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Constituent Bioconcentration in Rainbow Trout Exposed to a Complex Chemical Mixture

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Recent work illustrates the effects of complex chemical mixtures upon environmental fate processes such as bioconcentration (Southworth et al. 1980; Landrum 1982; Linder and Bergman 1982; Muir et al. 1982; McCarthy 1983). Classically, aquatic contaminant fate models predicting a chemical's bioconcentration factor (BCF) are based upon single-compound derived models (Branson et al. 1975; Neely et al. 1974; Bishop and Maki 1980), yet such BCF predictions may deviate from observed BCFs when physicochemical interactions or biological responses to complex chemical mixture exposures are not adequately considered in the predictive model.

For example, biological responses affecting bioconcentration were noted by Southworth et al. (1980) when depuration rates were enhanced in fathead minnows (Pimephales promelas) exposed to mixtures of azaarenes characteristic of synfuel process water; the increased depuration was suggested to result from induction of detoxifying enzyme systems in the exposed animals. Similarly, uptake rates of chemicals from aquatic systems can be affected by physicochemical interactions and biological responses consequent to complex chemical mixture exposures, and subsequent BCF predictions for constituents of the mixture may deviate from estimates based upon single-compound models. Landrum (1982), for example, used defined chemical mixtures, while Linder and Bergman (1982) used nontoxic dilutions of an oil-shale retort water, yet each study noted that uptake rates of constituents in such chemical mixtures differed from uptake rates for those same constituents in single-compound exposures. The mechanisms mediating uptake rate differences may be based upon physicochemical processes (Landrum 1982) or competitive interactions for transport of bioconcentrated constituents of complex chemical mixtures (Linder and Bergman 1982), or perhaps a combination of these processes. Noting these deviations from single-compound predictions, we recently evaluated a hazard assessment strategy (Bergman and Meyer 1982) in laboratory exposures of rainbow trout (Salmo gairdneri) to a model complex chemical mixture, an oil-shale retort water. In their suggested protocols Bergman and Meyer (1982) have accounted for biological responses resulting from exposures to complex chemical mixtures, and hence consider a source of variability inadequately regarded in classical models based upon physicochemical characteristics of chemicals or mathematical models developed in single-compound systems.

MATERIALS AND METHODS

Animals and exposure system. Rainbow trout (42.9 ± 9.3 gm; $\bar{X}$ ± S.D.) were obtained from the Wyoming Department of Fish and Game and were held in the aquarium facilities at the University of Wyoming. Photoperiod was maintained at 16:8 L:D, and water temperatures were held at 12-13°C during pre-experimental holding periods and the 15-week exposure. Fish were fed daily (Silver Cup, Murray, UT) and the flow-through exposures of four concentrations (0.002% and 0.004%, and 0.001% and 0.01% dilutions) of a model complex chemical mixture (oil-shale retort water, see below) bracketed the projected no observable-effects-concentration (NOEC) of 0.0045% (Marcus and Bergman 1980). These exposure concentrations were maintained by metering retort water directly into mixing boxes positioned above the exposure aquaria, preceding the 1.0 L/minute discharge outlet for each concentration and control. Both control (dilution water) and exposure (oil-shale retort water dilutions) stock reservoirs were metered into the mixing boxes via silicon tubing, and the metering rate was controlled by a cassette pump calibrated to maintain discharge from the mixing boxes at the desired exposure concentrations. Control and diluent water for complex chemical mixture exposures was a combination of well water and dechlorinated city water (Table 1), and the oil-shale retort water was obtained from the Laramie Energy Technology Center, Laramie, WY.

Table 1. General water quality characteristics of dilution water.

Water quality parameter	Value[a]
Dissolved Oxygen	6.5
pH (units)	8.3
Conductivity (µmho/cm at 25°C)	820
Alkalinity, total ($CaCO_3$)	160
Hardness ($CaCO_3$)	470

[a]Values in mg/L unless otherwise noted.

