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Resorcinol as "endocrine disrupting chemical": Are thyroid-related adverse effects adequately documented in reptiles? *In vivo* experimentation in lizard *Podarcis siculus*

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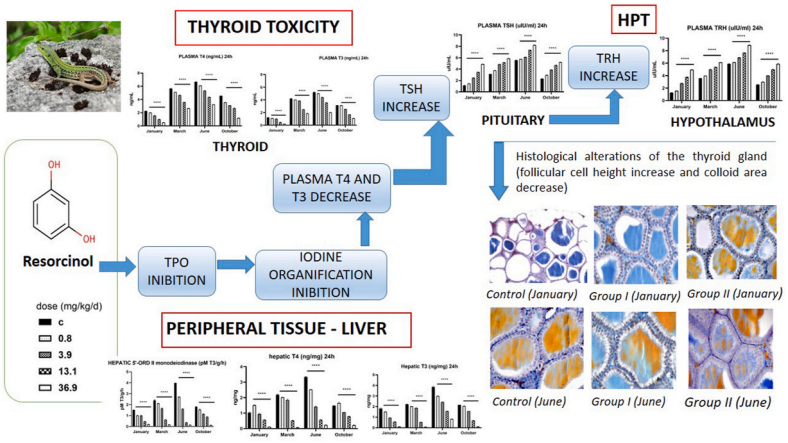
HIGHLIGHTS

- Resorcinol seriously compromises the thyroid gland.
- Plasma T4 and T3 levels were significantly decreased in the resorcinol exposure.
- HPT axis is correlated with the exposure of resorcinol.
- Resorcinol exposure mainly disrupted hepatic dio 2 and T_4 and T_3 contents.

GRAPHICAL ABSTRACT



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ABSTRACT

The endocrine system and particularly thyroid hormones regulate almost all physiological processes in a timely manner in all vertebrates, from fish to reptiles to mammals, so risk assessment of endocrine disrupting chemicals (EDCs) is extremely important given their persistent presence in all environmental matrices. Resorcinol, as well as nonylphenol, octylphenol, and bisphenol A, F, S, are non-Halogenated Phenolic (non-HPCs) Chemicals known as EDCs. Resorcinol is a particular example in that most studies are based exclusively on humans while animal studies are few and often inadequate. The aim of this study was to assess the effects of exposure to different doses of resorcinol on the thyroid gland of the lizard *Podarcis siculus* during different periods of the thyroid gland

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activity cycle. Our results showed histopathologic changes in thyroid (follicular cell height increase and colloid area decrease), a thyroid weight increase in combination with serum T_4 and T_3 decrease, serum TSH, TRH increase in male lizards treated with 0.8,3.9,13.1, and 36.9 mg/kg/d of resorcinol. Besides, we also investigated the impacts of resorcinol treatments on hepatic 5′ORD (type II) deiodinase and hepatic content of T_3 and T_4. Our findings showed that they are in agreement with *in vivo* in humans and in rodents data and therefore, resorcinol in reptiles may meet the WHO definition of ECDs.

1. Introduction

Resorcinol (1,3-dihydroxybenzene) is used in the production of rubber products, wood adhesives, flame retardants, UV stabilizers, and dyes (Schmiedel and Decker, 2000; Pasquier et al., 2023), but it is also a component of many personal care products, such as hair dyes, anti-acne preparations, and peels (WHO, 2013). The global resorcinol market stood at nearly 70 thousand tons in 2022 and is expected to grow steadily by 3.94 percent over the forecast period to 2032 (Hercog et al., 2019). Therefore, this increasing availability of resorcinol leads to an increase in its load in wastewater systems and, therefore, in the aquatic and terrestrial environment (ECHA, 2020). Besides, the following percent distribution was calculated (ECHA, 2020), according to which resorcinol will be distributed mainly into soil and water compartments when loading to all compartments was applied: Air 0.00222 %, Water 36.1 %, Soil 63.8 % and Sediment 0.07 %. Resorcinol, as well as nonylphenol (NP), octylphenol (OP), and bisphenol A, F, S, is non-Halogenated Phenolic (non-HPC) Chemical belonging to Endocrine Disrupting Chemicals (EDCs) (ECHA, 2020; Kabir et al., 2015; Ismanto et al., 2022; Pasquier et al., 2023).

There are many clinical studies on the adverse effects of non-HPCs on male and female reproductive functions (Welsch et al., 2008; Gore et al., 2015; Shahidehnia, 2016; Sifakis et al., 2017; Massányi et al., 2020; Thacharodi et al., 2023), while little is known about the morphological and functional alterations of the thyroid gland (Sciarrillo et al., 2010, 2021).

Thyroid hormones (THs) regulate growth, development and metabolism in all vertebrates (Forrest and Visser, 2013). A very recent comparative analysis of studies published in the National Center for Biotechnology Information (NCBI) regarding alterations of endocrine glands by endocrine disruptors in different classes of vertebrates reported that 77 percent of these involved mammals, 18 percent fish, 6 percent amphibians, 5 percent birds, and only 2 percent reptiles (Thambirajah et al., 2022).

In this context, the endocrine toxicity assessment of resorcinol is a striking example in non-mammalian vertebrates. Because resorcinol has been widely used as a drug in humans, particularly for the treatment of skin ulcers (Lynch et al., 2002), it has long been described as interfering with human thyroid function (Fawcett and Kirkwood, 1953; Lynch et al., 2002; Caturegli et al., 2014; Tukes, 2017; Pasquier et al., 2023). In fact, available studies on resorcinol have allowed the French Agency for Food, Environment and Occupational Health and Safety (ANSES) (ANSES, 2020) to name resorcinol as a “substance of very high concern” due to its properties (REACH, 2020). Resorcinol has been shown to inhibit peroxidase (TPO) activity in a dose-dependent manner resulting in altered iodine uptake in humans *in vivo* with clinical hypothyroidism, and from the numerous *in vitro* studies (Cooksey et al., 1985; Lindsay et al., 1992; Paul et al., 2014; Paul Friedman et al., 2016; Dong et al., 2020).The large number of human studies in the literature are clinical studies that unequivocally demonstrate that resorcinol can dramatically alter the thyroid gland depending on the dose used; ecotoxicological studies demonstrating the effect of resorcinol in the context of more likely environmental exposure in the population are entirely lacking. Given the wide distribution of resorcinol in the environment, it is surprising that no biomonitoring studies on human exposure to resorcinol are available. Only a few occupational studies on resorcinol exposure are available that even suggest that occupational exposure to resorcinol is not sufficient to cause obvious effects on the thyroid gland (Lynch et al., 2002; Porras et al., 2018).

There are only a few studies on environmental resorcinol exposure and thyroid physiology in non-mammalian vertebrates that may be exposed to environmental pollution through various routes, including ingestion of contaminated food, polluted water or soil. These experimental animal studies besides being limited have too many methodological shortcomings to be completely conclusive about the effects produced by resorcinol exposure. However, the only potentially thyroid-related adverse effects of resorcinol that have been examined in experimental animal studies concern neurodevelopmental outcomes (Welsch et al., 2008).

Reptiles are the most sensitive groups of terrestrial vertebrates to contaminants but have received extremely limited attention (Wang et al., 2020; Rosenfeld et al., 2017; Sparling et al., 2010; Tan and Zoeller, 2007). *Podarcis siculus* has been chosen as sentinel species because it is the most abundant reptile species in Southern Italy, living in both cultivated fields and open country. Moreover, reptiles are used as bio-indicators because of their persistence in many habitats, high sensitivity to contaminants like birds and mammals and great ability to bio-accumulate and bio-magnify pollutants (Capaldo et al., 2012; De Falco et al., 2004, 2007, 2010; Sciarrillo et al., 2010, 2021, 2022).

