Section A7.4.2 Bioconcentration in aquatic organisms (fish)

Annex Point IIA, VII.7.5 *Lepomis macrochirus*

Official use only

1 REFERENCE

1.1	**Reference**	[redacted], 1991, Dichlofluanid: Bioconcentration in fish, [redacted], Report No. BF-006 (unpublished), 1991-08-15
1.2	**Data protection**	Yes
1.2.1	Data owner	Bayer Crop Science AG
1.2.2	Companies with letter of access	Bayer Chemicals AG
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes, EPA guideline72-6, 165-4 (1982)
2.2	**GLP**	Yes
2.3	**Deviations**	No, with regard to OECD guideline No. 305

3 MATERIALS AND METHODS

3.1	**Test material**	[Ring-UL-^{14}C]-dichlofluanid, specific radioactivity: 370 kBq/mg (10 µCi/mg)
3.1.1	Lot/Batch number	Batch No.: [redacted]
3.1.2	Specification	[Ring-UL-^{14}C]-dichlofluanid
3.1.3	Purity	[redacted]
3.1.4	Further relevant properties	Water solubility (a.i.): 1.3 mg/l
3.1.5	Radiolabelling	[Ring-UL-^{14}C]-dichlofluanid
3.1.6	Method of analysis	Samples were analysed in accordance with the following conditions. <u>Processing of fish samples for radioassay</u> Fish samples were collected and dissected into edible (body, muscle, skin, skeleton, fins) and non-edible (head, internal organs) portions. Samples were transferred into weighed polystyrene vials suitable for further handling. After determining the wet weight of the samples they were lyophilized, reweighed and homogenized. Aliquotal parts were taken for radioactivity measurement. <u>Radioactivity measurement</u> Normally triplicate sub samples were analysed due to inhomogeneities in the freeze-dried tissues caused mainly by the scales. The radioactivity of extracts and solutions was determined by means of liquid scintillation measurement (LS-measurement); solid samples were combusted before the LS-measurement.
3.2	**Reference substance**	No

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3.2.1	Method of analysis for reference substance	-	
3.3	**Testing/estimation procedure**		
3.3.1	Test system/ performance	Test animals	X

The bluegill sunfish (*Lepomis macrochirus*) (lot 3/90) used in this 42-day dynamic study were obtained from [redacted]. The fish were identified to species by the supplier. All test fish were held in culture tanks on a 16-hour daylight photoperiod and observed for at least 14 days prior to testing. Fish culture techniques were basically those described by Brauhn et al. During the acclimation and test periods, the fish received a Kronen-Fisch, Typ I (Rheinkrone, D-4230 Wesel) fish food once daily ad libitum. Upon arrival a prophylactic treatment with oxytetracycline for fin rot disease was performed. No mortality occurred in the fish lot used 14 days prior to the introduction of the fish in the test medium.

Test system

A dosing system comprising a Hamilton ®Microlab MT dispenser with a 250 µl-syringe for each aquarium controlled by an EPSON HX20 computer (for dosing of stock solution) and flow-meters (for water flow control) was used for the introduction of [^{14}C]-dichlofluanid and diluent water into the 100 litre test aquaria. Aerated reconstituted water was delivered to the glass aquaria at an average rate of approx. 25 l per hour per aquarium during the exposure period (28 days), an amount sufficient to replace the approximately 100 litre test volume about 6 times in a 24-hour period and stock solution ([^{14}C]-dichlofluanid in acetone) was dosed at a rate of 50 µl every 72 seconds (= 2.5 ml/h). Water (continuously 25 l/h) and aliquots of [^{14}C]-dichlofluanid stock solution (50 mg/l, 0.05 ml every 72 sec) were delivered to a 2000 ml-mixing cell to yield a nominal exposure concentration of 5.0 µg/l. The mixture was running continuously from the mixing vessel into the respective aquarium.

The control aquarium received an amount of acetone solvent (0.1 ml/l) as the exposure aquarium.

The exposure system consisted of one 5.0 µg/l nominal concentration aquarium and one control aquarium. The aquaria were labelled with the study number and treatment level.

