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Uptake, Elimination, and Metabolism of Three Phenols by Fathead Minnows

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Abstract. Uptake rates of total ^{14}C in fathead minnows (*Pimephales promelas*) exposed to sublethal concentrations of radiolabeled test compounds followed the order: phenol > 2,4,5-trichlorophenol > *p*-nitrophenol. Mean whole body ^{14}C concentration factors were 15,800, 1,850, and 180 for phenol, 2,4,5-trichlorophenol, and *p*-nitrophenol exposures, respectively. Only minor amounts of tissue ^{14}C was parent compound after 28 days of exposure in fish exposed to phenol and *p*-nitrophenol, while 78.6% of the ^{14}C was parent compound in 2,4,5-trichlorophenol exposed fish. Tissue ^{14}C in fish exposed to 2,4,5-trichlorophenol was eliminated at a faster rate than in fish exposed to phenol or *p*-nitrophenol. Observed mean ^{14}C depuration half-lives for lower and higher exposures combined were 387, 150, and 12 hours for phenol, *p*-nitrophenol, and 2,4,5-trichlorophenol, respectively. Parent compound comprised 1.5, 2.7, and 0.7% of total ^{14}C for phenol, 2,4,5-trichlorophenol, and *p*-nitrophenol, respectively, after 28 days of depuration.

The percentage of acetone-unextractable ^{14}C increased from the end of uptake to the end of depuration for phenol and 2,4,5-trichlorophenol, and decreased slightly for *p*-nitrophenol. ^{14}C contribution from polar metabolites increased relative to total ^{14}C during the depuration phase for 2,4,5-trichlorophenol and *p*-nitrophenol.

Increased demands for energy as well as for natural and synthetic products have increased the potential for contamination of aquatic ecosystems with a variety of organic chemicals. Potential pollutants may exist in the form of raw unrefined chemicals, as organic synthesis intermediates, or as products of synthesis, refinement, and use. Many of these compounds are produced and used on a high annual tonnage basis. Phenol, 2,4,5-trichlorophenol, and *p*-nitrophenol are three compounds of high production volume.

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Phenol is a common toxic component of various industrial wastewaters (Mitrovic *et al.*, 1968; Kobayashi and Akitake, 1975a) and of oil refinery waste streams (Reynolds *et al.* 1975). It has antiseptic properties and is a major intermediate in the manufacture of numerous derivatives, including dyes, medical products, resins, and other industrial compounds (Stirton 1977). 2,4,5-Trichlorophenol is a germicide, but more commonly used as an intermediate in the synthesis of several products, including certain herbicides, insecticides, and disinfectants (Doedens 1964). *p*-Nitrophenol is a major intermediate in the production of phosphorus-containing pesticides, dyes, antipyretics, photographic chemicals, and leather preservatives (Matsugama 1967); and is a breakdown product of certain organophosphate pesticides (Bhagwat *et al.* 1974).

Fish readily take up many types of pollutants from their environment, concentrating certain compounds in their tissues several orders of magnitude higher than ambient water concentrations. This ability to concentrate chemicals from the environment has both human health and ecological implications.

Chemicals may be accumulated by fish through direct uptake from the water or from ingested food items (Hamelink and Spacie 1977). However, bioconcentration of toxicants from water alone is presumed to roughly approximate the net accumulation potential from all sources (Hamelink 1977). The present study was conducted to describe the uptake, metabolism, and elimination of phenol, 2,4,5-trichlorophenol (TCP), and *p*-nitrophenol (PNP) by fish following exposure to sub-lethal water concentrations under flow-through conditions.

Materials and Methods

Exposure System

Glass test chambers, with inside dimensions of 61.0 × 29.8 × 30.5 cm and a water depth of 15.0 cm, contained 27.3 L water per chamber. Glass covers were placed over each chamber, and the outer sides were covered with fiber board to minimize disturbance.

The water delivery system consisted of the water metering cell portion (w cells) of a proportional diluter (Mount and Brungs 1967) constructed to deliver one L of dilution water per cycle to mixing chambers before entering exposure and control chambers. The mixing chambers (one per tank) were one L beakers with self-starting siphons which began to empty when nearly one L of water was present. Concurrent with delivery of water to the mixing chamber, test compounds dissolved in methyl alcohol were added by chemical metering pumps (Fluid Metering Incorporated, Model RPC, 0-1.6 $ml \cdot min^{-1}$) to the same chambers, mixed, and then administered through the self-starting siphon to exposure chambers beneath the water surface in a strong downward jet. This effected a mixing action within the test chambers. Water flow-rates to the test chambers averaged one L every four min. Pumps were adjusted to deliver 0.1 $ml \cdot min^{-1}$ and pumping time was set at 60.0 ± 3.0 sec. per diluter cycle. Water from the drain of each test chamber was passed through a bed of activated charcoal to remove chemicals before final discharge.

Dilution water obtained from Lake Superior was adjusted to the desired temperature of 22°C. Headbox water was continuously circulated over nylon screen to allow for release of excess dissolved gases. Mean water temperatures in the test tanks were 21.9 ± 0.2°C for phenol, PNP and respective controls, and 22.0 ± 0.6°C for TCP and its control. Total alkalinity, pH, and dissolved oxygen were measured three times in the control tanks by standard methods (APHA 1971). Total alkalinity (as $CaCO_3$) ranged from 40.0 to 43.2 $mg \cdot L^{-1}$, pH from 7.36 to 7.62, and dissolved oxygen (Winkler Method) from 8.02 to 8.42 $mg \cdot L^{-1}$.

Test Compounds

^{14}C labeled test compounds (98% radiochemical pure) were supplied by California Bionuclear Corporation. All compounds were uniformly ring-labeled. Unlabeled compounds were supplied by the same company at the same levels of purity. Labeled and unlabeled stocks of each compound were mixed in proportions necessary to yield a minimal activity of approximately 1000 counts · min^{-1} in the volume of water sampled for quantitation of compound concentration.

