DRAFT PROTOCOL (15th May 2009)

CHLORINATED TETRADECANE, FISH BIOCONCENTRATION

Guidelines:

OECD Guideline for Testing of Chemicals 305
Bioconcentration: Flow through Fish Test

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PROPOSED DATES

Start of Analytical Method Development:……..……
Experimental Start Date:…….
Experimental Termination Date:……
Project Number:……..
Test Concentration:……..
Test Substance Number:…….

PROTOCOL APPROVAL

STUDY DIRECTOR…….. DATE……

CLIENT MANGER……. DATE……

SPONSOR'S REPRESENTATIVE……. DATE…….

INTRODUCTION

Brixham Environmental Laboratory will experimentally determine the bioconcentration factor for chlorinated n-tetradecane in rainbow trout (*Onchorhynchus mykiss*). The study will be performed based on the procedures in OECD Guideline for testing of Chemicals, Guideline 305: 'Bioconcentration: flow-through in fish' (OECD, 1996). This procedure is described in Brixham Environmental Laboratory Standard Operating Procedure BA331:04.

OBJECTIVES

To investigate the potential for bioconcentration of a low-chlorinated, low-chainlength (C_{14}) constituent of medium-chain chlorinated paraffins (MCCP) in relation to the EU PBT criteria.

SUMMARY

This protocol describes a fish bioconcentration study based on OECD Guideline 305 (1996) (except as otherwise noted), employing a radiolabelled single-chainlength (C_{14}) chlorinated paraffin. The exposure (accumulation) phase will be 28 days (or as described below) followed by a depuration (clean water) phase of 28 days (or as described below). Samples of water and fish tissue will be taken at intervals for analysis for the test substance based on radioactivity.

EXPERIMENTAL DESIGN

Test substance

[^{14}C]-n-tetradecane, of known purity, chlorinated to approximately 45% by weight (approximately $C_{14}H_{25.5}Cl_{4.5}$).

Test species

The test species used in the study will be Rainbow trout (*Onchorhynchus mykiss*), with a length between 4 and 12 cm (subject to analytical requirements).

The fish will be acclimated for at least two weeks in water at the test temperature and feed throughout on a sufficient diet and of the same type to be used during the test. Following a 48-hour settling-in period, mortalities will be recorded and the following criteria will be applied:

- mortalities of greater than 10% of population in seven days: reject the entire batch;
- mortalities of between 5 and 10% of population in seven days: acclimate for seven additional days;
- mortalities of less than 5% of population in seven days: accept the batch - if more than 5% mortality during second seven days reject the entire batch.

Every effort will be made to ensure that the fish used in test are free from observable diseases and abnormalities. Any diseased fish will be discarded. Fish used within the test will not have received treatment for disease in the two weeks preceding the test, or be treated for disease during the test.

The weight and length of the fish at the start of the study will be determined on a representative sample of the fish.

Test system and conditions

The test will be conducted in a flow-through system, with a water flow rate sufficient to provide a minimum of 1 litre per gram of fish per day. Addition of the test substance will be by continuous dosing from a stock solution in organic solvent (dimethylformamide, DMF). The stock solution will be mixed with dilution water in a mixing cell, stirred at high speed to maximise dissolution of the test substance.

Test temperature maintained at 15 ± 2ºC, photoperiod of 16 h light: 8 h dark.

Exposure concentration

A single test substance exposure concentration (0.5 μg l^{-1}) and a solvent control will be tested (assuming that the specific activity of the radiolabel is approximately 80 MBq g^{-1}). A single test substance concentration is a deviation from OECD 305; this is justified because the principal reason for a second (lower) concentration is to act as contingency against toxicity occurring at the higher level, whereas MCCP is known to be non-toxic to fish at concentrations up to and exceeding the water solubility limit. The single concentration to be tested is significantly below the estimated water solubility of the substance and is the minimum that can be tested whilst maintaining adequate analytical sensitivity in terms of water exposure concentration and tissue residues. A second exposure concentration (as required by OECD) would, by necessity, be higher (typically by a factor of ten) and therefore closer to the water solubility, and is considered superfluous in this case.

