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## INTRODUCTION

Metronidazole (MNZ) belongs to the group of 5-nitroimidazoles. In aquaculture this compound appears to be highly effective as an oral medication for the treatment of hexamitosis, spironucleosis and ichthyobodosis. MNZ has been classified as suspected mutagen and carcinogen and no MRL has been established for this substance. As a consequence it was banned for use in food-producing animals in 1998 and inserted into Annex IV of Regulation (EEC) No. 2377/90 repealed by Commission Regulation (EU) No. 37/2010 where it is listed in table 2. In fish MNZ is rapidly metabolised to hydroxymetronidazole (MNZOH) which may have comparable toxicity with the parent form. Limited data on MNZ residue depletion in fish are available and no reports on the residue distribution of MNZ in different fish tissues have been published. Thus, the aim of the study was to evaluate tissue distribution and residue depletion of MNZ in rainbow trout muscle, skin, kidney, liver and gill after multiple oral administration. Moreover, the influence of sampling and storage conditions on the level of MNZ and its metabolite in muscle and skin were investigated.

## MATERIALS AND METHODS

180 rainbow trout (*Oncorhynchus mykiss*) with an average body weight of  $150 \pm 20$  g were orally treated with MNZ in feed at the average dose of  $25 \text{ mg kg}^{-1}$  body weight  $\text{day}^{-1}$  for 7 days at  $11 \pm 2^\circ\text{C}$ . Samples of muscle with and without adhering skin in natural proportions, skin alone, kidney, liver and gill were collected from 15 fish, which had been euthanised at scheduled time points (1, 2, 3, 5, 8, 11, 14, 21, 28, 36 and 42 days posttreatment). The samples were placed immediately into ice and transferred to  $-25^\circ\text{C}$  within 15 min. In addition, the samples of muscle without skin and skin alone were first stored at RT for 2 h, then at  $4^\circ\text{C}$  for 18 h and transferred to  $-25^\circ\text{C}$ . Moreover, 15 rainbow trout were euthanised and killed before the start of drug administration and used as the control group. The concentrations of MNZ and MNZOH in fish tissues were determined by LC-MS/MS.

## RESULTS

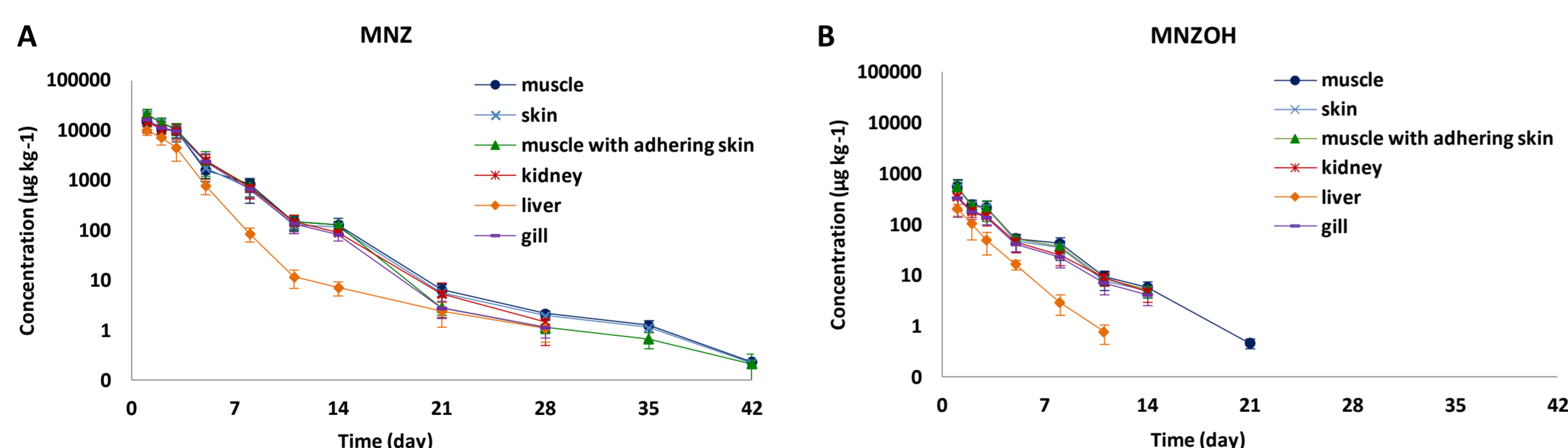


Fig. 1. Depletion of (A) MNZ and (B) MNZOH in tissues of rainbow trout orally treated with a dose of  $25 \text{ mg kg}^{-1}$  bw  $\text{day}^{-1}$  for 7 days (mean  $\pm$  standard error of the mean,  $n=15$ ).

Tab. 1. The elimination half-lives of MNZ and MNZOH in tissues of rainbow trout after oral administration of metronidazole in feed at a dose of  $25 \text{ mg kg}^{-1}$  bw  $\text{day}^{-1}$  for 7 days.

Tissue	Elimination half-life (day)	
	MNZ	MNZOH
Muscle	2.53	2.05
Skin	2.47	1.70
Muscle with adhering skin	2.37	1.98
Kidney	1.93	2.12
Liver	1.91	1.24
Gill	1.83	2.06



## RESULTS

MNZ was detected at the level close to the decision limit ( $0.20 \mu\text{g kg}^{-1}$ ) in muscle, skin and muscle with adhering skin up to 42 days, while MNZOH was present in muscle alone up to 21 days post-administration (Fig. 1). The elimination half-lives of MNZ and MNZOH in rainbow trout tissues were 1.83-2.53 and 1.24-2.12 days, respectively (Tab. 1). When muscle without skin was analysed, higher MNZ and MNZOH concentrations were detected, and for longer period of time, than in muscle with adhering skin (Tab. 2). As indicated in Tab. 3, less careful sampling and storing conditions as those simulated in this study (muscle without skin and skin alone after sampling were kept at room temperature for 2 h following storing at  $4^\circ\text{C}$  for 18 h prior to deep-freezing at  $-25^\circ\text{C}$ ) could significantly influence the concentrations of both MNZ and MNZOH in target tissues.

