Fish may be analysed as whole fish or as portions, e.g. edible portion (muscle), viscera, remaining carcass, etc. Specific organs may also be analysed if sufficient biomass is available. If results based on body portions are desired, after the fish is killed it should be eviscerated, taking care not to puncture any parts of the visceral portion which could leak body fluids and possibly cause contamination of the remaining portions. The edible portion or muscle may be removed with a scalpel, blotted dry, and weighed before storing or analysis. The remaining carcass should be weighed before being stored or analysed. It is necessary to record data for each portion for each individual fish so that whole body data can be reconstructed based on the sum of the parts.

After weighing, the sample is usually ground or homogenised to promote extraction of test material or to enhance solution of the tissue. Procedures for grinding, extraction, separation of impurities, determination of lipid content, etc., are described in the U.S. Food and Drug Administration's Pesticide Analytical Manual (10) or the U.S. Environmental Protection Agency's Manual of Analytical Methods for the Analysis of Pesticide Residues in Human and Environment Samples (11).

When determining the bioconcentration of test materials which concentrate in lipids, it is often desirable to determine the percent of the total tissue weight made up by lipids. Results between samples are frequently less variable when based on lipid weight rather than on total weight (12).

Organism samples can be wrapped in acetone-rinsed foil, placed in glass jars and frozen if they are not to be analysed immediately.

- Analytical guidelines

Prior to analysing fish or water for the test substance, control samples should be spiked with several different concentration of the test substance and then analysed. Final values of C_w and C_f should be corrected for recoveries and background.

Analytical detection limits of test substance in both fish and water should be determined before the bioconcentration test begins and should be documented in the protocol. As a guideline, the limit of detection may be defined as a signal 2.5 times higher than the background noise level.

If possible, results reported as "not detected at the limit of detection" should be minimised by pre-test method development and experimental design. These results cannot be used for rate constant calculations.

The units of C_w and C_f should both be expressed either as ppm or ppb.

3. DATA AND REPORTING

- Treatment of results

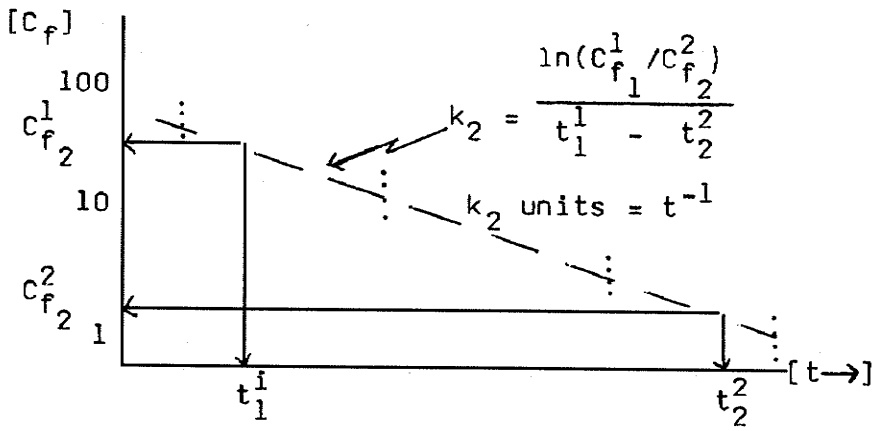
(estimation of rate constants from raw time concentration data)

Model discrimination

Most bioconcentration data can "reasonably" be described with a simple two-compartment/two-parameter model as shown by a "straight line" depuration profile plotted on semi-log paper. If the depuration profile does not appear to be a straight line, then more complex models can be employed (14). Typical variations from the simple model include a third parameter to describe the rate of metabolism of the parent compound or two additional parameters to describe redistribution of the parent compound within the body of the fish. If the "best model" is in question, it may be worthwhile to estimate parameters for the models in question and to compare the "likelihood index" of each model according to statistical tests (14).