The concentration of solvent (DMF) used as carrier for dosing the substance would not exceed 0.1 ml/l (if possible ≤0.01 ml/l). A solvent control will also be tested containing the same concentration of DMF.

Duration

Unless otherwise specified by amendment, the test will comprise 28 days exposure (accumulation phase) followed by 28 days elimination in clean water (depuration phase). The exposure and/or depuration phases may be extended if considered necessary to define reliably the uptake and/or depuration rates. The duration of the exposure phase may be reduced to a minimum of 21 days if steady state (or no detectable bioconcentration) has been demonstrated. The duration of the depuration phase may be reduced if tissue concentrations have declined below detection before 28 days.

Feeding

The population of fish in each tank will be fed a proprietary trout food at a rate which is intended to maintain body weight and lipid levels in the fish. To achieve this, the feeding level is intended to be slightly in excess of a maintenance ration; the daily ration to each tank is 2% of total fish weight in that tank, which is measured initially and estimated thereafter.

The total initial weight of fish in each tank will be determined by bulk weighing, within 24 hours prior to the start of the exposure. Every 7 days, the total weight of fish remaining in each tank is estimated as follows:

Remaining weight = W - M

where
W = Total fish weight estimated or measured 7 days previously
M = Total weight of fish sampled or removed as mortalities since previous estimate.

In addition, the estimate of remaining weight may be adjusted to allow for growth that has occurred, based on the weights of the fish sampled, using the data from 2 or more sampling occasions, and if necessary pooling the data from all treatments; any such adjustment will be recorded in the study book.

The final estimate of remaining weight is used to set the daily ration for the following seven days.

All batches of food used will be analysed for pesticides and heavy metals. Analysis of the food for the test substance (if applicable) will be described in the study plan. The lipid and total protein content of the food should be known.

Analytical sampling for the test substance in water

Water sampled from the test substance treatment will be analysed at least twice before addition of the fish to ensure correct operation of the dosing system and at least weekly during the exposure phase, to

coincide with fish sampling occasions. Appropriate volumes of water (dependant upon the specific activity of the test material) will be extracted with hexane prior to Liquid Scintillation Counting (LSC) analysis, to measure total radioactivity, expressed as equivalent concentration of the test substance.

Samples from the solvent control will be taken on the same occasions, to confirm normal background levels of radioactivity. The recovery efficiency for the solvent extraction procedure will be reported for each sample point and the appropriate recovery factor will be applied to the test data.

Analytical sampling for the test substance in fish tissue

Fish sampled from the test substance treatment will be analysed on at least 5 occasions during the exposure phase, and at least 4 occasions during depuration. A minimum of 5 fish will be sampled per occasion. A double sample (10 fish) will be taken on the change-over day from exposure to depuration. A further sample of 10 fish will be snap frozen and retained after the exposure period for further analysis if required at the end of the study. The tissue samples will be analysed for radioactive residues by sample oxidation (combustion) followed by LSC, and the results expressed as as equivalent concentration of the test substance. The same numbers of fish will be sampled from the solvent control on each occasion, but fewer may be analysed, sufficient to define the background radioactivity levels.

The wet weights of the individual fish sampled will be recorded.

Lipid content of fish

The lipid content of the fish will be determined on additional fish sampled at the start of the study, at the end of the accumulation phase and at the end of the study, with a minimum of 3 analyses per occasion.

Water quality parameters monitored

The following parameters will be measured daily in each tank for the first three days; pH, oxygen, temperature and flow rates. Thereafter, flow rates and temperature will be measured three times per week and pH and oxygen twice per week until the end of the test.

The pH, alkalinity, hardness, conductivity and NPOC will be measured in one replicate of the control and one concentration of the test substance, once per week.

The temperature in one test vessel will be recorded continuously.