The aim of this study was to assess the effects of exposure to resorcinol on the thyroid gland of the lizard *P. siculus* during different periods of the thyroid gland activity cycle: January for the winter stasis, March for the recovery period, June for the activity period, and October for the autumn stasis (Sciarrillo et al., 2000) through histopathological analysis of thyroid gland (follicular cell height, colloid area and thyroid weight), plasma thyroid hormones level evaluation (TRH, TSH, T_3 and T_4), content of thyroid system in liver (hepatic T_3 and T_4 contents and deiodinase types II (5′ORD2) activity).

2. Material and methods

2.1. Animals and experimental procedures

Animal experiments were performed according to the ethical provisions imposed by the European Union and permitted by the National Committee of the Italian Ministry of Health on *in vivo* experimentation.

Sexually mature male specimens of the lizard *Podarcis siculus* have been captured in Campania (southern Italy; Latitude: 41° 19′54 ″N; Longitude: 13° 59′29 ″E) and precisely in the area near the metropolitan city of Naples which is the one with the highest population density in Europe, during different periods of the thyroid gland activity cycle: January for the winter stasis, March for the recovery period, June for the activity period, and October for the start of the winter stasis (Sciarrillo et al., 2000). The animals were collected in the same year.

Then, we selected ten lizards for each period (without significant differences in body mass) and placed them in large soil-filled terraria containing heather and indoor subjected to photoperiod (11 h of daylight) and natural temperature (15–24 °C); moreover, they were daily fed honeycomb moth (*Galleria mellonella*) caterpillars, water was available *ad libitum* and to reverse capture-related stress conditions, an acclimatization period of 15 days was allowed before starting the treatment (Di Lorenzo et al., 2021).

All of the lizards used for analysis were about the same age and were weighed on a precision balance before and after the experimental tests.

The experiments were approved by institutional committees (Ministry of Health, Italy) and organized to minimize the number of animals used. The animals were sacrificed by decapitation after deep anaesthesia with ketamine hydrochloride (Parke-Davis, Berlin, Germany) 325 pg/g of body weight (Sciarrillo et al., 2021, 2022).

During the four experimental periods, the lizards were divided into three experimental groups according to the following scheme (n = number of samples used in each experiment).

For the choice of doses to be used, reference was made to the very few available experimental animal studies concerning the potentially thyroid adverse effects of resorcinol; particularly in rats exposed to average daily doses of resorcinol in which there are signs of alterations in behaviors.

Control Group: Untreated control lizards was intraperitoneally injected with 50 μL of corn oil for 28 times. From this group, five lizards were sacrificed 24 h after the last injection and five 15 days after last injection.

Group I: Lizards were treated with 14 intraperitoneally injections (ip) of 0.8–3.9-13.1–36.9 mg/kg/d of Resorcinol (CAS 108-46-3) Merck KGaA, Darmstadt, Germany and sacrificed 24 h after the last injection.

Group II: Lizards were treated with 14 ip injections of 0.8–3.9-13.1–36.9 mg/kg/d of Resorcinol and sacrificed 15 days after the last injection (recovery group).

2.2. Biochemical analysis

2.2.1. Plasma TRH (thyrotropin-releasing hormone), TSH (thyroid stimulating hormone) and thyroid hormones assays

TRH and TSH levels were determined by immunoradiometric assay (IRMA) as previously reported in Sciarrillo et al. (2022). Sample serum and standards were added to anti-ligand coated tubes. The tracer/-capture reagent, a blend of ligand-tagged TSH-rabbit antibody and 125I labeled (10 pCi), 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 was 0.01 μIU/ml, with an accuracy close to 100% and a mean intra-assay and inter-assay variance of 5.0% and 7.5%, respectively. Cross-reactivity studies were performed using substances, which could theoretically interfere with the performance of the assay. The cross-reactivity for FSH, hCG and LH in TSH IRMA was less than 0.001 and therefore was not considered for data calculations (Sciarrillo et al., 2000).

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T_3 and T_4 levels were determined using radioimmunoassay (RIA) (Byk-Sangtec Diagnostica, Dietzenbach, Germany) (Sciarrillo et al., 2022).

In the T3 assay, a measured amount of sample serum and standards was added to a tube coated with anti-T3 rabbit antibody, along with a trace amount of radioactively labeled T3 ([125I]-T3, 165 kBq; 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, USA) to release T3 from serum binding proteins. The 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%, while the inter-assay variance ranged between 3.9 and 5.7% in 12 assays (Sciarrillo et al., 2000).

For T4, a measured amount of sample serum and standards was added to a tube coated with anti-T4 rabbit antibody, along with a trace amount of radioactively labeled T4 ([125I]-T4, 165 kBq; Byk-Sangtec Diagnostica) 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.) to release T4 from serum binding proteins. The sensitivity was 0.45 ng/ml with an accuracy close to 100%; the mean intra-assay and inter-assay coefficients of variation were 4.6 and 4.3%, respectively. The cross-reactivity for T4 in the T3 RIA (1.3%) was not considered for data calculations, neither was that for T3 in the T4 RIA (0.1%) (Sciarrillo et al., 2000).

2.2.2. Hepatic thyroid hormones and 5′ORD (type II) monodeiodinase

Livers were removed and flushed in a buffer composed of MOPS and EDTA at pH 7.4. The contents of T_3 and T_4 in hepatic tissue were determined by radioimmunoassay RIA and were expressed as ng/mg of tissue (fresh weight) (Sciarrillo et al., 2022). The activity of the enzyme is expressed as pM T_3/g (of liver)/h) (Sciarrillo et al., 2022).

2.3. Histological analysis

Thyroid glands were removed, weighed and immediately fixed in Bouin's fixative and processed for light microscopy (LM). Serially cut paraffin sections (7 μm) were stained with Galgano stain and observation was performed using a Zeiss Axioskop microscope (Milano, Italy). The height of the follicular cells (μm) and the diameter of the follicles (μm) were measured in 30 cells every 3 slides and always on the second section of both normal and treated samples using a digital system of image (KS 300) (Zeiss, Milano, Italy) (Sciarrillo et al., 2010, 2021, 2022).

2.4. Statistical analysis

The obtained data have been averaged prior to calculating the experimental group mean and the standard error of the mean. As revealed by the χ^2 test (chi-square test), data were not different from the normal distribution. The control and experimental data of all the groups were tested together for significance using two-way ANOVA, followed by Bonferroni's for multi-group comparison using GraphPad Prism version 8.00 for Windows, GraphPad Software (La Jolla, CA, USA). Differences were considered significant at $^{****}p < 0.001$.

Doses (mg/kg/d)	Experimental periods							
	January		March		June		October	
	Group I sacrificed 24 h after the last injection	Group II sacrificed 15d after the last injection	Group I sacrificed 24 h after the last injection	Group II sacrificed 15d after the last injection	Group I sacrificed 24 h after the last injection	Group II sacrificed 15d after the last injection	Group I sacrificed 24 h after the last injection	Group II sacrificed 15d after the last injection
Control Group	n=5	n=5	n=5	n=5	n=5	n=5	n=5	n=5
0.8	n=5	n=5	n=5	n=5	n=5	n=5	n=5	n=5
3.9	n=5	n=5	n=5	n=5	n=5	n=5	n=5	n=5
13.1	n=5	n=5	n=5	n=5	n=5	n=5	n=5	n=5
36.9	n=5	n=5	n=5	n=5	n=5	n=5	n=5	n=5

3. Results

3.1. Sign of toxicity and animal mortality

All animals were observed twice a day, at 9:30 a.m. and 3 p.m., to check general appearance and behavior as well as to detect any signs of toxicity related to resorcinol intake. The behaviors observed were (i) inactivity, (ii) hyperactivity, (iii) climbing, (iv) walking, and (v) eating. The behavioral variables tested showed significant differences between the treatment groups, especially the animals in the group with 36.9 mg/kg/d resorcinol assumed a posture with the limbs close to the body. Hyperactivity and climbing were also reduced, and walking speed decreased over time, especially in the group with the highest dose of resorcinol. Clear signs of toxicity, such as decreased locomotor activity and crawling, were repeatedly observed during the resorcinol administration period as early as after the first dose. A similar pattern of toxicity was observed in all groups of lizards treated with the different dosages of resorcinol. No deaths were observed; however, lizards in the high-dose group showed clinical signs of exposure, which persisted up to 15 days after exposure.

