PAHs produced by the isolated trout hepatocytes were similar to those found in trout *in vivo*, this preparation may be useful in toxicity tests. The use of isolated cell preparations for toxicity tests allows not only the testing of more than one chemical with only one animal but also allows for the manipulation of the testing system apart from influences from the whole animal.

# Part VI Appendix

Table A.1. Acute toxicity of 2,4-DCP to trout.

| Concentration of 2,4-DCP | No. of test trout | Number of fish dead at 24h | 48h | 72h | 96h |
|---|---|---|---|---|---|
| 0.16 | 11 | 0 | 0 | 0 | 0 |
| 0.52 | 11 | 0 | 0 | 0 | 0 |
| 1.03 | 11 | 0 | 0 | 1 | 1 |
| 2.07 | 11 | 0 | 1 | 2 | 2 |
| 2.46 | 11 | 1 | 3 | 3 | 4 |
| 3.12 | 11 | 2 | 4 | 5 | 6 |
| 4.06 | 11 | 5 | 5 | 9 | 10 |
| 5.00 | 11 | 11 | 11 | 11 | 11 |
| 10.00 | 11 | 11 | 11 | 11 | 11 |
| $LC_{50}$ estimated by Probit Analysis | | 3.741 | 3.396 | 2.831 | 2.630 |
| 95% confidence limit | | 3.356<br>4.254 | 2.945<br>4.024 | 2.356<br>3.385 | 2.182<br>3.108 |
| Slope of probit line | | 10.153 | 6.375 | 4.982 | 5.277 |

Table A.2. Acute toxicity of 2,4,6-TCP to trout.

| Concentration of 2,4,6-TCP (ppm) | No. of test trout | Number of fish dead at 24h | 48h | 72h | 96h |
|---|---|---|---|---|---|
| 0.10 | 11 | 0 | 0 | 0 | 0 |
| 0.49 | 11 | 0 | 0 | 0 | 0 |
| 1.12 | 11 | 0 | 0 | 1 | 1 |
| 1.51 | 11 | 0 | 0 | 1 | 3 |
| 2.02 | 11 | 1 | 3 | 5 | 6 |
| 3.06 | 11 | 4 | 6 | 9 | 9 |
| 4.07 | 11 | 7 | 9 | 9 | 10 |
| 5.00 | 11 | 11 | 11 | 11 | 11 |
| 10.00 | 11 | 11 | 11 | 11 | 11 |
| $LC_{50}$ estimated by Probit Analysis | | 3.304 | 2.786 | 2.249 | 1.991 |
| 95% confidence limit | | 2.835<br>3.820 | 2.374<br>3.242 | 1.866<br>2.684 | 1.635<br>2.381 |
| Slope of probit line | | 7.776 | 6.960 | 5.243 | 5.143 |

Table A.3. Acute toxicity of 2,3,4,6-TTCP to trout

| Concentration of 2,3,4,6-TTCP (ppm) | No. of test trout | Number of fish dead at 24h | 48h | 72h | 96h |
|---|---|---|---|---|---|
| 0.03 | 11 | 0 | 0 | 0 | 0 |
| 0.20 | 11 | 0 | 0 | 0 | 0 |
| 0.29 | 11 | 0 | 0 | 2 | 5 |
| 0.36 | 11 | 1 | 2 | 5 | 6 |
| 0.45 | 11 | 5 | 5 | 7 | 9 |
| 0.51 | 11 | 6 | 8 | 9 | 10 |
| 0.72 | 11 | 9 | 11 | 11 | 11 |
| 1.00 | 11 | 11 | 11 | 11 | 11 |
| 10.00 | 11 | 11 | 11 | 11 | 11 |
| $LC_{50}$ estimated by Probit Analysis | | 0.506 | 0.442 | 0.386 | 0.334 |
| 95% confidence limit | | 0.449<br>0.578 | 0.395<br>0.498 | 0.339<br>0.435 | 0.287<br>0.377 |
| Slope of probit line | | 8.032 | 9.235 | 8.061 | 7.711 |

Table A.4. Acute toxicity of PCP to trout.

| Concentration of PCP (ppm) | No. of test trout | Number of fish dead at 24h | 48h | 72h | 96h |
|---|---|---|---|---|---|
| 0.01 | 11 | 0 | 0 | 0 | 0 |
| 0.10 | 11 | 0 | 0 | 1 | 1 |
| 0.13 | 11 | 1 | 1 | 2 | 3 |
| 0.16 | 11 | 2 | 4 | 5 | 6 |
| 0.20 | 11 | 4 | 7 | 9 | 9 |
| 0.31 | 11 | 8 | 9 | 10 | 10 |
| 0.62 | 11 | 11 | 11 | 11 | 11 |
| 1.04 | 11 | 11 | 11 | 11 | 11 |
| $LC_{50}$ estimated by Probit Analysis | | 0.236 | 0.193 | 0.161 | 0.153 |
| 95% confidence limit | | 0.201<br>0.296 | 0.164<br>0.235 | 0.136<br>0.194 | 0.126<br>0.183 |
| Slope of probit line | | 6.441 | 5.891 | 5.668 | 5.373 |

Table A.5. Acute toxicity of 2-MN to trout.

| Concentration of 2-MN | No. of test trout | Number of fish dead at 24h | 48h | 72h | 96h |
|---|---|---|---|---|---|
| 0.10 | 11 | 0 | 0 | 0 | 0 |
| 0.53 | 11 | 0 | 0 | 1 | 1 |
| 1.12 | 11 | 0 | 1 | 2 | 3 |
| 2.02 | 11 | 2 | 4 | 5 | 7 |
| 2.47 | 11 | 6 | 7 | 8 | 9 |
| 3.01 | 11 | 9 | 10 | 10 | 10 |
| 5.00 | 11 | 11 | 11 | 11 | 11 |
| $LC_{50}$ estimated by Probit Analysis | | 2.443 | 2.080 | 1.694 | 1.456 |
| 95% confidence limit | | 2.127<br>2.771 | 1.694<br>2.446 | 1.278<br>2.137 | 1.070<br>1.841 |
| Slope of probit line | | 10.605 | 6.230 | 3.790 | 3.798 |

Table A.6. Acute toxicity of fluorene to trout.

| Concentration of Fluorene (ppm) | No. of test trout | Number of fish dead at 24h | 48h | 72h | 96h |
|---|---|---|---|---|---|
| 0.5 | 11 | 0 | 0 | 0 | 0 |
| 1.0 | 11 | 0 | 0 | 0 | 0 |
| 2.0 | 11 | 0 | 1 | 2 | 2 |
| $LC_{50}$ | | >2.00 | >2.00 | >2.00 | >2.00 |

Table A.7.. Acute toxicity of pyrene to trout.

| Concentration of Pyrene (ppm) | No. of test trout | Number of fish dead at 24h | 48h | 72h | 96h |
|---|---|---|---|---|---|
| 0.50 | 11 | 0 | 0 | 0 | 0 |
| 1.01 | 11 | 0 | 0 | 0 | 0 |
| 2.00 | 11 | 0 | 0 | 0 | 0 |
| $LC_{50}$ | | >2.00 | >2.00 | >2.00 | >2.00 |

Table A.8. Concentration of DCP in blood of trout following an intraarterial administration of 10mg/kg.

ug DCP/ml blood

| Fish No. | 1 | 2 | 3 | 4 | 5 | 6 | Mean ± S.D. |
|---|---|---|---|---|---|---|---|
| Time (min) | | | | | | | |
| 5 | 184 | 133 | 155 | 101 | 204 | 195 | 162.4 ± 39.9 |
| 10 | 17.8 | 8.6 | 10.1 | 6.3 | 17.2 | 5.4 | 10.9 ± 5.4 |
| 15 | 15.4 | 6.4 | 11.4 | 4.0 | 4.7 | 4.9 | 7.8 ± 4.6 |
| 30 | 6.6 | 3.9 | 5.6 | 3.7 | 4.4 | 3.4 | 4.6 ± 1.2 |
| 45 | 5.3 | 3.7 | 4.3 | 3.5 | 4.3 | 2.9 | 4.0 ± 0.83 |
| 60 | 1.2 | 2.4 | 2.7 | 2.2 | 1.2 | 1.7 | 1.9 ± 0.63 |
| 90 | 2.1 | 2.0 | 2.0 | 1.9 | 1.8 | 1.0 | 1.8 ± 0.40 |
| 120 | 1.0 | 1.5 | 1.2 | 1.1 | 0.6 | 0.6 | 1.0 ± 0.35 |
| 150 | 0.75 | 0.65 | 1.1 | 0.97 | 0.16 | 0.21 | 0.64 ± 0.39 |
| 180 | 0.72 | 1.1 | 1.0 | 0.65 | 0.31 | 0.21 | 0.67 ± 0.36 |
| 210 | 0.67 | 0.08 | 1.2 | 0.49 | 0.27 | 0.35 | 0.51 ± 0.39 |
| 240 | 0.52 | 0.42 | 1.4 | 0.32 | 0.25 | 0.27 | 0.53 ± 0.44 |
| 270 | 0.17 | 0.22 | 0.37 | 0.36 | 0.13 | 0.25 | 0.25 ± 0.10 |
| 300 | 0.16 | 0.19 | 0.35 | 0.27 | 0.10 | 0.19 | 0.21 ± 0.09 |
| 330 | 0.13 | 0.34 | 0.22 | 0.20 | 0.07 | 0.06 | 0.17 ± 0.11 |
| 360 | 0.11 | 0.31 | 0.11 | 0.09 | 0.06 | 0.04 | 0.12 ± 0.10 |
| 390 | 0.07 | 0.43 | 0.02 | 0.08 | 0.01 | 0.02 | 0.10 ± 0.16 |
| 420 | 0.08 | 0.32 | 0.11 | 0.03 | 0.01 | 0.03 | 0.09 ± 0.12 |

Table A.9. Concentration of TCP in blood of trout following an intraarterial administration of 10mg/kg.