Test compounds were dissolved in reagent grade methyl alcohol at concentrations of 50 and 500 mg · L^{-1} for low and high toxicant concentrations, respectively. Approximately 0.1 ml (±5%) of stock solution containing the test compound was mixed with one L of water before entering each chamber (control excluded) containing fish. This gave an approximate concentration of 100 mg · L^{-1} of methyl alcohol to all chambers, and 5 or 50 $\mu g \cdot L^{-1}$ of test compound to low and high exposures, respectively.

Test Animals

Fathead minnows (*Pimephales promelas*) hatched from EPA Environmental Research Laboratory-Duluth brood stock eggs were raised in the laboratory at approximately 22.0°C. Fish were from 28 to 42 days of age and were fed freshly hatched brine shrimp (*Artemia* sp.) in equal volumes during the tests. Occasionally, brine shrimp hatches were poor and test fish diets were supplemented with frozen adult brine shrimp. Artificial lighting was supplied by fluorescent lamps (Duro-Test Vita-Lite®) with a 12 L:12 D photoperiod.

At the start of exposure for each compound, 80 fish were randomly withdrawn from a common pool of fish and placed into each of the two test and single control chambers. Exposures lasted for 28 days, after which five fish were saved for metabolite characterization, and the remainder transferred to clean water for depuration studies. During depuration, the chambers were continually flushed at the same flow rate as during exposure. Depuration was followed over 28 days, after which the remaining fish were frozen for metabolite analyses. Five fish were randomly sampled from control and test chambers on days 1, 2, 4, 7, 14, 21 and 28 during both the uptake and depuration phases of the study.

Water Analyses

Water samples were collected in duplicate with a glass siphon from near the center of the test chambers and approximately mid-depth in the water columns. Sampling dates coincided with fish sampling dates, but with an additional 5 to 6 samplings during the uptake phase after day 7 when fish sampling occurred at weekly intervals. Phenol, TCP and PNP were extracted with benzene:isobutanol (1:1, v/v) based on a procedure by Bhagwat *et al.* (1974) for PNP. Test water (100 ml) was acidified with 2 drops of 1 N H_2SO_4 and extracted with 25 ml of 1:1 benzene:isobutanol in a 250 ml separatory funnel. The upper solvent layer was dried with oven-heated anhydrous sodium sulfate, and a 5.0 ml aliquot was withdrawn and placed into a 20 ml scintillation vial, to which 15.0 ml of scintillation fluid was added. Scintillation fluid consisted of 60 ml of concentrated liquid scintillator (Spectrafluor®, Amersham/Searle Corp.) · L^{-1} of toluene (Fisher®, Scintalyzed grade).

Extraction efficiencies were determined several times during the testing period for each compound. Water was fortified with known quantities of the radiolabeled stock solutions and extracted. Radioactivity from the water extractions was compared to radioactivity from stock solutions added directly to a mixture of 15.0 ml scintillation fluid and 5.0 ml of extraction solvent. Mean percentage recoveries and standard deviations of phenol, TCP, and PNP from water were 83.8 ± 1.7 (n = 15), 96.5 ± 2.4 (n = 14), and 90.4 ± 3.5 (n = 15), respectively.

Fish Analyses

Fish were blotted dry on paper toweling and weighed to the nearest 0.1 mg. Whole fish were individually digested in tissue solubilizer (Soluene®, Packard Inst. Co. or NCS®, Amersham/Searle Corp.), which was used at the rate of 1 ml per 100 mg fish. Samples were heated in capped 20 ml

scintillation vials at 50°C for 3 hr to accelerate digestion. Upon complete digestion (excluding minute bone remnants), 4.0 ml of benzene were added to the samples in the vials, followed by the addition of 15.0 ml of scintillation fluid. When more than one ml of tissue solubilizer was used, the solution was diluted with benzene to give a 1:4 ratio (1 ml solubilizer plus tissue:4 ml benzene), then subdivided into 5 ml aliquots and placed into additional vials. Extraction efficiencies were determined several times during the testing period for each compound. Fish were injected intramuscularly with 1.0 μl of radioactive stock solution and immediately dissolved in tissue solubilizer. The same procedures described for test fish were used to prepare the fish for recovery determinations. Mean percentage recoveries and standard deviations of phenol, TCP, and PNP from fish were 98.4 $\pm$ 7.5 (n= 23), 96.5 $\pm$ 3.4 (n = 23), and 99.8 $\pm$ 3.0 (n = 20), respectively. Measurements of ^{14}C in fish tissue during uptake and depuration are regarded as equivalents of parent compounds, rather than as intact parent compounds. Relative contributions of parent compounds and metabolites toward total observed ^{14}C were determined at the ends of uptake and depuration, as described later.

Radioactivity Measurements

Samples were counted for five min along with known concentrations of fortified samples using a Packard Tricarb® liquid scintillation counter. Background and ^{14}C quench standards were run with each set of samples. A computer program utilized the standard curve for each compound and sample type to correct for quenching and to calculate absolute activity of ^{14}C in water and fish.

Fish with unusually high or low concentrations of test compounds (differing from the mean by more than one standard deviation) were statistically analyzed for outlying results ($p \leq 0.05$) by the method of Grubbs and Beck (1972). This resulted in the elimination of data on 6 fish out of the 414 fish analyzed, with 2, 0, and 4 fish eliminated from the phenol, TCP, and PNP tests, respectively.

