

# Thyroid Hormones as Potential Early Biomarkers of Exposure to Nonylphenol in Adult Male Lizard (*Podarcis sicula*)

Rosaria Sciarriello<sup>\*1</sup>, Anna Capaldo<sup>2</sup>, Salvatore Valiante<sup>2</sup>, Flaminia Gay<sup>2</sup>, Anna Sellitti<sup>2</sup>,  
Vincenza Laforgia<sup>2</sup> and Maria De Falco<sup>2</sup>

<sup>1</sup>Department of Biological and Environmental Sciences, University of Sannio, Benevento, Italy

<sup>2</sup>Department of Evolutive and Comparative Biology, University of Naples "Federico II," Naples, Italy

**Abstract:** The thyroid has been shown to be a target organ of environmental chemicals, specifically endocrine disrupting contaminants. Reptiles are particularly suitable as contaminant biomonitors, due to their persistence in a variety of habitats, wide geographic distribution, longevity, and, in many cases, site fidelity. Nonylphenol, an estrogenic-like compound, can induce vitellogenin synthesis in males and immature reptilian species, but little is known about its effects on thyroid hormones balance. The present study evaluated the potential effects of an acute exposure to nonylphenol (i.p. injected) on the thyroid of the lizard *Podarcis sicula*.

Nonylphenol induced a significant decrease of T4 and T3 plasma levels, in agreement with the decrease of the epithelial cell height; the nuclei of the thyroid cells were small and elongated, with dense chromatin and a greatly reduced cytoplasm. The colloid was retracted with few reabsorption vacuoles. Moreover, nonylphenol administration significantly inhibited plasma thyroid-stimulating hormone levels, thereby altering the thyroid function.

This study highlights how the structural and functional disruption of the thyroid gland in non-target organisms as the lizard might also have an environmental aetiology. In conclusion, nonylphenol was suspected to inhibit the thyroid hormones balance, suggesting the thyroid should be included among the other endocrine glands, susceptible to endocrine disruption.

**Keywords:** Histology, nonylphenol, *Podarcis sicula*, thyroid hormones.

## INTRODUCTION

Endocrine-disrupting chemicals are a broad group of substances that alter the functions of the endocrine systems in wildlife and humans (European Commission 1997). The endocrine disruptors are widespread in the environment and food chains and include some common environmental contaminants such as pesticides, plastic ingredients, dioxins, and biocides [1-3]. The possible impact of these endocrine-disrupting chemicals needs to be considered, because many of the compounds accumulate due to their persistence in the environment. Moreover, the endocrine-related adverse effects can occur at lower dose levels than those causing tumorigenicity or teratogenicity [4] with long-term consequences on health [5,6].

Over the past decade there has been an increasing focus on the effects of synthetic chemicals on human endocrine systems, especially on effects related to androgen and estrogen homeostasis. However, there is increasing evidence from animal and *in vitro* studies, that also the thyroid is vulnerable to endocrine-disrupting effects. Environmental chemicals may interfere with thyroid homeostasis through many mechanisms of action, i.e. at the receptor level, in binding to transport proteins, in cellular uptake mechanisms

or in modifying the metabolism of thyroid hormones (THs). Several environmental chemicals have a high degree of structural resemblance to the THs thyroxine (T<sub>4</sub>) and triiodothyronine (T<sub>3</sub>), and therefore interfere with binding of THs to receptors or transport proteins [7].

Nonylphenol (NP) and octylphenol are industrial additives used in a wide variety of detergents, plastics and pesticides [8-10]. NP may be one of the more critical compounds due to its toxicity, persistence and estrogenic effects [11-16]. Interests toward this endocrine disruptor on thyroid hormones (THs) balance [17-23] are just beginning to be taken into consideration as shown by the *in vitro* effects of NP inhibiting thyroid peroxidases, catalysing iodination of triiodothyronine (T<sub>3</sub>) and thyroxine (T<sub>4</sub>) in ovariectomized rat [19].