ug TCP/ml blood

| Fish No. / Time (min) | 1 | 2 | 3 | 4 | 5 | 6 | Mean ± S.D. |
|---|---|---|---|---|---|---|---|
| 5 | 102.7 | 212.4 | 125.5 | 135 | 110.4 | 122.2 | 134.7 ± 39.7 |
| 10 | 14.3 | 38.5 | 20.1 | 11.7 | 10.4 | 16.6 | 18.6 ± 10.5 |
| 15 | 9.7 | 17.1 | 12.2 | 8.9 | 7.6 | 10.5 | 11.0 ± 3.4 |
| 20 | 6.5 | 16.6 | 6.4 | 6.2 | 5.2 | 3.5 | 7.4 ± 4.7 |
| 30 | 4.7 | 9.2 | 4.2 | 4.7 | 4.3 | 4.7 | 5.3 ± 1.9 |
| 45 | 4.2 | 8.2 | 4.0 | 4.0 | 3.6 | 3.6 | 4.6 ± 1.8 |
| 60 | 0.98 | 2.4 | 3.1 | 3.7 | 1.7 | 0.75 | 2.1 ± 1.2 |
| 90 | 1.1 | 0.72 | 1.7 | 2.1 | 1.0 | 1.2 | 1.3 ± 0.51 |
| 120 | 0.96 | 0.49 | 1.1 | 1.4 | 0.75 | 1.4 | 1.0 ± 0.42 |
| 150 | 0.67 | 1.1 | 0.75 | 0.96 | 0.65 | 0.65 | 0.80 ± 0.23 |
| 180 | 0.42 | 0.74 | 0.62 | 0.62 | 0.59 | 0.49 | 0.58 ± 0.12 |
| 210 | 0.31 | 0.39 | 0.41 | 0.4 | 0.42 | 0.41 | 0.39 ± 0.04 |
| 240 | 0.2 | 0.33 | 0.52 | 0.35 | 0.21 | 0.075 | 0.28 ± 0.15 |
| 270 | 0.16 | 1.03 | 0.31 | 0.27 | 0.09 | 0.12 | 0.33 ± 0.35 |
| 300 | 0.19 | 0.28 | 0.0 | 0.26 | 0.11 | 0.1 | 0.19 ± 0.07 |
| 330 | 0.15 | 0.58 | 0.09 | 0.18 | 0.075 | 0.062 | 0.19 ± 0.20 |
| 360 | 0.17 | 0.77 | 0.1 | 0.21 | 0.021 | 0.054 | 0.22 ± 0.28 |
| 390 | 0.07 | 0.36 | 0.13 | 0.15 | 0.017 | 0.051 | 0.13 ± 0.12 |
| 420 | 0.04 | 0.27 | 0.07 | 0.12 | 0.008 | 0.032 | 0.09 ± 0.10 |

Table A.10. Concentration of TTCP in blood of trout following an intraarterial administration of 10mg/kg.

ug TTCP/ml blood

| Fish No. | 1 | 2 | 3 | 4 | 5 | 6 | Mean ± S.D. |
|---|---|---|---|---|---|---|---|
| Time (min) | | | | | | | |
| 5 | 267.9 | 183.3 | 217.2 | 178.8 | 185.3 | 141.7 | 196.2 ± 42.6 |
| 10 | 5.69 | 4.98 | 9.91 | 5.21 | 6.05 | 6.92 | 6.46 ± 1.82 |
| 15 | 2.43 | 2.07 | 3.81 | 2.53 | 2.97 | 3.23 | 2.84 ± 0.63 |
| 30 | 1.76 | 1.43 | 2.59 | 1.47 | 2.24 | 2.57 | 2.01 ± 0.53 |
| 45 | 1.43 | 1.12 | 3.39 | 1.06 | 1.93 | 1.69 | 1.77 ± 0.86 |
| 60 | 1.07 | 1.07 | 3.15 | 1.65 | 1.47 | 1.55 | 1.66 ± 0.86 |
| 90 | 0.89 | 0.85 | 2.61 | 0.57 | 0.96 | 1.32 | 1.20 ± 0.73 |
| 120 | 0.43 | 0.56 | 1.86 | 0.32 | 0.82 | 0.87 | 0.81 ± 0.56 |
| 150 | 0.56 | 0.47 | 1.01 | 0.41 | 0.61 | 0.68 | 0.62 ± 0.21 |
| 180 | 0.39 | 0.34 | 1.18 | 0.29 | 0.37 | 0.43 | 0.50 ± 0.34 |
| 210 | 0.36 | 0.29 | 0.84 | 0.21 | 0.34 | 0.61 | 0.44 ± 0.25 |
| 240 | 0.27 | 0.27 | 0.35 | 0.17 | 0.37 | 0.39 | 0.32 ± 0.08 |
| 270 | 0.20 | 0.21 | 0.49 | 0.17 | 0.27 | 0.34 | 0.28 ± 0.12 |
| 300 | 0.14 | 0.21 | 0.17 | 0.17 | 0.12 | 0.27 | 0.18 ± 0.05 |
| 330 | 0.11 | 0.11 | 0.24 | 0.09 | 0.19 | 0.22 | 0.16 ± 0.06 |
| 360 | 0.08 | 0.09 | 0.29 | 0.06 | 0.13 | 0.18 | 0.14 ± 0.08 |
| 390 | 0.08 | 0.11 | 0.48 | 0.4 | 0.11 | 0.10 | 0.15 ± 0.16 |
| 420 | 0.07 | 0.05 | 0.27 | 0.9 | 0.12 | 0.07 | 0.10 ± 0.09 |

Table A.11. Concentration of PCP in blood of trout following an intraarterial administration of 10mg/kg.

ug PCP/ml blood

| Fish No. | 1 | 2 | 3 | 4 | 5 | 6 | Mean ± S.D. |
|---|---|---|---|---|---|---|---|
| Time (min) | | | | | | | |
| 5 | 114.7 | 90.7 | 33.6 | 62.1 | 95.7 | 83.2 | 80.1 ± 28.5 |
| 10 | 35.5 | 43.5 | 24.3 | 40 | 47.7 | 40.6 | 38.6 ± 8.1 |
| 20 | 5.6 | 7.6 | 1.7 | 3.9 | 6.21 | 0.19 | 4.2 ± 2.8 |
| 30 | 1.4 | 3.3 | 1.5 | 3.0 | 3.2 | 5.0 | 2.9 ± 1.3 |
| 45 | 2.4 | 3.2 | 1.4 | 2.4 | 3.4 | 4.0 | 2.8 ± 0.92 |
| 60 | 2.0 | 3.0 | 1.1 | 1.1 | 2.7 | 2.7 | 2.1 ± 0.84 |
| 90 | 1.7 | 1.4 | 0.97 | 0.79 | 1.0 | 1.3 | 1.2 ± 0.34 |
| 120 | 2.0 | 2.4 | 0.96 | 1.0 | 1.1 | 5.1 | 2.1 ± 1.6 |
| 150 | 1.9 | 1.8 | 0.87 | 1.1 | 1.2 | 2.1 | 1.5 ± 0.50 |
| 180 | 1.8 | 1.7 | 0.75 | 1.2 | 1.0 | 2.6 | 1.5 ± 0.67 |
| 240 | 1.0 | 1.4 | 0.62 | 0.91 | 0.85 | 3.0 | 1.3 ± 0.87 |
| 270 | 1.0 | 1.0 | 0.5 | 0.94 | 0.92 | 2.8 | 1.2 ± 0.81 |
| 300 | 1.1 | 1.1 | 0.31 | 0.86 | 0.76 | 1.4 | 0.9 ± 0.37 |
| 330 | 1.2 | 1.2 | 0.27 | 0.76 | 0.82 | 3.6 | 1.3 ± 1.2 |
| 360 | 1.0 | 1.1 | 0.43 | 0.42 | 0.71 | 2.3 | 1.0 ± 0.70 |
| 480 | 1.1 | 0.65 | 0.35 | 0.39 | 0.69 | 2.5 | 0.95 ± 0.81 |
| 720 | 0.57 | 0.67 | 0.12 | 0.45 | 0.42 | 1.5 | 0.62 ± 0.47 |
| 1200 | 0.31 | 0.41 | 0.084 | 0.28 | 0.5 | 0.096 | 0.28 ± 0.17 |
| 1440 | 0.08 | 0.22 | 0.065 | 0.12 | 0.12 | 0.42 | 0.17 ± 0.13 |

Table A.12. Concentration of 2-MN in blood of trout following an intraarterial administration of 10mg/kg.

ug 2-methylnapthalene/ml blood

| Fish No. | 1 | 2 | 3 | 4 | 5 | 6 | Mean ± S.D. |
|---|---|---|---|---|---|---|---|
| Time (min) | | | | | | | |
| 5 | 14.62 | 35.67 | 45.62 | 49.61 | 48.93 | 32.35 | 37.8 ± 13.4 |
| 10 | 4.35 | 21.24 | 29.29 | 21.62 | 47.54 | 21.76 | 24.3 ± 14.0 |
| 15 | 3.62 | 16.75 | 20.01 | 20.75 | 51.66 | 19.21 | 22.0 ± 15.9 |
| 30 | 2.72 | 9.32 | 16.8 | 18.75 | 39.46 | 14.35 | 16.9 ± 12.5 |
| 45 | 2.53 | 8.77 | 12.35 | 13.43 | 25.5 | 9.4 | 12.0 ± 7.6 |
| 60 | 2.45 | 7.72 | 10.21 | 10.21 | 37.51 | 8.7 | 12.8 ± 12.4 |
| 90 | 1.78 | 5.45 | 6.75 | 9.55 | 37.17 | 6.5 | 11.2 ± 12.9 |
| 120 | 1.45 | 7.73 | 6.32 | 7.24 | 5.26 | 3.2 | 5.2 ± 2.4 |
| 150 | 1.20 | 4.25 | 5.41 | 6.35 | 31.36 | 2.4 | 8.5 ± 11.3 |
| 180 | 0.89 | 4.37 | 4.89 | 5.97 | 3.78 | 1.7 | 3.6 ± 1.9 |
| 420 | 0.75 | 4.2 | 4.44 | 4.98 | 4.87 | 1.5 | 3.4 ± 1.8 |
| 1440 | 0.41 | 1.24 | 0.57 | 0.65 | 0.34 | 0.45 | 0.6 ± 0.3 |

Table A.13. Concentration of fluorene in blood of trout following ar administration of 10mg/kg.

