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Paraquat and menadione exposure of rainbow trout (*Oncorhynchus mykiss*)—Studies of effects on the pentose-phosphate shunt and thiamine levels in liver and kidney

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Abstract

Possible xenobiotic interactions with thiamine were studied in salmonid fish, by repeatedly injecting two model substances, paraquat and menadione, into juvenile rainbow trout (*Oncorhynchus mykiss*). These two substances were chosen because of their well-known ability to redox-cycle and cause depletion of NADPH in several biological systems. Depletion of NADPH increases metabolism through the pentose-phosphate shunt and may thereby increase the need for thiamine diphosphate by heightened transketolase activity. A special food was produced with lower thiamine content than commercial food, usually enriched with thiamine, which could mask an effect on the thiamine level. After 9 weeks of exposure, glucose-6-phosphate dehydrogenase, transketolase, glutathione reductase and ethoxyresorufin *O*-deethylase were analysed in liver and kidney cellular sub-fractions as well as analysis of total thiamine concentrations in liver, kidney and muscle. The results showed that paraquat caused a large increase in hepatic glutathione reductase activity and induced hepatic glucose-6-phosphate dehydrogenase activity, i.e., the rate-limiting enzyme in the oxidative part of the pentose-phosphate shunt. Despite this paraquat exposure did not affect transketolase activity

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and total thiamine concentration.

Keywords: Vitamin B_1; Thiamine; Paraquat; Fish reproduction; Glutathione reductase; Glucose-6-phosphate dehydrogenase; Transketolase; M74; Early mortality syndrome

1. Introduction

Salmon (*Salmo salar*) and brown trout (*Salmo trutta*) in the Baltic Sea and a number of salmonid species (*Salmo salar*, *S. Trutta*, *Oncorhynchus kisutch*, *O. tshawytscha*, *O. mykiss* and *Salvenius namaycush*) in urbanised and industrialized areas in North America suffer from severe reproductive disturbances characterized by an insufficient level of thiamine (vitamin B_1) transmitted from the females to the oocytes, causing high yolk-sac fry mortality [1–6]. This syndrome is designated M74 in the Baltic Sea and Early mortality syndrome or Cayuga syndrome in North America. The time of death of the offspring is correlated to their thiamine status, lower levels result in earlier mortality among the developing larvae [7]. A low thiamine level in the eggs is a reflection of a low level in the parent female [2]. The development of the syndrome can be inhibited by injecting thiamine into broodfish or directly into the fertilised eggs, or by the immersion of eggs or yolk-sac fry into a solution of thiamine [1,5,8–10].

Thiamine is a water-soluble vitamin that is essential for vertebrae animals. The major intracellular active fraction in most tissues is the diphosphorylated form, thiamine diphosphate, which is the cofactor for at least three enzymes—transketolase, pyruvate dehydrogenase and α-ketoglutarate dehydrogenase. Transketolase is situated in the non-oxidative part of the pentose-phosphate shunt in the cytosol, its function is to metabolise pentose sugars. Pyruvate dehydrogenase catalyses the conversion of pyruvate to acetyl-CoA, a primary substrate for oxidation in the citric acid cycle in the mitochondria. α-Ketoglutarate dehydrogenase transforms α-ketoglutarate to succinyl-CoA in the citric acid cycle.

Baltic salmon yolk-sac fry suffering from low thiamine levels show reduced hepatic transketolase and α-ketoglutarate dehydrogenase activities. The activities of both these enzymes have been found to be significantly correlated to the amount of thiamine in the fry below a threshold level of about 1.3–1.5 nmol/g (whole body wet weight), above which the enzyme activities reach a maximum [11].