Exposure of rats to NP dose-dependently increased thyroid stimulating hormone (TSH) [24], but no consistent effects on peripheral hormones were found [24,25]. In addition, another study performed in the rat, showed that NP increased T<sub>3</sub> and T<sub>4</sub> levels, without modifying TSH in ovariectomized rats. This pattern was not consistent with *in vitro* studies of protein extracts in which NP supplied inhibitory effects on thyroperoxidase (TPO) activity [19]. Interestingly, NP might also exert a notable impact during the development of fish and tadpoles as displayed by clearly decreased TH [21] along with the reduced rate of metamorphic progression and tail reabsorption in bullfrog tadpoles [26]. Conversely, fish treatment with dietary 4-NP

\*Address correspondence to this author at the Department of Biological and Environmental Sciences, University of Sannio, Via Port'Arsa, 11-82100 Benevento, Italy; Tel: 39- 0824-305156; Fax: 39- 0824-305156; E-mail: sciarrillo@unisannio.it

during smoltification did not significantly influence plasma T<sub>3</sub> and T<sub>4</sub> concentrations in fresh water coho salmon [27].

Only recent study [23] evaluated the potential effects of a single acute exposure to NP on the thyroid and reproductive axis of adult male shubunkins (*Carassius auratus*). Reptiles are particularly suitable as contaminant biomonitors due to their persistence in a variety of habitats, wide geographic distribution, longevity, and, in many cases, site fidelity [28].

The aims of the present study were (1) to develop a biological model for monitoring the ecotoxic effects of NP in the environment of the Campania region, based on a sentinel species, *Podarcis sicula*, because it is the most abundant species living in the open country and in cultivated fields and (2) to evaluate the possible adverse effects of NP on an endocrine organ such as the thyroid gland. Thus the sensitivity of the sentinel species to NP and the effects of this industrial additive on the thyroid gland morphology and thyroid hormones plasma levels were handled in the present study.

## MATERIAL AND METHODS

### Animals and Housing Conditions

Adult male lizards of *P. sicula* (weighing 13–15 g) were live-captured in the neighbourhood of Naples in June (n=20), when the thyroid gland was in full functional activity [29]. After capture, the animals were housed in large soil-filled terraria containing heather, and exposed to natural temperature and photoperiod. Water dishes were present in the terraria, and the animals were fed on live fly larvae daily. Captivity lasted 20 days to reverse capture-related stress [30]. All animals have been captured with the authorization of 06/01/2000 no. SCN/2D/2000/9213 of Italian Ministry of Environment.

### Experimental Procedure

The animals received i.p. injections of Nonylphenol (Nonylphenol, CAS 84852-15-3, Sigma Aldrich, St. Louis, MO). Nonylphenol was dissolved in 50 µl of peanut oil, with an injection volume of 0.1 ml. Injections were between 8.00 a.m. and 8.30 a.m.. The specimens were divided into three groups, each consisting of 20 animals, in order to obtain an adequate plasma volume.

- Group 1. The animals received three weekly i.p. injections of NP (1.72 µg/100g body wt) for 7 days and were sacrificed 24 hr after the last injection.
- Group 2. The animals received five weekly i.p. injections of NP (1.72 µg/100g body wt) for 14 days and were sacrificed 24 hr after the last injection.
- Group 3. The animals received five weekly i.p. injections of NP (1.72 µg/100g body wt) for 28 days and were sacrificed 24 hr after the last injection.

A control group for each NP-treated group was kept under the same conditions as the treated ones, but it was intraperitoneally injected with peanut oil. Animals were sacrificed 24 hr after the last injection. The animals were anaesthetized by hypothermia, chilling them in chipped ice. Blood samples were collected by intracardiac puncture and put into heparinized tubes. Blood collection lasted less than 3 min; plasma obtained by centrifuging (2,500 g for 10 min at 4°C) the blood samples, was stored at -20°C until assay.

### Light Microscopy

Immediately after collection of blood samples, the animals were decapitated, and the thyroid glands were removed and fixed in Bouin's fixative and processed for light microscopy (LM). Serially cut paraffin sections (7 µm) were stained by Galgano I stain [31]. Observations were performed using a Zeiss Axioskop microscope; images were captured with a camera attached to an IBM computer running the Kontron Elektronik KS 300 image analysis system and then they were processed by Adobe Photoshop. The height of the follicular cells was measured in 30 cells every 3 slides, always on the second section of normal and treated specimens using a digital system of image (KS 300).

### Hormone Assay

Plasma levels of T<sub>3</sub> and T<sub>4</sub> were determined by radioimmunoassay (RIA) [32,33]. In the T<sub>3</sub> assay, a measured amount of sample serum and standards was added to a tube coated with anti-T<sub>3</sub> rabbit antibody, with a trace (4.4 Ci) amount of radioactively labeled T<sub>3</sub> (<sup>125</sup>I T<sub>3</sub>) (Byk-Sangtec Diagnostica, Dietzenbach, Germany) and an agent-blocking Tris buffered saline 4 mM, ANS (8-anilino-1-naphthalenesulfonic acid) 6 mM sodium salicylate with 0.2% sodium azide as a preservative (Sigma Chemical Co., St. Louis, MO) to release T<sub>3</sub> from serum-binding proteins. Sensitivity was 0.1 ng/mL with an accuracy of about 97%. The range of intra-assay variance in 20 assays was 1.0–2.6%, whereas the interassay variance ranged between 3.9% and 5.7% in 12 assays.