Lipid Analysis

Separate groups of fish were raised under the same conditions of water temperatures and photoperiod as the treatment fish for the purpose of lipid analysis; feeding schedule and food type was the same as for the test fish. Samples of 3 to 18 fish were collected weekly and frozen until analysis. Samples were weighed and collectively homogenized with Na_2SO_4. The mixture was extracted for four hr in a Soxhlet apparatus with 1:1 hexane: methylene chloride. The extract was concentrated to 20 ml, and an aliquot placed in a dried and preweighed weighing pan. Solvent was evaporated at 100°C, and percent lipids calculated.

Uptake, Equilibrium and Depuration Equations

Kinetic expressions were based on the assumption of Branson *et al.* (1975) that uptake and clearance of a chemical in fish from water can be described by the reversible reaction model

$$C_w \underset{b_2}{\overset{b_1}{\rightleftarrows}} C_f \tag{1}$$

where

C_w = concentration of chemical in water
C_f = concentration of chemical in fish
b_1, b_2 = rate constants for movement of chemical into and out of fish

The equation used in this study to describe uptake when C_w was assumed to be constant was

$$y = a + b'_1 x \tag{2}$$

where

y = concentration of ^{14}C parent compound equivalents in fish ($\mu g \cdot g^{-1}$)
a = ordinate intercept for plot of y versus x
b'_1 = uptake rate constant with units of (μg/g of fish)$\cdot hr^{-1}$
x = time (hr)

The equation used to describe the initial phase of biphasic depuration and the entire monophasic depuration was:

$$\text{Ln } y = a - b_2 x \tag{3}$$

where

y and x are defined as above
a = ordinate intercept for plot of Ln y versus x
b_2 = depuration rate constant with units of hr^{-1}

The following equation describes the change of C_f with time during fish exposure to concentration C_w:

$$\frac{dC_f}{dt} = b_1 C_w - b_2 C_f \tag{4}$$

If C_w is held constant, integration of equation 4 gives

$$C_f = (b_1/b_2)\, C_w\, (1 - e^{-b_2 t}) \tag{5}$$

Under conditions of steady state, as exposure time t approaches infinity, equation 5 is transformed to

$$\frac{C_f}{C_w} = \frac{b_1}{b_2} = CF \tag{6}$$

Where CF is the concentration factor at steady state of ^{14}C—labeled parent compound equivalents. Depuration half-lives of ^{14}C were calculated using the transformed depuration equation (eq. 3)

$$x_{1/2} = \frac{a - \text{Ln } y_{1/2}}{b_2} \tag{7}$$

where $x_{1/2}$ = half-life of ^{14}C in fish tissue
Ln $y_{1/2}$ = natural logarithm of one-half the ^{14}C in fish at the last day of uptake
a and b_2 are defined as above

Metabolite Characterization

Fish from the high-exposure and control tanks were collected at the ends of the uptake and depuration phases, weighed, and frozen for later metabolite studies. Samples were shipped on dry ice to the University of Illinois, for metabolite studies. Fish were homogenized by a Polytron® homogenizer in an ice bath, extracted 3 times with 10 ml acetone, and centrifuged at 1500 G for 5 min. Supernatants were collected, pooled, and 0.5 ml aliquots were radioassayed for total acetone-extractable ^{14}C with a Packard Tricarb scintillation spectrometer. Centrifuged tissue residues were dried overnight in a hood, subsamples were oxidized (Packard Sample Oxidizer Model 306), and counted to determine acetone-unextractable ^{14}C.

Supernatants were dehydrated overnight with anhydrous sodium sulfate and concentrated for thin-layer chromatography (TLC) and radioautography. Radioassay techniques were similar to the methods of Metcalf *et al.* (1971) and Lu and Metcalf (1975). TLC plates were prepared by coating a slurry of 33 g of silica gel GF-254 (type 60) in 60 ml distilled water onto 0.25 mm-thick glass plates. Plates were air-dried and activated at 110°C for one hr prior to use. Sample extracts were applied and TLC plates were developed in a 0°C cold room with chloroform:benzene:ethyl acetate (65:15:15 v/v) for fish exposed to phenol, TCP and PNP.

The behavior of parent compounds and metabolites on TLC plates was determined by radioautography. X-ray film (Kodak no-screen, NS-54T) was exposed, and subsequently developed and fixed by Kodak developer and fixer for 2 and 5 minutes, respectively. R_f values of parent compounds and metabolites were determined from the radioautograms. Authentic standards of parent compounds and metabolites were used for characterization, and metabolite identifications were confirmed by co-chromatography. For quantitative determinations of parent compounds and metabolites, silica gel from portions of the TLC plates corresponding to darkened areas of the

radioautograms was scraped into scintillation vials containing 10 ml cocktail D (100 g naphthalene and 5 g PPO·L^{-1} dioxane) and counted. Quenching was corrected by the channel ratios method (Bush 1963). Percentage contributions of parent compounds and metabolites were determined and converted to actual quantities in fish tissue from the previously determined concentrations of parent compound equivalents.

Results and Discussion

Mean (ranges in parentheses) water concentrations ($\mu g \cdot L^{-1}$) measured in the uptake phase for respective low and high exposures were: Phenol, 2.5 (1.4-4.0) and 32.7 (25.4-44.6); TCP, 4.8 (4.2-5.7) and 49.3 (42.2-55.9); and PNP, 4.1 (3.0-5.7) and 44.1 (34.0-61.3). Control tank ^{14}C concentrations were similar to background levels. Fish survival and growth were not affected by the test compounds at these concentrations. Observations of the fish did not indicate any abnormal behavior such as loss of equilibrium, mobility, or schooling tendency.

Measurements of radioactivity in fish for determinations of uptake rates, elimination rates, and animal concentration of test compounds from water were of total ^{14}C, and not necessarily of parent compounds. Characterization of total ^{14}C in fish at the ends of uptake and depuration indicate the relative contributions of parent compounds at those times.

