AQUATIC TOXICITY OF NICLOSAMIDE (2',5-Dichloro-4'-nitrosalicylanilide) AND RESIDUE BEHAVIOUR IN RAINBOW TROUT

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ABSTRACT

The purpose of this work was to characterize the bioaccumulation potential of the antithelmintic drug niclosamide in rainbow trout as well as the possible adverse action of this chemical on other aquatic organisms. It was found that this substance is very toxic to invertebrates as well as to aquatic vertebrates. In comparison, the BCF of approximately 20 as evaluated for muscle tissue is very small. Liver, however, accumulates niclosamide approximately 300 fold.

INTRODUCTION

The use of drugs in fish culturing is neccessary to cure infectious diseases or to prevent their appearance. Such a treatment not only results in residues in edible fish but leads to an exposure of other aquatic organisms.

This problem is examplarily investigated for niclosamide which has good efficiacy against cestode infections in fish. It is proven that niclosamide diffuses rapidly from medicated food into the surrounding water. Therefore in addition to the determination of its tissue accumulation rates in rainbow trouts its action on most relevant aquatic organisms was determined.

METHODS

The effects of niclosamide on the different organisms were tested according to the respective OECD-guidelines No. 203 (Brachydanio rerio), No. 204 part I and II (Daphnia magna), No. 201 (Scenedesmus subsp.).

The bioaccumulation assay was performed with rainbow trout (salmo gairdneri) in a continuous flow through system. The flow rate was 10 l/h, the loading with fish was approx. 3 g/l. Niclosamide was delivered to the test containers in a concentration of 0.01 and 0.02 mg/l, respectively, over a time period of 11 days. At each time point of the exposure phase and the depuration phase 4 fishes were sampled. Immediately after killing muscle and liver samples were separated and frozen until they were analysed.

The determination of niclosamide was carried out as follows.

a) water samples (modified from [1]):
After concentration and clean up on solid phase extraction columns (RP-18) and elution with methanol samples were analysed by HPLC. The chromatographic conditions are listed in table 1.

b) liver and muscle samples:
Dissected liver tissue of about 0.8g and muscle tissue of about 7g was suspended in 60 ml acetonitrile and homogenized with an UltraTurrax. The pellet resulting from centrifugation was extracted once more by 20 ml acetonitrile. After adding sodium hydroxide and shaking with hexane a liquid-liquid-partition with dichloromethane under acidic conditions was carried out. The acetonitrile/dichloromethane phase was dried with sodium sulphate. The organic phase was evaporated and the dried residue was dissolved in methanol. The conditions of the following HPLC are shown in table 1.

H P L C c o n d i t i o n s		
samples:	water	liver, muscle
column:	O D S, 5 µm, I.D. 5 mm, length 25 cm	
moblie phase:	17% methanol 58% acetonitrile 25% acetic acid (1%)	85% methanol 15% acetic acid (1%)
detection:	UV, 320 nm	UV, 328 nm
determination limit:	1 µg/l	liver: 0.2 mg/kg muscle: 0.02 mg/kg

Tab. 1: HPLC conditions

R E S U L T S A N D D I S C U S S I O N

Parameters comprising the mortality of parent animals of Daphnia magna, their reproduction rate and the appearance of the first offspring and judged according to the "no observed effect concentration" ("NOEC") revealed as the most sensitive if compared with the short-term toxicity parameters evaluated with this or the other test species (Table 2).

Brachydanio rerio	LC_{50} (96 h)	0.113 mg/l
Daphnia magna	EC_{50} (24 h)	0.16 mg/l
Daphnia magna	$NOEC_{50}$ (21 d)	0.02 mg/l
Scenedesmus subsp.	IC_{10} (168 h)	0.8 mg/l
Scenedesmus subsp.	IC_{50} (168 h)	8.4 mg/l

Tab. 2: Aquatic toxicity of niclosamide

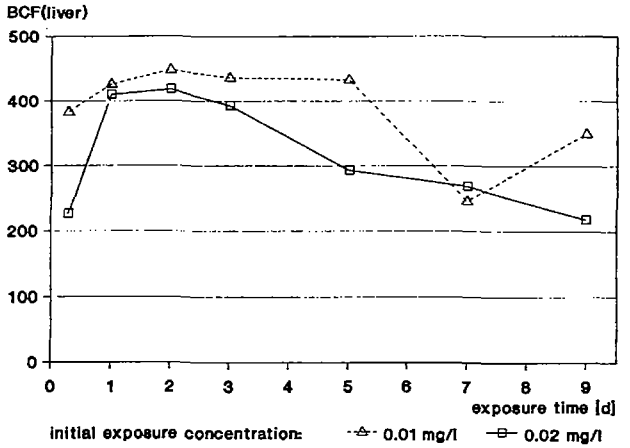

Fig. 1: Bioconcentration factors in liver

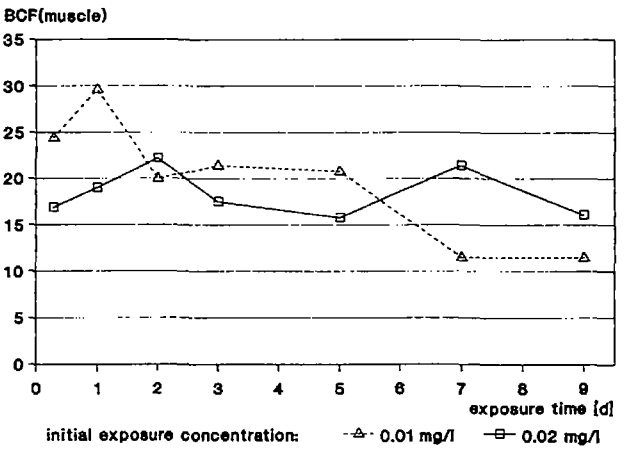

Fig. 2: Bioconcentration factors in muscle

A value of 8 for the ratio of EC_{50}/NOEC points to a delayed/cumulating action of niclosamide in Daphnia magna. A delayed appearance of letality also occurs in Brachydanio rerio if its incidences at 24 and 96 hours were compared. After 96 hours a LC_{50} value of 0.113 mg/l was found. However even at the highest test concentration of 0.175 mg/l any letal effect couldn't be observed 24 hours after starting the exposure. Since the substance is being absorbed very rapidly as shown by rainbow trouts exposed under conditions of the bioaccumulation assay it is verified, that its toxic action is delayed. The toxicity of niclosamide appears lower for plant species like green algae than for animals (Table 2).

In the initial phase of exposure the concentration of niclosamide in the exposure medium declines very rapidly what could be observed also in the respective tissue samples. According to the bioconcentration factors (BCF) evaluated for liver (Fig. 1) or muscle (Fig. 2) a steady state is rapidly reached. The BCF values for liver and muscle have the order of magnitude of 300 and 20, respectively. The apparent decline of the BCFs with time points to an induction of degrading enzymes.
The disappearance of niclosamide from liver and muscle during the depuration phase can be characterised by half-lives of 10 and 7 hours, respectively (Table 3).

	liver	muscle
BCF	~300	~20
k_2 [1/h]	0.07	0.09
$t_{½}$ [h]	10	7

Tab. 3: Kinetic parameters

REFERENCE

1. V.K. Dawson; A rapid HPLC method for simultaneously determining the concentrations of TFM and Bayer 73 in water during lampricide treatments;
J. Fish. Res. Board Can. 39 (1982), 778-782