The cause of the thiamine deficiency syndrome is unknown. Low concentrations in eggs of the carotenoid astaxanthin, which may act as an antioxidative protector, are related to M74 syndrome [12]. Levels of the membrane-bound antioxidants α-tocopherol and ubiquinone are lower in eggs that develop M74 than in eggs that do not [13]. Increased levels of cholesterol oxidation are found in M74 eggs [14]. Elevated hepatic activities of glutathione reductase and glutathione peroxidase are reported in yolk-sac fry developing M74 [15]. Yolk-sac fry sampled before developing M74 have lower EROD activities in livers than healthy fry [11,15], while newly-hatched yolk-sac fry that later are developing M74 have higher activities than

healthy fry, indicating a correlation between organic pollutants, thiamine status and the cytochrome P4501A biotransformation system [16,17]. Taken together, the above cited information suggest that oxidative stress due to biotransformation of xenobiotics may interfere with normal thiamine metabolism in a way that increases thiamine consumption, resulting in thiamine deficiency.

NADPH is a crucial compound, directly and indirectly involved in numerous steps in the enzymatic biotransformation of anthropogenic substances and in reductive biosynthesis. The oxidative part of the pentose-phosphate shunt is a major source of NADPH in the cell. The first reaction is catalysed by glucose-6-phosphate dehydrogenase and oxidizes glucose-6-phosphate to 6-phospho-glucono δ-lactone. When the metabolic need for the cell is NADPH-generation the non-oxidative part of the shunt, where transketolase is situated, generates sugars that can be reconverted to glucose-6-phosphate [18]. An increased metabolism through the pentose-phosphate shunt may result in increased consumption of thiamine diphosphate. The pentose-phosphate shunt might be effectively induced by redox-cycling substances that generate reactive oxygen species via single-electron reduction by NADPH cytochrome P-450 reductase, thereby consuming NADPH. A preliminary investigation found decreased liver transketolase activities in rainbow trout (*O. mykiss*) larvae exposed to substances known to be metabolised to reactive oxygen species [19].

The aim of this study was to investigate if xenobiotics, which during biotransformation consume the reducing equivalent NADPH via a redox-cycling mechanism, might induce both the oxidative and non-oxidative parts of the pentose-phosphate shunt and finally, via this metabolic pathway give rise to an increased consumption of thiamine. This hypothesis suggests that induction of the non-oxidative part of the shunt leads to elevated transketolase activity and thereby an enhanced need for thiamine diphosphate, which after long-term exposure might be reflected in decreased thiamine content in fish tissue. Rainbow trout juveniles were used as experimental animals and two model compounds were chosen, paraquat (1,1′-dimethyl-4,4′-bipyridinium) and menadione (2-methyl-1,4-naphtho-quinone).

2. Materials and methods

2.1. Chemicals and equipment

Paraquat [1,1′-dimethyl-4,4′-bipyridinium dichloride (M-2254)], menadione [2-methyl-1,4-naphtho-quinone (M-5625)], tricaprylin [1,2,3-trioctanoylglycerol (T-9126)], thiamine hydrochloride (T-4625), xylulose 5-phosphate (X-3750), triosephosphate isomerase (EC 5.3.1.1, T-2391), α-glycerophosphate dehydrogenase (EC 1.1.1.8, G-6751), NADH (N-8129), glucose-6-phosphate (G-7879), $NADP^+$ (N-0505), NADPH, (N-7505), glutathione disulfide (G-4626), ethoxyresorufin (E-3763), resorufin (R-3257) and bovine serum albumin (A-4378) were all obtained from Sigma Chemical CO., St. Louis, MO. Taka-Diastase was bought from Pfaltz and

Bauer, Chemicon, Stockholm, Sweden. All other chemicals were obtained from common commercial sources and were of analytical purity.

Enzyme assays were run on a Hitachi U-3763 spectrophotometer or a Jasco FP-777 spectrofluorometer. The automated pre-column derivation technique for conversion of thiamine to a fluorescent thiochrome compound consisted of an ASPEC liquid-handling robot with a Model 401 dilutor (Gilson, Villiers-le-Bel, France). The high-pressure liquid chromatography (HPLC) system consisted of an LC-10AD pump, an RF-551 spectro-fluorometric detector, a Chromatopac C-R5A integrator (all from Shimadzu, Tokyo, Japan) and a Model 7010 injector (Rheodyne, Cotati, CA). The column (5 μm packing material; 150×4.1 mm^2 i.d.) was a polymer-based PRP-1 (Hamilton Co., Reno, NV).