The test aquaria arranged in a lab room were kept at 22 °C (± 1) by adding diluent water electronically thermostated to that temperature. Temperature was measured once daily on working days and the range of temperature deviations was followed by a mercury-minimum-maximum-thermometer, which was reset after each reading.

The diluter system was calibrated by volumetric measurements of syringe dispenser aliquots and flow-rate of flow meters.

Preparation of the test substance

The stock solution for the treatment of the fish were prepared as follows:

The radioactive material (ca. 37 MBq, 370 kBq/mg delivered in a 2 l bottle) was diluted in 2 l acetone p.a. by adding the solvent to the tracer. Thus the concentration in the stock solution was 50 mg/l with a specific radioactivity of 370 kBq/mg or 22200 dpm/µg (equivalent to 777 dpm per 7 ml-sample of test medium).

Test procedure

Uptake phase

The uptake phase was initiated by transferring groups of 56 randomly selected and previously acclimated fish (length 6.66 ± 0.66 cm) to the test chamber. The initial loading was 2.3 g fish/l and 0.38 g fish/l/day. The fish were observed initially and every 24 hours on working days thereafter during the exposure period of 28 days for mortality and/or adverse behaviour. At the same intervals pH and dissolved oxygen were measured in all aquaria. The temperature was recorded hourly in the control tank. Water and fish were sampled throughout the uptake period on day 0, 1, 3, 8, 10, 14, 21 and 28. The water and fish samples were radioassayed and analysed according to the indicated conditions.

Depuration phase

On day 28 of the exposure period, the addition of the [^{14}C]-dichlofluanid test material ceased. At the beginning of the depuration phase, the aquaria were cleaned mechanically, emptied by suction to a water height of ca. 5 cm, and filled with uncontaminated diluent water (22 °C). During that procedure the fish remained in the aquaria. The fish were then exposed to flowing uncontaminated diluent water (22 °C) for 14 days. During the depuration period, water and fish were sampled on day 29, 31, 35, 38 and 42. The samples were radioassayed according to the indicated conditions. The fish were observed initially and every 24 hours on working days during the depuration period of 14 days for mortality and/or adverse behaviour. At the same intervals pH and dissolved oxygen were measured in all aquaria. The temperature was recorded hourly in the control tank.

Sampling

Fish

Fish were sampled on day 0, 1, 3, 8, 10, 14, 21, 28, 29, 31, 35, 38 and 42. On these dates, four fish from each chamber were collected and processed individually. The fish were dissected into edible and viscera/non-edible parts. Samples were treated and measured according to the indicated conditions. Fresh weight and dry weight of the fish portions were determined.

Water

At each sampling day, 3 samples of 7 ml of water were removed from each aquarium. The concentrations of ^{14}C calculated as [^{14}C]-dichlofluanid in water were calculated by liquid scintillation counting of triplicate 7 ml-samples pipetted directly from each control and test tank. 7 ml scintillation cocktail were added to each sample.

Chemical and physical test parameters

Water quality parameters of dissolved oxygen and pH were measured

initially and throughout the study at least on working days in the control and exposure chambers. The temperature was recorded hourly. The test chambers were not aerated throughout the test. Dissolved oxygen levels remained at or above 96% saturation.

3.3.2 Estimation of bioconcentration

Calculation of results

In evaluating the data obtained from the bioconcentration study, a steady-state approach was used. This consists of a two compartment model (water and fish) which is used to describe the movement of the test material in and out of the test fish. This approach is used to determine the steady-state bioconcentration factor (BCF), the uptake rate constant and the depuration rate constant.

The water concentration of dichlofluanid was calculated by the formula:

Net dpm/ml = Activity of test water – Activity of control water (dpm/ml)

$$\text{Water concentration } \mu g/l = \frac{(\text{net dpm/ml}) * 1000}{\text{specific activity of parent compound (dpm/}\mu g)}$$

The tissue concentration of dichlofluanid was calculated by the formula:

Net dpm/g = Activity of test tissue – Activity of control tissue (dpm/g)

$$\text{Tissue concentration mg/kg} = \frac{(\text{net dpm/g}) * 1000}{\text{specific activity of parent compound (dpm/mg)}}$$