Resorcinol treatment caused a significant decrease in the body weight (g) of treated lizards compared with controls (for each period), a decrease that was dose-and time-dependent (Table 1).This change in body weight being present in all groups of treated lizards showed that the diet and care of the animals were adequate and that the health status did not influence the results of resorcinol toxicity on the body weight of treated lizards.

3.2. Plasma levels of hormones belonging to the HPT axis after resorcinol treatment

The lizards treated with 14 ip injections at different doses of resorcinol and sacrificed after 24 h from the last injection (Group I) showed a significant dose-dependent increase in TRH level. In all experimental periods, we showed almost three times increase at the highest dose compared with the control group (Fig. 1A). Specifically, in the group treated with the highest dose of resorcinol (36.9 mg/kg/d) during the period of thyroid gland inactivity (January), the TRH level increased from 1.25 ± 0.04 to 4.96 ± 0.01 μUI/mL; in June, the period of maximum thyroid gland activity, the TRH concentration reaches a significantly high level (8.89 ± 0.05 μUI/mL; $p < 0.001$) compared with that of the control group (5.87 ± 0.05 μUI/mL). In the intermediate periods of thyroid gland activity (March and October) the increase in TRH levels was confirmed although with more modest values (March: 6.12 ± 0.01 μUI/mL *vs.* 3.56 ± 0.02 μUI/mL; October: 5.87 ± 0.05 μUI/mL *vs.* 2.55 ± 0.03 μUI/mL) (Fig. 1A).

This increase in TRH stimulated the anterior pituitary gland to produce TSH; in fact, plasma TSH levels increased in all groups treated with the different doses of resorcinol during all periods of the trial (Fig. 1B). During the period of maximum thyroid gland activity (June), TSH concentration increased significantly (5.77 ± 0.01 μUI/mL *vs.* 5.57 ± 0.05 - control group; $p < 0.001$) from the lowest dose of resorcinol (0.8 mg/kg/d) until reaching the highest concentration in the plasma of animals treated with the highest concentration of resorcinol (36.9 mg/kg/d) (8.24 ± 0.05μUI/mL *vs.* 5.57 ± 0.05 μUI/mL - control group; $p < 0.001$) (Fig. 1B). In winter (January) when TSH levels in control specimens are low (1.13 ± 0.04 μUI/mL), exposure to resorcinol from the lowest dose (0.8 mg/kg/d) induced an increase in TSH levels reaching a significantly ($p < 0.001$) high value (4.89 ± 0.01 μUI/mL) in specimens exposed to the highest dose of resorcinol for 14 days (Fig. 1B).

Plasma levels of thyroid hormones in the lizard were affected by the different doses of resorcinol after 14 days of treatment. In fact, the level of circulating T_4 and T_3 were found to be dose-dependently decreased in all treatment groups during all periods of the trial (Fig. 1C and D). In winter (January), when thyroid activity is inhibited, animals treated with the highest concentration of resorcinol (36.9 mg/kg/d) showed a decrease in plasma T_3 ($p < 0.001$) from 1.23 ± 0.03 in control specimens to 0.22 ± 0.01 ng/mL in treated animals. Same decrease in plasma T_3 concentrations are also found in June when the thyroid gland is in full activity (control group: 5.20 ± 0.05 ng/mL *vs.* treated group: 2.04 ± 0.01 ng/mL) (Fig. 1C). During the recovery period (March) and the onset of winter stasis (October) of the thyroid gland, in animals exposed to 36.9 mg resorcinol/kg/d, plasma T_3 decreased ($p < 0.001$) from 4.21 ± 0.03 in control specimens to 1.87 ± 0.01 ng/mL in March and from 3.15 ± 0.01 in control specimens to 1.11 ± 0.01 ng/mL in October (Fig. 1C).

Same decreasing trend in plasma T_4 levels was found in a dose-dependent manner in all specimens treated with the different doses of resorcinol during the four different periods of thyroid gland activity (Fig. 1D). In June, when the thyroid gland is in full secretory activity, T_4 levels significantly decreased in animals treated from the lowest dose of resorcinol (control group: 6.68 ± 0.05 ng/mL *vs.* treated group: 6.11 ± 0.01 ng/mL) to the highest dose of resorcinol (3.24 ± 0.01 ng/mL) (Fig. 1D). Even in January when thyroid gland secretory activity is inhibited, T_4 levels significantly decreased ($p < 0.001$) in a dose-dependent manner, from an already very low value in controls (2.25 ± 0.03 ng/mL) to a very low value (0.52 ± 0.01 ng/mL) in specimens treated with the highest dose of resorcinol (Fig. 1D).

The trend of HPT axis hormones was the same in the specimens treated with 14 injections of resorcinol and sacrificed 15 days after the last injection (recovery group - Group II). In fact, a smaller increase in TRH levels was observed in all specimens treated with different doses of resorcinol during the four periods of thyroid gland activity when compared with specimens sacrificed 24 h after the last injection; however, this increase in TRH levels remained significantly higher than the level recorded in the control specimens (Fig. 1E). Specifically, in June, when the thyroid gland is stimulated, plasma TRH levels increased significantly in animals treated from the lowest dose of resorcinol (control group: 5.88 ± 0.05 μUI/mL vs. treated group: 6.10 ± 0.03 μUI/

Table 1
Body weight (g) change in *Podarcis siculus* subjected to 14 intraperitoneally injections of 0.8–3.9-13.1–36.9 mg/kg/d of Resorcinol and sacrificed 24 h after the last injection (**Group I)** and sacrificed 15 days after the last injection (**Group II**) in the four experimental periods.

Doses (mg/kg/d)	Experimental periods							
	January		March		June		October	
	Group I sacrificed 24 h after the last injection	Group II sacrificed 15d after the last injection	Group I sacrificed 24 h after the last injection	Group II sacrificed 15d after the last injection	Group I sacrificed 24 h after the last injection	Group II sacrificed 15d after the last injection	Group I sacrificed 24 h after the last injection	Group II sacrificed 15d after the last injection
Control Group	7.61 ± 2.06	7.59 ± 2.04	8.39 ± 1.89	8.44 ± 1.55	8.86 ± 2.44	8.89 ± 2.34	7.81 ± 1.46	7.82 ± 1.14
0.8	7.01 ± 2.03	7.05 ± 2.05	8.12 ± 1.51	8.15 ± 1.41	8.72 ± 2.01	8.83 ± 2.03	7.55 ± 2.15	7.59 ± 2.02
3.9	6.99 ± 1.04	6.99 ± 1.03	7.67 ± 1.55	7.74 ± 1.45	7.62 ± 1.33	7.52 ± 1.41	6.15 ± 1.51	6.10 ± 1.61
13.1	5.46 ± 1.23	5.41 ± 1.22	6.62 ± 1.02	6.68 ± 1.06	6.36 ± 2.02	6.49 ± 2.02	5.88 ± 1.12	5.99 ± 1.13
36.9	4.76 ± 1.51*	5.60 ± 0.28**	5.11 ± 1.35**	5.21 ± 1.45**	5.14 ± 1.01**	5.25 ± 1.22**	4.68 ± 1.41*	4.72 ± 1.51*

(****p < 0.001, in the comparison with the different controls). A more detailed description is in the text (see Materials and Methods section).