2.2. Food preparation

Commercial fish food is often enriched with unrealistic levels of thiamine compared to the natural diet. For instance, the commercial food used in part of this investigation from Aller Aqua, Södertälje, Sweden, contains 15 mg/kg (50 nmol/g) thiamine. To avoid feeding the experimental animals unnaturally high levels of dietary thiamine, a custom food was prepared for this investigation. Atlantic herring was homogenised and mixed with standard concentrations of vitamins. Carbohydrates were added to constitute 10% of the homogenate and it was incubated for 3 h at room temperature to facilitate the breakdown of thiamine by the enzyme thiaminase. The homogenate was thereafter heated and pelleted. The food was stored in a −20 °C freezer to maintain a constant thiamine level for the entire feeding period.

2.3. Fish material and exposure

Juvenile rainbow trout were obtained from a local hatchery, Näs Fiskodling AB, By Kyrkby, Sweden. The fish were placed in regular fish tanks with a diameter of 1.4 m and a water depth of 0.45 m. The basins were supplied with a continuous flow of charcoal filtrated and aerated tap water. The water temperature was 10–12 °C and the water flow 1–1.5 l/min. The light followed the annual fluctuation for the time of the year (April–August), controlled by an astronomic clock. Twelve weeks before the start of the exposure period the fish were separated into two groups. One group was fed the commercial food (2% of body weight/day, 5 days/week), while the other group received the custom food with the lower thiamine content (0.5% of body weight/day, 5 days/week). After 12 weeks, at start of the exposure period, a random sub-sample from each fish group was investigated for influence of the different diets on the thiamine levels in liver, trunk kidney and muscle as well as the enzymatic variables analysed in this study during the exposure period (see below).

The group receiving the custom food was then randomly separated into three groups (time 0 weeks for the exposure period) and placed in separate basins. One group was injected intraperitoneally with tricaprylin (control group), the second group was injected with paraquat dissolved in 0.9% NaCl (30 nmol/g body weight)

and the third with menadione dissolved in tricaprylin (5 nmol/g body weight). The injected volumes were 0.2 ml. These injections were repeated in all three groups after 2 and 4 weeks. At 8 weeks a randomly selected sub-sample of fish from each group were moved to new identical basins and injected with thiamine, buffered with sodium hydroxide to a pH of 6.5 and dissolved in 0.9% NaCl, to a final concentration of 15 nmol/g body weight. During the 9-week exposure period all the fish were fed the low thiamine custom food at the rate of 1% of body weight/day, 5 days/week.

2.4. Sampling procedures

In addition to the sampling prior to the exposure period (described above), individuals from each group were sampled 1 and 9 weeks after the first exposure injection. Total length, body and liver weight were recorded for each fish. Although the fish were juvenile, it was possible in most cases to determine the sex, by dissecting the gonad and placing it directly under a microscope. The whole liver from each individual fish was homogenised in an equal weight of 0.25 M sucrose in a Potter-Elvehjelm homogeniser by using four strokes up and down at 400 rpm. The homogenate was divided into two aliquots, one for thiamine analysis and one for enzyme analysis. The latter was diluted to a 10% homogenate in 0.25 M sucrose and centrifuged at 9000 *g* for 7 min. The obtained supernatant was used for enzymatic activity measurements. Samples were kept on ice during preparation and were thereafter directly frozen in liquid nitrogen and subsequently stored at −140 °C until analysis. The trunk kidney was prepared in the same way as the liver, but was homogenised in 9 times its weight with eight strokes up and down at 420 rpm. An epaxon muscle tissue was also dissected and placed in liquid nitrogen for later thiamine analysis.