Phenol

^{14}C uptake by fathead minnows exposed to radiolabeled phenol was the highest of the compounds tested in this study. Uptake and depuration curves for the two exposure levels of 2.5 and 32.7 $\mu g \cdot L^{-1}$ are presented in Figure 1. At the lower exposure, a rapid uptake occurred at the rate of approximately 0.7 $\mu g \cdot g^{-1} \cdot hr^{-1}$ (Table 1), and a maximum tissue concentration was attained on day 7. A tissue plateau level of ^{14}C was maintained between days 14 and 28, with a mean concentration factor (CF) of 14,500 for this time interval.

At the higher phenol exposure, uptake of ^{14}C was also rapid (approximately 5.6 $\mu g \cdot g^{-1} \cdot hr^{-1}$ with an apparent plateau concentration in the fish beginning on day 4 and continuing through day 21. The mean CF for this plateau was 17,000. During the last 7 days of exposure, tissue concentrations of ^{14}C increased 2.5 times from the previous measurement (day 21) and achieved a concentration above water of 53,000 on day 28. This increase may have been caused by an increased water concentration of phenol on day 21, since depuration of ^{14}C was slow from these fish.

At the lower phenol exposure, an initial 14 day depuration phase occurred with a rate constant of $0.003 \cdot hr^{-1}$. This was followed by an apparent stabilization of ^{14}C tissue residues throughout the remaining 14 days of depuration. Calculated and observed ^{14}C tissue half-lives, were 385 and 336 hr, respectively. Depuration at the higher exposure was monophasic over the 28 days measured, with a rate constant of approximately $0.001 \cdot hr^{-1}$. Calculated and observed ^{14}C tissue half-lives were 497 and 438 hr, respectively, at the higher phenol exposure.

Phenol was readily metabolized by fathead minnows (Table 2). Acetone-unextractable ^{14}C comprised 78.5% and 89.1% of total radioactivity at the ends

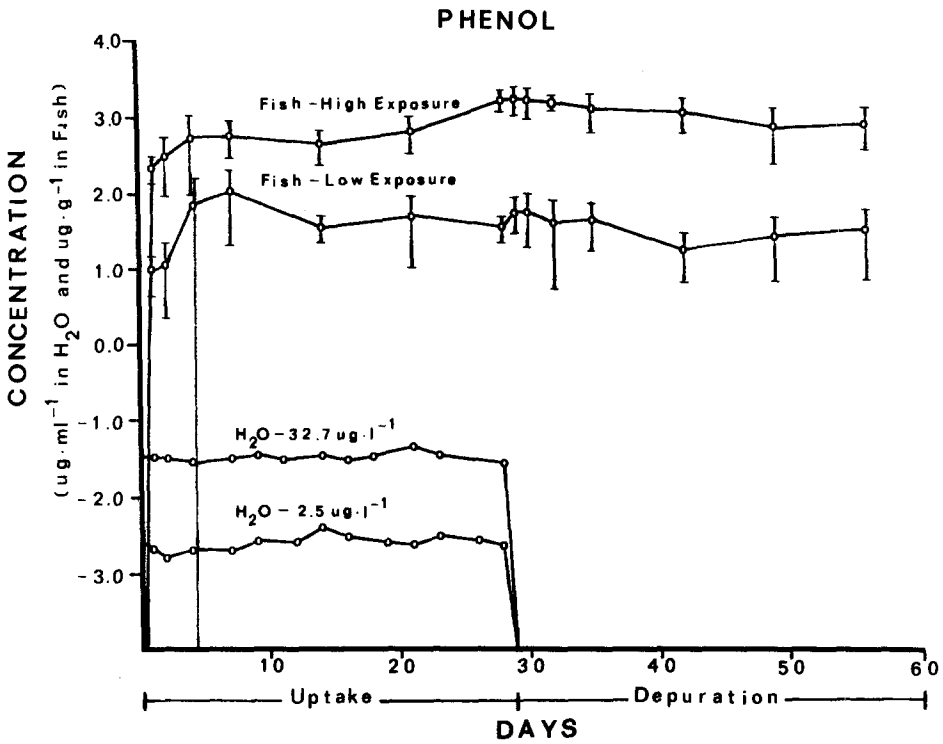

Fig. 1. Log mean exposure water concentrations of ^{14}C-tagged phenol ($\mu g \cdot ml^{-1}$) and log mean ($\pm$SD) whole fish total ^{14}C residues ($\mu g \cdot g^{-1}$) during uptake and depuration phases

of uptake and depuration phases, respectively. At the ends of uptake and depuration, phenol comprised 8.8 and 1.5%, respectively, of the total ^{14}C.

The results indicate a high retention of certain phenolic metabolites or conjugation products. Phenol has a low *n*-octanol/water partition coefficient (log P = 1.47), and would not be expected to be highly accumulative as parent compound in animal tissue. Goldfish (*Carassius auratus*) exposed to phenol at concentrations from 5 to 100 $mg \cdot L^{-1}$ for up to 5 days concentrated phenol only slightly, from 1.2 to 2.3 times (Kobayashi and Akitake 1975b). Rainbow trout (*Salmo gairdneri*) exposed to phenol at concentrations from 1-11 $mg \cdot L^{-1}$ for 24 hr contained phenol in whole blood, muscle, and brain at concentrations similar to and approximately twice the exposure water concentrations (Swift 1978).