µg fluorene/ml blood

| Fish No. | 1 | 2 | 3 | 4 | 5 | 6 | Mean ± S.D. |
|---|---|---|---|---|---|---|---|
| Time (min) | | | | | | | |
| 5 | 48.1 | 18.9 | 10.7 | 20.3 | 9.2 | 24.2 | 21.4 ± 12.9 |
| 10 | 31.9 | 10.7 | 4.3 | 9.24 | 3.9 | 16.7 | 12.8 ± 10.5 |
| 15 | 23.3 | 6.4 | 2.9 | 7.65 | 2.1 | 13.5 | 9.3 ± 8.0 |
| 30 | 6.8 | 6.2 | 3.2 | 6.7 | 0.98 | 9.7 | 5.6 ± 3.1 |
| 45 | 6.3 | 4.3 | 3.4 | 4.8 | 1.6 | 8.4 | 4.8 ± 2.4 |
| 60 | 11.7 | 4.0 | 2.4 | 4.5 | 1.3 | 6.7 | 5.1 ± 3.7 |
| 90 | 6.1 | 3.2 | 1.7 | 3.4 | 1.0 | 4.4 | 3.3 ± 1.8 |
| 120 | 11.2 | 3.0 | 1.4 | 3.2 | 0.87 | 3.7 | 3.9 ± 3.7 |
| 150 | 7.8 | 2.7 | 2.1 | 3.0 | 0.65 | 3.0 | 3.2 ± 2.4 |
| 180 | 11.4 | 2.3 | 1.0 | 2.8 | 1.12 | 2.4 | 3.5 ± 3.9 |
| 240 | 7.3 | 2.0 | 0.96 | 2.1 | 0.41 | 2.1 | 2.6 ± 2.5 |
| 420 | 4.3 | 1.2 | 0.66 | 1.1 | 0.39 | 2.0 | 1.6 ± 1.4 |
| 1440 | 2.8 | 0.52 | 0.21 | 0.34 | 0.07 | 1.4 | 0.89 ± 1.0 |

Table A.14. Concentration of pyrene in blood of trout following an intraarterial administration of 10mg/kg.,

ug pyrene/ml blood

| Fish No. / Time (min) | 1 | 2 | 3 | 4 | 5 | 6 | Mean ± S.D. |
|---|---|---|---|---|---|---|---|
| 5 | 12.6 | 24.2 | 35.6 | 31.4 | 21.5 | 45.7 | 28.5 ± 11.6 |
| 10 | 11.9 | 6.7 | 8.4 | 7.7 | 8.6 | 12.5 | 9.3 ± 2.4 |
| 15 | 5.2 | 6.0 | 2.7 | 6.3 | 5.2 | 10.2 | 5.9 ± 2.7 |
| 30 | 5.0 | 2.4 | 5.2 | 10.6 | 5.9 | 8.7 | 6.3 ± 2.9 |
| 45 | 12.8 | 3.2 | 4.6 | 2.2 | 4.7 | 1.3 | 4.8 ± 4.1 |
| 60 | 4.0 | 0.2 | 4.3 | 4.5 | 4.0 | 9.0 | 4.3 ± 2.8 |
| 90 | 3.0 | 4.5 | 1.1 | 1.2 | 3.2 | 11 | 4.0 ± 4.6 |
| 120 | 3.7 | 3.2 | 1.83 | 0.97 | 3.4 | 7.3 | 3.4 ± 2.2 |
| 150 | 3.4 | 2.7 | 1.25 | 1.95 | 3.0 | 6.9 | 3.2 ± 2.0 |
| 180 | 3.0 | 1.9 | 0.22 | 0.98 | 2.5 | 5.2 | 2.3 ± 1.7 |
| 240 | 2.5 | 2.0 | 0.45 | 0.75 | 2.3 | 4.0 | 2.0 ± 1.3 |
| 420 | 2.1 | 1.2 | 0.18 | 0.62 | 1.1 | 2.0 | 1.2 ± 0.75 |
| 1440 | 1.12 | 0.85 | 0.07 | 0.49 | 1.2 | 1.97 | 0.95 ± 0.65 |

Table A.15. Concentration of DCP in blood of trout exposed to 0.5mg/L in water.

ug DCP/ml blood

| Fish No. | 1 | 2 | 3 | Mean ± S.D. |
|---|---|---|---|---|
| Time (min) | | | | |
| 5 | 0.22 | 0.31 | 0.19 | 0.24 ± 0.06 |
| 10 | 0.73 | 0.67 | 0.37 | 0.89 ± 0.19 |
| 15 | 0.55 | 0.92 | 0.42 | 0.63 ± 0.26 |
| 30 | 0.43 | 0.51 | 0.59 | 0.51 ± 0.09 |
| 45 | 0.56 | 0.63 | 0.52 | 0.57 ± 0.06 |
| 60 | 0.28 | 0.71 | 0.57 | 0.52 ± 0.22 |
| 90 | 0.75 | 0.65 | 0.4 | 0.61 ± 0.18 |
| 120 | 0.26 | 0.51 | 0.43 | 0.42 ± 0.16 |
| 150 | 0.48 | 0.31 | 0.32 | 0.37 ± 0.11 |
| 180 | 0.46 | 0.32 | 0.3 | 0.36 ± 0.09 |
| 210 | 0.11 | 0.25 | 0.19 | 0.18 ± 0.08 |
| 240 | 0.18 | 0.29 | 0.16 | 0.21 ± 0.07 |
| 270 | 0.19 | 0.27 | 0.14 | 0.21 ± 0.07 |
| 300 | 0.23 | 0.24 | 0.11 | 0.19 ± 0.08 |
| 330 | 0.09 | 0.19 | 0.08 | 0.12 ± 0.06 |
| 360 | 0.05 | 0.16 | 0.15 | 0.12 ± 0.06 |
| 390 | 0.09 | 0.12 | 0.06 | 0.09 ± 0.03 |
| 420 | 0.09 | 0.11 | 0.02 | 0.07 ± 0.05 |

Table A.16. Concentration of TCP in blood of trout exposed to 0.5mg/L in water.

ug TCP/ml blood

| Fish No. | 1 | 2 | 3 | Mean ± S.D. |
|---|---|---|---|---|
| Time (min) | | | | |
| 5 | 0.36 | 0.09 | 0.24 | 0.23 ± 0.14 |
| 10 | 0.57 | 0.25 | 0.47 | 0.43 ± 0.16 |
| 15 | 0.72 | 0.45 | 0.75 | 0.64 ± 0.17 |
| 30 | 0.82 | 0.77 | 0.88 | 0.82 ± 0.06 |
| 45 | 0.61 | 0.43 | 0.68 | 0.57 ± 0.13 |
| 60 | 0.55 | 0.5 | 0.75 | 0.61 ± 0.13 |
| 90 | 0.41 | 0.4 | 0.57 | 0.46 ± 0.11 |
| 120 | 0.35 | 0.27 | 0.49 | 0.37 ± 0.11 |
| 150 | 0.31 | 0.31 | 0.47 | 0.36 ± 0.09 |
| 180 | 0.27 | 0.2 | 0.31 | 0.26 ± 0.06 |
| 210 | 0.24 | 0.21 | 0.21 | 0.22 ± 0.02 |
| 240 | 0.13 | 0.20 | 0.06 | 0.13 ± 0.07 |
| 270 | 0.09 | 0.07 | 0.13 | 0.096 ± 0.03 |
| 300 | 0.06 | 0.12 | 0.06 | 0.08 ± 0.04 |
| 330 | 0.04 | 0.06 | 0.14 | 0.086 ± 0.05 |
| 360 | 0.03 | 0.05 | 0.1 | 0.063 ± 0.04 |
| 390 | 0.08 | 0.09 | 0.04 | 0.074 + 0.03 |
| 420 | 0.05 | 0.04 | 0.03 | 0.04 ± 0.01 |

Table A.17. Concentration of TTCP in blood of trout exposed to 0.5mg/L in water.

ug TTCP/ml blood

| Fish No. | 1 | 2 | 3 | Mean ± S.D. |
|---|---|---|---|---|
| Time (min) | | | | |
| 5 | 0.24 | 0.57 | 0.48 | 0.43 ± 0.21 |
| 10 | 1.03 | 0.62 | 0.51 | 0.72 ± 0.27 |
| 15 | 0.74 | 0.75 | 0.61 | 0.71 ± 0.08 |
| 30 | 1.03 | 1.13 | 0.78 | 0.98 ± 0.18 |
| 45 | 1.69 | 1.0 | 0.82 | 1.17 ± 0.46 |
| 60 | 1.74 | 0.72 | 0.54 | 1.01 ± 0.65 |
| 90 | 1.23 | 0.73 | 0.71 | 0.89 ± 0.31 |
| 120 | 1.07 | 0.67 | 0.6 | 0.78 ± 0.25 |
| 150 | 0.85 | 0.57 | 0.47 | 0.63 ± 0.22 |
| 180 | 0.55 | 0.45 | 0.2 | 0.42 ± 0.18 |
| 210 | 0.52 | 0.47 | 0.24 | 0.41 ± 0.15 |
| 240 | 0.31 | 0.3 | 0.17 | 0.26 ± 0.08 |
| 270 | 0.08 | 0.21 | 0.16 | 0.15 + 0.07 |
| 300 | 0.24 | 0.24 | 0.09 | 0.19 ± 0.09 |
| 330 | 0.24 | 0.2 | 0.04 | 0.16 ± 0.11 |
| 360 | 0.15 | 0.17 | 0.09 | 0.14 ± 0.04 |
| 390 | 0.08 | 0.12 | 0.11 | 0.11 ± 0.02 |
| 420 | 0.12 | 0.02 | 0.07 | 0.07 ± 0.05 |

Table A.18. Concentration of PCP in blood of trout exposed to 0.5mg/L in water.