2.5. Enzyme activity analysis

Transketolase activity was analysed with a coupled method according to Smeets et al. [20] and Tate and Nixon [21]. The incubation volume was 1 ml and the temperature was 30 °C. The incubation media consisted of 100 mM Tris–HCl buffer pH 7.6, $MgCl_2$ 1.2 mM, D-xylulose 5-phosphate 0.8 mM, NADH 0.2 mM, 8 U triosephosphate isomerase/ml and 0.8 U α-glycerophosphate dehydrogenase/ml. The transketolase activity is presented in nmol NADH oxidized/min/mg protein. Glucose-6-phosphate dehydrogenase activity was measured at 30 °C according to Taketa and Watanabe [22] with the following modifications: 50 mM Tris–HCl buffer pH 7.75, $MgCl_2$ 20 mM, $NADP^+$ 0.5 mM and glucose-6-phosphate 1 mM. The glucose-6-phosphate dehydrogenase activity is presented as nmol NADPH formed/min/mg protein. Ethoxyresorufin *O*-deethylase activity was measured according to Prough et al. [23] at 22 °C. The results are presented as pmol resorufin formed/min/mg protein. Glutathione reductase activity was measured according to Carlberg and Mannervik [24]. The measurements were performed at 30 °C and the activities are presented as nmol NADPH oxidized/min/mg protein. Enzyme assays were carried out on quickly thawed samples by vigorous shaking at approximately

0 °C. All enzyme assays were demonstrated to be linear with time and protein under the conditions used. Appropriate background and control incubations were performed. Enzyme determinations were routinely run in duplicate or triplicate with the values for these samples agreeing to within 15%. The protein content was determined according to Lowry et al. [25] with bovine serum albumin as standard.

2.6. Thiamine analysis

Total thiamine was analysed based on the method by Roser et al. [26] and was performed as described by Amcoff et al. [2]. The samples were homogenised in 0.1 M HCl and extracted by acid hydrolysis at 121 °C after which the extract was cooled to 23 °C and the pH adjusted to 4.0. Thiamine phosphate esters were enzymatically dephosphorylated by Taka-Diastase when added at a ratio of 0.1 g/g sample and incubated at 45 °C for 4 h. The extracted thiamine was converted to the fluorescent compound thiochrome by means of automated pre-column derivatization, and analysed by HPLC. Known amounts of thiamine and thiamine diphosphate were added to liver, kidney and muscle samples and analysed in order to verify a high recovery efficiency of the Taka-Diastase preparation. All samples were analysed in duplicate and presented on a wet weight basis.

2.7. Statistical analysis

Statistical differences in biodata, thiamine content and enzyme activities between the group of fish fed commercial food and the group fed the custom food were analysed using Student's t-test. Statistical differences between exposed groups and respective control group were analysed using one-way ANOVA and multiple comparison by Tukey Honestly Significant Difference test. The results in Graph and Tables are presented as means $\pm$ 95% confidence intervals and are considered statistically different at a significance level of 0.95, expressed as a P-value; $P < 0.05$.

3. Results

3.1. Feeding with custom food

A total of 98 rainbow trout juveniles were used in this investigation. The gender ratio was close to 50%, since no significant gender differences were observed among the investigated variables (not shown) the results were pooled. Control analysis showed that the concentration of total thiamine in the custom food held 32 nmol/g. Compared to the rainbow trout that were fed the commercial food, those receiving the custom food had, after 12 weeks, a significantly lower ($P < 0.05$) condition factor, as well as reduced hepatic ethoxyresorufin O-deethylase and transketolase activities (Table 1). As expected, the liver thiamine content also showed a significant decrease ($P < 0.05$), about 30%. The thiamine content in the kidney did not show any decrease, neither was transketolase activity affected. Reduced ethoxyresorufin

Table 1
Biodata, enzyme activities and thiamine concentration in rainbow trout juveniles fed commercial food or custom food during a 12-week period

Variable	Commercial food		Custom food	
Total weight (g)	57±19 (7)		39±7.2 (10)	
Total length (cm)	17±1.5 (7)		16±0.93 (10)	
Condition factor	1.1±0.076 (7)		0.88±0.034* (10)	
Liver somatic index (%)	1.5±0.39 (7)		1.2±0.15 (10)	
	Liver	Kidney	Liver	Kidney
Glucose-6-phosphate dehydrogenase[a]	172±33 (7)	264±92 (7)	139±20 (10)	350±86 (10)
Transketolase[a]	46±11 (7)	45±15 (7)	22±2.7* (10)	55±13 (10)
Glutathione reductase[a]	9.0±1.9 (7)	93±28 (7)	9.5±2.4 (10)	89±21 (10)
Ethoxyresorufin *O*-deethylase[b]	29±15 (7)	5.7±1.7 (7)	6.7±4.1* (10)	2.6±1.1* (10)
Thiamine concentration (nmol/g)	21±1.3 (4)	17±1.2 (4)	15±2.0* (4)	15±1.5 (4)

Figures are means±95% confidence intervals from (n) fish. Condition factor: (body weight (g))/(length (cm)^3) × 100. Liver somatic index: (liver weight (g))/(body weight (g)) × 100.