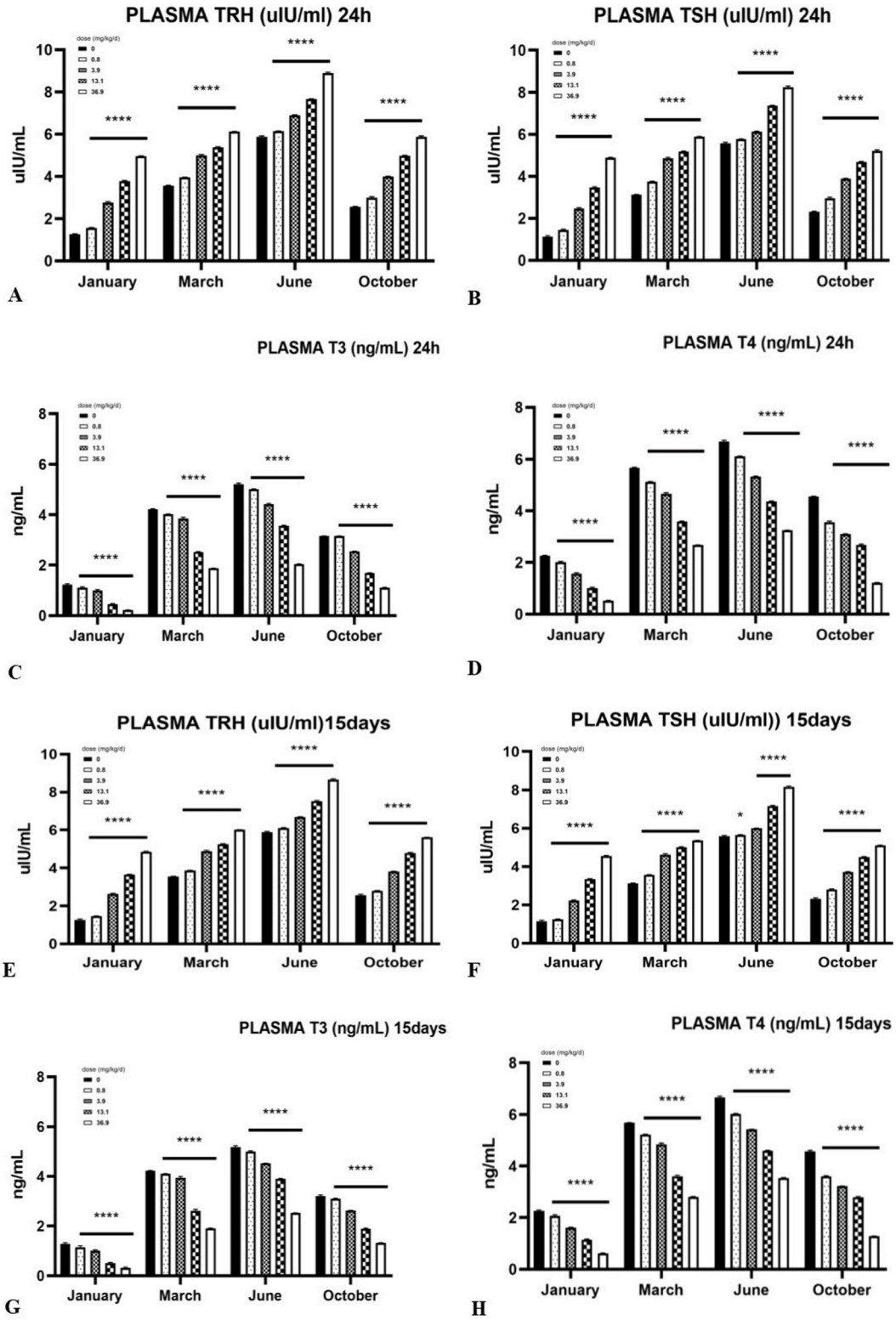

Fig. 1. Plasma TRH (A), TSH (B), T_3 (C), and T_4 (D) levels in *Podarcis siculus* subjected to 14 intraperitoneally injections of 0.8–3.9-13.1–36.9 mg/kg/d of Resorcinol and sacrificed 24 h after the last injection (**Group I**). Plasma TRH (E), TSH (F), T_3 (G), and T_4 (H) levels in *Podarcis siculus* subjected to 14 intraperitoneally injections of 0.8–3.9-13.1–36.9 mg/kg/d of Resorcinol and sacrificed 15 days after the last injection (**Group II**). (****$p < 0.001$, in the comparison with the different controls). A more detailed description is in the text (see Materials and Methods section).

mL) to the highest 8.65 ± 0.05) (Fig. 1E). Same increase in TRH levels was also found in winter when the thyroid gland is inhibited (Fig. 1E).

For the other HPT axis hormones, the trend is also the same as that found for animals treated with the different doses of resorcinol at different times of the year but sacrificed after 24 h (Fig. 1F, G, H).

3.3. Hepatic thyroid hormones content and 5-T4 ORD (type II) monodeiodinase activity after resorcinol treatment

Resorcinol-treated lizards had lower hepatic concentrations of T_3, T_4, and 5′ORD (type II) activity than control lizards at all doses of resorcinol and at all times of the year (Fig. 2). Indeed, in January a decrease in 5′ORD (type II) activity was observed in lizards exposed to 14 injections of resorcinol and sacrificed 24 h after the last injection (Group I) both at the lowest dose (1.01 ± 0.03 pM T_3/g/h) and at the maximum dose of resorcinol (0.21 ± 0.01 pM T_3/g/h) (Fig. 2A).

Even in June, when 5-T4 ORD monodeiodinase activity is highest, treatment with resorcinol causes a significant decrease in enzyme activity in specimens treated with the highest dose of resorcinol (0.14 ± 0.01 pM T_3/g/h) compared with control specimens (3.98 ± 0.01 pM T_3/g/h) (Fig. 2A).

Hepatic T_4 and T_3 contents decreased in all the resorcinol treated groups (dose-dependent), becoming particularly low in animals treated with 14 injections of resorcinol and sacrificed 24 h after the last injection in all times of the year considered (Group I) (Fig. 2C–E). Specifically, in June when hepatic thyroid hormone contents are high (T_4: 3.33 ± 0.04 ng/mg; T_3: 3.85 ± 0.07 ng/mg), hepatic T_4 and T_3 content significantly decreased ($p < 0.001$) from the lowest to the highest dose of resorcinol (T_4: 0.24 ± 0.01 ng/mg; T_3: 0.84 ± 0.01 ng/mg) (Fig. 2C–E). Even in winter when liver contents of T_3 and T_4 are low (T_4: 1.63 ± 0.03 ng/mg; T_3: 1.82 ± 0.05 ng/mg) significant ($p < 0.001$) decreases were found in specimens treated with both low (T_4: 1.01 ± 0.03 ng/mg; T_3: 1.51 ± 0.03 ng/mg) and high doses of resorcinol (T_4: 0.11 ± 0.01 ng/mg; T_3: 0.21 ± 0.01 ng/mg) (Fig. 2C–E).

Also in lizards sacrificed after 15 days after the last injection of the different doses of resorcinol (Group II) there was a significant ($p < 0.001$) decrease in 5′ORD (type II) activity and hepatic T_4 and T_3 contents in all times of the year considered (Fig. 2B–D, F).

3.4. Thyroid gland Histology after resorcinol treatments

The thyroid gland is located transversely about halfway down the trachea in Podarcis siculus specimens, with a ribbon-like structure composed of follicles connected by interfollicular connective tissue containing blood vessels. The gland is covered by a capsule of superficial connective tissue that branches out to form a network surrounding the follicles. Each follicle is formed by an epithelium, composed of thyrocytes containing a medium-sized colloidal mass (Sciarrillo et al., 2000).

