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Toxic effects of selenium on marine fish

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Abstract—— This paper deals with the toxic effects of selenium uptakes in the tissues of marine fish. The selenium concentration in skin, liver, muscle and gill of the fish were determined by proton induce X-ray emission (PIXE). The enzyme levels in liver tissues also were measured and evaluated . The results are shown that 50% lethal dose was LD_{50}=0. 29μg/g and it was found independent on the injected doses. The enzymes in serviving fish showed a gradual and complete recovery during the observation period. A very low Se uptake in muscle was found. The biological half lives of Se concentration in liver and gill were nearly identical, while the skin exhibited a half life 2. 5 times greater.

Keywords: toxic level; enzyme activity; nuclear analysis.

1 Introduction

During the past three decades there has been an increasing interest in the effect of trace elements on human health(Combs, 1986). Selenium (Se) is an essential trace element and studies on the chemical reactivity of Se containing compounds against peroxides and oxygen centered radicals led to the concept that Se could be considered an antioxidant. Moreover, Se has been shown to be an integral part of one of the antioxidant glutathione peroxidase enzymes (GPx). However, Se high levels can be acutely toxic(Tallandini, 1989). Se interactions with other elements and enzyme, homeostatic mechanisms and metabolic routes need further studies specially in order to understand Se toxicity causes. In this study results on Se acute toxicity, bioaccumulation and biochemical interactions in marine fish after Na_2SeO_3 intraperitoneal injection(0. 1—1. 0 μg/g body weight) are reported.

2 Materials and methods

Adult marine fish, average wet weight 41. 7g(range 17—73 g), 80% males, were collected from the lagoon of Venice in October. The fish were acclimated in glass tanks with recirculating marine water (T= 21. 0±0. 2℃), pH = 8. 2—8. 3, 32% salinity) for a mini-

mum of 15 days prior to experiment. The fish were fasted for 5 days and then fed polychaetes once daily. After acclimatization, fish were anaesthetized and treated with a single intraperitoneal injection of sodium selenite in physiological solution at seven doses from 0.1 to 1.0 μg/g (Se μg/body weight g). About twenty fish were sampled for each sampling dose (included controls). The control fish were injected with the physiological solution alone. The fish treated at different doses were kept in separate water tank and the surviving ones were sacrificed by spinal transection at the base of the head at the 4th , 8th, and 28th day, after the injection. Liver, gill, skin, and part of muscle, were removed and frozen until assayed.

The microsomal liver fraction was separated in order to measure the Cyt. P450 levels according to Estabrook and Werringloer(Estabrook, 1978). In the postmicrosomal supernatant Se- and non Se-dependent Glutathione Peroxidase (GPx) specific activities were assessed according to Grunzler *et al.* (Grunzler, 1987).

In order to assay Se content, the tissues were lyophilized and an amount of about 50 mg (dry weight) was acid digested (in 0.3 ml of HNO_3) at 70℃ for 5 min. Palladium (100μg) was added as internal standard. A drop (40μl) of the final solution was deposited on a kapton backing and vacuum dried at room temperature. Standard PIXE measurements were achieved through a 2 MeV proton beam from Napoli Tandem Accelerator(Moro, 1988).

The results are expressed as mean value and standard error ($M \pm SE$). Correlations and differences between mean values were statistically analyzed by the r-test and t-test at a significance level of $P < 0.05$.

3 Results

3.1 Lethal dose

Acute toxic effects of Se were measured following both the Se dose as a function of the 50% lethal time (LT_{50}) and the percent lethality at 96 h after injection as a function of the Se dose (LD_{50}). The data are shown in Fig. 1 and Fig. 2. The 50% lethal dose was $LD_{50} = 0.29 \mu g/g$, as determined by graphic interpolation.