ug PCP/ml blood

| Fish No. | 1 | 2 | 3 | Mean ± S.D. |
|---|---|---|---|---|
| Time (min) | | | | ± |
| 5 | 0.08 | 0.29 | 0.2 | 0.19 ± 0.11 |
| 15 | 0.21 | 0.65 | 0.25 | 0.37 ± 0.24 |
| 30 | 0.39 | 0.95 | 1.06 | 0.80 ± 0.36 |
| 60 | 0.57 | 0.41 | 2.77 | 1.25 ± 1.32 |
| 120 | 0.92 | 1.43 | 1.7 | 1.35 ± 0.40 |
| 150 | 1.17 | 1.57 | 0.56 | 1.10 ± 0.51 |
| 180 | 0.84 | 1.21 | 0.86 | 0.97 ± 0.21 |
| 210 | 0.62 | 1.04 | 0.5 | 0.85 ± 0.28 |
| 240 | 0.71 | 1.0 | 1.14 | 0.95 ± 0.22 |
| 270 | 0.6 | 0.99 | 0.63 | 0.74 ± 0.22 |
| 300 | 0.61 | 0.9 | 0.92 | 0.81 ± 0.17 |
| 360 | 0.59 | 0.85 | 0.72 | 0.72 ± 0.13 |
| 480 | 0.8 | 0.64 | 0.38 | 0.44 ± 0.18 |
| 720 | 0.24 | 0.53 | 0.64 | 0.47 ± 0.21 |
| 900 | 0.11 | 0.33 | 0.22 | 0.22 ± 0.11 |
| 1080 | 0.08 | 0.29 | 0.23 | 0.20 ± 0.11 |
| 1200 | 0.01 | 0.2 | 0.18 | 0.13 ± 0.10 |
| 1400 | 0.006 | 0.1 | 0.07 | 0.06 ± 0.05 |

Table A.19. Concentration of 2-MN in blood of trout exposed to 0.5mg/L in water.

ug 2-methylnapthalene/ml blood

| Fish No. | 1 | 2 | 3 | Mean ± S.D. |
|---|---|---|---|---|
| Time (min) | | | | |
| 5 | 2.01 | 0.43 | 0.02 | 0.82 ± 1.05 |
| 10 | 3.24 | 0.97 | 1.04 | 1.75 ± 1.29 |
| 15 | 5.07 | 1.21 | 1.07 | 2.45 ± 2.27 |
| 60 | 4.91 | 1.02 | 1.06 | 2.33 ± 2.23 |
| 150 | 3.75 | 1.47 | 0.78 | 2.00 ± 1.55 |
| 180 | 3.23 | 1.69 | 1.47 | 2.13 ± 0.95 |
| 240 | 2.15 | 0.74 | 0.74 | 1.21 ± 0.81 |
| 300 | 1.65 | 0.55 | 0.71 | 0.97 ± 0.59 |
| 1440 | 0.87 | 0.21 | 0.12 | 0.40 ± 0.40 |

Table A.20. Concentration of fluorene in blood of trout exposed to 0.5mg/L in water.

ug fluorene/ml blood

| Fish No. | 1 | 2 | 3 | Mean ± S.D. |
|---|---|---|---|---|
| Time (min) | | | | |
| 5 | 0.75 | 2.04 | 0.24 | 1.01 ± 0.93 |
| 10 | 1.01 | 3.75 | 0.85 | 1.87 ± 1.63 |
| 15 | 1.84 | 4.21 | 1.45 | 2.51 ± 1.49 |
| 60 | 2.26 | 3.92 | 1.17 | 2.45 ± 1.38 |
| 120 | 3.95 | 1.21 | 0.09 | 1.75 ± 1.98 |
| 180 | 2.01 | 1.31 | 0.98 | 1.43 ± 0.52 |
| 240 | 1.53 | 0.54 | 0.93 | 1.01 ± 0.51 |
| 300 | 1.67 | 0.62 | 1.34 | 1.21 ± 0.53 |
| 1440 | 0.29 | 0.02 | 0.04 | 0.12 ± 0.15 |

Table A.21. Concentration of pyrene in blood of trout exposed to 0.5mg/L in water.

ug pyrene/ml blood

| Fish No. | 1 | 2 | 3 | Mean ± S.D. |
|---|---|---|---|---|
| Time (min) | | | | |
| 15 | 5.45 | 1.64 | 0.92 | 2.67 ± 2.43 |
| 30 | 7.49 | 2.02 | 1.74 | 3.75 ± 3.24 |
| 45 | 4.93 | 2.33 | 0.27 | 2.51 ± 2.33 |
| 120 | 5.93 | 3.17 | 3.05 | 4.05 ± 1.63 |
| 200 | 6.44 | 2.09 | 0.98 | 3.17 ± 2.88 |
| 240 | 6.83 | 2.42 | 2.33 | 3.86 ± 2.57 |
| 480 | 5.48 | 2.0 | 1.49 | 2.99 ± 2.17 |
| 720 | 2.29 | 0.99 | 1.01 | 1.43 ± 0.75 |
| 1200 | 0.97 | 0.54 | 0.65 | 0.72 ± 0.22 |
| 1440 | 1.37 | 0.5 | 0.41 | 0.76 ± 0.53 |

Table A.22. Concentration of DCP in tissues of trout following a single intraarterial administration of 10 mg/kg.

µg DCP/g (x10)

| Time | 2h | | | | 4h | | | | 6h | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fish No. | 1 | 2 | 3 | Mean ± S.D. | 4 | 5 | 6 | Mean ± S.D. | 7 | 8 | 9 | Mean ± S.D. |
| Tissue | | | | | | | | | | | | |
| Liver | 2.27 | 2.35 | 0.51 | 1.71 ± 1.04 | 0.91 | 2.78 | 0.43 | 1.37 ± 1.29 | 1.21 | 0.94 | 0.05 | 0.73 ± 0.61 |
| Kidney | 0.89 | 1.21 | 0.31 | 0.81 ± 0.45 | 0.08 | 1.24 | 0.81 | 0.71 ± 0.28 | 0.32 | 0.47 | 0.32 | 0.37 ± 0.09 |
| Fat | 4.82 | 2.46 | 1.21 | 2.83 ± 1.83 | 2.72 | 3.41 | 1.43 | 2.52 ± 1.01 | 1.79 | 1.98 | 4.63 | 2.81 ± 1.56 |
| Gill | 0.51 | 0.51 | 0.07 | 0.36 ± 0.25 | 0.35 | 0.21 | 0.41 | 0.32 ± 0.11 | 0.32 | 0.37 | 0.15 | 0.28 ± 0.12 |
| Muscle | 0.011 | 0.024 | 0.002 | 0.012 ± 0.011 | 1E-4 | 0.007 | 0.035 | 0.014 ± 0.018 | 0.001 | 0.006 | 0.015 | 0.007 ± 0.008 |
| Skin | 0.098 | 0.061 | 0.011 | 0.056 ± 0.044 | 0.021 | 0.053 | 0.062 | 0.045 ± 0.022 | 0.031 | 0.042 | 0.053 | 0.035 ± 0.011 |
| Intestine | 0.154 | 0.092 | 0.001 | 0.082 ± 0.077 | 0.087 | 0.061 | 0.065 | 0.071 ± 0.014 | 0.045 | 0.051 | 0.058 | 0.051 ± 0.007 |
| Stomach | 0.018 | 0.031 | 0.017 | 0.022 ± 0.008 | 0.023 | 0.016 | 0.021 | 0.021 ± 0.004 | 0.008 | 0.031 | 0.031 | 0.023 ± 0.013 |

| Time | 8h | | | | 10h | | | |
|---|---|---|---|---|---|---|---|---|
| Fish No. | 10 | 11 | 12 | Mean ± S.D. | 13 | 14 | 15 | Mean ± S.D. |
| Tissue | | | | | | | | |
| Liver | 0.71 | 0.88 | 0.21 | 0.61 ± 0.35 | 0.57 | 0.35 | 0.37 | 0.43 ± 0.12 |
| Kidney | 0.31 | 0.18 | 0.12 | 0.21 ± 0.09 | 0.24 | 0.27 | 0.06 | 0.19 ± 0.09 |
| Fat | 0.95 | 2.11 | 3.99 | 2.35 ± 1.59 | 1.21 | 0.98 | 3.25 | 1.81 ± 1.05 |
| Gill | 0.21 | 0.01 | 0.21 | 0.14 ± 0.12 | 0.52 | 0.69 | 0.88 | 0.69 ± 0.19 |
| Muscle | 0.001 | 0.002 | 0.003 | 0.002 ± 0.001 | 9E-4 | 7E-4 | 0.002 | 0.001 ± 0.0011 |
| Skin | 0.022 | 0.049 | 0.027 | 0.032 ± 0.015 | 0.026 | 0.001 | 0.028 | 0.018 ± 0.015 |
| Intestine | 0.078 | 0.054 | 0.001 | 0.044 ± 0.041 | 0.041 | 0.041 | 0.009 | 0.051 ± 0.018 |
| Stomach | 0.021 | 0.001 | 0.073 | 0.031 ± 0.037 | 0.001 | 0.007 | 0.029 | 0.012 ± 0.015 |

Table A.23. Concentration of TCP in tissues of trout following a single intraarterial administration of 10 mg/kg.

µg TCP/g (x10)

| Time | 2h | | | | 4h | | | | 6h | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fish No. | 1 | 2 | 3 | Mean ± S.D. | 4 | 5 | 6 | Mean ± S.D. | 7 | 8 | 9 | Mean ± S.D. |
| Tissue | | | | | | | | | | | | |
| Liver | 1.02 | 1.46 | 1.78 | 1.42 ± 0.38 | 0.97 | 1.57 | 0.79 | 1.11 ± 0.41 | 0.46 | 1.41 | 0.65 | 0.84 ± 0.51 |
| Kidney | 0.14 | 0.29 | 0.35 | 0.26 ± 0.11 | 0.18 | 0.32 | 0.07 | 0.19 ± 0.13 | 0.11 | 0.21 | 0.12 | 0.14 ± 0.06 |
| Fat | 2.07 | 2.85 | 2.01 | 2.31 ± 0.47 | 3.63 | 0.79 | 0.32 | 1.58 ± 1.79 | 1.77 | 0.78 | 1.22 | 1.25 ± 0.51 |
| Gill | 0.13 | 0.03 | 0.17 | 0.11 ± 0.07 | 0.08 | 0.21 | 0.02 | 0.11 ± 0.09 | 1.02 | 0.82 | 0.64 | 0.82 ± 0.19 |
| Muscle | 0.074 | 0.092 | 0.095 | 0.087 ± 0.012 | 0.163 | 0.076 | 0.004 | 0.081 ± 0.081 | 0.005 | 0.013 | 0.012 | 0.011 ± 0.004 |
| Skin | 0.002 | 0.034 | 0.021 | 0.019 ± 0.012 | 0.032 | 0.031 | 0.021 | 0.028 ± 0.006 | 0.42 | 0.07 | 0.17 | 0.22 ± 0.18 |
| Intestine | 0.021 | 0.064 | 0.011 | 0.032 ± 0/028 | 0.025 | 0.024 | 0.022 | 0.023 ± 0.003 | 0.048 | 0.021 | 0.012 | 0.027 ± 0.018 |
| Stomach | 0.032 | 0.015 | 0.001 | 0.015 ± 0/015 | 0.031 | 0.002 | 0.011 | 0.014 ± 0.014 | 0.017 | 0.011 | 0.012 | 0.013 ± 0.004 |