For T<sub>4</sub>, a measured amount of sample serum and standards was added to a tube coated with anti-T<sub>4</sub> rabbit antibody, along with a trace amount of radioactively labeled T<sub>4</sub> (<sup>125</sup>I T<sub>4</sub>), 4.4 Ci, (Byk-Sangtec Diagnostica, Dietzenbach, Germany) and a blocking agent, Tris-buffered saline 4 mM, ANS 6 mM sodium salicylate with 0.2 % sodium azide as a preservative (Sigma Chemical Co. St. Louis, MO) to release T<sub>4</sub> from serum-binding proteins. Sensitivity was 0.45 ng/mL, with an accuracy close to 100%; the mean intra-assay and inter-assay coefficients of variance were 4.6% and 4.3%, respectively. A logit-log curve fit using a % B/Bo calculation was used. T<sub>4</sub> and T<sub>3</sub> concentrations were determined by computing the % B/Bo for each sample and then finding the results on the standard curve. Crossreactivity for T<sub>4</sub> in the T<sub>3</sub> RIA (1.3%) was not considered for data calculations, neither was that for T<sub>3</sub> in the T<sub>4</sub> RIA (0.1%).

Plasma TSH was determined by immunoradiometric assay (IRMA) [32,33]. Sample serum and standards were added to antigen-coated tubes. The Tracer/Capture Reagent, a blend of ligand-tagged TSH specific antibody and <sup>125</sup>I-labeled TSH (10 µCi), was added to each tube. A cubic spline function with the zero standard as one of the standard points was used for calculations. The minimum detectable dose (MDD) was 0.01 µIU/mL, with an accuracy close to 100%, and the mean intra-assay and interassay coefficients of variance were 5.0% and 7.5%, respectively.

### Statistical Analysis

All data were expressed as means ± standard error of mean (SEM). The control and the experimental data of all

**Table 1.** Variations of Epithelium Height of the Follicular Cells Of the Thyroid Gland in *P. sicula* Subjected to NP Treatment (see Materials and Methods Section). Note: Values are Shown as Means  $\pm$  SEM. \*  $P < 0.05$  from Control Specimens, \*\*  $P < 0.001$  from Control Specimens

Group	Treatment ( $\mu\text{g}/100\text{g}$ Body Weight/Day)	Height of Follicular Epithelium ( $\mu\text{m}$ )
Control	Peanut oil	$15.1 \pm 0.02$
1	$1.72 \mu\text{g}/100\text{g}$ wt/7d	$8.32 \pm 0.05^*$
2	$1.72 \mu\text{g}/100\text{g}$ wt/14d	$5.10 \pm 0.04^{**}$
3	$1.72 \mu\text{g}/100\text{g}$ wt/28d	$3.02 \pm 0.02^{**}$

the groups were tested together for significance using one-way analysis of variance (ANOVA), followed by Duncan's test for multigroup comparison and Student's t-test for between group comparison. Differences were considered significant at  $P < 0.05$ .