[a] nmol/min/mg protein.

[b] pmol/min/mg protein.

* Significantly different from corresponding value in fish fed commercial food ($P < 0.05$, Student's t-test).

O-deethylase activity was observed in the kidney although the decrease was small compared with that in the liver. Both dietary groups showed similar glucose-6-phosphate dehydrogenase and glutathione reductase activities in both liver and kidney.

3.2. Exposure period for paraquat or menadione

During the 9-week exposure period, all rainbow trout were fed the custom food including thiamine injected groups. The fish did not increase in weight or length during this period, thus, the condition factors were not affected (Table 2). However, after 9 weeks of exposure the group exposed to paraquat showed a significant decrease ($P < 0.05$) in the liver somatic index.

After 1 week of exposure to paraquat, the glucose-6-phosphate dehydrogenase activity in the liver was significantly elevated ($P < 0.05$) compared with that of the control group. After 9 weeks the results indicated that the activity was not significantly elevated ($P = 0.053$) (Table 3). No effect of paraquat exposure was seen on the glucose-6-phosphate dehydrogenase activity in the kidney. Menadione exposure did not affect the glucose-6-phosphate dehydrogenase activity or any of the other, investigated variables at the used dosages. At both sampling occasions a 10- to 14-fold increase in glutathione reductase activity was found in the liver in the group exposed to paraquat (Fig. 1), but no change in activity was found in the kidney samples (Table 3). The activity was not affected by the thiamine injections. The ethoxyresorufin *O*-deethylase activity was low and similar to the control values during the entire exposure period. However, in the paraquat group, after 9 weeks of

Table 2
Weight, length, condition factor and liver somatic indices in rainbow trout juveniles injected with tricaprylin (control), paraquat (PQ) or menadione (MD)

Variable	1 week			9 weeks			9 weeks		
	Control	PQ	MD	Control	PQ	MD	Control+T	PQ+T	MD+T
Total weight (g)	40±5.2 (6)	35±9.0 (6)	36±9.6 (6)	38±5.1 (16)	35±4.7 (16)	34±4.7 (13)	32±6.9 (6)	35±9.3 (4)	33±9.8 (5)
Total length (cm)	17±0.52 (6)	16±1.4 (6)	16±1.3 (6)	16±0.82 (16)	16±0.56 (16)	16±0.59 (13)	15±0.87 (6)	16±1.6 (4)	16±1.2 (5)
Condition factor	0.88±0.056	0.89±0.074	0.85±0.099	0.88±0.034	0.86±0.054	0.88±0.046	0.91±0.10	0.85±0.047	0.84±0.090
Liver somatic index (%)	1.3±0.23 (6)	1.3±0.25 (6)	1.6±0.24 (6)	1.0±0.083 (16)	0.83±0.093* (16)	1.1±0.10 (13)	1.3±0.40 (6)	0.98±0.072 (4)	1.1±0.15 (5)

Values shown are for fish sampled 1 week after a single i.p. injection and for fish injected 3 times and sampled 9 weeks after the first injection. +T denotes a sub-sample of the latter group injected with thiamine 1 week before sampling. Figures are means±95% confidence interval from (n) fish. Condition factor: (body weight (g))/(length (cm)^3) × 100. Liver somatic index: (liver weight (g))/(body weight (g)) × 100.

* Significantly different from control ($P < 0.05$, one-way ANOVA, Tukey).