| Time | 8h | | | | 10h | | | |
|---|---|---|---|---|---|---|---|---|
| Fish No. | 10 | 11 | 12 | Mean ± S.D. | 13 | 14 | 15 | Mean ± S.D. |
| Tissue | | | | | | | | |
| Liver | 0.09 | 0.97 | 0.65 | 0.57 ± 0.45 | 0.13 | 0.35 | 0.42 | 0.31 ± 0.15 |
| Kidney | 0.01 | 0.19 | 0.17 | 0.12 ± 0.11 | 0.081 | 0.054 | 0.091 | 0.075 ± 0.019 |
| Fat | 1.62 | 0.88 | 1.12 | 1.22 ± 0.35 | 0.53 | 0.61 | 0.78 | 0.78 ± 0.13 |
| Gill | 0.67 | 0.24 | 0.32 | 0.41 ± 0.29 | 0.17 | 0.23 | 0.29 | 0.29 ± 0.06 |
| Muscle | 0.089 | 0.072 | 0.041 | 0.067 ± 0.025 | 0.041 | 0.031 | 0.061 | 0.061 ± 0.015 |
| Skin | 0.07 | 0.31 | 0.22 | 0.21 ± 0.12 | 0.012 | 0.001 | 0.031 | 0.031 ± 0.015 |
| Intestine | 0.008 | 0.021 | 0.017 | 0.015 ± 0.006 | 0.011 | 0.018 | 0.007 | 0.007 ± 0.006 |
| Stomach | 0.002 | 0.011 | 0.022 | 0.011 ± 0.009 | 0.001 | 0.028 | 0.002 | 0.002 ± 0.016 |

Table A.24. Concentration of TTCP in tissues of trout following a single intraarterial administration of 10 mg/kg.

µg TTCP/g (x10)

| Time | 2h | | | | 4h | | | | 6h | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fish No. | 1 | 2 | 3 | Mean ± S.D. | 4 | 5 | 6 | Mean ± S.D. | 7 | 8 | 9 | Mean ± S.D. |
| Tissue | | | | | | | | | | | | |
| Liver | 2.51 | 1.42 | 1.02 | 1.65 ± 0.77 | 1.47 | 1.73 | 0.76 | 1.32 ± 0.51 | 0.41 | 1.21 | 0.65 | 0.75 ± 0.41 |
| Kidney | 1.05 | 0.91 | 0.45 | 0.81 ± 0.31 | 0.84 | 0.93 | 0.42 | 0.73 ± 0.26 | 0.16 | 0.61 | 0.42 | 0.39 ± 0.23 |
| Fat | 4.32 | 2.92 | 1.31 | 2.85 ± 1.51 | 0.92 | 3.97 | 2.61 | 2.51 ± 1.52 | 2.04 | 2.04 | 1.62 | 2.87 ± 1.82 |
| Gill | 0.41 | 0.47 | 0.21 | 0.36 ± 0.14 | 0.37 | 0.47 | 0.12 | 0.32 ± 0.18 | 0.31 | 0.31 | 0.17 | 0.27 ± 0.09 |
| Muscle | 0.003 | 0.021 | 0.012 | 0.012 ± 0.0087 | 0.023 | 0.004 | 0.015 | 0.014 ± 0.0091 | 0.008 | 0.008 | 0.004 | 0.007 ± 0.002 |
| Skin | 0.047 | 0.072 | 0.043 | 0.054 ± 0.016 | 0.052 | 0.063 | 0.032 | 0.049 ± 0.016 | 0.021 | 0.021 | 0.014 | 0.034 ± 0.029 |
| Intestine | 0.095 | 0.095 | 0.065 | 0.085 ± 0.017 | 0.081 | 0.088 | 0.041 | 0.071 ± 0.025 | 0.017 | 0.017 | 0.024 | 0.036 ± 0.027 |
| Stomach | 0.023 | 0.031 | 0.012 | 0.022 ± 0.011 | 0.01 | 0.039 | 0.021 | 0.021 ± 0.018 | 0.013 | 0.013 | 0.021 | 0.023 ± 0.013 |

| Time | 8h | | | | 10h | | | |
|---|---|---|---|---|---|---|---|---|
| Fish No. | 10 | 11 | 12 | Mean ± S.D. | 13 | 14 | 15 | Mean ± S.D. |
| Tissue | | | | | | | | |
| Liver | 0.72 | 0.42 | 0.66 | 0.61 ± 0.16 | 0.31 | 0.75 | 0.21 | 0.42 ± 0.29 |
| Kidney | 0.31 | 0.17 | 0.12 | 0.22 ± 0.12 | 0.22 | 0.17 | 0.17 | 0.18 ± 0.02 |
| Fat | 2.04 | 0.99 | 4.29 | 2.44 ± 1.69 | 1.47 | 1.53 | 1.92 | 1.64 ± 0.24 |
| Gill | 0.12 | 0.12 | 0.15 | 0.13 ± 0.017 | 0.02 | 0.12 | 0.09 | 0.07 ± 0.04 |
| Muscle | 0.002 | 7E-4 | 0.004 | 0.002 ± 0.0019 | 0.001 | 9E-4 | 0.002 | 0.001 ± 0.0005 |
| Skin | 0.013 | 0.043 | 0.019 | 0.025 ± 0.016 | 0.012 | 0.342 | 0.021 | 0.018 ± 0.191 |
| Intestine | 0.002 | 0.057 | 0.072 | 0.041 ± 0.037 | 0.041 | 0.029 | 0.024 | 0.031 ± 0.008 |
| Stomach | 0.031 | 0.021 | 0.046 | 0.032 ± 0.013 | 0.011 | 0.002 | 0.025 | 0.012 ± 0.013 |

Table A.25. Concentration of PCP in tissues of trout following a single intraarterial administration of 10 mg/kg.

µg PCP/g (x10)

| Time | 4h | | | | 8h | | | | 12h | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fish No. | 1 | 2 | 3 | Mean ± S.D. | 4 | 5 | 6 | Mean ± S.D. | 7 | 8 | 9 | Mean ± S.D. |
| Tissue | | | | | | | | | | | | |
| Liver | 3.49 | 1.80 | 2.21 | 2.50 ± 0.83 | 2.47 | 2.55 | 1.88 | 2.30 ± 0.37 | 3.00 | 1.42 | 188 | 2.10 ± 0.87 |
| Kidney | 2.12 | 1.35 | 1.69 | 1.72 ± 0.39 | 2.10 | 1.08 | 1.92 | 1.70 ± 0.57 | 1.35 | 1.07 | 178 | 1.40 ± 0.36 |
| Fat | 8.16 | 4.97 | 5.47 | 6.20 ± 1.72 | 4.31 | 5.98 | 5.10 | 5.13 ± 0.84 | 9.20 | 4.20 | 823 | 7.21 ± 2.65 |
| Gill | 0.29 | 0.82 | 0.42 | 0.41 ± 0.12 | 0.47 | 0.02 | 0.47 | 0.32 ± 0.26 | 0.31 | 0.25 | 0.25 | 0.27 ± 0.03 |
| Muscle | 0.009 | 0.047 | 0.040 | 0.030 ± 0.020 | 0.032 | 0.011 | 0.032 | 0.025 ± 0.012 | 0.024 | 0.013 | 0.011 | 0.016 ± 0.007 |
| Skin | 0.918 | 0.065 | 0.052 | 0.045 ± 0.024 | 0.041 | 0.049 | 0.021 | 0.037 ± 0.014 | 0.03 | 0.016 | 0.014 | 0.020 ± 0.008 |
| Intestine | 0.06 | 0.21 | 0.09 | 0.12 ± 0.08 | 0.87 | 1.34 | 0.64 | 0.95 ± 0.36 | 0.24 | 0.09 | 0.0 | 0.11 ± 0.12 |
| Stomach | 0.53 | 0.098 | 0.052 | 0.061 ± 0.015 | 0.055 | 0.066 | 0.041 | 0.054 ± 0.012 | 0.061 | 0.034 | 0.046 | 0.047 ± 0.013 |

| Time | 16h | | | | 24h | | | |
|---|---|---|---|---|---|---|---|---|
| Fish No. | 10 | 11 | 12 | Mean ± S.D. | 13 | 14 | 15 | Mean ± S.D. |
| Tissue | | | | | | | | |
| Liver | 1.15 | 3.42 | 1.43 | 2.00 ± 1.24 | 0.78 | 1.43 | 1.15 | 1.12 ± 0.33 |
| Kidney | 0.95 | 2.14 | 1.02 | 1.37 ± 0.67 | 0.83 | 1.00 | 0.87 | 0.90 ± 0.09 |
| Fat | 5.68 | 3.21 | 4.04 | 4.31 ± 1.26 | 2.04 | 5.24 | 4.93 | 4.07 ± 1.76 |
| Gill | 0.27 | 0.16 | 0.17 | 0.20 ± 0.06 | 0.12 | 0.17 | 0.0 | 0.11 ± 0.09 |
| Muscle | 0.125 | 0.082 | 0.084 | 0.097 ± 0.024 | 0.033 | 0.052 | 0.050 | 0.045 ± 0.010 |
| Skin | 0.035 | 0.009 | 0.013 | 0.019 ± 0.014 | 0.011 | 0.018 | 0.10 | 0.013 ± 0.004 |
| Intestine | 0.073 | 0.081 | 0.077 | 0.077 ± 0.004 | 0.055 | 0.073 | 0.058 | 0.062 ± 0.010 |
| Stomach | 0.071 | 0.0 | 0.061 | 0.044 ± 0.038 | 0.021 | 0.0 | 0.047 | 0.026 ± 0.024 |

Table A.26. Concentration of 2-MN in tissues of trout following a single intraarterial administration of 10 mg/kg.