Bioconcentration factors for fish portions were determined by dividing the [^{14}C]-tissue radioactivity by the mean [^{14}C]-water radioactivity up to and including that day.

dpm/ml (water) = (dpm (treated) - dpm (control))/sample volume

Sample volume in all water radioactivity measurements was 7 ml.

$$\text{dpm/g (fish portion)} = \frac{(\text{dpm/g dry weight (treated) - dpm/g dry weight (control)})}{(\text{sample weight (fresh) / sample weight (dry)})}$$

$$\text{Bioconcentration factor} = \frac{\text{dpm/g (fish portion)}}{\text{dpm/ml (water)}}$$

Bioconcentration factor for whole fish were determined by the following calculation:

$$BCF(T) = \frac{(BCF(E) * \text{fresh weight}(E)) + (BCF(V) * \text{fresh weight}(V))}{(\text{fresh weight}(E) + \text{fresh weight}(V))}$$

(T) = whole fish
(E) = edible portion
(V) = non-edible portion

Statistics

The uptake rate constant and depuration rate constant were determined by the Dow BIOFAC computer program. The BCF at steady state, the time to reach 90% of steady state and the time to reach 1/2 of test compound clearance (depuration) were also calculated from the estimated rate constants. A measure of the variability of the estimated parameters was provided by the standard deviation of each estimate. The measured bioconcentration factors in the fish samples were multiplied with the mean exposure concentration of 4.4 µg/l.

4 RESULTS

4.1 Experimental data

4.1.1	Mortality/behaviour	Fish showed no abnormal behaviour throughout the test.
4.1.2	Lipid content	Lipid content was not determined
4.1.3	Concentrations of test material during test	Individual values of radioactivity and concentration of the test substance in test water are given in the report (table 2). Water concentrations ranged from 4.1 µg/l to 4.7 µg/l through 28 days of the bioconcentration (uptake) phase. The average water concentration (using the mean value for each sample) during the uptake phase was 4.4 (± 0.2) µg/l. Results of radioanalysis (individual and mean values) of [^{14}C]-dichlofluanid for all sampling times in edible tissue and non-edible tissue during 28 days of constant exposure to [^{14}C]-dichlofluanid and 14 days of depuration in clean water are summarised in table 9-14 of the report. A graph showing the uptake and depuration of the test material in the test organism (whole fish) and the time to steady-state is given in figure 3 of the report. The mean tissue residues at steady state were calculated to be 0.27 mg/kg for edible tissue, 0.38 mg/kg for viscera and 0.32 mg/kg for whole fish. The depuration half-lives are provided in table A7_4_2-1.
4.1.4	Bioconcentration factor (BCF)	The bioconcentration factors of the whole fish and the edible parts are 72 (± 14) and 61 (± 9), respectively. The BIOFAC calculated BCF values for whole fish and edible parts corresponded well with the respective average steady-state bioconcentration factors of 73 X and 62 X for [^{14}C]-dichlofluanid for days 8, 10, 14, 21 and 28. Time to reach 90% of steady state in the whole fish was 0.8 days.

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4.1.5	Uptake and depuration rate constants	See table A7_4_2-1
4.1.6	Depuration time	See table A7_4_2-1
4.1.7	Metabolites	No metabolites identified
4.1.8	Other Observations	-
4.2	**Estimation of bioconcentration**	Bioconcentration factor is based on measurements.

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5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

A dynamic 42-day study (28 days of exposure, 14 days for the depuration part) was conducted according to EPA guideline 72-6, 165-4 (1982) to evaluate the bioconcentration of [^{14}C]-dichlofluanid by bluegill sunfish (*Lepomis macrochirus*). A computer-controlled dosing system allowed approximately 6 renewals of each 100 l aquaria in a 24 hour-period. The test fish were exposed to one solvent control and one treated group (nominal concentration: 5.0 µg/l). Acetone (0.1 ml/l) was used as solvent.
Radioanalysis (^{14}C-CO_2) of edible and non-edible portions of individual fish was performed at different time points throughout the exposure and depuration period. At the same time points radioactivity in water samples was measured. Bioconcentration factors (BCF) were calculated for total [^{14}C]-residues. BCFs for whole fish were calculated from BCFs of edible and non-edible portions. The kinetic data were calculated using a computer program (BIOFAC).