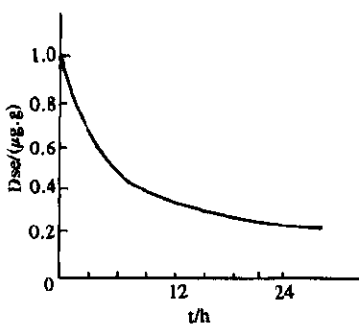

Fig. 1 Se dose as a function of the 50% lethal time

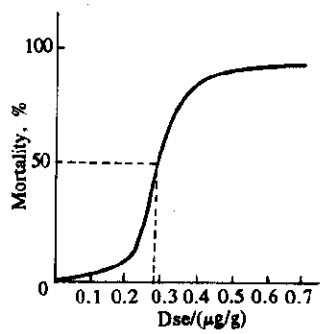

Fig. 2 Percent mortality at 96 h as a function of the Se dose

3.2 Effects of Se on enzymes

At doses of 0.1 and 0.2 μg/g, essentially all fish survived to the treatment. They have been sacrificed 4, 8 and 28 days after injection, to follow the enzyme levels in the liver. No significant differences among the values measured at different sampling times for each treatment were detected. The mean values are reported in Table 1.

Table 1 Enzyme levels, averaged on the sampling time, in fish injected 0.1 and 0.20 μg/g and in the control group

Dose, μg/g	0	0.1	0.2
Cyt. P450[a]	0.129±0.007	0.125±0.012	0.119±0.012
Se-GPx[b]	488±28	463±34	675±50
Total GPx[c]	675±50	602±40	577±20

a. Concentration in nmol/mg protein; b. specific activity units (nmol NaDPH ox./min mg protein). Substrate H_2O_2; c. specific activity units (nmol NADPH ox./min mg protein). Substrate CHP(Cumene Hydroperoxide)

The uniformity of values again suggests the presence of a threshold in the action of the injected selenite.

At 0.3, 0.4 and 0.5 μg/g doses, the number of fish dead within 48 h after injection was increasing. The measured enzyme levels in the dead fish are reported in Table 2.

Table 2 Enzyme levels in fish dead within 48 h after 0.3, 0.4 and 0.5 μg/g injection

Dose, μg/g	0.3	0.4	0.5	Mean
Cyt. P450[a]	0.044±0.005	0.055±0.011	0.049±0.008	0.049±0.005
Se-GPx[b]	387±25	293±36	351±21	339±19
Total GPx	582±75	440±93	432±29	483±46

No significant differences among the enzyme levels in the dead fish at the three doses were detected. On the contrary, all levels were significantly lower than corrresponding values in the control group. We may attribute to the measured values in the dead fish (averaged on the doses in the last column of Table 2), the meaning of minimum viable value.

The values of enzyme levels in fish survived after receiving 0.3 and 0.4 μg/g doses and sacrificed 4, 8 and 28 days after injection, are reported in Table 3.

Table 3 Enzyme levels, averaged on 0.3 and 0.4 injected doses, in fish sacrificed at different time after injection

Sampling time, day	4	8	28
Cyt. P450[a]	0.102±0.015	-	0.144±0.007
Se-GPx[b]	387±28	511±58	418±104
Total GPx[c]	680±106	618±98	513±141

Notes: For units refer to Table 1

The behavior of enzyme levels in relation with sampling time is shown in Fig. 3. The 0-day points represent the values measured in the dead fish, and the normal values are reported

(conventionally) as 50-day points.

A trend of recovering is evident in the three tested enzymes, although at different rates.

3.3 Se uptake by tissue

The tissue Se concentration were found unchanged in fish injected 0.1 and 0.2 μg/g with respect to the control values, at all sampling times. The mean values are shown in Table 4.

Table 4 Tissue Se concentrations (μg/g dry weight), averaged on the sampling time, in fish injected 0.1 and 0.2 μg/g and in the control group

Dose, μg/g	0	0.1	0.2
Liver Se	2.4±0.4	-	2.6±0.4
Gill Se	3.9±0.3	4.7±0.6	4.8±0.3
Skin Se	2.0±0.2	2.1±0.2	2.2±0.2
Muscle Se	2.0±0.2	1.9±0.1	1.8±0.1

These results give further evidence to the presence of a threshold. Also the results show that the fish under 0.29μg/g dose are able to endure the toxic effects of Se. The Se concentrations, measured in fish dead within 48h and in the sacrificed ones at 4, 8 and 28 days after 0.3 and 0.4 μg/g injection, are reported in Table 5.