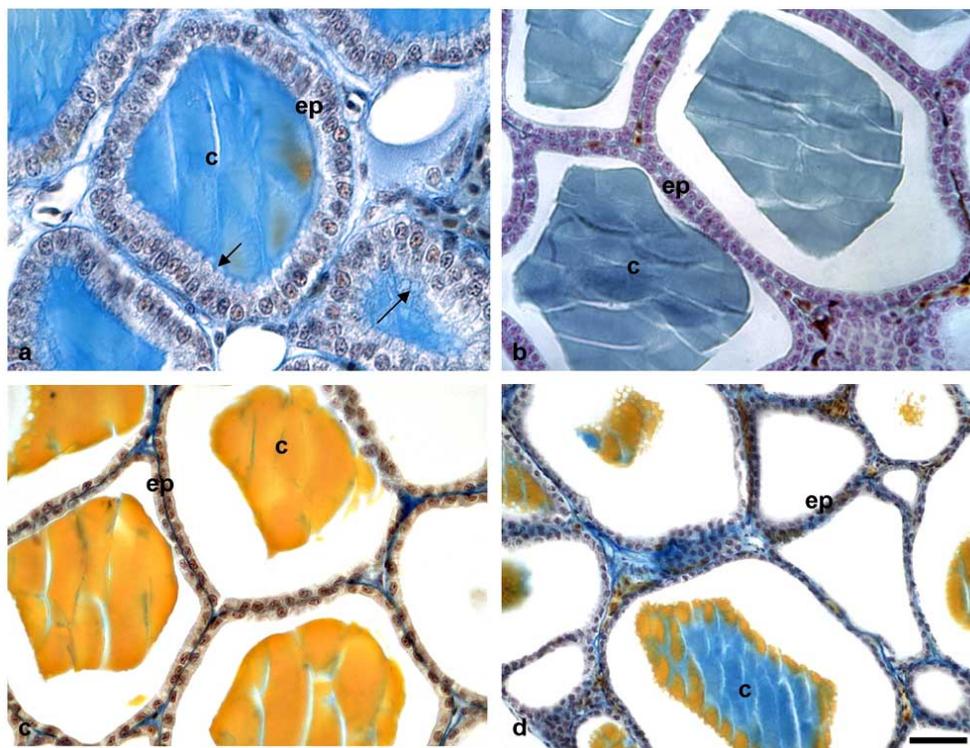
## RESULTS

### Morphological Observations of the Thyroid Gland

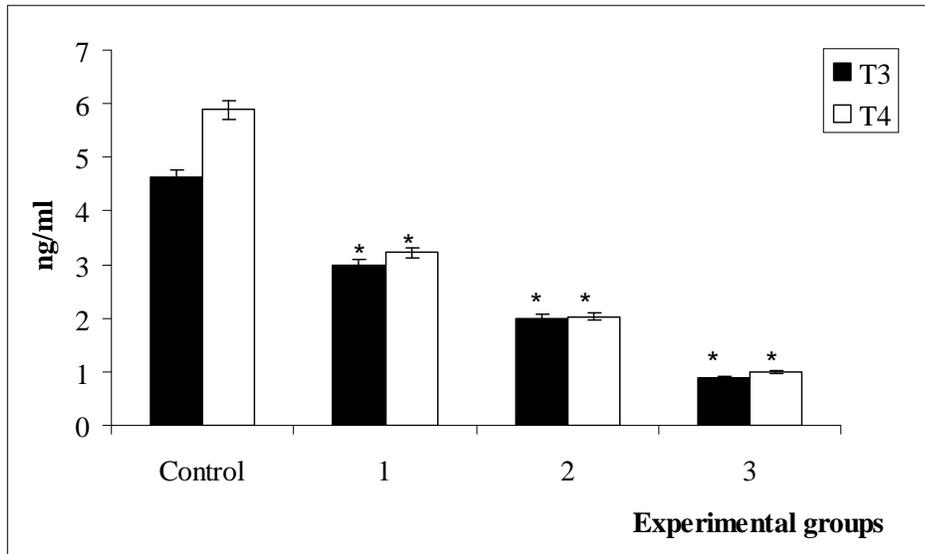
The thyroid gland of *P. sicula* control specimens is a single discrete ribbon-like structure, which transversely crosses the middle of the trachea. It is formed by follicles, surrounded by an epithelium formed by thyrocytes, and containing the colloid; the follicles are connected by an inter-follicular connective tissue, containing blood vessels. A

superficial connective tissue capsule envelops the gland and sends branches which form a network surrounding the follicles. Control thyroids showed a medium-high follicular epithelium ( $15.1 \pm 0.02 \mu\text{m}$ ) (Table 1; Fig. 1a).

The thyroid gland of NP-treated lizards showed dose-dependent morphological changes. Indeed, in Groups 1 and 2, the follicular epithelium was low and the nuclei of the thyrocytes were small and elongated with dense chromatin and a greatly reduced cytoplasm. The colloid was retracted with few reabsorption vacuoles (Figs. 1b-1c). In Group 3, the thyroid gland showed very evident signs of poor functional activity. The height follicular epithelium was very low and the thyrocyte nuclei were small and elongated with dense chromatin and greatly reduced cytoplasm. The colloid showed rare reabsorption vacuoles (Fig. 1d). Data about the



**Fig. (1).** Normal and NP-treated thyroids of exposed lizards *P. sicula* (stain Galgano I); **a.** Normal specimen; note the cuboidal follicular epithelial cells (ep), the colloid (c) and the reabsorption vacuoles (arrow); **b.** specimen treated with NP ( $1.72 \mu\text{g}/100\text{g}$  body wt) for 7 days and sacrificed 24 hr after the last injection; the follicular epithelium (ep) is lower than in normal specimen; **c.** specimen treated with NP ( $1.72 \mu\text{g}/100\text{g}$  body wt) for 14 days and sacrificed 24 hr after the last injection; the follicular epithelium (ep) is very low; **d.** specimen treated with NP ( $1.72 \mu\text{g}/100\text{g}$  body wt) for 28 days and sacrificed 24 hr after the last injection; note the follicular epithelium (ep) very low and no reabsorbing vacuoles in the colloid. Scale bar:  $20 \mu\text{m}$ .