In the seasonal lizard *P. siculus*, the thyroid gland undergoes a marked annual cycle (Sciarrillo et al., 2000), characterized by a functional stasis, starting in autumn to become full stasis in December–January. In this period, the follicular epithelium was low, while the colloid was compact and devoid of reabsorption vacuoles. In spring, there was a thyroid activity resumption that reached its maximum in May–June; the follicular epithelium was very high and colloid was retracted with clear signs of reabsorption. The thyroid activity decreases

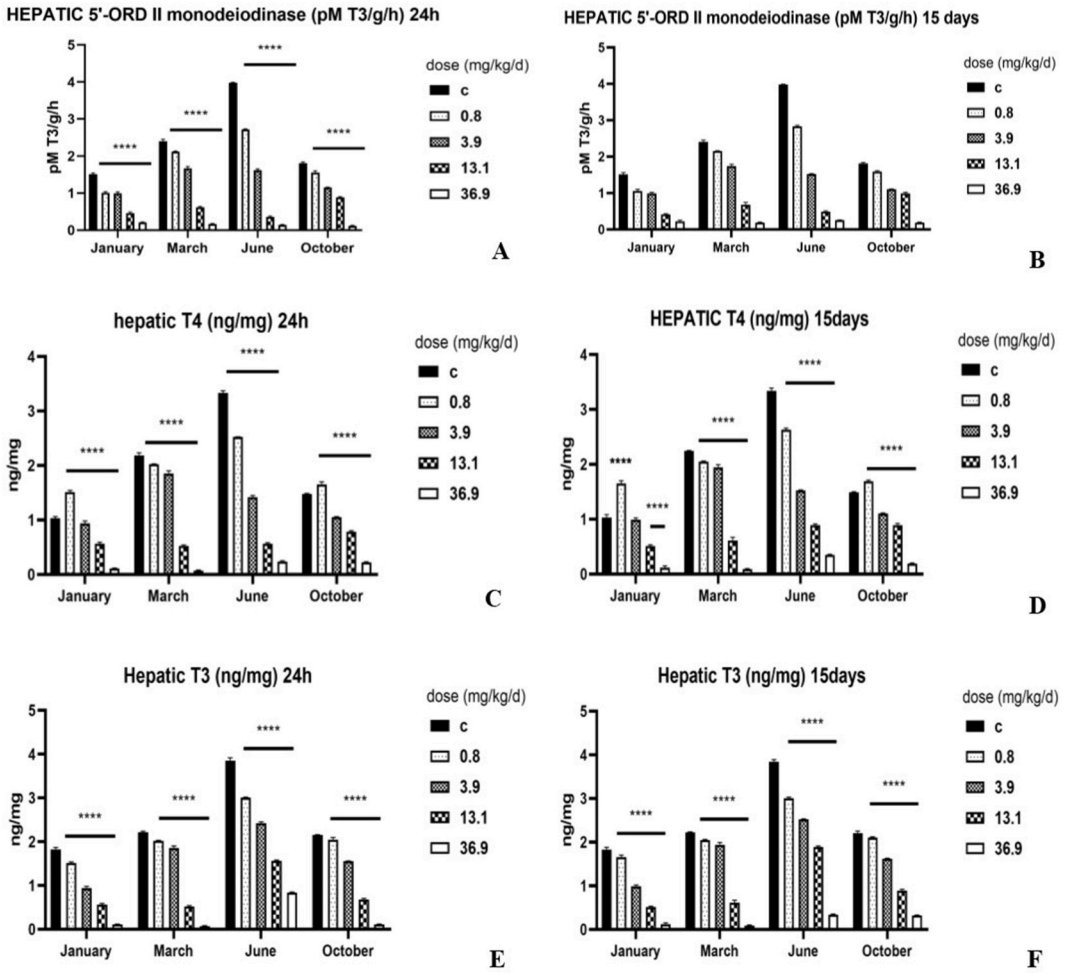

Fig. 2. Hepatic 5′ORD-monodeiodinase (type II) activity(A) and hepatic T_3(C), T_4 (E) content in *Podarcis siculus* subjected to 14 intraperitoneally injections of 0.8–3.9-13.1–36.9 mg/kg/d of Resorcinol and sacrificed 24 h after the last injection (**Group I**). Hepatic 5′ORD-monodeiodinase (type II) activity (B) and hepatic T_3(D), T_4 (F) content subjected to 14 intraperitoneally injections of 0.8–3.9-13.1–36.9 mg/kg/d of Resorcinol and sacrificed 15 days after the last injection (**Group II**). (****$p < 0.001$, in the comparison with the different controls). A more detailed description is in the text (see Materials and Methods section).

again afterwards (Sciarrillo et al., 2000),

Winter stasis (January): Animals in the control group had moderate body weight (7.61 ± 2.06 g) (Table 1), and thyroid weight was highest (0.233 ± 0.13 g) (Table 2) compared with the other three different periods considered). The thyroid revealed maximal follicular diameter (462 ± 52 μm) (Table 3) and minimal epithelial height (2.15 ± 0.06 μm) (Table 4). The gland showed a low follicular epithelium and retracted compact colloid and devoid of reabsorption vacuoles; the nuclei of the thyrocytes were small and elongated with dense chromatin and a greatly reduced cytoplasm (Fig. 3A).

The thyroid gland of lizards treated with 14 injections of resorcinol at the maximum dose (36.9 mg/kg/d) and sacrificed 24 h after the last injection, appeared richly vascularized with medium follicular epithelium (6.26 ± 0.51 μm) (Table 4); thyrocytes had still a cubic shape and colloid showed various reabsorption vacuoles (Fig. 3B). After 14 injections of resorcinol at the maximum dose and sacrificed 15 days after the last injection, lizards showed a thyroid gland with the same morphological aspects (follicular epithelium: 6.60 ± 0.25 μm (Table 4); follicular diameter: 160 ± 25 μm (Table 3) of lizards treated with the same dose of resorcinol but sacrificed after 24 h after the last injection (Group I) (Fig. 3C).

Group I animals at the highest dose of resorcinol had a decrease in body weight (4.76 ± 1.51 g) (Table 1) and thyroid weight significantly increased (0.281 ± 0.01 g) (Table 2), in Group II, the lizards had a slightly higher body weight (5.60 ± 0.28 g; Table 2) and slightly lower thyroid weight (0.276 ± 0.01 g) than in Group I (Table 2).

Recovery period (March): the thyroid follicles of control groups showed a medium-high follicular epithelium (6.10 ± 0.02 μm) (Table 4) and a follicular diameter of 232 ± 30 μm (Table 3); they presented numerous reabsorbing vacuoles in the colloid (Fig. 3D).

The thyroid gland of lizards treated with 14 injections of resorcinol at the maximum dose and sacrificed 24 h after the last injection appeared richly vascularized with high follicular epithelium (9.21 ± 0.35 μm) (Table 4); the thyrocytes still had a cylindrical shape, and the colloid showed many resorption vacuoles (Fig. 3E). After 14 injections of resorcinol at the maximum dose and sacrificed 15 days after the last injection, lizards showed a thyroid gland with the same morphological aspects (follicular epithelium: 9.35 ± 0.45; follicular diameter: 106 ± 16 μm) of lizards treated with the same dose of resorcinol but sacrificed 24 h after the last injection group (Fig. 3F).

At the highest dose of resorcinol, group I lizards had a decrease in body weight (control group: 8.39 ± 1.89 *vs.* treated group: 5.11 ± 1.35 g) (Table 1) and thyroid weight increased significantly (control group: 0. 125 ± 0.06 *vs.* treated group: 0.197 ± 0.01 g) (Table 2); on the other hand, group II lizards had slightly higher body weight (5.21 ± 1.45 g) (Table 1) and slightly lower thyroid weight (0.191 ± 0.01g) than group I lizards (Table 2).

Activity period (June): During this period, there was the highest stimulation of the thyroid gland, which revealed an increase in follicular epithelium (8.10 ± 0.04 μm) (Table 4) and a follicular diameter of 244 ± 26 μm (Table 3). The thyrocytes had a prominent continuous epithelium, the chromophobe droplets were seen in the colloid at the periphery (Fig. 4A).