Graph paper method for depuration rate constant

Plot each concentration of the test material found in fish at each sampling time on semi-log paper. The slope of that line is k_2:



Graph paper method for uptake rate constant

Given k_2, calculate k_1 as follows:

$$k_1 = \frac{C_f\, k_2}{C_w\,(1 - e^{-k_2 t})}$$

The value of C_f is read from the smooth uptake/depuration curve near the uptake mid-point on semi-log paper.

Computer method for calculating uptake and depuration rate constant

The preferred means for obtaining the bioconcentration factor and k_1 and k_2 rate constants is to use non-linear parameter estimation methods on a digital computer. Two such programs are BIOFAC (Dow) and NONLIN (Proctor and Gamble). These programs find values for k_1 and k_2 given a set of sequential time concentration data and the model:

$$C_w \frac{k_1}{k_2}\,(1 - e^{-k_2 t}) \qquad 0 \le t \le t_c$$

$$C_w \frac{k_1}{k_2}\left[e^{-k_2\,(t - t_c)} - e^{-k_2\, t_c}\right] \qquad t > t_c$$

This approach provides standard deviation estimates of k_1 and k_2, and BIOFAC statistically weights the analytical and biological variation of the fish concentration data. These and other non-linear parameter estimation programs are readily available for most computers accepting the Fortran IV language or can be made available from a time-sharing service bureau; they are currently being used by many bioconcentration testing laboratories.

- Test report

The test report should include the following information:

- name of test, investigator, laboratory, and date test was begun;
- a detailed description of the test material, including its sources, lot number, composition (identity and concentration of major ingredients and major impurities), known physical and chemical properties, and identity and concentration of any carriers (solvents) or other additives used;

- the source of the dilution water, its chemical characteristics, and a description of any pretreatment;

- detailed information about the test organisms, including scientific name and how verified (and strain for salmonids when appropriate), weight (wet, blotted dry), standard length of fish, height of bivalve molluscs, age, life stage, source, history, observed diseases, treatments, acclimation procedure, and food used;

- a description of the experimental design and metering system;

- description of tissue and water samples analysed, and methods used to obtain, prepare and store them;

- methods used for, and results (with standard deviation) of, all chemical analyses of water quality and concentration of test material in tissue and water, including validation studies and reagent blanks;

- the steady-state bioconcentration factor, the uptake and depuration rate constants, the confidence margin (± standard deviation) and the method of computations/data analysis;

- anything unusual about the test, any deviation from these procedures, and any other relevant information.

• Interpretation of results

Scientific judgment rather than rigid criteria should be exercised in accepting or rejecting bioconcentration test results.

Calculated BCF values, based on an octanol/water partition coefficient, have a very wide confidence margin (greater than ± 100 per cent), but the quality of the value may be better (narrower confidence margin) than an experimental value for a poorly designed study. Generally, the confidence margins for well-designed studies approach ± 20 per cent. Acceptable bioconcentration data should be reported with confidence margins.

Other criteria for judging the quality of bioconcentration data include the following guideline:

- Percent mortality or adverse effect in control or treated organisms (suggested guideline, 10 per cent).

- Percent effect of dose on uptake/depurature rate constants (suggested guideline, 20 per cent).

- Percent variation in C_w (suggested guideline, 20 per cent) except for the initial dip that may approach 50 per cent during the first few days of exposure.

- Temperature and dissolved oxygen should not vary more that ± 1°C and ± 3 mg/litre.

- The importance of actually visualising an apparent plateau has been a subject of recent debate. It is suggested that 80 per cent of steady-state (k_1/k_2) in any tissue with a confidence margin of ± 20 per cent is more than sufficient to estimate high quality rate constants for compounds with BCF ≤ 10.000. For compounds with BCF > 10.000, it may be desirable and acceptable to terminate the uptake phase after a few days not to exceed 28 days - even though < 80 per cent of steady-state was reached.

- A clearly defined uptake/depurature profile is an indicator of high quality bioconcentration data.

4. LITERATURE

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