

## BACKGROUND LEVELS OF IODINATED THYRONINES AND TYROSINES IN WHOLE BODY HOMOGENATES OF ADULT MALE AND FEMALE ZEBRAFISH (*DANIO RERIO*) VIA LC/ICP-MS

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### *Introduction*

In the last three decades the interference of organic and inorganic pollutants with the endocrine system of men and wildlife has stepwise come into our consciousness. This is documented by the number of research activities and programs which has multiplied significantly during that period<sup>1, 2</sup>. Especially in the 1990s, increasing concern about endocrine disrupting chemicals (EDCs) and their sublethal effects has developed as the growing body of scientific evidence confirmed continuously that a range of man-made chemicals are producing adverse health effects in humans and wildlife by interfering with the functioning of natural hormone systems. It is to be expected that developing organisms like foetus, newborn, child and juvenile are most susceptible to those adverse effects arising from exposure to EDCs<sup>3</sup>. There is also a concern about increased incidences of certain types of cancer (breast, testis, prostate), which are suspected to be related to preceding endocrine disruption. The issues surrounding EDCs are extremely complex, which is partly due to the fact that the endocrine system is sensitive to molecular pollutants with an extremely high structural diversity resulting in a growing list of suspected endocrine disruptors.

Disruption of normal thyroid function by xenobiotic chemicals is an important ecological issue. It has been shown for example that thyroid hormone levels were affected in to chlorinated dioxines and furanes exposed fish<sup>4</sup>. In the zebrafish (*Danio rerio*) exposure to goitrogens resulted in inhibition of transition from larva to juvenile including the formation of scales and pigment patterning. It also hindered the growth of paired fins<sup>5</sup>. Theoretically, normal thyroid hormone (TH) homeostasis and action can be disrupted at several sites in the synthetic and elimination pathways (c.f.<sup>6</sup>). Indeed, xenobiotic chemicals, which are known to interfere with normal thyroid hormone homeostasis and action, have been shown to act primarily by (A) up-regulation of catabolism of TH, (B) inhibition of iodide uptake, (C) inhibition of TH synthesis, or (D) inhibition and/or up-regulation of deiodinases. However, the most commonly used measurements of thyroid status are limited to circulating concentrations of thyroid stimulating hormone (TSH) and the two thyroid hormones (3,5,3',5'-tetraiodo-thyronine; T<sub>4</sub>, and 3,5,3'-triiodo-thyronine; T<sub>3</sub>). These measurements alone are insufficient to determine the mechanism by which thyroid disruption occurs.

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Recently a new approach for the analysis of iodinated organic species in human serum has been developed using liquid chromatography/inductively coupled plasma-mass spectrometry (LC/ICP-MS). This method is able to quantify iodide,  $T_4$  and  $T_3$ , as well as inactivated TH, reverse  $T_3$  ( $rT_3$ ), and the synthetic precursors of TH, monoiodo-tyrosine (MIT) and diiodo-tyrosine (DIT) in a single injection. In this work, the LC/ICP-MS approach was used to analyze whole body homogenates of adult male and female zebrafish (*Danio rerio*) to establish the respective background levels.

## Material and Methods

**Zebrafish (*Danio rerio*).** Adult wild type zebrafish (AB, 7-8 months old) were taken from the local zebrafish facility.

**Whole body homogenates.** The animals were killed by putting them on ice. The fish were homogenized individually in four times of the body weight buffer (250 mM sucrose, 10 mM Tris, pH 7.4). The homogenates were centrifuged at 4°C at 24,000 g for 15 min, the supernatant collected and the core discarded.

**Iodine Species Analysis.** The Method has been developed for analysis of human serum and has been published previously<sup>7,8</sup>. It mainly consists of a digestion step with protease to destroy the thyroid hormone binding proteins followed by the analysis with HPLC online coupled to an ICP/MS-system. The analytes were quantified by comparing the integrated peak-areas with corresponding values from standard-solutions with known concentrations. The total iodine content was determined by ICP/MS and compared to the sum of the individual species.

**Reliability and Reproducibility.** The intrinsic variation of the HPLC-method was tested by ten repeated injections of one selected standard solution and one sample, respectively (Fig. 1b).

## Results

**Reliability and Reproducibility.** Figure 1a shows a typical chromatogram. The peaks are baseline separated and can be assigned to individual iodine species unequivocally. Here we see three unidentified peaks ( $U_{1-3}$ ). Fig. 1b shows the reproducibility of the quantitative iodine speciation analysis. The overall standard deviation between runs of the same sample was less than 3% for the organic