Model complex chemical mixture. Oil-shale retort waters are complex chemical mixtures, highly alkaline and characteristically, quite toxic to aquatic biota (Marcus and Bergman 1980). For example, Table 2 summarizes a brief chemical analysis of the process water used in these exposures (Occidental-6 retort water), and a reverse-phase high performance liquid chromatography (HPLC) fingerprint (Figure 1) illustrates the complexity of the organic constituents in such a model mixture. While it is unlikely aquatic biota will be exposed to such untreated synfuel process water, it nonetheless models the potential biological effects precluded by exposure to complex chemical mixtures such as solid waste leachates, agricultural runoff, and industrial process waste waters (Bergman

and Meyer 1982). Toxicity bioassays projected a 0.0045% dilution of Occidental-6 retort water as the NOEC (Marcus and Bergman 1980), and this concentration was bracketed in modeling the biological effects of complex chemical mixture exposures upon environmental processes such bioconcentration.

Table 2. General water quality characteristics of Occidental-6 oil-shale retort water.[a]

Water quality parameter	Value[a]
Alkalinity, total ($CaCO_3$)	6,700
Bicarbonate (HCO_3^-)	6,100
Carbonate ($CO_3^=$)	1,000
Dissolved organic carbon	2,800
Total organic carbon	2,900
Chemical oxygen demand	7,900
Conductivity (μmhos/cm at 25°C)	12,800
Ammonia (total ammonia as NH_3)	1,000
Total Kjeldahl nitrogen (N)	1,000
pH (units)	8.9
Total dissolved solids	10,200

[a]Values in mg/L unless otherwise noted.

Residue analysis. As indicated by the Occidental-6 retort water fingerprint (Figure 1) and its preliminary chemical analysis in Table 2, the extraction procedure for tissue residue analysis considered the heterogeneity of the complex chemical mixture. Briefly, an exhaustive steam distillation (Veith and Kiwus 1977) was adapted to effectively extract a range of organic compounds from fish tissue, water, and sediment (Meyer et al. 1981); the liquid-liquid reservoir of the distillation column was charged with an aqueous layer (Fisher, HPLC grade water) and a mixed extraction solvent layer (70:25:5; HPLC grades, n-butyl ether: cyclohexane:n-octanol), and ensured efficient extraction of compounds ranging from polar phenolics to the nonpolar, hydrophobic polynuclear aromatic hydrocarbons.

In the current work fish samples were taken following 1, 3, 6, 9, 12 and 15 weeks of continuous-flow exposure. Four fish were sampled from each exposure or control, two being retained whole and the others being dissected for bile and liver samples. All fish samples (whole and dissected) were homogenized in a Waring blender, then extracted using the exhaustive steam distillation or frozen for later analysis. Homogenized fish tissue or liver was added to 400 ml 1% H_2SO_4 and exhaustively steam distilled for 16-18 hours. Following exaustive steam distillation of experimental and control samples, both aqueous and mixed extraction solvent components of the liquid-liquid reservoir were analyzed for extractable, bioconcentrated organics. Bile samples collected by tuberculin syringe

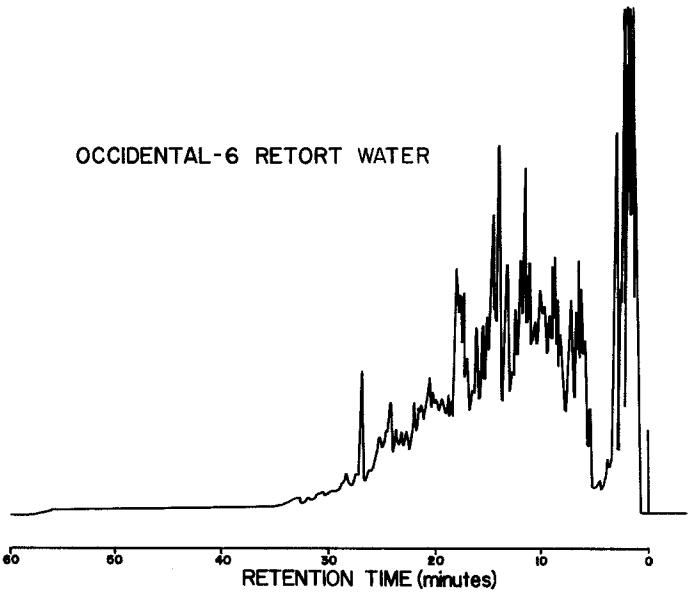

Figure 1. HPLC fingerprint of Occidental-6 retort water. See text for chromatographic conditions and Table 2 for general water quality characteristics.

were deproteinized with HPLC grade methanol (Fisher), and Waters HPLC with both UV (254 nm) and fluorescence (313 nm excitation: 425 nm emission) detectors was used in analyses of all aqueous and mixed extraction solvent samples as well as deproteinized bile samples. Preliminary analyses reported here compare experimental and control chromatographic profiles for each sample based upon retention time similarities.

