# DEVELOPMENT OF A BIOASSAY FOR THYROID DISRUPTION IN PATIENTS EXPOSED TO ENDOCRINE DISRUPTING CHEMICALS

Isabel Cerrillo[1], Patrocinio Molinero[2], Patricia Araque[1], Alicia Granada[1], Begoña Olmos[1], Esther Cáceres[3], Jorge Rosell[3], Fernando Escobar[4], Nicolas Olea[1]

[1]Lab. Medical Investigations, Hospital Clínico, University of Granada
[2]Biochemistry Dpt. University of Seville, Spain.
[3]Surgery Dpt., Hospital Clínico, University of Granada. Granada. Spain.
[4]Endocrinology Service. Hospital Clínico. University of Granada

## Introduction

The increase in hypothyroidism among the general population[1,2] and among inhabitants of areas characterised by the accumulation of industrial residues[3] may be due to environmental factors, including the presence of chemical substances that can alter thyroid hormonal homeostasis. Despite international interest in investigating this issue and in establishing a reliable battery of tests for thyroid disruption, few studies have been developed to date. In this context, studies on human exposure to environmental compounds have focused on compounds that produce goitre, and most assays are designed for chemicals that alter the ligand binding and activation of the thyroid hormone receptor. We present a new bioassay that investigates the inhibitory effects of chemicals on the activity of 5'-deiodinase type II enzyme (5'DII). Thyroxin 5'-deiodinase is responsible for the conversion of $T_4$ to $T_3$. Its type II iso-enzyme (5'DII), which is under the control of regulatory factors such as the level of circulating thyroid hormones, plays an important role in maintaining intracellular levels of $T_3$ and could serve as a defence against thyroid hormone deficiency. We also quantified endocrine disrupters from the cervical fat tissue of patients with different thyroid diseases.

## Material and methods

*Determination of chemicals in adipose tissue*

Sixty fat samples were analyzed for organochlorine compounds (OCs) using gas chromatography with an electron capture detector (GC-ECD). The OCs were extracted from adipose tissue by a previously described method [4]. An aliquot of 200 mg adipose tissue was dissolved in hexane and eluted in a glass column filled with Alumina Merck 90 (70-230 mesh, nº 1097) that had been dried at 600 ºC for 4 h followed by the addition of 5 % water. The eluate obtained was concentrated at reduced pressure and then under a stream of nitrogen to a volume of 1 ml. The organic extract obtained was purified with the use of silica Sep-Pak after the prior treatment of the cartridge with 2 ml hexane. The extract was eluted with 10ml hexane and then with 10ml hexane:methanol:isopropanol (45:40:15; v/v/v). Both eluates were collected and dried in a stream of nitrogen. The dry residue was dissolved in 1ml hexane and analyzed with GC/ECD by an internal standard method using p-p´dichlorobenzophenone.

*Bioassay for thyroid disruption*

The bioassay for thyroid disruption was developed in the lab for these chemicals. First, labelling with $^{125}I$ of $T_3$ hormone was carried out using the chloramine-T method described by Nakamura [5]. This $T_4^*$ was incubated for 60 minutes at 37 ºC with phosphate buffer, dithiothreitol and a homogenate of brown adipose tissue from rats previously treated with methimazole, which had induced hypothyroidism in the animals:


The reaction was stopped by adding bovine serum albumin and trichloroacetic acid. It was centrifuged for 10 min at 3000 rpm, and 0.5 mL of supernatant was passed through an ionic exchange column, separating the radioactively labelled reaction products ($^{125}$ I and remains of $T_4$*). The radioactivity, measured with a gamma radioactivity counter, corresponds to the $^{125}$ I and is directly proportional to the activity of 5'deiodinase type II enzyme. The results are expressed as % activity 5^d-II enzyme. We tested in this bioassay all the compounds found in the adipose tissue of the patients and some other endocrine disrupters such as bisphenol A, bisphenol F, buthyl-hydroxy anisole and 4-phenoxyphenol, to which humans are also widely exposed.

**Results and Discussion**

Results of organochlorine pesticides content in the fat samples are listed in Table 1. All the samples studied were positive for one or more chemicals. The predominant chlorinated pesticides were DDE and HCB, present in 96 % and 92 % of the tested samples, respectively. The frequency of appearance was, in decreasing order: p,p'DDE, HCB, endosulphan-ether, chlordane, aldrin, endosulphan-diol, lindane, endosulphan-lactone, o,p'DDT, p,p'DDT, endosulphan-II (E-II), endosulphan-I (E-I), endrin, dieldrin and endosulphan sulphate.

**Table 1.** Organochlorine molecules in patient samples (ng/g lipid)

| | Mean | SD | %Frequency |
|---|---|---|---|
| **o,p´DDT** | 23.3 | 9.16 | 16.3 |
| **p,p´DDT** | 99.2 | 51.3 | 16.2 |
| **p,p'DDE** | 332.2 | 325.4 | 93.1 |
| **Lindane** | 13.5 | 14.2 | 27.9 |
| **HCB** | 139.1 | 107.1 | 72.1 |
| **Aldrin** | 57.3 | 47.6 | 46.5 |
| **Endrin** | 92.4 | 65.5 | 4.65 |
| **Dieldrin** | 65.9 | - | 2.32 |
| **E-I** | 3.43 | 1.49 | 6.97 |
| **E-II** | 31.1 | 20.9 | 9.30 |
| **E-ether** | 1.14 | 1.02 | 48.8 |
| **E-lactone** | 7.61 | 6.55 | 23.2 |
| **E-diol** | 10.7 | 7.84 | 36.7 |
| **E-sulphate** | 26.1 | - | 2.32 |

The bioassay for thyroid disruption measures the release of radioiodine from labelled (5'-$^{125}$I) $T_4$ either in the absence of competitors or in the presence of testing chemicals. The method is specific for 5'DII activity because the substrate contains $^{125}$I only in position 5'[3]. The $^{125}$I measured is directly proportional to the activity of the enzyme and is fully suppressed by iopanoic acid (IA: positive control of inhibition). We investigated the inhibitory effects of the chemicals on the activity of 5'DII enzyme. Results are shown in Table 2 and expressed as % of inhibition of 5'D-II activity, which is directly proportional to the $^{125}$I measured. Most of the tested chemicals were effective in inhibiting the release

of radioiodine: the inhibitory activity on the 5'D-II of most chemicals was significant at concentrations as low as 0.1 mM. The assay is easy to perform and may be useful for risk assessment.

**Table 2.** Inhibitory effects of chemicals on activity of 5'DII enzyme. Results are expressed as 5'D-II activity in fmol/mg protein/h.

| **Compound (Concentration M)** | Inhibition of 5'DII activity |
|---|---|
| **Absence of competitor** | 100 |
| **IA 4mM** | 9.94 |
| **BHA 1mM** | 55.1 |
| **BHA 0.1 mM** | 75.6 |
| **BPF 1mM** | 37.3 |
| **4-Phenoxyphenol 1mM** | 17.2 |
| **BPA 0.1 mM** | 54.3 |
| **BPA Cl 0.1 mM** | 18.9 |
| **BPA $Cl_2$ 0.1 mM** | 31.1 |
| **BPA $Cl_3$ 0.1mM** | 2.27 |
| **BPA$Cl_4$ 0.1 mM** | 55.3 |

**IA:** Iopanoic acid

Human exposure to chemicals that disrupt thyroid hormone system has to be evaluated as thoroughly as have been sex-steroid hormones. Effects of organohalogenated compounds on the activity of 5'D-II is evidence of possible thyroid disruption.

**References**

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