Figure 1a: Iodine Speciation Analysis via HPLC

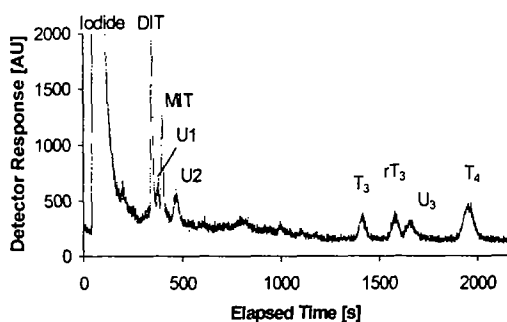
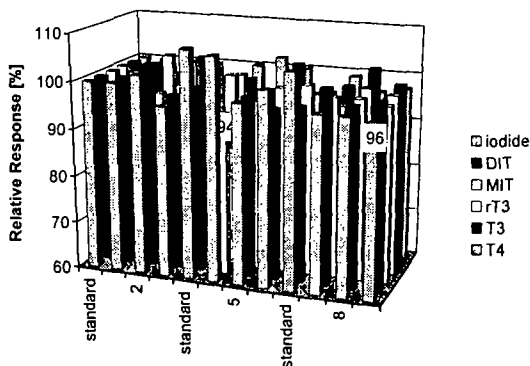


Figure 1b: Reproducibility of Analysis



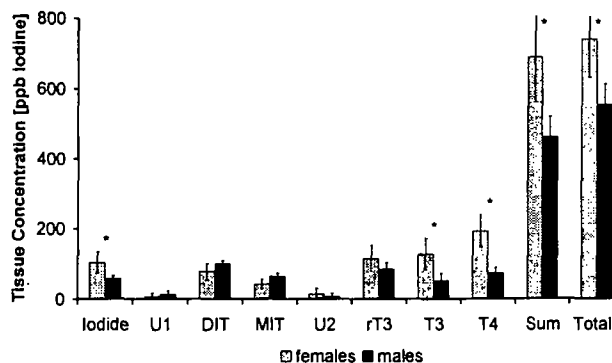
iodine species and less than 6 % for iodide. Table 1 shows the data for all species and individuals along with the calculated standard deviation. The variability between different individuals expressed as standard deviation of each n=5 samples lay between 18 (DIT in males) and 224% (U2 in both sexes).

**Table 1:** Iodine species concentrations [ppm iodine] in whole body homogenates of *Danio rerio*

females	Iodide	U1	DIT	MIT	U2	rT3	T3	T4	Sum	Total I
AB050700W01	53	0	101	50	0	157	122	230	712	820
AB050700W02	171	0	80	43	0	53	69	137	553	662
AB050700W03	107	0	97	54	75	218	271	302	1123	1077
AB050700W04	37	40	0	0	0	104	133	229	542	614
AB050700W05	157	0	117	72	0	49	46	70	511	521
mean	105	8	79	44	15	116	128	193	688	739
standard deviation	60	18	46	27	34	72	87	91	255	218
deviation +	30	9	23	13	17	36	44	45	128	109
deviation -	30	9	23	13	17	36	44	45	128	109
percentage	57%	224%	58%	61%	224%	62%	68%	47%	37%	30%
males	Iodide	U1	DIT	MIT	U2	rT3	T3	T4	Sum	Total I
AB160600M06	43	0	91	86	0	145	92	101	558	636
AB160600M07	84	42	134	87	40	78	57	65	588	673
AB160600M08	58	0	95	52	0	77	90	99	471	598
AB160600M09	57	0	96	47	0	78	13	77	368	437
AB160600M10	54	27	92	54	0	52	13	23	322	425
mean	59	14	101	65	8	86	53	74	461	554
standard deviation	15	20	18	19	18	35	39	29	116	115
deviation +	8	10	9	10	9	17	20	15	58	58
deviation -	8	10	9	10	9	17	20	15	58	58
percentage	26%	142%	18%	30%	224%	40%	74%	39%	25%	21%

In general the sampling size n=5 for each female and male zebrafish, respectively, does not allow to detect differences for all species. Nevertheless the data show statistically significant higher T<sub>4</sub>, T<sub>3</sub>, rT<sub>3</sub>, and total iodine levels for female *D. rerio* than for male ones (Fig. 2). This sex-specific difference in TH-levels points to a possible link between the thyroid-axis on the one hand and the estrogen- and/or androgen-axes on the other hand.

**Figure 2:** Thyroid Hormone Levels of *D. Rerio*



The significance of the higher concentrations of T<sub>3</sub>, U<sub>5</sub>, and U<sub>4</sub> is not clear yet and has to be investigated in future research projects. Nevertheless, the data demonstrate that the LC/ICP-MS method was successful at measuring I<sup>-</sup>, MIT, DIT, T<sub>4</sub>, T<sub>3</sub> and rT<sub>3</sub> in these two species. Furthermore, the method also detected 5 additional iodinated compounds, which are currently unidentified.

## Outlook

Future work will focus on identifying the unidentified iodinated compounds observed in this preliminary study and determining the effects of known thyroid antagonists on the concentrations of the iodinated compounds in *D. rerio*. Also part of our future research plans is the characterization of the link between the thyroid endocrine axis and the endocrine regulation of reproduction.

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