The study shows no significant deviations from test guideline.

5.2 Results and discussion

Fish showed no abnormal behaviour throughout the test.

Temperature remained at 22 °C; the dissolved oxygen concentrations ranged between 7.3 and 9.6 mg/l, corresponding to 83 – 109% saturation at the respective temperature and were considered adequate for testing; the pH values of the treated chamber were consistent with the control throughout the study and ranged from 6.9 to 7.7.

Results of the study (calculated with BIOFAC) are given in table A7_4_2-1.

The bioconcentration factors of the whole fish and the edible parts are 72 (± 14) and 61 (± 9), respectively.
The BIOFAC calculated BCF values for edible parts and whole fish corresponded well with the respective average steady-state bioconcentration factors of 62 X and 73 X for [^{14}C]-dichlofluanid for days 8, 10, 14, 21 and 28. These values correspond to calculated steady-state total residue levels of 0.27 and 0.32 mg [14C]-dichlofluanid equivalents/kg for edible parts and whole fish, respectively.

Time to reach 90% of steady state in the whole fish was 0.8 days.

24 hours after cessation of exposure 84, 86 and 85% of the maximum measured plateau residues were depurated from edible portions, non-edible portions and whole fish, respectively. After seven days in uncontaminated water more than 99% of the maximum plateau radio-activity was depurated from edible portions, non-edible portions and whole fish, respectively.

The half life for clearance from whole fish was 0.24 days.

5.3 Conclusion

Validity criteria can be considered as fulfilled.

Dichlofluanid is accumulated very rapidly by bluegill sunfish with a total residue bioconcentration factor of 73 X for whole fish. When exposure ceases, the residues are depurated quickly with a half-life of less than 6 hours. Accumulation in edible parts is less (62 X) than in whole fish (73 X).

Time to reach 90% of steady state in the whole fish was 0.8 days.

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		The bioconcentration factor for dichlofluanid may be overestimated in this study because all calculations refer to radioactivity. Thus the BCFs given in this report refer to total residues from exposure to a constant concentration of dichlofluanid.
5.3.1	Reliability	1
5.3.2	Deficiencies	No, with regard to the OECD guideline No. 305 but: Study was performed with one concentration of the test substance instead of at least two; No replicates

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Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	13/12/04
Materials and Methods	Accept applicant's version noting the following minor deviation: **3.3.1** Only one test concentration was used instead of 2, this was highlighted as a deficiency (5.3.2).
Results and discussion	Accept applicant's version with the following comment: The results refer to analysis of [^{14}C]-dichlofluanid, whereas the analysis measured total radioactivity, which is unable to distinguish between dichlofluanid and it's breakdown products.
Conclusion	Accept applicant's version with the following comment: The BCF for dichlofluanid is overestimated in the study because all the calculations refer to radioactivity which includes any breakdown products and does not exclusively represent dichlofluanid (see Remarks).
Reliability	Reliability = 1
Acceptability	Acceptable
Remarks	The applicant has submitted additional comment on the study which was originally used to address concerns from the Finnish CA when they evaluated the study (it does not say what they evaluated the study for) ([redacted], 2004). The applicant defends the judgement to measure the bioconcentration of degradation products rather than just dichlofluanid. The applicant states that 'the reported BCF may not be reliable as an intrinsic value for dichlofluanid, but it can be used in the risk assessment as a worst case'. All endpoints and data presented in the summary and tables have been checked against the original summary and are correct.
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Findings	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_4_2-1: Results of the study (calculated with BIOFAC)

	edible	whole fish	viscera
Bioconcentration factor (BCF)	61 (±9)	72 (±14)	87 (±13)
Time to reach 90% of Steady-State, [days]	0.82 (±0.09)	0.80 (±0.11)	1.27 (±0.14)
t(1/2) for clearance, [days]	0.25 (±0.03)	0.24 (±0.03)	0.38 (±0.04)
Uptake rate constant, [1/day]	172 (±18)	209 (±28)	156 (±17)
Clearance rate constant, [1/day]	2.8 (±0.3)	2.9 (±0.4)	1.8 (±0.2)