µg 2-MN/g (x10)

| Time | 16h | | | | 24h | | | | 48h | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fish No. | 1 | 2 | 3 | Mean ± S.D. | 4 | 5 | 6 | Mean ± S.D. | 7 | 8 | 9 | Mean ± S.D. |
| Tissue | | | | | | | | | | | | |
| Liver | 8.32 | 1.49 | 5.31 | 5.04 ± 3.42 | 3.86 | 4.35 | 1.39 | 3.29 ± 1.59 | 1.75 | 0.92 | 1.92 | 1.53 ± 0.54 |
| Kidney | 0.67 | 0.80 | 2.04 | 1.17 ± 0.76 | 1.28 | 0.76 | 0.90 | 0.98 ± 0.30 | 0.81 | 0.63 | 0.90 | 0.78 ± 0.14 |
| Fat | 43.5 | 15.3 | 11.7 | 23.5 ± 17.4 | 32.8 | 14.3 | 29.41 | 25.5 ± 9.8 | 17.62 | 2.45 | 27.03 | 15.7 ± 12.41 |
| Gill | 0.99 | 0.46 | 0.20 | 0.55 ± 0.40 | 0.32 | 0.50 | 0.62 | 0.48 ± 0.15 | 0.0 | 0.79 | 0.17 | 0.32 ± 0.42 |
| Muscle | 0.021 | 0.015 | 0.009 | 0.015 ± 0.006 | 0.044 | 0.027 | 0.019 | 0.030 ± 0.013 | 0.025 | 0.003 | 0.014 | 0.014 ± 0.011 |
| Skin | 0.071 | 0.044 | 0.017 | 0.044 ± 0.027 | 0.053 | 0.041 | 0.002 | 0.032 ± 0.03 | 0.062 | 0.012 | 0.067 | 0.047 ± 0.03 |
| Spleen | 0.73 | 0.11 | 0.21 | 0.35 ± 0.33 | 0.03 | 0.65 | 0.10 | 0.26 ± 0.34 | 0.024 | 0.21 | 0.19 | 0.14 ± 0.10 |
| Intestine | 0.092 | 0.046 | 0.045 | 0.061 ± 0.027 | 0.11 | 0.12 | 0.71 | 0.32 ± 0.34 | 0.23 | 0.07 | 0.12 | 0.14 ± 0.08 |
| Stomach | 0.31 | 1.08 | 0.23 | 0.54 ± 0.50 | 1.17 | 0.27 | 0.15 | 0.53 ± 0.56 | 0.34 | 0.43 | 0.31 | 0.36 ± 0.06 |

| Time | 72h | | | | 144h | | | |
|---|---|---|---|---|---|---|---|---|
| Fish No. | 10 | 11 | 12 | Mean ± S.D. | 13 | 14 | 15 | Mean ± S.D. |
| Tissue | | | | | | | | |
| Liver | 1.21 | 0.41 | 1.59 | 1.07 ± 0.60 | 0.31 | 1.11 | 1.34 | 0.92 ± 0.34 |
| Kidney | 0.57 | 0.98 | 0.31 | 0.62 ± 0.34 | 0.17 | 0.27 | 0.46 | 0.30 ± 0.15 |
| Fat | 1.76 | 17.69 | 11.45 | 13.6 ± 3.5 | 9.19 | 10.41 | 12.9 | 10.8 ± 1.9 |
| Gill | 0.31 | 0.19 | 0.10 | 0.20 ± 0.11 | 0.02 | 0.12 | 0.13 | 0.09 ± 0.06 |
| Muscle | 0.001 | 0.001 | 0.00 | 9E-4 ± 0.0009 | 5E-4 | 6E-4 | 4E-4 | 5E-4 ± 0.0001 |
| Skin | 0.021 | 0.001 | 0.020 | 0.014 ± 0.001 | 0.002 | 0.002 | 0.002 | 0.002 ± 0.0 |
| Spleen | 0.012 | 0.257 | 0.001 | 0.09 ± 0.14 | 0.009 | 0.001 | 0.014 | 0.008 ± 0.007 |
| Intestine | 0.13 | 0.08 | 0.06 | 0.09 ± 0.04 | 0.008 | 0.0 | 0.052 | 0.020 ± 0.028 |
| Stomach | 0.32 | 0.43 | 0.03 | 0.26 ± 0.21 | 0.14 | 0.42 | 0.34 | 0.30 ± 0.14 |

Table A.27. Concentration of fluorene in tissues of trout following a single intraarterial administration of 10 mg/kg.

µg Fluorene/g (x10)

| Time | 16h | | | | 24h | | | | 48h | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fish No. | 1 | 2 | 3 | Mean ± S.D. | 4 | 5 | 6 | Mean ± S.D. | 7 | 8 | 9 | Mean ± S.D. |
| Tissue | | | | | | | | | | | | |
| Liver | 9.29 | 4.42 | 0.0 | 4.57 ± 4.64 | 2.12 | 4.35 | 2.71 | 3.06 ± 1.12 | 1.32 | 0.31 | 2.09 | 1.17 ± 0.89 |
| Kidney | 1.14 | 1.35 | 0.99 | 1.16 ± 0.18 | 1.35 | 1.00 | 0.56 | 0.97 ± 0.25 | 0.63 | 0.14 | 1.42 | 0.73 ± 0.65 |
| Fat | 20.2 | 43.7 | 16.2 | 26.7 ± 14.8 | 4.65 | 25.5 | 32.25 | 21.8 ± 15.0 | 13.3 | 17.6 | 29.8 | 20.2 ± 8.6 |
| Gill | 0.31 | 0.69 | 0.35 | 0.45 ± 0.21 | 0.34 | 0.30 | 0.05 | 0.23 ± 0.13 | 0.05 | 0.24 | 0.01 | 0.10 ± 0.12 |
| Muscle | 0.007 | 0.011 | 0.018 | 0.012 ± 0.006 | 0.031 | 0.011 | 0.00 | 0.014 ± 0.007 | 0.010 | 0.010 | 0.007 | 0.009 ± 0.002 |
| Skin | 0.008 | 0.001 | 0.03 | 0.013 ± 0.015 | 0.021 | 0.024 | 0.006 | 0.017 ± 0.009 | 0.013 | 0.010 | 0.004 | 0.009 ± 0.004 |
| Spleen | 0.92 | 0.43 | 0.63 | 0.66 ± 0.25 | 0.92 | 0.17 | 0.17 | 0.42 ± 0.43 | 0.03 | 0.29 | 0.07 | 0.13 ± 0.004 |
| Intestine | 0.35 | 0.20 | 0.71 | 0.42 ± 0.26 | 0.37 | 0.20 | 0.06 | 0.21 ± 0.16 | 0.15 | 0.13 | 0.20 | 0.16 ± 0.04 |
| Stomach | 0.21 | 0.69 | 0.72 | 0.54 ± 0.29 | 0.45 | 0.39 | 0.24 | 0.36 ± 0.11 | 0.39 | 0.21 | 0.33 | 0.31 ± 0.09 |

| Time | 72h | | | | 144h | | | |
|---|---|---|---|---|---|---|---|---|
| Fish No. | 10 | 11 | 12 | Mean ± S.D. | 13 | 14 | 15 | Mean ± S.D. |
| Tissue | | | | | | | | |
| Liver | 0.34 | 0.76 | 2.02 | 1.04 ± 0.87 | 0.33 | 0.16 | 1.10 | 0.53 ± 0.50 |
| Kidney | 0.18 | 0.39 | 0.69 | 0.42 ± 0.26 | 0.47 | 0.37 | 0.24 | 0.36 ± 0.12 |
| Fat | 30.9 | 12.2 | 11.2 | 18.1 ± 11.0 | 2.72 | 7.28 | 2.09 | 4.03 ± 2.83 |
| Gill | 0.04 | 0.24 | 0.08 | 0.12 ± 0.11 | 0.17 | 0.01 | 0.06 | 0.08 ± 0.08 |
| Muscle | 0.002 | 0.012 | 0.004 | 0.006 ± 0.005 | 0.002 | 0.002 | 0.005 | 0.003 ± 0.002 |
| Skin | 0.006 | 0.003 | 0.006 | 0.005 ± 0.003 | 0.003 | 0.003 | 0.0 | 0.002 ± 0.002 |
| Spleen | 0.04 | 0.10 | 0.07 | 0.07 ± 0.03 | 0.033 | 0.015 | 0.048 | 0.032 ± 0.016 |
| Intestine | 0.04 | 0.01 | 0.10 | 0.05 ± 0.05 | 0.005 | 0.041 | 0.035 | 0.027 ± 0.019 |
| Stomach | 0.08 | 0.15 | 0.07 | 0.10 ± 0.04 | 0.02 | 0.0 | 0.025 | 0.017 ± 0.013 |

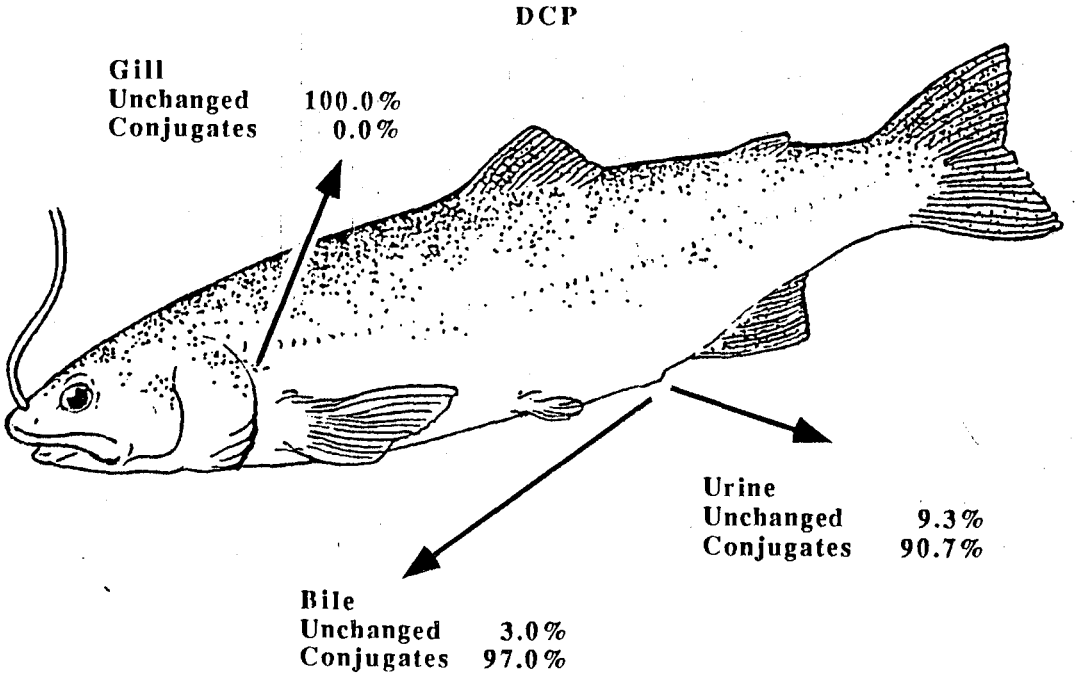
Table A.28. Concentration of pyrene in tissues of trout following a single intraarterial administration of 10 mg/kg.