RESULTS AND DISCUSSION

Chromatographic analyses of aqueous and nonaqueous liquid-liquid reservoir components yielded differences in mixed extraction solvent HPLC profiles of whole fish exposed for 1 and 3 weeks to the highest dilution of the complex chemical mixture when compared to their corresponding control (Figure 2), yet subsequent whole fish extractions at 6, 9, 12 and 15 weeks into exposure demonstrated no qualitative differences between control and exposed fish. At these times (6, 9, 12 and 15 weeks), however, when whole fish extractions showed no differences between exposed and control chromatographic profiles, liver extractions and deproteinized bile samples from exposed fish were qualitatively different than their corresponding controls (Figures 3 and 4). Behavioral observations performed during the initial 10 weeks of exposure demonstrated no difference between exposed and control animals in avoidance behavior or coughing frequency, and similarly, growth effects were undetected throughout the 15-week experiment. In total then, these results support the projected NOEC (0.0045% dilution), although the differences in bioconcentration profiles suggest hazard assessment strategies

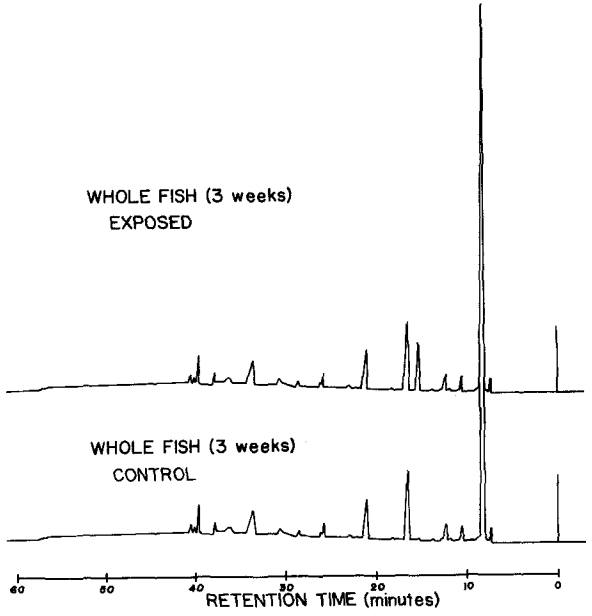

Figure 2. Whole fish extractions yielded a bioconcentration profile with differences noted at 16 minutes. See text for explanation of extractions and HPLC conditions. UV absorbance illustrated above.

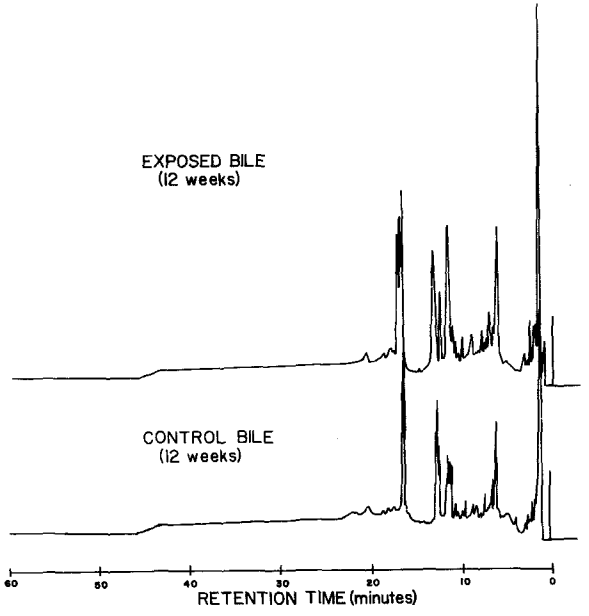

Figure 3. Deproteinized bile profiles displayed differences between controls and exposed fish. UV absorbance illustrated above. See text for HPLC conditions and time course relationships, Figures 2 - 4.