The dissolved oxygen concentration in the test vessels will be maintained above 60% of the air saturation value.

Calculation of BCF

Depending on the data obtained, the BCF will be calculated as the quotient of the steady state tissue concentration and the water exposure concentration and/or as the quotient of the uptake and depuration rate constants (k_1/k_2), as per OECD 305.

Sample Handling and Safety

In order for Brixham Environmental Laboratory to comply with relevant health, safety and environmental regulations the Sponsor shall ensure Brixham Environmental Laboratory is provided with all available information regarding known or potential hazards associated with any substances supplied to Brixham Environmental Laboratory by, or on behalf of, the Client.

Retention of Materials

The quoted price(s) will include a charge for the retention of material and/or data used or produced by the Project in an archive for a period as determined by UKGLP, any applicable legislation concerning laboratory practice or any notice of a regulatory authority, such period currently being three (3) years. During such period the Client may request access to, or the repatriation of the material and/or the data,

which will be supplied by Brixham Environmental Laboratory following the receipt of reasonable postage and packaging charges.

Final Report

A final report of the results of the study will be prepared by Brixham Environmental Laboratory. The report will include, but will not be limited to the following:

- Name and address of the facility performing the study.

- Dates upon which the study was initiated and completed, and the definitive experimental start and termination dates.

- A statement of compliance signed by the Study Director addressing any exceptions to Good Laboratory practice Standards

- Test substance:

  - physical nature, purity and, where relevant, physicochemical properties;

  - chemical identification data (including the organic carbon content, if appropriate);

- Test species:

  - scientific name, strain, source, any pretreatment, acclimation, age, size-range, etc.

- Test conditions:

  - test procedure used (e.g. flow-through conditions);

  - type and characteristics of illumination used and photoperiod(s);

  - test design (e.g. number and size of test chambers, water volume replacement rate, number of replicates, number of fish per replicate, length of uptake and depuration phases, sampling frequency for fish and water samples); method of preparation of stock solutions and frequency of renewal (the solubilising agent, its concentration and its contribution to the organic carbon content of test water must be given, when used);

  - the nominal test concentration, the means of the measured values and their standard deviations in the test vessels and the method by which these were attained;

  - source of the dilution water, description of any pretreatment, results of any demonstration of the ability of test fish to live in the water, and water characteristics: pH, hardness, temperature, dissolved oxygen concentration, residual chlorine levels (if measured), and total organic carbon;

  - water quality within test vessels, pH, hardness, TOC, temperature and dissolved oxygen concentration;

  - detailed information on feeding (e.g. type of food(s), source, composition - at least lipid and protein content if possible, amount given and frequency);

  - information on the treatment of fish and water samples, including details of preparation, storage, extraction and analytical procedures (and precision) for the test substance and lipid content (if measured).

- Results:
  - results from any preliminary studies performed;

- mortality of the control fish and the fish in each exposure chamber and any observed abnormal behaviour;

- the lipid content of the fish;

- extraction efficiencies for the hexane-water solvent extractions;

- curves, (including all measured data,) showing the uptake and depuration of the test chemical in the fish, the time to steady-state;

- Concentration in fish (Cf) and concentration in water (Cw) (with standard deviation and range, if appropriate) for all sampling times (Cf expressed in mg Kg^{-1} wet weight of whole body, and Cw in mg l^{-1} (or µg l^{-1}) .;

- the steady-state bioconcentration factor, (BCF_{ss}), and/or kinetic concentration factor (BCF_K) and if applicable, 95% confidence limits for the uptake and depuration (loss) rate constants (all expressed in relation to the whole body, confidence limits and standard deviation (as available) and methods of computation/data analysis for each concentration of test substance used;

References:

OECD. (1996). OECD Guidelines for Testing of Chemicals. Bioconcentration: flow-through fish test.

BA331:04. Brixham Environmental Laboratory Standard Operating Procedure. Dynamic Bioconcentration study with rainbow trout (*Oncorhynchus mykiss*)