The thyroid of lizards treated with 14 injections of resorcinol at the maximum dose and sacrificed 24 h after the last injection exhibited a very high follicular epithelium (15.1 ± 1.01 μm) (Table 4); the thyrocytes still had a cylindrical shape, and the colloid showed numerous resorption vacuoles (Fig. 4B). After 14 injections of resorcinol at the maximum dose and sacrificed 15 days after the last injection, the lizards showed a thyroid with the same morphological aspects (follicular epithelium: 15.5 ± 1.22 μm (Table 4); follicular diameter: 109 ± 20 μm (Table 3) as the lizards treated with the same dose of resorcinol but sacrificed 24 h after the last injection (Fig. 4C).

Lizards treated with the highest dose of resorcinol (Group I) had a decrease in body weight (control group: 8.86 ± 2.44 vs. treated group: 5.14 ± 1.01g) (Table 1) and a significant increase in thyroid weight (control group: 0. 208 ± 0.2 vs. treated group: 0.244 ± 0.01g) (Table 2); in Group II, the lizards had a slightly higher body weight (5.25 ± 1.22 g) (Table 1) and a slightly lower thyroid weight (0.240 ± 0.01 g) than in group I (Table 2).

Start of the winter stasis (October): During the beginning of the winter stasis, the thyroid gland begun to reduce its secretory activity; in fact, the height of the follicular epithelium was reduced (3.81 ± 0.06 μm) (Table 4) and a follicular diameter was increased (362 ± 52 μm) (Table 3) (Fig. 4D).

The thyroid of lizards treated with 14 injections of resorcinol at the maximum dose and sacrificed 24 h after the last injection showed a high follicular epithelium (8.68 ± 0.41 μm) (Table 4); the thyrocytes assumed a cubic shape and the colloid showed many resorption vacuoles (Fig. 4E). After 14 injections of resorcinol at the highest dose and sacrificed 15 days after the last injection, the lizards showed a thyroid with the same morphological aspects (follicular epithelium: 8.72 ± 0.51 μm (Table 4); follicular diameter: 109 ± 13 μm (Table 3) as the lizards treated with the same dose of resorcinol but sacrificed 24 h after the last injection (Fig. 4F).

The body weight of Group I lizards at the highest dose of resorcinol had a decrease compared with that of untreated lizards (control group: 7.81 ± 1.46 vs. treated group: 4.68 ± 1.41g) (Table 1) and thyroid weight increased significantly (control group: 0.208 ± 0.2 vs. treated group: 0.244 ± 0.01g) (Table 2); besides, the body weight is slightly higher (4.72 ± 1.51 g) (Table 1) and thyroid weight is slightly lower (0.290 ± 0.01 g) of Group II lizards than Group I lizards (Table 2).

4. Discussion

The resorcinol has a similar structure to other non-HPCs, such as NP, OP, and Bisphenol A, F, S, known as EDCs. The ecotoxicity of resorcinol has attracted much attention in recent years given its wide use in

Table 2
Thyroid weight (g) change in *Podarcis siculus* subjected to 14 intraperitoneally injections of 0.8–3.9-13.1–36.9 mg/kg/d of Resorcinol and sacrificed 24 h after the last injection (**Group I**) and sacrificed 15 days after the last injection (**Group II**) in the four experimental periods.

Doses (mg/kg/d)	Experimental periods							
	January		March		June		October	
	Group I sacrificed 24 h after the last injection	Group II sacrificed 15d after the last injection	Group I sacrificed 24 h after the last injection	Group II sacrificed 15d after the last injection	Group I sacrificed 24 h after the last injection	Group II sacrificed 15d after the last injection	Group I sacrificed 24 h after the last injection	Group II sacrificed 15d after the last injection
Control Group	0.233 ± 0.13	0.234 ± 0.11	0.125 ± 0.06	0.125 ± 0.06	0.208 ± 0.02	0.208 ± 0.02	0.220 ± 0.13	0.221 ± 0.13
0.8	0.244 ± 0.03	0.241 ± 0.03	0.129 ± 0.01	0.126 ± 0.01	0.212 ± 0.01	0.210 ± 0.01	0.235 ± 0.05	0.230 ± 0.05
3.9	0.269 ± 0.01*	0.261 ± 0.04*	0.167 ± 0.05*	0.163 ± 0.05*	0.232 ± 0.03*	0.230 ± 0.03*	0.255 ± 0.01*	0.250 ± 0.01*
13.1	0.276 ± 0.03**	0.270 ± 0.03**	0.182 ± 0.02**	0.180 ± 0.02**	0.236 ± 0.02**	0.230 ± 0.02**	0.288 ± 0.02**	0.280 ± 0.02**
36.9	0.281 ± 0.01***	0.276 ± 0.01***	0.197 ± 0.01***	0.191 ± 0.01***	0.244 ± 0.01***	0.240 ± 0.01***	0.292 ± 0.01***	0.290 ± 0.01***

(****$p < 0.001$, in the comparison with the different controls). A more detailed description is in the text (see Materials and Methods section).

Table 3
Follicular diameter (μm) change in *Podarcis siculus* subjected to 14 intraperitoneally injections of 0.8–3.9-13.1–36.9 mg/kg/d of Resorcinol and sacrificed 24 h after the last injection (**Group I**) and sacrificed 15 days after the last injection (**Group II**) in the four experimental periods.

Doses (mg/kg/d)	Experimental periods							
	January		March		June		October	
	Group I sacrificed 24 h after the last injection	Group II sacrificed 15d after the last injection	Group I sacrificed 24 h after the last injection	Group II sacrificed 15d after the last injection	Group I sacrificed 24 h after the last injection	Group II sacrificed 15d after the last injection	Group I sacrificed 24 h after the last injection	Group II sacrificed 15d after the last injection
Control Group	462 ± 52	465 ± 50	232 ± 30	235 ± 32	244 ± 26	248 ± 25	362 ± 52	366 ± 56
0.8	420 ± 45	424 ± 45	222 ± 51	225 ± 41	262 ± 31	273 ± 33	268 ± 41	272 ± 51
3.9	379 ± 54**	399 ± 53**	177 ± 35**	174 ± 25**	172 ± 23**	182 ± 24**	198 ± 12**	199 ± 13**
13.1	216 ± 37***	221 ± 22***	156 ± 45***	156 ± 45***	123 ± 42***	129 ± 42***	168 ± 12***	149 ± 23***
36.9	126 ± 51****	160 ± 25****	102 ± 12****	106 ± 16****	103 ± 22****	109 ± 20****	108 ± 12****	109 ± 13****

(****$p < 0.001$, in the comparison with the different controls). A more detailed description is in the text (see Materials and Methods section).

Table 4
Epithelial height (μm) change in *Podarcis siculus* subjected to 14 intraperitoneally injections of 0.8–3.9-13.1–36.9 mg/kg/d of Resorcinol and sacrificed 24 h after the last injection (**Group I**) and sacrificed 15 days after the last injection (**Group II**) in the four experimental periods.

Doses (mg/kg/d)	Experimental periods							
	January		March		June		October	
	Group I sacrificed 24 h after the last injection	Group II sacrificed 15d after the last injection	Group I sacrificed 24 h after the last injection	Group II sacrificed 15d after the last injection	Group I sacrificed 24 h after the last injection	Group II sacrificed 15d after the last injection	Group I sacrificed 24 h after the last injection	Group II sacrificed 15d after the last injection
Control Group	2.15 ± 0.06	2.16 ± 0.05	6.10 ± 0.02	6.06 ± 0.06	8.1 ± 0.04	8.1 ± 0.04	3.81 ± 0.06	3.81 ± 0.06
0.8	2.20 ± 0.03	2.24 ± 0.05	6.22 ± 0.51	6.25 ± 0.41	8.62 ± 0.01	8.73 ± 0.03	4.68 ± 0.41	4.72 ± 0.51
3.9	3.79 ± 0.04**	3.99 ± 0.03**	7.17 ± 0.55**	7.24 ± 0.45**	9.72 ± 0.33**	9.82 ± 0.41**	5.68 ± 0.12**	5.89 ± 0.13**
13.1	5.16 ± 0.07***	5.21 ± 0.22***	8.62 ± 1.02***	8.68 ± 1.06***	10.3 ± 0.02***	10.9 ± 0.02***	6.48 ± 0.12***	6.69 ± 0.13***
36.9	6.26 ± 0.51****	6.60 ± 0.25****	9.21 ± 0.35****	9.35 ± 0.45****	15.1 ± 1.01****	15.5 ± 1.22****	8.68 ± 0.41****	8.72 ± 0.51****

(****$p < 0.001$, in the comparison with the different controls). A more detailed description is in the text (see Materials and Methods section).

consumer products (Acconcia et al., 2017; Forte et al., 2016, 2019; In et al., 2015; Liu et al., 2017, 2019; Ismanto et al., 2022; Pasquier et al., 2023) such that its global demand has increased to 10.6 million tons by the end of 2022 (Hercog et al., 2019).