Table 3
Enzyme activities and thiamine concentration in juvenile rainbow trout injected with tricaprylin (control), paraquat (PQ) or menadione (MD)

Variable	1 week			9 weeks			9 weeks		
	Control	PQ	MD	Control	PQ	MD	Control+T	PQ+T	MD+T
Liver									
Glucose-6-phosphate dehydrogenase[a]	120±29 (6)	220±46*(6)	151±31 (6)	155±22 (16)	206±45 (16)	157±26 (13)	146±25 (6)	179±85 (4)	160±23 (5)
Transketolase[a]	19±3.4 (6)	17±5.0 (6)	17±4.6 (6)	18±3.5 (16)	17±2.7 (16)	18±2.9 (13)	17±3.9 (6)	15±7.1 (4)	14±3.7 (5)
Ethoxyresorufin *O*-deethylase[b]	4.2±9.2 (6)	1.9±0.75 (6)	0.49±0.30 (6)	2.2±1.0 (16)	2.9±0.99 (16)	1.7±0.89 (13)	1.1±1.2 (6)	1.5±2.0 (4)	1.4±0.64 (5)
Thiamine concentration (nmol/g)	n.a.	n.a.	n.a.	9.5±1.7 (16)	11±0.92 (16)	10±1.3 (13)	16.5 (1)	n.a.	10.3 (1)
Kidney									
Glucose-6-Phosphate dehydrogenase[a]	355±170 (6)	385±97 (6)	365±132 (6)	423±62 (16)	386±70 (16)	415±49 (13)	429±190 (6)	327±74 (4)	514±91 (5)
Transketolase[a]	52±16 (6)	59±19 (6)	66±14 (6)	64±11 (16)	49±10 (16)	65±6.5 (13)	57±27 (6)	40±18 (4)	68±19 (5)
Glutathione reductase[a]	105±24 (6)	110±28 (6)	116±20 (6)	120±13 (16)	129±27 (16)	114±14 (13)	115±26 (6)	117±36 (4)	128±22 (5)
Ethoxyresorufin *O*-deethylase[b]	2.9±0.59 (6)	1.5±1.6 (6)	3.2±0.98 (6)	2.1±0.37 (16)	1.7±0.23 (16)	2.1±0.30 (13)	2.4±0.33 (6)	4.4±0.48* (4)	3.1±0.91 (5)
Thiamine concentration (nmol/g)	n.a.	n.a.	n.a.	14±1.5 (16)	14±1.0 (16)	15±0.79 (13)	n.a.	n.a.	n.a.
Muscle									
Thiamine concentration (nmol/g)	n.a.	n.a.	n.a.	4.2±0.59 (16)	5.2±0.66 (16)	4.6±0.42 (13)	n.a.	n.a.	n.a.

Values shown are for fish sampled 1 week after a single i.p injection and for fish injected 3 times and sampled 9 weeks after the first injection. +T denotes a sub-sample of the latter group injected with thiamine 1 week before sampling. Figures are means±95% confidence interval from (n) fish. n.a.: not analysed.

[a] nmol/min/mg protein.

[b] pmol/min/mg protein.

* Significantly different from control ($P < 0.05$, one-way ANOVA, Tukey).