Tab. 2. MNZ and MNZOH concentrations in rainbow trout muscle with and without adhering skin, and their ratio after oral administration of metronidazole in feed at a dose of  $25 \text{ mg kg}^{-1}$  bw  $\text{day}^{-1}$  for 7 days.

Time (day)	MNZ			MNZOH		
	Muscle ( $\mu\text{g kg}^{-1}$ )	Muscle with skin ( $\mu\text{g kg}^{-1}$ )	Ratio <sup>a</sup> (%)	Muscle ( $\mu\text{g kg}^{-1}$ )	Muscle with skin ( $\mu\text{g kg}^{-1}$ )	Ratio <sup>a</sup> (%)
1	14998.90 $\pm$ 2869.14	21533.33 $\pm$ 4906.19	1.44	553.10 $\pm$ 216.42	539.33 $\pm$ 218.00	0.98
2	10722.22 $\pm$ 2295.03	13818.33 $\pm$ 3751.12	1.29	244.56 $\pm$ 54.86	237.17 $\pm$ 61.30	0.97
3	9917.78 $\pm$ 3894.52	19315.00 $\pm$ 3176.87	1.04	214.33 $\pm$ 76.04	204.25 $\pm$ 90.09	0.95
5	1603.67 $\pm$ 647.31	2510.00 $\pm$ 1287.87	1.57	53.00 $\pm$ 13.02	51.53 $\pm$ 12.32	0.97
8	841.85 $\pm$ 261.01	763.50 $\pm$ 296.30	0.91	41.68 $\pm$ 14.16	36.25 $\pm$ 9.35	0.87
11	150.05 $\pm$ 43.18	149.10 $\pm$ 48.35	0.99	9.52 $\pm$ 2.90	8.60 $\pm$ 2.43	0.90
14	129.18 $\pm$ 41.67	127.50 $\pm$ 21.02	0.99	5.73 $\pm$ 1.65	5.11 $\pm$ 1.44	0.89
21	6.33 $\pm$ 1.65	2.84 $\pm$ 0.81	0.45	0.46 $\pm$ 0.10	n.d.	
28	2.13 $\pm$ 0.29	1.21 $\pm$ 0.24	0.53	n.d.	n.d.	
35	1.25 $\pm$ 0.32	0.67 $\pm$ 0.24	0.54	n.d.	n.d.	
42	0.23 $\pm$ 0.07	0.21 $\pm$ 0.12	0.91	n.d.	n.d.	

<sup>a</sup> Ratio of the compound concentration in muscle with adhering skin to the compound concentration in muscle alone; n.d., not detectable.

Tab. 3. MNZ and MNZOH concentrations in rainbow trout muscle and skin after oral administration of metronidazole in feed at a dose of  $25 \text{ mg kg}^{-1}$  bw  $\text{day}^{-1}$  for 7 days kept at different storage conditions (mean,  $n=15$ ).

Time (day)	Muscle				Skin			
	Directly frozen at $-25^\circ\text{C}$		Stored at room temperature for 2 hours following storing at $4^\circ\text{C}$ for 18 hours prior deep-freezing at $-25^\circ\text{C}$		Directly frozen at $-25^\circ\text{C}$		Stored at room temperature for 2 hours following storing at $4^\circ\text{C}$ for 18 hours prior deep-freezing at $-25^\circ\text{C}$	
	MNZ	MNZOH	MNZ	MNZOH	MNZ	MNZOH	MNZ	MNZOH
1	14998.90	553.10	14755.56	534.78	20268.86	503.75*	20115.43	377.96*
2	10722.22	244.56	10482.22	237.33	14518.48	238.00	12337.23	206.57
3	9917.78	214.33	8033.33	209.25	10144.16	212.25*	9418.17	137.70*
5	1603.67	53.00	1520.33	49.67	1760.00	47.15	1710.00	42.15
8	841.85	41.68	689.87	35.83	701.08	34.24*	664.78	14.60*
11	150.05	9.52	147.86	7.99	127.40	7.90	120.21	6.41
14	129.18	5.73	123.13	5.26	117.00	5.00	112.71	4.85
21	6.33*	0.46*	3.85*	0.29*	5.65*	ND	3.37*	ND
28	2.13	ND	1.91	ND	1.92	ND	1.61	ND
35	1.25*	ND	0.34*	ND	1.11	ND	1.01	ND
42	0.23	ND	ND	ND	0.22	ND	0.22	ND

\*Statistically significant difference (paired t-test,  $p < 0.05$ ); n.d., not detectable.

## CONCLUSIONS

- MNZ is well distributed in rainbow trout muscle, skin, kidney, liver and gill and is rapidly converted into MNZOH after oral administration of MNZ in feed.
- MNZ occurs at higher concentrations and is more persistent than MNZOH in all trout tissues, thus it is the most relevant marker residue.
- Muscle alone could be more appropriate than muscle with adhering skin for the effective residue control of MNZ in rainbow trout.
- It is essential to ensure direct cooling immediately after sampling since MNZ and its metabolite degrade in fish muscle and skin stored in non-freezing conditions.