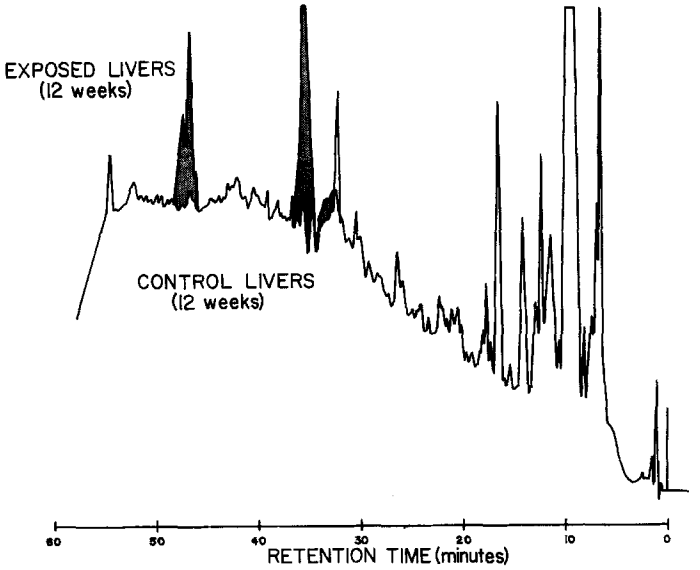

Figure 4. Liver extractions displayed differences in comparisons between control and exposed fish as indicated by stippling. See text for explanations.

such as Bergman and Meyer (1982) may be useful in evaluating environmental fate processes associated with complex chemical mixtures.

While these preliminary results reflect qualitative data, they suggest, nonetheless, that biological responses of aquatic biota exposed to complex chemical mixtures must not be oversimplified in evaluations of the environmental fate of chemicals entering aquatic systems. Tentatively, these results suggest future work addressing problems of bioconcentration of constituents in a complex chemical mixture. Specifically, a not unfounded interpretation of these findings suggests that despite the nontoxic exposure concentrations, constituents of the model complex chemical mixture are bioconcentrated by fish exposed to steady-state concentrations of the mixture. Such conclusions are supported by the differences in whole fish tissue extractions after 1 and 3 weeks of exposure (Figure 2) and chromatographic differences observed in liver extractions and bile samples from weeks 6-15. While the residue accumulations in exposed whole fish were apparently transient, the absence of difference in whole fish extraction chromatograms after 3 weeks of exposure may result from two interrelated factors, one analytical and the other biological.

First, the chemical characterization of tissue extractions has analytical limitations, largely dictated by the low exposure concentrations of the model complex chemical mixture. Under these nontoxic exposure conditions the concentration of the constituents in the mixture approached the detection limits of the HPLC systems used

in these preliminary analyses, and only those constituents present in sufficiently high concentration would be detected in tissue extractions following bioconcentration. Hence, both 1 and 3 weeks after exposure whole fish displayed a single bioconcentrated residue in the extractions at 16 minutes (Figure 2). Many other constituents may have been bioconcentrated, but owing to extremely low water concentrations and low bioconcentration factors, these residues were not detected in tissue extractions. Similar arguments may be applied to the samples collected subsequent to those at weeks 1 and 3.

Second, the apparent absence of the bioconcentrated residues in whole fish after 3 weeks may be explained by altered rates of uptake and depuration of xenobiotics or their metabolites. While recent work offers explanations for altering uptake rates (Landrum 1982; Linder and Bergman 1982), the current work suggests that depuration rate differences may be influential in these preliminary results. For example, enzyme induction may effect residue concentrations as suggested by Southworth et al. (1980) and may be mediating the changes in bioconcentration profiles in the current work. The differences, for example, between exposed and control liver extractions and deproteinized bile samples suggest that decreased whole fish residues may be accounted for in part by enhanced depuration of bioconcentrated constituents or their metabolites.