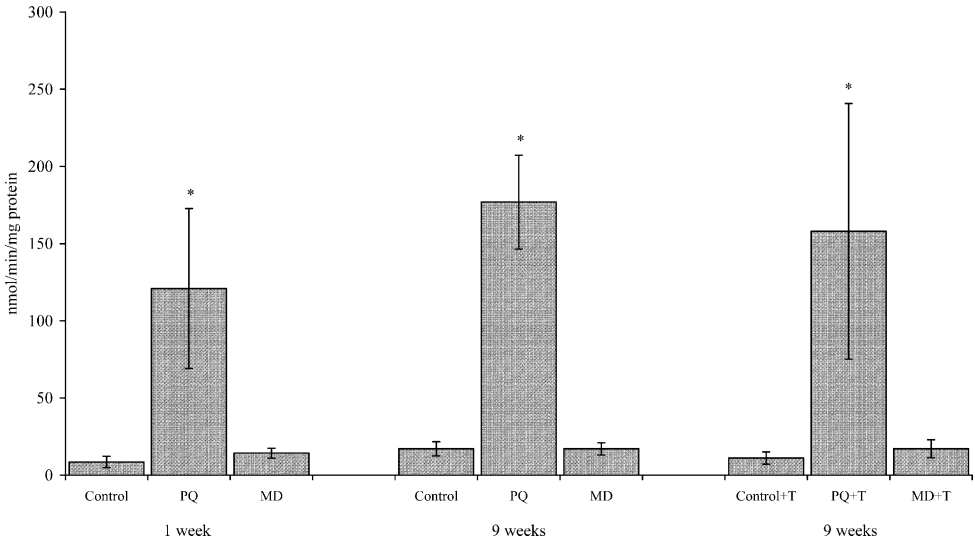

Fig. 1. Glutathione reductase activity (nmol/min/mg protein) in juvenile rainbow trout livers. Values shown are for fish sampled 1 week after a single i.p.injection of tricaprylin (Control), paraquat (PQ) or menadione (MD), and for fish injected 3 times and sampled 9 weeks after the first injection. +T means a sub-sample of the latter group injected with thiamine 1 week before sampling. Figures show mean values ± 95% confidence interval. *Significantly different from respective control group at the $P < 0.05$ level (one-way ANOVA, Tukey).

exposure and with supplemental thiamine injections during the last week, a slight increase was seen in the kidney compared with the control group.

Thiamine concentrations were measured at the start of exposure and after 9 weeks (Tables 1 and 3). A significant ($P < 0.05$) decrease in thiamine concentration in the liver was seen in the control group during this period, from 15 ± 2.0 to 9.5 ± 1.7 nmol/g. A corresponding decrease in kidney tissue could not be observed—the analysed results were 15 ± 1.5 nmol/g in the beginning of exposure and 14 ± 1.5 nmol/g after 9 weeks. Furthermore, neither paraquat nor menadione exposure affected the total thiamine levels in liver and kidney (Table 3).

4. Discussion

The custom food, with lower thiamine levels than commercial food, led to a decrease in the liver's thiamine concentration, though probably not below the critical level for saturation of the transketolase protein with thiamine diphosphate. In this study, the thiamine content in the liver of the control group was 15 nmol/g after 12 weeks on the custom food (Table 1). A diet of raw herring during 30 weeks resulted in a decrease of liver thiamine diphosphate in the rainbow trout from about 12 nmol/g to about 5 nmol/g, which affected the erythrocyte transketolase activity but not the hepatic activity [27]. Results from earlier studies show that Baltic salmon yolk-sac fry

have a threshold limit interval of 1.3–1.5 nmol/g for full activity of transketolase in the liver [11]. The lower hepatic transketolase activity in the fish fed the custom food, compared with those fed the commercial food, may instead of a too low thiamine content be attributed to a lower carbohydrate content in the custom food. The non-oxidative part of the pentose-phosphate shunt is mainly regulated by the availability of D-ribulose 5-phosphate [28]. We might conclude that the fish produced for the exposure part of this study were not overloaded with thiamine but still had a thiamine content above the level causing effects on transketolase activity.

The ethoxyresorufin *O*-deethylase activity in the group fed the custom food was 75% lower in the liver and 50% lower in the kidney, compared to the group fed the commercial food. The specific activity in the liver is in agreement with earlier comparisons made at our laboratory between commercial and home-made food based on cod (*Gadus morhua*) from the Barents Sea [29]. An explanation is that commercial food contains inducers of the cytochrome P 450 system [30].

No significant changes in weight, length, condition factor, liver somatic index or enzyme activities were found in the controls during the 9-week long experimental period. The food supply was not enough (1% of body weight/day) for adequate growth but sufficient to keep the enzyme activities at a constant level. Food deprivation in rainbow trout is reported to have a negative affect on the liver somatic index and the hepatic enzyme activities of glutathione reductase and ethoxyresorufin *O*-deethylase [31]. Glucose-6-phosphate dehydrogenase activity in juvenile rainbow trout has also been reported to be negatively affected after 5 weeks of food deprivation [32].

A potential mechanism of paraquat toxicity in biological systems has been suggested by Bus and Gibson [33]. A cyclic single-electron redox reaction causes the depletion of cellular NADPH and the generation of superoxide anion and the parent compound. Superoxide ions may form hydrogen peroxide and hydroxyl radicals, the latter being an extremely potent oxidant that may damage nucleic acids, proteins and polysaccharides. Hydroxyl radicals can also form lipid peroxides which can be enzymatically reduced to lipid alcohols by glutathione peroxidase. This reaction consumes glutathione and may increase glutathione reductase activity.