Table 5 Tissue Se concentrations (μg/g dry weight), both in fish dead within 48 h and in the sacrificed ones at different times after injection of 0.3 or 0.4 μg/g

	Sampling time, day			
	Dead, 48h	4	8	28
Liver Se	6.2±0.5	3.4±0.9	2.6±0.7	2.6±0.6
Gill Se	14.1±1.0	6.9±0.5	4.1±0.3	4.8±0.8
Skin Se	4.8±0.4	4.0±0.4	2.7±0.5	2.2±0.3
Muscle Se	2.3±0.1	2.3±0.3	2.2±1.5	2.2±0.2

The behavior of tissue Se concentrations in relation with sampling time is shown in Fig.

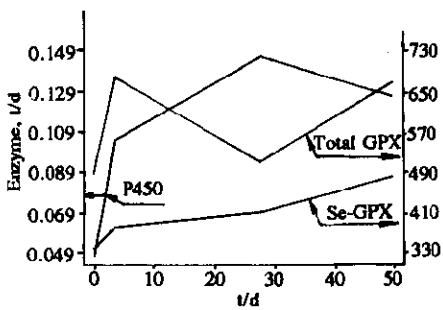

Fig. 3 Behavior of the enzyme levels as a function of the sampling time

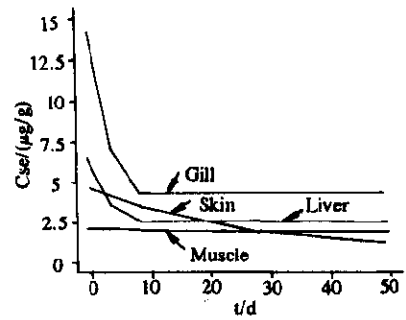

Fig. 4 Tissue Se concentrations as a function of the sampling time

The 0-day points represent the values measured in the dead fish, and the normal values are reported (conventionally) as 50-day points.

The Se concentration in muscle can be considered unaffected by the Se injection, in the limit of our experiment.

In liver, gill and skin, the Se concentration is well represented by an exponential decay towards the normal value (NV).

By fitting the concentration (Y) through the model :

$$Y = NV + A.\exp(-Bt). \quad (1)$$

We have obtained the values of the parameters reported in Table 6. In the last column, the biological elimination half lives, $T_{1/2} = 0.693/B$, are calculated.

Table 6 Parameters calculated by fitting the tissue Se concentrations with the exponential model

Tissue	NV, μg/g	A, μg/g	B, d^{-1}	$T_{1/2}$, d
Liver	2.4	3.70±0.12	0.33±0.03	2.1±0.2
Gill	3.9	10.2±0.6	0.33±0.05	2.1±0.3
Skin	2.0	2.88±0.25	0.13±0.03	5.3±1.2

Notes: $T_{1/2}$ is the biological elimination half life; NV is the normal value; A is a factor; B is the reciprocal of days

4 Discussion

The very sharp increase of the mortality curve in a narrow concentration range suggests the presence of a threshold in the action of Se injected.

This evidence is also supported by the behavior of Cyt. P450 concentration in liver microsomes and of the specific activities of liver Se and not Se-dependent Glutathione Peroxidases. Indeed, these enzymes remained at normal values in fish injected 0.1 and 0.2 μg/g, while significantly fallen out in fish injected over the 50% lethal dose.

The enzyme levels in fish dead following the injection, were found independent on the injected dose. The minimum viable value for Cyt. P450 level and GPx activities was accordingly determined.

In the surviving fish the three tested enzymes showed a gradual and complete recovery during the observation period.

The muscle Se content was found totally unaffected by the Se injection, even at lethal doses. This implies a very low Se uptake in this tissue.

The Se absorption in liver, gill and skin occurs in a very short time, as demonstrated by the maximum burdens found in fish dead within 48 hours after injection. The goodness of the fitting for the exponential elimination seems to indicate that only one metabolic pool is involved for these organs.

The biological half lives of Se concentration in liver and gill were nearly identical, while the skin exhibited an elimination half life 2.5 times greater.

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