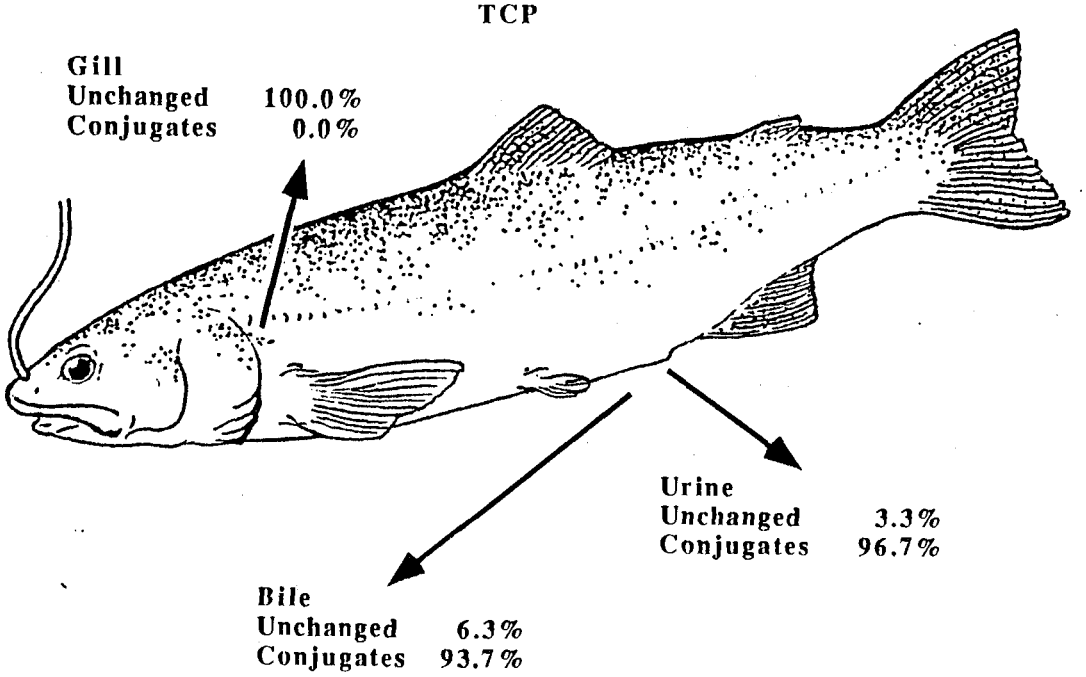
µg Pyrene/g (x10)

| Time | 16h | | | | 24h | | | | 48h | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fish No. | 1 | 2 | 3 | Mean ± S.D. | 4 | 5 | 6 | Mean ± S.D. | 7 | 8 | 9 | Mean ± S.D. |
| Tissue | | | | | | | | | | | | |
| Liver | 2.13 | 6.43 | 8.99 | 5.85 ± 3.47 | 1.04 | 3.24 | 3.4 | 2..56 ± 1.32 | 0.98 | 3.97 | 4.74 | 3.23 ± 1.99 |
| Kidney | 2.22 | 1.94 | 2.38 | 2.18 ± 0.22 | 0.66 | 0.35 | 0.55 | 0.52 ± 0.16 | 0.57 | 0.5 | 0.43 | 0.51 ± 0.04 |
| Fat | 38.7 | 55.3 | 30.8 | 41.6 ± 12.5 | 19.6 | 41.7 | 36.2 | 32.5 ± 11.5 | 25.4 | 30.5 | 26.6 | 27.5 ± 2.67 |
| Gill | 0.21 | 0.19 | 0.74 | 0.38 ± 0.31 | 0.17 | 0.35 | 0.11 | 0.21 ± 0.12 | 0.2 | 0.11 | 0.08 | 0.13 ± 0.06 |
| Muscle | 0.001 | 0.015 | 0.041 | 0.019 ± 0.021 | 0.012 | 0.056 | 0.034 | 0.034 ± 0.022 | 0.011 | 0.037 | 0.021 | 0.023 ± 0.013 |
| Skin | 0.015 | 0.006 | 0.006 | 0.009 ± 0.005 | 0.004 | 0.02 | 0.012 | 0.012 ± 0.008 | 0.014 | 0.006 | 0.006 | 0 |
| Spleen | 0.63 | 0.24 | 0.81 | 0.56 ± 0.29 | 0.39 | 0.42 | 0.18 | 0.33 ± 0.13 | 0.17 | 0.04 | 0.09 | 0.11 ± 0.07 |
| Intestine | 0.49 | 0.13 | 0.28 | 0.31 ± 0.18 | 0.21 | 0.05 | 0.3 | 0.13 ± 0.08 | 0.3 | 0.21 | 0 | 0.17 ± 0.15 |
| Stomach | 0.3 | 0.02 | 0.61 | 0.31 ± 0.32 | 0.09 | 0.37 | 0.26 | 0.24 ± 0.14 | 0.25 | 0.18 | 0.14 | 0.19 ± 0.06 |

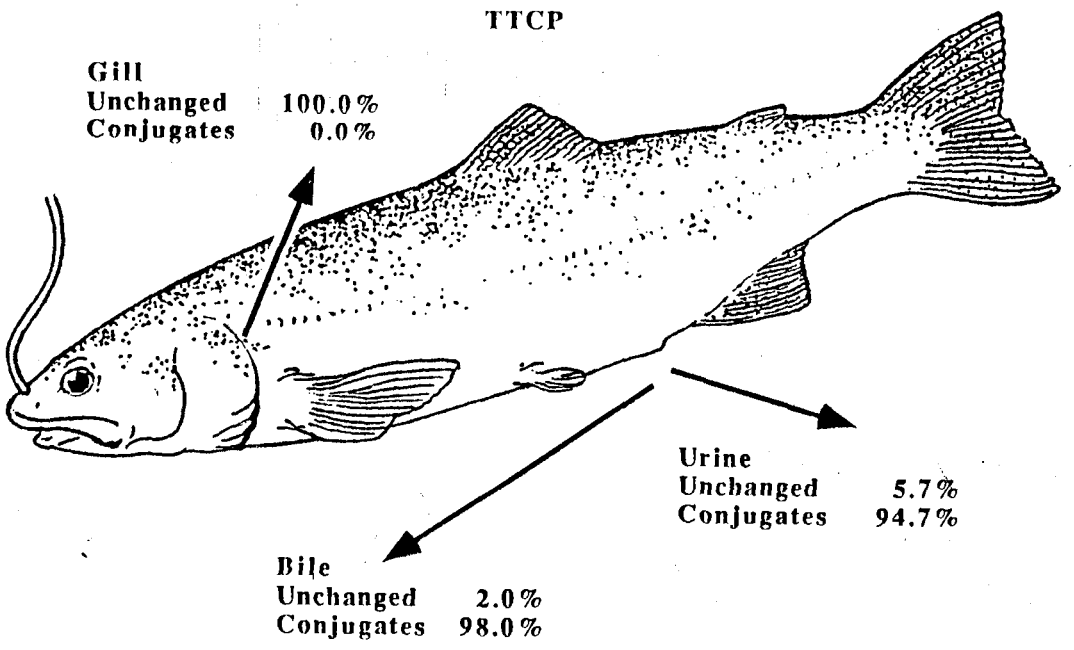
| Time | 72h | | | | 144h | | | |
|---|---|---|---|---|---|---|---|---|
| Fish No. | 10 | 11 | 12 | Mean ± S.D. | 13 | 14 | 15 | Mean ± S.D. |
| Tissue | | | | | | | | |
| Liver | 0.47 | 1.65 | 1.39 | 1.17 ± 0.62 | 0.21 | 1.02 | 1.62 | 0.95 ± 0.71 |
| Kidney | 0.49 | 0.45 | 0.47 | 0.47 ± 0.02 | 0.45 | 0.37 | 0.62 | 0.48 ± 0.13 |
| Fat | 32.1 | 19.7 | 24.7 | 25.5 ± 6.21 | 13.7 | 20.5 | 12.9 | 15.7 ± 4.1 |
| Gill | 0.11 | 0.23 | 0.02 | 0.12 ± 0.11 | 0.13 | 0.02 | 0.12 | 0.09 ± 0.06 |
| Muscle | 0.017 | 0.021 | 0.001 | 0 ± 0.011 | 0.002 | 0.004 | 0.002 | 0.003 ± 0.0018 |
| Skin | 0.006 | 0.014 | 0.003 | 0.008 ± 0.01 | 0.003 | 0.006 | 0.004 | 0.005 ± 0.001 |
| Spleen | 0.06 | 0.12 | 0 | 0.06 ± 0.06 | 0.009 | 0.024 | 0 | 0.011 ± 0.012 |
| Intestine | 0.19 | 0.07 | 0.25 | 0.17 ± 0.09 | 0.047 | 0.078 | 0.163 | 0.096 ± 0.06 |
| Stomach | 0.01 | 0.09 | 0.29 | 0.13 ± 0.14 | 0.021 | 0.01 | 0.209 | 0.08 ± 0.11 |