The stimulation of the pentose-phosphate shunt, as reflected by elevated glucose-6-phosphate activity following the first week of exposure to paraquat in this study, is a well-known effect of paraquat exposure in mammals. Rats had significantly elevated glucose-6-phosphate dehydrogenase activity in lung tissue after 3 weeks of paraquat exposure [34]. Stimulation of the oxidative part of the pentose-phosphate shunt in paraquat-exposed rat lung has been shown by measuring the CO_2-production of the shunt [35,36]. Glucose-6-phosphate dehydrogenase is regulated by the NADPH/$NADP^+$ ratio [28] and the increased activity is probably a consequence of reduced NADPH concentration. The cellular content of NADPH was found to be decreased in perfused rat liver after exposure to paraquat, while the sum of NADPH and $NADP^+$ remained the same [37]. The source for NADPH-generation in the pentose-phosphate shunt is glucose-6-phosphate and the decreased liver somatic indices observed after 9 weeks in this study might be due to lowered glycogen content.

Paraquat exposure caused high glutathione reductase activity, which is in accordance with the results of a previous study in which the paraquat treatment of rats caused both glutathione reductase and glutathione peroxidase induction in lung tissue [38]. A significant decrease in glutathione has been found in mice liver after paraquat exposure [34]. Other investigations have studied the effects of paraquat exposure in fish. Indications of oxidative damage to membrane lipids measured by the increase in soluble thiobarbituric acids have been observed in gilthead sea breams (*Sparus aurata*) [39]. Increased glutathione peroxidase activity in erythrocytes from carp (*Cyprinus carpio*) and crucian carp (*Carassius carassius*), but not in erythrocytes from tench (*Tinca tinca*), demonstrates species specific differences [40]. The high increase in glutathione reductase activity, found in this study, has not been reported earlier. Glutathione reductase activities of about 250% of control level have been reported in rainbow trout liver after 20 weeks exposure to PCB [41] and after injections with phenobarbital or 2,3-dimetoxynaphtoquinone [42]. Treatment with β-naphthoflavone gave only a weak increase in glutathione reductase activity after 1 week [43].

This study indicates a different metabolic route for menadione compared with paraquat. The lack of effect of menadione on the pentose-phosphate shunt could be seen as an indication that menadione is metabolised by other means than using NADPH as electron donor. In an in vitro study in which flounder (*Platichthys flesus*) and perch (*Perca fluviatilis*) microsomes were incubated in the presence of menadione, hydroxyl radicals were produced at the same rate whether they were stimulated with NADPH or NADH [44]. Menadione may also be reduced by two-electron reduction to hydroquinone by DT-diaphorase, preventing redox-cycling [45]. An alternative explanation may be that the dose was too low or the duration of response was shorter than 1 week.

The dose of paraquat chosen for this study was higher than the chosen menadione dose based on the results from a preliminary study where rainbow trout larvae were exposed to three different doses of paraquat or menadione (0.3, 3 and 30 nmol/g). The results showed that both 3 and 30 nmol/g menadione caused high mortality, while paraquat only slight mortality. Considering that early developmental stages are more sensitive to xenobiotics than later stages and that the doses should be near those causing mortality, the paraquat dose was chosen to be 6 times higher than the menadione dose (3×30 and 3×5 nmol/g, respectively).

Since we analysed thiamine in liver, trunk kidney and muscle tissue without detecting any differences it is unlikely that a decrease in thiamine on a whole animal basis occur after paraquat exposure. In conclusion, despite the induction of the oxidative part of the pentose-phosphate shunt after the paraquat treatments, no effects on transketolase activities were found. This suggests that the oxidative and the non-oxidative parts of the shunt may act separately depending on the metabolic needs. In this case, the redox-cycling of anthropogenic substances accompanied by increased consumption of NADPH prioritize the oxidative part of the pentose-phosphate shunt and leave the thiamine containing part not affected.

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