**Fig. (2).** Variations of T<sub>3</sub> and T<sub>4</sub> levels in the plasma of *P. sicula* subjected to different experimental treatments (see Materials and Methods section). Values are shown as means  $\pm$  SEM. \*  $P < 0.05$  from control specimens, \*\*  $P < 0.001$  from control specimens.

height of follicular epithelium after acute treatment are shown in Table 1.

#### T<sub>4</sub> and T<sub>3</sub> Plasma Levels

Plasma levels of thyroid hormones in the lizard *P. sicula* were affected by the different NP doses after 28 days of treatment. In fact, the level of circulating T<sub>4</sub> and T<sub>3</sub> dose-dependently decreased in all treatment groups. Plasma T<sub>4</sub> decreased ( $p < 0.05$ ) from  $5.89 \pm 0.04$  ng/ml in the control specimens to  $3.22 \pm 0.03$  ng/ml in animals of Group 1, and to  $2.03 \pm 0.02$  ng/ml in animals of Group 2 and reached its minimum value ( $p < 0.001$ ) ( $1.00 \pm 0.02$  ng/ml) in animals exposed to five weekly injections i.p. of NP ( $1.72 \mu\text{g}/100\text{g}$  wt) for 28 days (Group 3).

Plasma T<sub>3</sub> decreased ( $p < 0.05$ ) from  $4.62 \pm 0.02$  ng/ml in the control specimens to  $2.89 \pm 0.04$  ng/ml in animals of Group 1 and  $1.59 \pm 0.05$  ng/ml in animals of Group 2 and

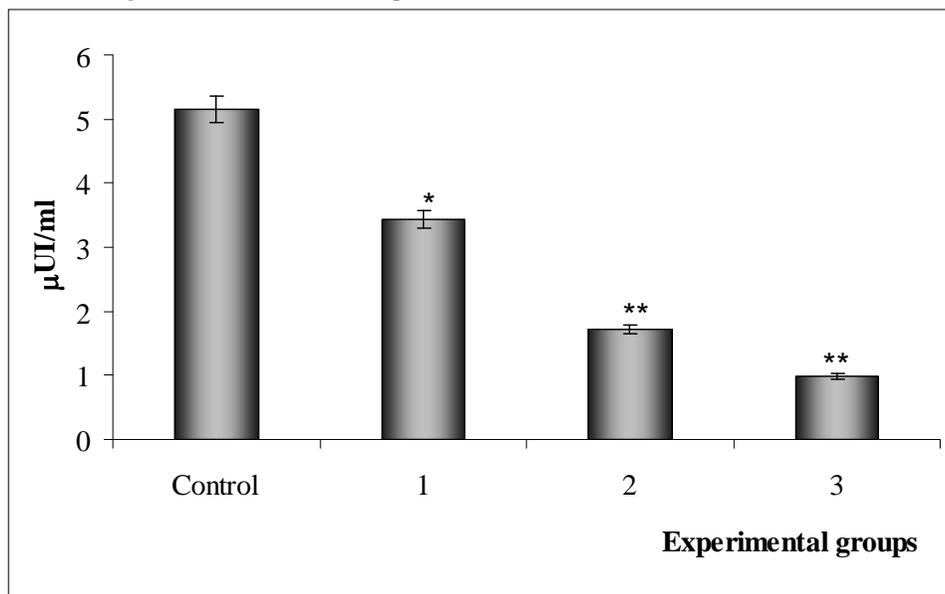
reached its minimum value ( $p < 0.001$ ) ( $0.09 \pm 0.02$  ng/ml) in animals of Group 3 (Fig. 2).