Phenyl sulfate is the major conjugated product of phenol in fish, as fish appear to have a greatly diminished capacity for formation of glucuronide conjugates of phenol (Kobayashi *et al.* 1975). Conjugates are generally more polar, more water-soluble, and less lipophilic than parent molecules, and as such are more readily eliminated from animals (Kaufman 1976). However, *in vivo* binding to plasma proteins has been demonstrated (Curtis *et al.* 1974) for some sulfate ester conjugates of phenol; and Swift (1978) determined that erythrocytes maintained a constant concentration of "phenolic compounds" when rainbow trout were exposed to phenol for 24 hr at concentrations below 10 $mg \cdot L^{-1}$. Such binding to both erythrocytes and plasma proteins would likely retard the

Table 1. ^{14}C uptake and depuration rates, concentration factors (CF), and depuration half-lives for the test compounds in fathead minnows

Compound	Mean exposure level ($\mu g \cdot ml^{-1}$)	Uptake equation[a] $y = a + b_1'x$	Depuration equation[b] $\ln y = a - b_2x$	CF: Calculated b_1'/b_2 Cw[c]	CF: Observed plateau tissue concentration Cw[d]	Depuration half-life (hr)[e]: Calculated	Depuration half-life (hr)[e]: Observed
Phenol	0.0025	−6.524 + 0.7005x r = 0.98[f] (7 days)[g]	3.950 − 0.0029x r = 0.81 (14 days)	96,621	14,500	385	336
Phenol	0.0327	38.614 + 5.6048x r = 0.99 (4 days)	7.392 − 0.0014x r = 0.97 (28 days)	122,429	17,000	497	438
2,4,5-Trichlorophenol	0.0048	0.918 + 0.1827x r = 0.94 (2 days)	2.166 − 0.0756x r = 1.00 (1 day)	503	1,900	9.2	12
2,4,5-Trichlorophenol	0.0493	0.000 + 3.4475x r = 1.00 (1 day)	4.850 − 0.1056x r = 1.00 (1 day)	662	1,800	6.6	12
p-Nitrophenol	0.0041	−0.136 + 0.0085x r = 0.92 (4 days)	−1.311 − 0.0094x r = 0.51 (4 days)	221	80	37	72
p-Nitrophenol	0.0441	3.486 + 0.0535x r = 0.49 (4 days)	2.928 − 0.0027x r = 0.89 (14 days)	449	280	206	228

[a] y = Concentration of compound equivalents in fish ($\mu g \cdot g^{-1}$), a = intercept (y-axis), b_1' = slope or linear rate constant ($\mu g \cdot g^{-1} \cdot hr^{-1}$), x = time (hr)
[b] b_2 is first order rate constant with units hr^{-1}
[c] Mean concentration ($\mu g \cdot ml^{-1}$) of test compound in water during entire exposure
[d] Running mean concentration ($\mu g \cdot ml^{-1}$) of test compound in water for seven day maximum time interval preceding fish sampling
[e] Calculated and observed times are based upon the mean concentration of compound equivalents in fish on the last day of exposure
[f] Regression correlation coefficient
[g] Time span used to evaluate constants

Table 2. Comparative metabolism of ^{14}C phenol, 2,4,5-trichlorophenol and *p*-nitrophenol in fathead minnows

Compound	End of uptake	End of depuration
Phenol		
Total ^{14}C	1607 ppm[b]	714 ppm
(i) Extractable	345 (21.5%)[c]	78 (10.9%)
Phenol (R_f 0.54)[a]	141	11
Polar (R_f 0.0)	204	67
(ii) Unextractable	1262 (78.5%)[d]	636 (89.1%)
2,4,5-Trichlorophenol		
Total ^{14}C	127.8 ppm	4.5 ppm
(i) Extractable	114.1 (89.2%)	0.94 (21.0%)
2,4,5-Trichlorophenol (R_f 0.63)	100.4	0.12
Polar (R_f 0.0)	13.7	0.82
(ii) Unextractable	13.7 (10.8%)	3.56 (79.0%)
p-Nitrophenol		
Total ^{14}C	31.6 ppm	5.4 ppm
(i) Extractable	2.5 (8.0%)	0.9 (16.6%)
Unknown (R_f 0.63)[a]	0.5	—
p-Nitrophenol (R_f 0.42)	1.3	0.04
p-Aminophenol (R_f 0.12)	0.2	0.20
Polar (R_f 0.0)	0.5	0.66
(ii) Unextractable	29.1 (92.0%)	4.5 (83.4%)

[a] TLC with chloroform:benzene:ethyl acetate = 65:15:15 v/v in 0°C cold room
[b] Total mean ^{14}C parent compound equivalents; ppm = $\mu g \cdot g^{-1}$
[c] Calculated tissue concentration based on extraction with acetone
[d] Calculated tissue concentration based on total combustion of tissue residues

overall rate of metabolite elimination, and may partially explain the high ^{14}C body residues and slow rates of elimination observed in this study.

The high ^{14}C levels and slow elimination may also be due to metabolism of phenol and subsequent incorporation of the radiolabel into endogenous substances. Since 78.5 and 89.1% of the radioactivity was acetone-unextractable at the ends of uptake and depuration, respectively, much of this may have been due to incorporation of ^{14}C into tissue components by synthetic pathways involving products of phenol degradation.

The high ^{14}C levels in whole fish may be partly due to an accumulation of phenol plus metabolites in the gall bladder. Gall bladder concentrations greatly exceeding water concentrations have been observed for several polar compounds in fish, including 3-trifluoromethyl-4-nitrophenol and pentachlorophenol (Statham *et al.* 1976). Certain phenolic compounds, such as 3-trifluoromethyl-4-nitrophenol, are conjugated in the liver and excreted through the gall bladder into the digestive tract (Lech and Costrini 1972; Lech 1973). Distended gall bladders have been observed in bream, *Abramis brama* (Waluga 1966) and rainbow trout (Mitrovic *et al.* 1968; Swift 1978) exposed to phenol. The slow rates of ^{14}C elimination may be due to liver and gall bladder accumulation of phenolic conjugates and subsequent gradual elimination through the bile.