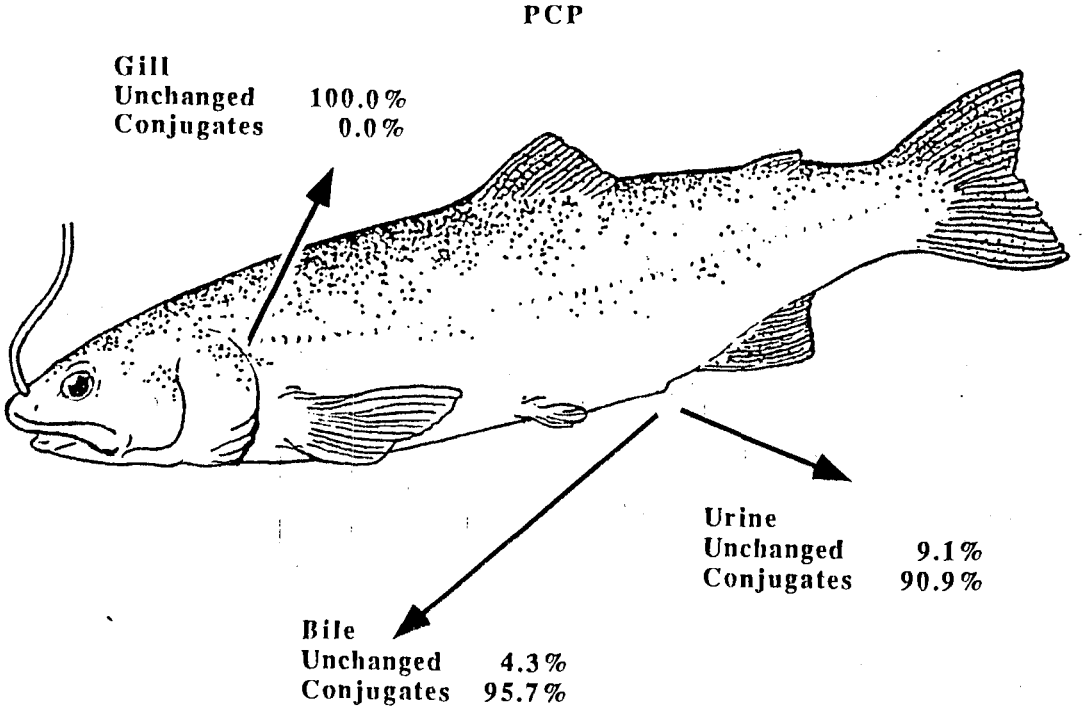
# Excretion of DCP and TCP



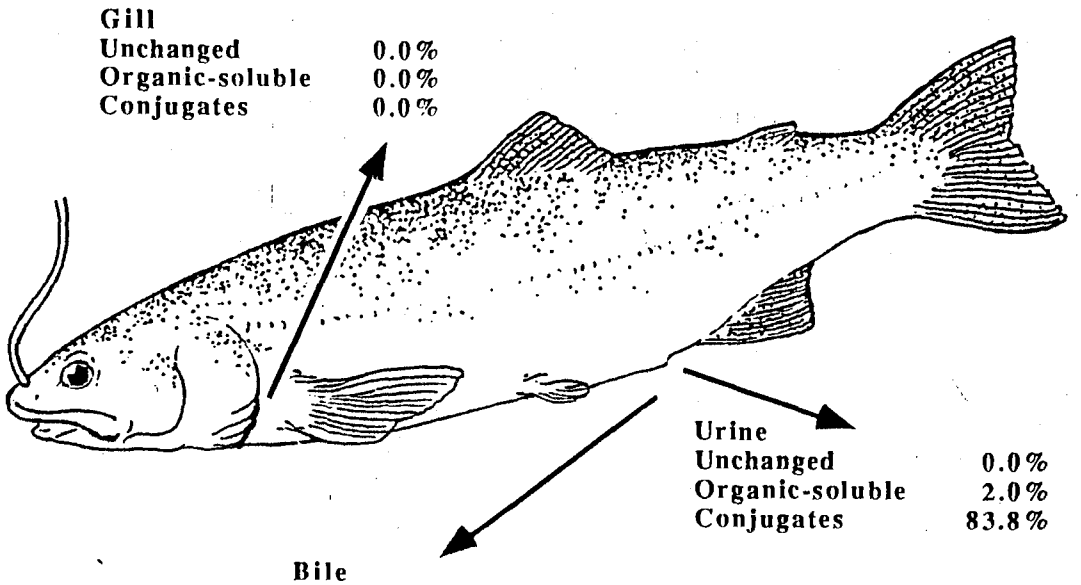
# Excretion of TTCP and PCP



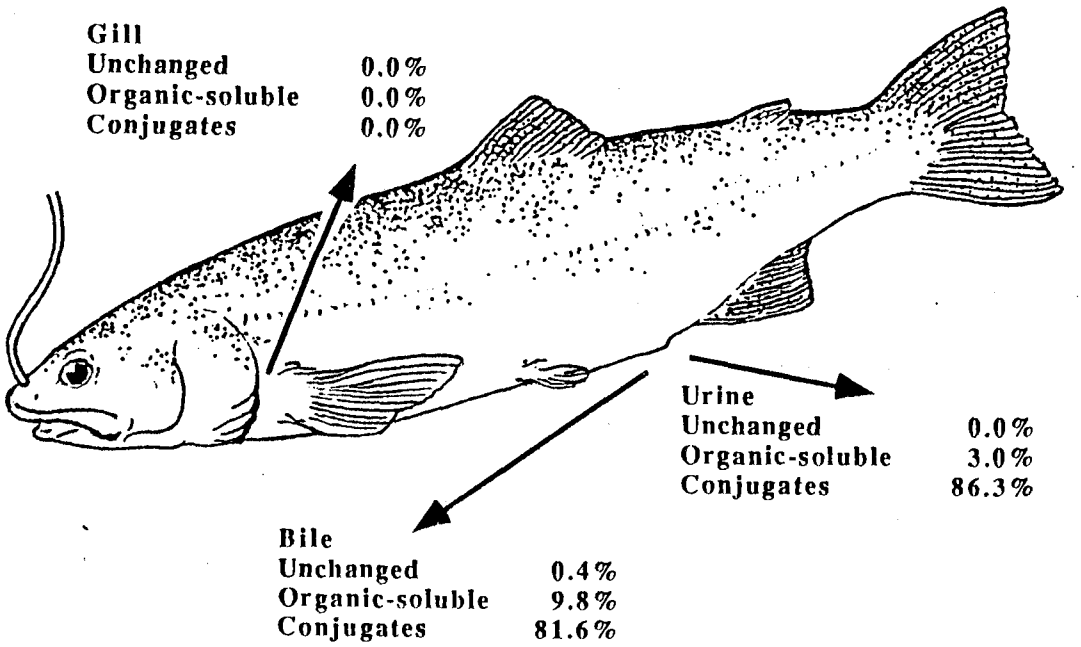



# Excretion of 2-MN and Fluorene

2-MN




Fluorene



# Excretion of Pyrene

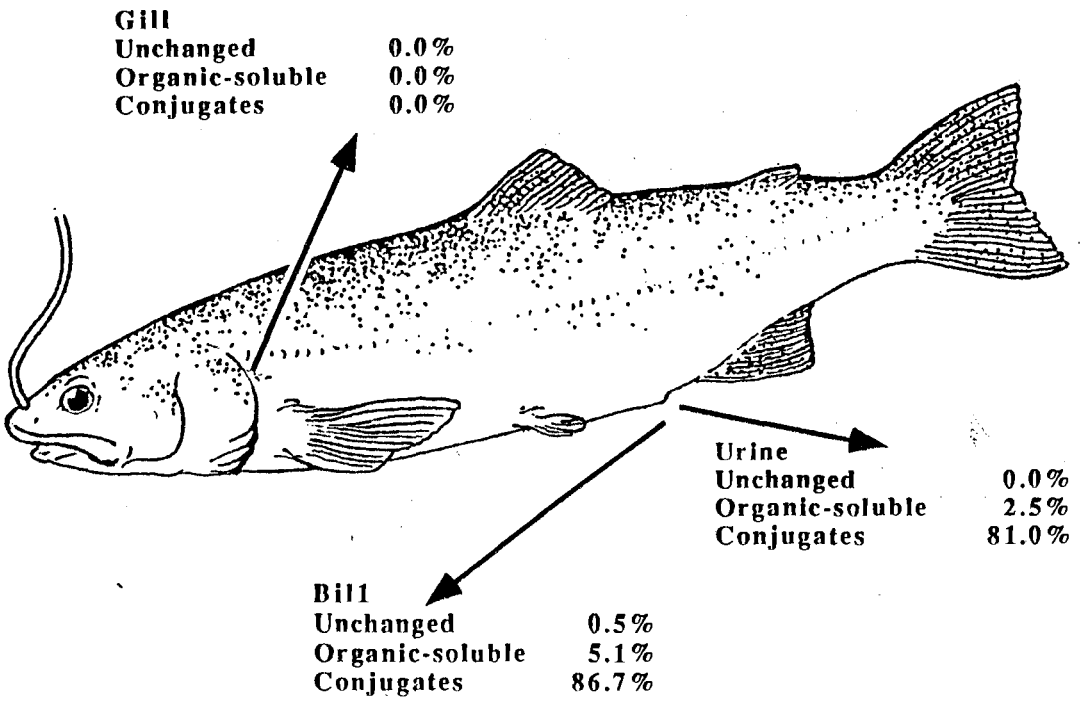


Table A.29. Composition of Media Used in the Isolation and Incubation of Rainbow Trout Hepatocytes.

Medium A (perfusion)

| Compound | Molecular Wt. | mM |
|---|---|---|
| NaCl | 58.44 | 124.0 |
| $KH_2PO_4$ | 228.23 | 0.44 |
| $NaHCO_3$* | 84.01 | 8.00 |
| $Na_2HPO_4$ | 141.97 | 0.35 |
| $MgSO_4$ | 120.39 | 0.81 |
| KCl | 74.56 | 5.40 |
| HEPES | 238.30 | 10.0 |
| Glucose | 180.16 | 5.0 |
| Lactate | 90.06 | 5.0 |
| $Na_2EDTA$ | 372.24 | 2.0 |

*$NaHCO_3$ is added after gassing

Medium B *(digestion)

Medium A to which is added

0.6 mg/ml collagenase(*Clostridium* sp.)
0.4 mg/ml hyaluronidase

Medium C *(washing and incubation)

Medium A to which is added

2% bovine serum albumin, fatty acid free
1 mM $CaCl_2$

*excluding EDTA and HEPES

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