#### TSH Plasma Levels

Plasma concentrations of TSH decreased in all treatment groups. A NP dose of  $1.72 \mu\text{g}/100\text{g}$  wt for 7 days (Group 1) produced a slight decrease in the level of TSH ( $3.43 \pm 0.03 \mu\text{IU}/\text{ml}$ ) with respect to the control group ( $5.15 \pm 0.03 \mu\text{IU}/\text{ml}$ ). A mild to significant ( $p < 0.05$ ) inhibition in the plasma levels of TSH was observed in lizards exposed to a dose of  $1.72 \mu\text{g}/100\text{g}$  wt for 14 days (Group 2) ( $2.09 \pm 0.04 \mu\text{IU}/\text{ml}$ ) and a dose of  $1.72 \mu\text{g}/100\text{g}$  wt for 28 days (Group 3) ( $0.98 \pm 0.04 \mu\text{IU}/\text{ml}$ ) (Fig. 3).

#### DISCUSSION AND CONCLUSIONS

The present study is the first report dealing with NP effects on the thyroid gland of the lizard *P. sicula*. NP is an



**Fig. (3).** Variations of TSH levels in the plasma of *P. sicula* subjected to different experimental treatment (see Materials and Methods section). Values are shown as means  $\pm$  SEM. \*  $P < 0.05$  from control specimens, \*\*  $P < 0.001$  from control specimens.

industrial additive used in a wide variety of detergents, plastics and pesticides [8- 10]. NP may be one of the more critical compounds due to its toxicity, persistence and estrogenic effects [11-16].

The results of this study indicate that both structural and functional differences in the thyroid gland of the lizard *P. sicula* exist, in the animals exposed to NP. Structurally, animals exposed to NP showed decreased epithelial cell height, and nuclei of the thyrocytes small and elongated, with dense chromatin and a greatly reduced cytoplasm. The colloid was retracted with few reabsorption vacuoles.

Functionally, the same animals exhibited decreased T<sub>4</sub> and T<sub>3</sub> plasma levels, compared to control animals. Both histological and hormonal data have been used to indicate thyroid endocrine disruption. Additionally, NP administration produced a significant inhibition on serum TSH levels. This result might have caused hypothyroidism induced by NP exposure; therefore, the authors suggest that the effects of NP could very well occur pituitary level *via* decreased TSH production accounting for a reduced thyroid activity.

Little is known about the effects of NP, and alkylphenols in general, on thyroid hormones (TH) balance [18-21, 27]. *In vitro* evaluation of several chlorinated phenols showed that NP had weak affinity for the transport protein TTR but not for thyroid receptor (TR) in chicken and bullfrog [18]. In addition, NP induced GH3 cell proliferation and has proved to inhibit thyroid peroxidases catalysing iodination of T<sub>3</sub> and T<sub>4</sub> [19, 20]. All *in vitro* studies have been performed on avian or mammal cells. To our knowledge, only three studies reported *in vivo* findings on fish species and in any case they consisted of contrasting results after two different treatments [21, 27]. McCormick and co-workers [21] found that intraperitoneal administration of 0.5 to 150 µg/g of 4-NP to juvenile Atlantic salmon led to a dose-dependent reduction of T<sub>4</sub> levels, while only higher doses reduced T<sub>3</sub> plasma levels. Both hormone levels were decreased by E<sub>2</sub> treatment at a 2 µg/g dose. On the contrary, Keen and co-workers [27] failed to observe such a reduction after dietary 4-NP treatment during smoltification in coho salmon, whereas a net decrease in T<sub>3</sub> levels was observed in E<sub>2</sub> treated animals. Recently, Zaccaroni and co-workers [23] evaluated the potential effects of a single acute exposure to nonylphenol (i.p. injected) on the thyroid and reproductive axis of 250 shubunkins (*Carassius auratus*). Nonylphenol induced a significant decrease of thyroxin levels, whereas no effect on triiodothyronine concentrations was detected. No histopathological changes were detected for thyroid or testes. Our data, in agreement with those of McCormick *et al.* [21] and Zaccaroni *et al.* [23], tend to point to a predominating effect of 4-NP on thyroid activity with T<sub>4</sub> turning out to be the most sensitive parameter of NP action. It has been suggested that NP could act on the thyroid through different mechanisms, like interference with the binding of T<sub>3</sub> to transthyretin, or antagonism to T<sub>3</sub> binding to TH receptors [18, 34]. Although these mechanisms have all been postulated for higher vertebrates, they may also apply to *Podarcis sicula*, confirming the direct effect of NP on the thyroid axis. These data confirm the existence of an interplay between NP and thyroid axis, suggesting the thyroid should

be included among the other endocrine glands susceptible to endocrine disruption.