Considering this significant increase, more attention should be paid to the accumulation of non-HPCs in aquatic and terrestrial environments and food chains to protect public and environmental health. EDCs alter the endocrine system in a timely and often non-monotonic manner posing a huge challenge because the endocrine system regulates almost all-physiological processes (Sciarrillo et al., 2010, 2022; De Falco et al., 2014; Di Lorenzo et al., 2021). Normally, the study focuses on the effects of such chemicals on male and female reproductive functions; the thyroid came to the fore much later, despite its influence in the growth, development and metabolism of vertebrate. Thyroid function is widely conserved among vertebrate species (Mullur et al., 2014; Sciarrillo et al., 2000, 2010, 2022) through the hypothalamus-pituitary-thyroid (HPT) axis in which the hypothalamus releases TRH to stimulate the anterior pituitary gland to produce TSH, which in turn stimulates the production of THs (T_3 and T_4) by the thyroid gland. THs are transported through various blood proteins to peripheral tissues (liver) via membrane transporters where, in the case of T_4, type 1 or type 2 ″outer ring" deiodinases, Dio 1 and Dio2, respectively, remove an iodine to peripherally convert it to T_3. This triiodinated form then binds to TRα or TRβ nuclear receptors, encoded by the *thra* and *thrb* genes, respectively, which regulate the expression of TH-responsive genes (Mullur et al., 2014; Virgilio et al., 2004; Sciarrillo et al., 2000, 2022). T_4 can also bind, albeit with lower affinity, to these TH receptors and recruit different cofactors, thus allowing for a differential gene expression (Virgilio et al., 2004; Schroeder et al., 2014).

In a recent study, Sciarrillo et al. (2021) demonstrated that lizards exposed to OP alone and in combination with NP affect THs activity through the regulation of multiple targets within the complex regulatory network of thyroid hormone metabolism and activity. This mechanism mediates gene regulation in response to T_3 deiodinase, which catalyzes the deiodination of T_4 to be converted to the biologically active form T_3 biologically active, and the HPT axis, which contains TRH-TSH-THs negative feedback (Sciarrillo et al., 2021).

To the authors' knowledge, no published data are available on the adverse effects of intraperitoneal administration of resorcinol on the thyroid gland and particularly on the hypothalamic-pituitary-thyroid axis on reptiles. The main objective of our study is to try to clarify the discrepancies between human clinical studies and experimental animal studies in order to evaluate the adverse effects on the thyroid gland. More specifically, we sought to shed light on why and how resorcinol is considered a "*substance of very high concern*" because of its toxic properties on the thyroid gland (ECHA, 2020). In addition, with reptiles and lizards in particular, our goal was also to provide insights for further improvements in the regulatory framework for the evaluation and testing of EDCs. For this study, *P siculus* was chosen as the sentinel species for ecotoxicological assessment of resorcinol because of its ability to distribute in a multitude of habitats, its longevity and its marked response to the adverse effects of environmental contaminants (Verderame et al., 2019; Sciarrillo et al., 2021, 2022).

Our analysis has shown that intraperitoneal administration of different doses of resorcinol at various stages of thyroid gland activity causes histological alterations of the thyroid gland (follicular cell height increase and colloid area decrease) and increase in thyroid weight in experimental animals, accompanied by a decrease in plasma levels of THs. Therefore, the effects of resorcinol on the thyroid gland are believed to be due to the inhibition of thyroid peroxidase (TPO) enzymes and the consequent disruption of TH synthesis. These results in a continuous and prolonged elevation of TSH and TRH. In this context, the

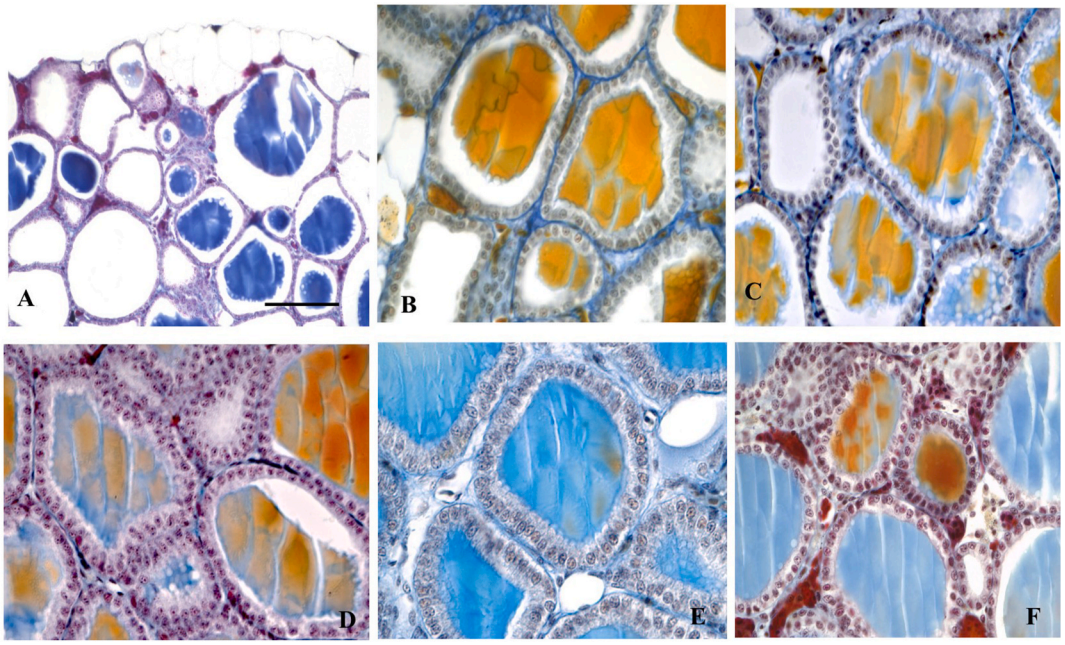

Fig. 3. Thyroid gland of lizard *Podarcis siculus* (stain Galgano I): scale bar: 20 μm. (**A**) Control lizard of the winter stasis (**January**): the follicular epithelium is low and the colloid is retracted with few reabsorption vacuoles. (**B**) Specimen treated with14 intraperitoneally injections of 36.9 mg/kg/d of Resorcinol and sacrificed 24 h after the last injection (**Group I**): the thyrocytes had still a cubic shape and colloid showed various reabsorption vacuoles. (**C**) Specimen treated with14 intraperitoneally injections of 36.9 mg/kg/d of Resorcinol and sacrificed 15 days after the last injection (**Group II**): the follicular epithelium is higher than normal, but the colloid is present in the follicles with an evident vascularization of the gland. (**D**) Control lizard of the recovery period (**March**): the thyroid follicles showed a medium-high follicular epithelium and the colloid present numerous reabsorbing vacuoles. (**E**) Specimen treated with14 intraperitoneally injections of 36.9 mg/kg/d of Resorcinol and sacrificed 24 h after the last injection (**Group I**): the thyrocytes had a cylindrical shape, and the colloid showed many resorption vacuoles. (**F**) Specimen treated with14 intraperitoneally injections of 36.9 mg/kg/d of Resorcinol and sacrificed 15 days after the last injection (**Group II**): the follicular epithelium is still higher than normal, the colloid present an evident reabsorbing vacuoles.