Efforts to identify the tissue residue(s) isolated from exposed whole fish after 1 and 3 weeks of exposure requires gas chromatographic-mass spectrometric techniques, but initial estimates of chemical characterization were derived from retention time similarities in HPLC elutions. Work with known standards suggest that tissue residue(s) belongs to the class of monocyclic hydrocarbons since the tissue residue(s) elutes with a retention time intermediate that of benzene and phenol. Again, analytical limitations may prohibit detection of bioconcentrated constituents in tissue extractions, particularly in the samples collected after 6, 9, 12 and 15 weeks of exposure. While differences between control and experimentals were not apparent in samples collected after 3 weeks exposure, both deproteinized bile and liver extraction samples suggest biotransformation of bioconcentrated constituents, and subsequently, metabolite depuration is increased and yields reduced body burdens of the constituents evident in whole fish at weeks 1 and 3. Preliminary analyses based on HPLC retention time similarities suggest the bioconcentrated residue(s) in exposed whole fish extractions at weeks 1 and 3 may be related to those chromatographic peaks noted in bile samples collected from exposed fish during subsequent weeks of exposure since differences in profiles occur in both whole fish and bile at 16 minutes. Chromatographic differences seen in liver profiles may suggest the role of that organ in the biotransformation process, and differences between control and exposed liver extractions are interpreted initially as large molecular weight complexes of bioconcentrated constituents or their metabolite(s). Indeed, the time course noted in the 15-week experiment suggest induction of xenometabolic enzyme systems may have occurred during the initial three weeks of exposure, and changes in chromatographic profiles may reflect altered distribu-

tions of tissue residues of bioconcentrated constituents or their metabolite(s).

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REFERENCES

Bergman HL, Meyer JS (1982) Complex effluent fate modeling. In: Dickson KL, Maki AW, Cairns, Jr. J (eds) Modeling the fate of chemicals in the aquatic environment. Ann Arbor Science, Ann Arbor, Michigan, p 247

Bishop WE, Maki AW (1980) A critical comparison of two bioconcentration test methods. In: Eaton JG, Parrish PR, Hendricks AC (eds) Aquatic toxicology, ASTM STP 707. American Society for Testing and Materials, Philadelphia, p 61

Branson DR, Blau GE, Alexander HC, Neely WB (1975) Bioconcentration of 2,2',4,4'-tetrachlorobiphenyl in rainbow trout as measured by an accelerated test. Trans Amer Fish Soc 4:785-792

Landrum PF (1982) The effect of co-contaminants on the bioavailability of polycyclic aromatic hydrocarbons to Pontoporeia hoyi. In: Polynuclear aromatic hydrocarbons: Combustion processes, environmental effects, and biochemistry, Seventh International Symposium

Linder GL, Bergman HL (1982) Anthracene bioconcentration in rainbow trout exposed to single compounds and complex chemical mixtures. In: Abstracts, Third Annual Meeting, Society of Environmental Toxicology and Chemistry. Arlington, Virginia, p 105

Marcus M, Bergman HL (1980) Quarterly progress report, Effects of aqueous effluents from in situ fossil fuel processing technologies. University of Wyoming, Laramie, Wyoming

Meyer JS, Linder G, Bergman HL (1981) An approach for bioaccumulation studies of complex organic effluents: extraction of representative organics from rainbow trout and water. In: Abstracts, Second Annual Meeting, Society of Environmental Toxicology and Chemistry. Arlington, Virginia, p 122

Muir DCG, Grift NP, Townsend BE, Metner DA, Lockhart WL (1982) Comparison of the uptake and bioconcentration of fluridone and terbutryn by rainbow trout and Chironomus tentans in sediment and water systems. Arch Environm Contam Toxicol 11:595-602

McCarthy JF (1983) Role of particulate organic matter in decreasing accumulation of polynuclear aromatic hydrocarbons by Daphnia magna. Arch Environm Contam Toxicol 12:559-568

Neely WB, Branson DR, Blau GE (1974) Partition coefficient to measure bioconcentration potential of organic chemicals in fish Environ Sci Tech 8:1113-1115

Southworth GE, Keffer CC, Beauchamp JJ (1980) Potential and realized bioconcentration. A comparison of observed and predicted bioconcentration of azaarenes in the fathead minnow (Pimephales promelas). Environ Sci Tech 14:1529-1531

Veith GD, Kiwus LM (1977) An exhaustive steam-distillation and solvent-extraction unit for pesticides and industrial chemicals. Bull Environm Contam Toxicol 17:631-636

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