In conclusion, the toxicological results disclose the inhibitory effect of NP on thyroid hormones balance after a single acute exposure. This observation suggests that TH may serve as a potential early biomarkers of NP endocrine disruption, as TH changes, namely decreased T<sub>4</sub> levels, occur before any others in all the tissues involved in thyroid hormone production and metabolism (i.e. thyroid, liver, kidney).

## REFERENCES

- [1] Latonnelle K, Le Menn F, Bennetau-Pelissero C. *In vitro* estrogenic effects of phytoestrogens in rainbow trout and Siberian sturgeon. *Ecotoxicology* 2000; 9: 115-25.
- [2] Kwak HI, Bae MO, Lee MH, *et al.* Effects of nonylphenol, bisphenol A, and their mixture on the viviparous swordtail fish (*Xiphophorus helleri*). *Environ Toxicol Chem* 2001; 20: 787-95.
- [3] Goksøyr A. Endocrine disruptors in the marine environment: mechanisms of toxicity and their influence on reproductive processes in fish. *J Toxicol Environ Health Part A* 2006; 69: 175-84.
- [4] Melnick R, Lucier G, Wolfe M. Summary of the National Toxicology Program's report of the endocrine disruptors low-dose peer review. *Environ Health Perspect* 2002; 110 : 427-31.
- [5] Davis DL, Bradlow HL, Wolff M, Woodruff T, Hoel DG, Anton-Culver H. Medical hypothesis: xenoestrogens as preventable causes of breast cancer. *Environ Health Perspect* 1993; 101: 372-77.
- [6] Foster PM, McIntyre BS. Endocrine active agents: implications of adverse and non-adverse changes. *Toxicol Pathol* 2002; 30: 59-65.
- [7] Boas M, Rasmussen UF, Skakkebaek NE, Main KM. Environmental chemicals and thyroid function. *Eur J Endocrinol* 2006; 154: 599-611.
- [8] Soto AM, Justicia H, Wray JW, Sonnenschein C. p-Nonyl-phenol: an estrogenic xenobiotic released from "modified" polystyrene. *Environ Health Perspect* 1991; 92: 167-73.
- [9] Liber K, Knuth ML, Stay FS. An integrated evaluation of the persistence and effects of 4 nonylphenol in an experimental littoral ecosystem. *Environ Toxicol Chem* 1999; 18: 357-62.
- [10] John DM, House WA, White GF. Environmental fate of nonylphenol atoxylates: differential absorption of homologs to components of river sediment. *Environ Toxicol Chem* 2000; 19: 293-300.
- [11] Arukwe A, Yadetie F, Male R, Goksøyr A. *In vivo* modulation of nonylphenol-induced zonagenesis and vitellogenesis by the antiestrogen, 3,3',4,4'-tetrachlorobiphenyl (PCB-77) in juvenile fish. *Environ Toxicol Pharmacol* 2001; 10: 5-15.
- [12] Ackermann GE, Schwaiger J, Negele RD, Fent K. Effects of long-term nonylphenol exposure on gonadal development and biomarkers of estrogenicity in juvenile rainbow trout (*Oncorhynchus mykiss*). *Aquat Toxicol* 2002; 60: 203-21.
- [13] Cionna C, Maradonna F, Olivetto I, Pizzonia G, Carnevali O. Effects of nonylphenol on juveniles and adults in the grey mullet, *Liza aurata*. *Reprod Toxicol* 2006; 22: 449-54.
- [14] Ishibashi H, Hirano M, Matsumura N, Watanabe N, Takao Y, Arizono K. Reproductive effects and bioconcentration of 4-nonylphenol in medaka fish (*Oryzias latipes*). *Chemosphere* 2006; 65: 1019-26.
- [15] Popek W, Dietrich G, Glogowski J, *et al.* Influence of heavy metals and 4-nonylphenol on reproductive function in fish. *Reprod Biol* 2006; 6: 175-88.
- [16] Vetillard A, Bailhache T. Effects of 4-n-Nonylphenol and tamoxifen on salmon gonadotropin-releasing hormone, estrogen receptor, and vitellogenin gene expression in juvenile rainbow trout. *Toxicol Sci* 2006; 92: 537-44.
- [17] Ishihara A, Nishiyama N, Sugiyama S, Yamauchi K. The effect of endocrine disrupting chemicals on thyroid hormone binding to Japanese quail transthyretin and thyroid hormone receptor. *Gen Comp Endocrinol* 2003; 134: 36-43.
- [18] Yamauchi K, Ishihara A, Fukazawa H, Terao Y. Competitive interactions of chlorinated phenol compounds with 3,3',5-triiodothyronine binding to transthyretin: detection of possible