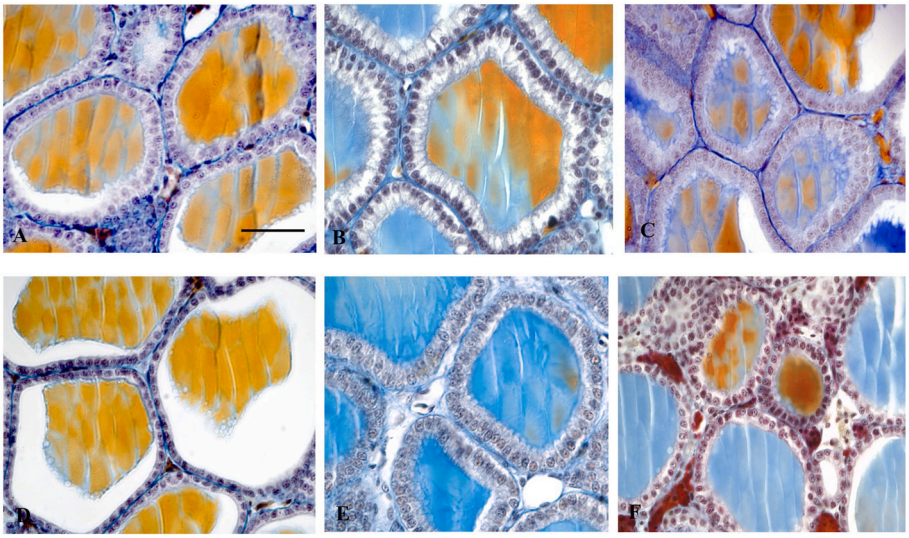

Fig. 4. Thyroid gland of lizard *Podarcis siculus* (stain Galgano I): scale bar: 20 μm. (A) Control lizard of the activity period (**June**): The thyrocytes have a prominent continuous epithelium, the chromophobe droplets were seen in the colloid at the periphery. (**B**) Specimen treated with14 intraperitoneally injections of 36.9 mg/kg/d of Resorcinol and sacrificed 24 h after the last injection (**Group I**): the cuboidal follicular epithelial cells, the colloid, and the reabsorption vacuoles are shown. (**C**) Specimen treated with14 intraperitoneally injections of 36.9 mg/kg/d of Resorcinol and sacrificed 15 days after the last injection (**Group II**): the follicular epithelium is high and the colloid is present in the follicles with an evident vascularization of the gland. (**D**) Control lizard of the start of the winter stasis (**October**): the thyroid gland begins to reduce its secretory activity; in fact, the height of the follicular epithelium is reduced and a follicular diameter is increased. (**E**) Specimen treated with14 intraperitoneally injections of 36.9 mg/kg/d of Resorcinol and sacrificed 24 h after the last injection (**Group I**): the thyrocytes assume a cubic shape and the colloid showed many resorption vacuoles. (**F**) Specimen treated with14 intraperitoneally injections of 36.9 mg/kg/d of Resorcinol and sacrificed 15 days after the last injection (**Group II**): the follicular epithelium is still higher than normal, the colloid present an evident reabsorbing vacuoles.

evaluation of resorcinol was also considered in peripheral tissues where thyroid hormones exert their action, such as the liver. Indeed, the expression of hepatic outer-loop Dio2 deiodinase decreased with the reduction of hepatic T_3 and T_4 content.

Our analysis focused on demonstrating the ability of resorcinol to alter thyroid function in non-mammalian vertebrates in order to know a plausible link between the adverse effect and the mechanism of action. The action of resorcinol on lizard thyroid function is clearly based on the inhibition of TPO activity resulting in a drastic reduction of plasma levels of thyroid hormones causing high and prolonged TSH release with consequent pictures of morphological stimulation of glandular tissue. In fact, thyroid glands in these lizards have reduced thyroid follicle size but follicular epithelial cells still express proteins primarily regulated by TSH and express colloid into the follicle lumen.

Such action of resorcinol on TPO has been shown in several *in vitro* studies using purified porcine thyroid TPO, human thyroid cell lines, rat thyroid microsomal fractions, or various cell lines transfected with the human TPO gene and with different substrates (Cooksey et al., 1985; Lindsay et al., 1992; Paul et al., 2014; Paul Friedman et al., 2016; Dong et al., 2020).

Resorcinol can therefore influence thyroid physiology in adult lizards via multiple mechanisms at the thyroid, pituitary, and hypothalamic levels as well as by altering the expression of peripheral tissue deiodinases.

Therefore, the reversibility, albeit minimal, of these effects when resorcinol treatment is discontinued for 15 days (Group II) can be considered tangible evidence of the cause-and-effect relationship between resorcinol exposure and the effects on thyroid disruption.

Our findings are in agreement with the *in vivo* data in humans and rodents (see review by Pasquier et al., 2023) and that therefore, resorcinol in reptiles may meet the WHO definition of ECDs.

Overall, the available rodent and human data suggest that either resorcinol can alter thyroid function when administered orally or parenterally "physiologically"; therefore, there are no major discrepancies between the few animal and human studies regarding the mode of action of resorcinol on thyroid function. Our analysis examined the regulation of thyroid function by resorcinol in reptiles, contrary to the general assumption that rodents are more sensitive to thyroid-disrupting chemicals than both humans and other non-mammalian vertebrates (Lynch et al., 2002). Our data on resorcinol suggested otherwise, showing that reptiles are more sensitive to TPO inhibition probably due to the pharmacodynamic difference in resorcinol, rather than to TPO-dependent iodine organification, which is one of the most conserved events in TH biosynthesis among vertebrates, Furthermore, given the presence in soil and water of resorcinol, an additional the goal of this work was to create interference strategies between human and rodent epidemiological and clinical studies with environmental studies in non -mammalian animal models regarding the definition of environmental endocrine disruptor.

5. Conclusion

It is noteworthy that in the case of resorcinol, animal studies have been limited, that more often experimental procedures have not been able to lead to clear conclusions about non-HPCs effects, and any related adverse effects.

In conclusion, our data showed that, even with discrepancies with experimental data in animals and based on experimental data in humans and rodents, in reptiles and particularly in the lizard, resorcinol meets the definition of EDCs and causes alteration of the thyroid gland with inhibition of TPO activity resulting in inhibition of iodine organification with a significant decrease in plasma levels of THs. In addition, this resorcinol-induced severe hypothyroidism causes a negative feedback on the hypothalamic-pituitary axis with a significant increase in serum TRH and TSH levels. This increase in TSH concentrations results in increased follicular epithelium height and stimulation in depletion of colloid resorption vacuoles with mild hyperplasia of the thyroid gland. Finally, the symptoms of severe induced hypothyroidism are evidenced by the reduced locomotor activity found mainly in lizards treated with the highest dose of resorcinol.

Author's statement

All authors have contributed to the manuscript that they approve for publication. The manuscript has no conflict of interest. In agreement with my co-authors, I declare that the submitted work is original research that has not been previously published and is not under consideration for publication elsewhere, in whole or in part.

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CRediT authorship contribution statement

Rosaria Sciarrillo: Writing – review & editing, Writing – original draft, Project administration. **Alessandra Falzarano:** Methodology, Investigation, Formal analysis. **Vito Gallicchio:** Methodology, Investigation. **Francesca Carrella:** Methodology, Investigation. **Teresa Chianese:** Methodology, Investigation. **Aldo Mileo:** Software, Methodology, Investigation. **Maria De Falco:** Supervision.

Declaration of competing interest

The authors have no competing financial interests or personal relationships that could influence the work reported in this article.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2024.143009.

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