Goldfish exposed to 20 $mg \cdot L^{-1}$ phenol for 24 hr and then transferred to

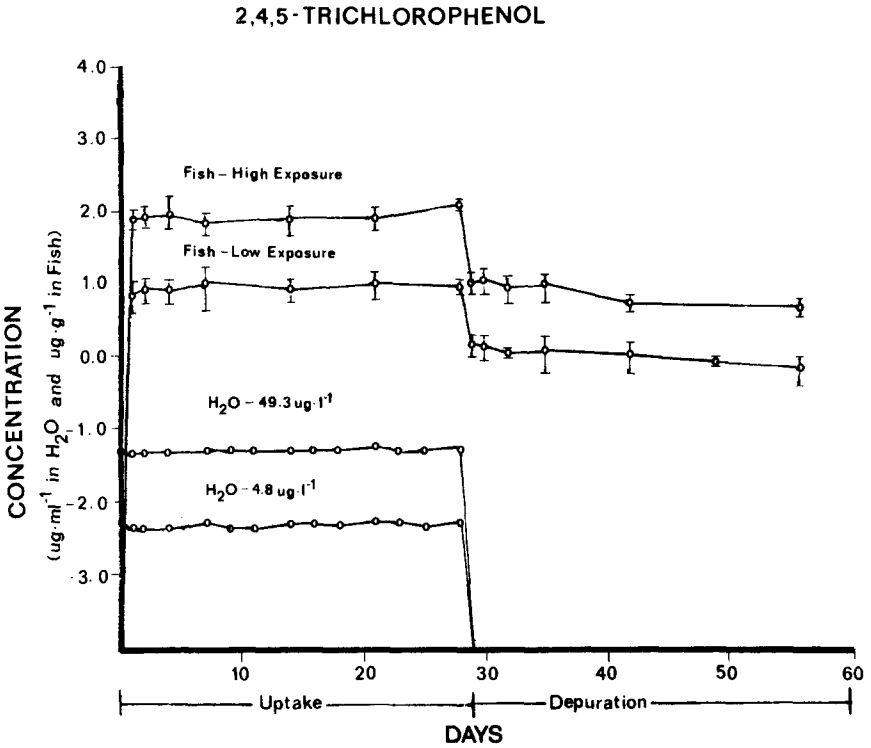

Fig. 2. Log mean exposure water concentrations of ^{14}C tagged-2,4,5-trichlorophenol ($\mu g \cdot ml^{-1}$) and log mean (±SD) whole fish total ^{14}C residues ($\mu g \cdot g^{-1}$) during uptake and depuration phases

clean water eliminated approximately 75% of their phenol body burdens within one hr mainly as free phenol, with a much slower subsequent elimination of phenol in a bound form (Kobayashi and Akitake 1975b). ^{14}C depuration curves and metabolism data of the present study indicate that very little phenol was present in a readily eliminated free form, but that most was present in a form that was slowly eliminated. The $\mu g \cdot L^{-1}$ exposures may have allowed for a more efficient binding of phenol than $mg \cdot L^{-1}$ exposures of other studies (Kobayashi and Akitake 1975b; Swift 1978). Further studies over a wide range of concentrations would be of interest.

2,4,5-Trichlorophenol

^{14}C uptake by fathead minnows from exposure to radiolabeled TCP was rapid at both lower (4.8 $\mu g \cdot L^{-1}$) and higher (49.3 $\mu g \cdot L^{-1}$) exposure levels. Uptake rates were approximately 0.2 and 3.4 $\mu g \cdot g^{-1} \cdot hr^{-1} \cdot$ for lower and higher exposures, respectively (Table 1).

Plateau concentrations of TCP within the fish were rapidly attained at both exposures (Figure 2). Mean plateau CFs were 1,900 for the lower exposure (days 2-28) and 1,800 for the higher (days 1-28).

The depuration half-life of ^{14}C from the fish was short, occurring in 12 hr (observed value) at both water concentrations. The initial phase of depuration

which occurred during the first day accounted for 84 to 92% of the ^{14}C in the fish. Depuration rate constants over a one-day period were approximately 0.08 and $0.1 \cdot hr^{-1}$ for lower and higher exposures, respectively. The second phase of depuration was quite slow and the half-life of the remaining ^{14}C was 21-28 days.

Due to the rapid elimination of TCP, depuration was significant even during the first day of exposure. This resulted in an underestimation of the uptake rate constant (b_1'), and poor agreement between calculated and observed concentration factors (Table 1). Calculation of b_1' from equation (5), which includes the depuration rate constant (b_2), provided a much closer agreement between calculated and observed concentration factors. At the lower TCP exposure, values of 1,800 and 1,900 were obtained for calculated and observed CF's, respectively; and values of 1,800 for both calculated and observed CF's at the higher exposure.

TCP was not readily metabolized. At the end of uptake, 78.6% of the total ^{14}C tissue residue was parent compound, and only 10.8% was acetone unextractable (Table 2). At the end of depuration, only 2.7% of total ^{14}C in the tissue was parent compound, while 79.0% was acetone-unextractable. Although the majority of ^{14}C was eliminated in the first day, the remaining residue was persistent and consisted of polar metabolites (18.2% of total ^{14}C) and possibly some persistent "bound" fractions.

Little information is available regarding TCP accumulation in fish. Considerably more research has focused on pentachlorophenol (PCP), which is discussed for comparative purposes.

Rainbow trout exposed to $0.026\ mg \cdot L^{-1}$ PCP for 24 hr rapidly accumulated residues in their tissues (Glickman *et al.* 1977). Liver, blood, fat, and muscle contained 16, 6.5, 6.0, and $1.0\ \mu g \cdot g^{-1}$, respectively. Elimination was also rapid, with ^{14}C half-lives of 23, 9.3, 6.9, and 6.2 hr for fat, liver, muscle, and blood, respectively. Similar rapid rates of uptake and elimination of TCP in fathead minnows were observed in the present study. Whole fish steady-state concentrations of ^{14}C were obtained by day 1-2 of uptake. Elimination half-lives of 9.2 and 6.6 hr at lower and higher TCP exposures, respectively, were similar to ^{14}C elimination half-lives in liver, muscle, and blood of PCP-exposed trout.