- thyroid-disrupting chemicals in environmental waste water. *Toxicol Appl Pharm* 2003; 187: 110-7.
- [19] Schmutzler C, Hamann I, Hofmann PJ, *et al.* Endocrine active compounds affect thyrotropin and thyroid hormone levels in serum as well as endpoints of thyroid hormone action in liver, heart and kidney. *Toxicology* 2004; 205: 95-102.
- [20] Ghisari M, Bonefeld-Jorgensen EC. Impact of environmental chemicals on the thyroid hormone function in pituitary rat GH3 cells. *Mol Cell Endocrinol* 2005; 244: 31-41.
- [21] McCormick SD, O'dea MF, Moeckel AM, Lerner DT, Björnsson BT. Endocrine disruption of parr-smolt transformation and seawater tolerance of Atlantic salmon by 4-nonylphenol and 17[ $\beta$ ]-estradiol. *Gen Comp Endocrinol* 2005; 142: 280-8.
- [22] Razia S, Maegawa Y, Tamotsu S, Oishi T. Histological changes in immune and endocrine organs of quail embryos: exposure to estrogen and nonylphenol. *Ecotoxicol Environ Saf* 2006; 65: 364-71.
- [23] Zaccaroni A, Gamberoni M, Mandrioli L, *et al.* Thyroid hormones as a potential early biomarker of exposure to 4-nonylphenol in adult male shubunkins (*Carassius auratus*). *Sci Total Environ* 2009; 407: 3301-06.
- [24] Nagao T, Wada K, Marumo H, Yoshimura S, Ono H. Reproductive effects of nonylphenol in rats after gavage administration: a two-generation study. *Reprod Toxicol* 2001; 15: 293-315.
- [25] Kim HS, Shin JH, Moon HJ, *et al.* Comparative estrogenic effects of p-nonylphenol by 3-day uterotrophic assay and female pubertal onset assay. *Reprod Toxicol* 2002; 16: 259-68.
- [26] Christensen JR, Richardson JS, Bishop CA, Pauli B, Elliott J. Effects of nonylphenol on rates of tail resorption and metamorphosis in *Rana catesbeiana* tadpoles. *J Toxicol Environ Health Part A* 2005; 68: 557-72.
- [27] Keen PL, Higgs DA, Hall KJ, Ikonou M. Effects of dietary exposure of 4-nonylphenol on growth and smoltification of juvenile coho salmon (*Oncorhynchus kisutch*). *Sci Total Environ* 2005; 349: 81-94.
- [28] Crain DA, Guillette LJ, Jr. Reptiles as models of contaminant induced endocrine disruption. *Anim Reprod Sci* 1998; 53: 77-86.
- [29] Sciarrillo R, Laforgia V, Cavagnuolo A, Varano L, Virgilio F. Annual variations of thyroid activity in the lizard *Podarcis sicula* (Squamata, Lacertidae). *Ital J Zool* 2000; 67: 263-67.
- [30] Manzo C, Zerani M, Gobbetti A, Di Fiore MM, Angelini F. Is corticosterone involved in the reproductive processes of the male lizard, *Podarcis s. sicula*? *Horm Behav* 1994; 28: 117-29.
- [31] Beccari N, Mazzi V. *Manuale di tecnica microscopica*. Rome: Societa' Editrice Libreria 1966.
- [32] Sciarrillo R, De Falco M, Virgilio F, *et al.* Morphological and functional changes in the thyroid gland of methyl thiophanate-injected lizards, *Podarcis sicula*. *Arch Environ Contam Toxicol* 2008; 55: 254-61.
- [33] Sciarrillo R, Capaldo A, Valiante S, Laforgia V, De Falco M. Localization and role of galanin in the thyroid gland of *Podarcis sicula* lizard (Reptilia, Lacertidae). *J Exp Zool Part A Ecol Genet Physiol* 2009; 311: 199-06.
- [34] Moriyama K, Tagami T, Akamizu T, *et al.* Thyroid hormone action is disrupted by bisphenol a as an antagonist. *J Clin Endocrinol Metab* 2002; 87: 5185-90.

---

Received: October 05, 2009

Revised: January 27, 2010

Accepted: February 25, 2010

© Sciarrillo *et al.*; Licensee *Bentham Open*.

This is an open access article licensed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted, non-commercial use, distribution and reproduction in any medium, provided the work is properly cited.