Fathead minnows exposed to radiolabeled TCP concentrated ^{14}C to steady-state concentration factors of 1,900 and 1,800 for lower and higher exposures, respectively. This level of ^{14}C concentration appears greater than that for PCP from other studies. Mosquitofish (*Gambusia* sp.) accumulated PCP in their tissues to an ecological magnification value of 296 after a 24 hr exposure (Lu and Metcalf 1975). PCP was concentrated approximately 400 times in adipose tissue of rainbow trout exposed to $0.026\ mg \cdot L^{-1}$ for 24 hr (Glickman *et al.* 1977). Bluegills (*Lepomis macrochrius*) exposed to $100\ \mu g \cdot L^{-1}$ PCP in a static exposure where the toxicant was daily replaced, concentrated the chemical in various tissues from 13 to 350 times after 8 days (Pruitt *et al.* 1977). Bluegill muscle, gills, digestive tract, and liver contained 1.3, 6, 21 and 35 $\mu g \cdot g^{-1}$, respectively.

Following the rapid initial loss of ^{14}C on day 1 from fathead minnows exposed to TCP in the present study, a persistent residue comprising between 3.6 and 16.3% of the day 28 uptake ^{14}C level remained throughout depuration. Similarly, in bluegills exposed to PCP for either 4 or 16 days, residues ranging from 0.02 to $0.6\ \mu g \cdot g^{-1}$ persisted after 16 days of depuration (Pruitt *et al.* 1977).

PCP residues persisted for at least 55 days in tissues of eels (*Anguilla anguilla* L.) that were exposed for 4 days and then transferred to clean water (Holmberg *et al.* 1972).

2,4,5-TCP and PCP have similar *n*-octanol/water partition coefficients, with log P values of 3.81 (Lu and Metcalf 1975) and 3.72 (Leo *et al.* 1971), respectively. On the basis of lipid solubility alone, similar levels of bioconcentration potential for TCP and PCP would be assumed. However, TCP accumulates to a somewhat greater extent.

A possible explanation for the apparent difference in bioconcentration potential between 2,4,5-TCP and PCP may be in their respective acidities. The uptake of acidic or basic compounds is strongly affected by the degree of ionization as regulated by pH (Hunn and Allen 1974). A pK_a value of 4.86 was reported for PCP (Kaila and Saarikoski 1977). Although a pK_a value for 2,4,5-TCP was not obtained from our review of the literature, 2,3,6-TCP and an unidentified TCP and pK_a values of 6.12 and 6.00, respectively (Kaila and Saarikoski 1977; Handbook of Chem. & Physics 1975). Thus, PCP would be more dissociated at neutral pH than the TCP's. The dissociation difference between 2,3,6-TCP and PCP was suggested as a possible basis for the greater toxicity of 2,3,6-TCP toward crayfish (*Astacus fluviatilis* L.) when exposed to water concentrations of both compounds (Kaila and Saarikoski 1977). PCP was more toxic to crayfish than TCP when injected directly into the animals.

PCP, as the parent compound, represented about 75% of the total ^{14}C accumulated in *Gambusia* sp. after a 24 hr exposure, while a polar product accounted for the remaining 25% (Lu and Metcalf 1975). 2,4,5-TCP comprised 78.6% of ^{14}C after 28 days of exposure in the present study. Phenolic conjugation was not considered an efficient process in *Gambusia,* due to the large quantities of free stored PCP. However, metabolism and conjugation of PCP do occur to some extent. Bile from a rainbow trout exposed to PCP for 24 hr contained high concentrations (250 $\mu g \cdot g^{-1}$) of PCP residues, mostly as the glucuronide conjugate (Glickman *et al.* 1977). Goldfish were reported to excrete PCP largely as a sulfate conjugate (Kobayashi and Akitake 1975a; Akitake and Kobayashi 1975). A polar product comprising 10.8% of total ^{14}C was observed in 2,4,5-TCP-exposed fish of the present study.

PARA-nitrophenol

^{14}C uptake by fathead minnows exposed to radiolabeled PNP was the lowest of the three test compounds. Uptake by fish at the lower exposure (4.1 $\mu g \cdot L^{-1}$) was characterized by a rate of approximately 0.008 $\mu g \cdot g^{-1} \cdot hr^{-1}$ (Table 1) for the first four days with no ^{14}C activity above background measured in the fish at the first 24 hr sampling. A decrease in ^{14}C concentration within the fish occurred on day 7 from the previous day 4 measurement (Figure 3). Concentration of PNP equivalents within the fish on days 7 and 14 were similar, and each sample contained a single fish with no measurable PNP. On day 21, an increase occurred in mean fish ^{14}C concentration which was maintained on day 28. The mean ^{14}C CF for days 7 through 28 was 80.

At the higher exposure (44.1 $\mu g \cdot L^{-1}$), the uptake rate constant for PNP was 0.05 $\mu g \cdot g^{-1} \cdot hr^{-1}$ for the first 4 days (Table 1). Fish ^{14}C concentrations reached a plateau after two days of uptake and remained relatively stable

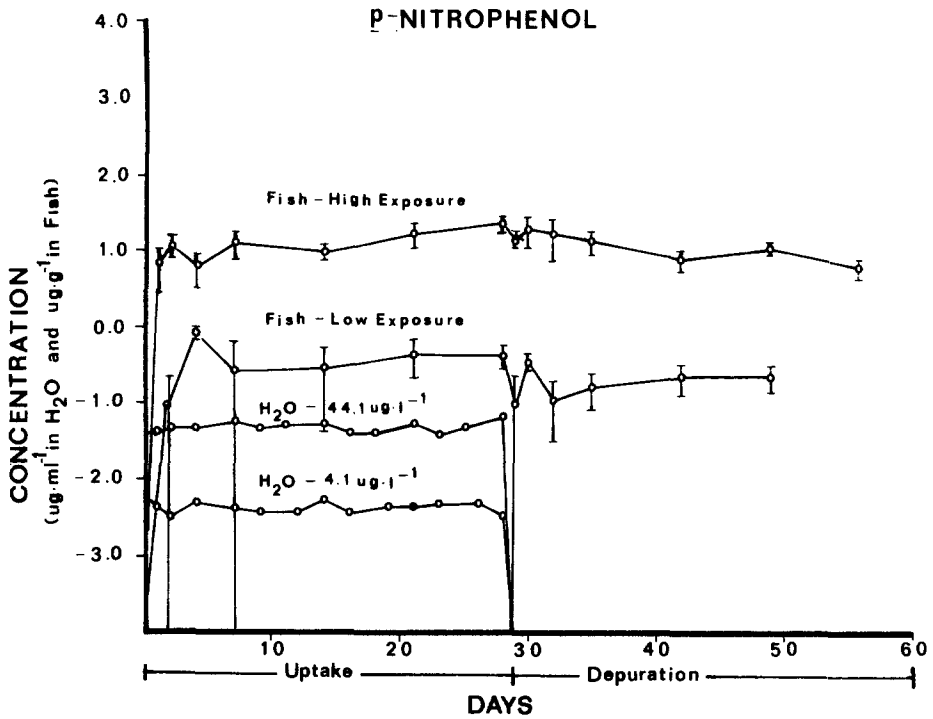

Fig. 3. Log mean exposure water concentrations of ^{14}C-tagged *p*-nitrophenol ($\mu g \cdot ml^{-1}$) and log mean (±SD) whole fish total ^{14}C residues ($\mu g \cdot g^{-1}$) during uptake and depuration phases

through day 14 (Figure 3). On day 21, an increase in whole body ^{14}C concentration ensued which continued to day 28. The mean CF for days 2 through day 28 was 280.

Depuration rate constants of 0.009 and 0.003 $\cdot hr^{-1}$ were calculated at lower and higher PNP exposures, respectively (Table 1). At the lower exposure, the half-life of ^{14}C within the fish was observed at 72 hr and calculated from the rate equation as 37 hr. At the higher exposure, depuration progressed at a more uniform rate, with the exception of fish measured on the first day of depuration which showed an unusually high clearance rate. Fish ^{14}C concentrations did not exhibit the high degree of variability observed with fish from the lower exposure. Calculated and observed ^{14}C tissue concentration half-lives were 206 and 228 hr, respectively.

The PNP that entered the fish was readily metabolized, as 92.0 and 83.4% was acetone-unextractable at the ends of uptake and depuration, respectively (Table 2). Only 4.1 and 0.7% of total tissue ^{14}C was parent compound at the ends of uptake and depuration, respectively. Some of the PNP was reduced to *p*-aminophenol, which was detected at the ends of both uptake and depuration.

Several factors may influence the low concentration potential of PNP. It has a relatively low *n*-octanol/water partition coefficient (log P = 1.91), and resultant low lipid solubility. Dissociation of phenolic compounds may also influence their tendency to bioconcentrate. Phenol, itself, is a weakly acidic substance, and the presence of nitro groups progressively enhances this acidity

Table 3. Percent lipids in fish tissues at different ages and weights

Age of fish in weeks	Group 1		Group 2	
	Mean fish Wt. (g) in sample	% Lipids	Mean fish Wt. (g) in sample	% Lipids
4	0.136	4.0	—	—
5	0.104	3.7	0.123	4.7
6	0.106	4.7	0.150	4.3
7	0.262	4.6	0.115	4.6
8	—	—	0.134	3.9
9	0.224	6.7	0.136	3.8
10	0.491	7.2	0.144	3.4
11	0.404	8.2	0.148	3.9
12	0.465	9.0	0.218	4.5

(Matsugama 1967). Phenol and PNP have pK values of 9.89 and 7.15, respectively (Handbook of Chemistry and Physics 1975). Non-ionized forms of compounds generally penetrate biological membranes more easily than ionized forms (Hunn and Allen 1974). PNP would be expected to have a lower membrane penetration potential than phenol on this basis.

Lipid Content

Analysis of fathead minnows for total lipid content (Table 3), showed that fish weight correlated better with lipid content than fish age. From the 2 groups combined at ages 4-12 wks, three weight classes with increasing lipid content were observed. The smallest fish averaging 0.10 to 0.15 g (n = 10 samples) had a mean lipid content of 4.1%. For the samples in which individual fish averaged 0.22 to 0.26 g (n = 3 samples), the mean lipid content was 5.3%; the samples in which individual fish averaged 0.40 to 0.49 g (n = 3 samples) the mean lipid content was 8.2%. Although there was considerable variation within and between these weight classes, it may be assumed that larger fish had a higher lipid content than smaller fish.

Fish from all exposures averaged less than 0.20 g throughout the entire 28 day uptake phase. From a review of the lipid content of the fish in combination with their mean weights during exposure, any changes in lipid content over this period of rather slow growth may not have greatly influenced the uptake pattern of the test compounds.

For most exposures, a greater weight increase occurred during the following 28 days of depuration. Fish from some of these groups averaged more than 0.40 g., and they probably had a higher lipid content than fish sampled from the same treatment groups at the end of uptake. This could have contributed to a slower elimination rate, particularly for compounds stored in the lipid compartment, than if the fish had been of more constant weight and lower lipid content.

The presence of methanol as a solvent carrier for these phenolic compounds may have had an effect upon their solubilities in water and subsequent

tendencies to partition into fish tissue. Comparative studies on bioconcentration of chemicals in aquatic organisms, both with and without solvent carrier as well as with several different carriers, would be of interest.

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