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The Effects of the Fungicide Methyl Thiophanate on Adrenal Gland Morphophysiology of the Lizard, *Podarcis sicula*

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Abstract. Endocrine-disrupting chemicals (EDCs) are a large group of substances able to modulate endocrine-signaling pathways, altering the normal function of the endocrine system. Although the fungicide methyl thiophanate (MT) is not considering a specific reproductive and developmental toxicant, it can induce histopathological damages in rat thyroid and adrenal glands that have a pivotal role in both processes. We investigated the MT effects on adrenal glands of *Podarcis sicula* lizard, the endemic species of Southern Italy living in open country and in cultivated fields. Reptiles are good bioindicators because they are easily harvested; they have a wide distribution and large populations. Moreover, they have good sensitivity to contaminants, and bioaccumulate and biomagnify pollutants to levels equal to or greater than those of birds and mammals. We used 1.5% MT/water to pollute terraria, food, and water twice a week for 15 and 30 days, and we evaluated adrenal toxicity through biochemical (adrenal and pituitary hormone plasma levels) and histological parameters (adrenal gland histopathology). We demonstrated a time-dependent increase of corticosterone plasma levels and a decrease of ACTH plasma levels, a hypertrophy of the steroidogenic tissue, and an enlargement of blood capillaries. Moreover, we observed a time-dependent increase of adrenaline plasma levels and adrenaline-producing cells, and an opposite trend of noradrenaline plasma concentrations. We also observed lymphocyte and macrophage infiltrations, signs of cell degeneration. Our findings on the bioindicator *P. sicula* provide an interesting basis to further elucidate the systemic mechanisms of EDCs.

Endocrine-disrupting chemicals (EDCs) include synthetic compounds able to change, mimic, or antagonize the normal functioning of the endocrine system by interfering with the synthesis, metabolism, and receptor binding and cellular responses of endogenous hormones (Neubert 1997). They include some common environmental contaminants such as pesticides, plastic ingredients, dioxins, and biocides. While this issue has gained high visibility in the scientific and in the public communities, the overall impact on human health and the environment is still unclear (Gelbke *et al.* 2004).

The main risk of EDCs is the ability to induce subtle effects, also at lower dose levels than those typically used in the standard testing paradigm of the U.S. EPA (Environmental Protection Agency) (Vadenbergh 2004), leading to detrimental effects in the reproductive and developmental processes (Choi and Jeung 2003) and also to cause tumorigenicity or teratogenicity (Maranghi *et al.* 2003). Moreover, it has been demonstrated that human exposure to EDCs results from dietary intake of these compounds as trace contaminants in food or high levels of endocrine-active phytochemicals in fruits, nuts, and vegetables (Safe 2005).

Among chemical compounds of environmental interest, methyl thiophanate (MT), a major member of the thiophanate derivatives, is widely used to control important fungal diseases of crops because it possesses a broader range of activity than most other available fungicides (Traina *et al.* 1998; Maranghi *et al.* 2003). MT is well absorbed by oral administration and distributed throughout the organism. It is metabolized by animals into benzimidazole compounds, including methyl-2-benzimidazole carbamate (carbendazim), through a cyclizing cleavage of the side chains (Traina *et al.* 1998; Maranghi *et al.* 2003). Although MT is not considered a specific developmental and reproductive toxicant, several studies on rodents showed that this fungicide is able to induce histopathological damages in thyroid and adrenal glands, which have a pivotal role in both processes (Barlas *et al.* 2002).

The evaluation of the ''biological impact'' of contaminants is of critical importance and the concept of ''bioindicator organism'' has become of great interest. Among several organisms, it has been shown that reptiles are species historically used as ''bioindicators'' because of their persistence in a variety of habitats, wide geographic distribution, their longevity and, in many cases, site fidelity (Crain and Guillette 1998). Additionally, reptiles exhibit sensitivity to contaminants similar to that reported for

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birds and mammals (Hall and Clark 1982); moreover, they are able to bioaccumulate and to biomagnify contaminants to levels equal to or greater than that reported for birds and mammals Bryan *et al.* 1987; Hall and Henry 1992).

The aim of the present study was to study the effects of MT exposure on adrenal glands of lizard *Podarcis sicula* by biochemical and histochemical approaches. This species has been chosen because it is the most abundant species in Southern Italy, living in the open country and in cultivated fields.

Moreover, although the majority of research in the field of endocrine toxicology has focused on estrogenicity (Harvey and Everett 2003), more recently it has been shown that there are many processes and toxicological targets in the endocrine system that could be subjected to chemical disruption and have equally important potential consequences for human health. In light of these preliminary observations, our attention has been focused on the adrenal gland for its central role in reproduction and development.

Materials and Methods

Compound

MT, working standard purity 96.2% (with adjuvant and thinners up to 100%), was obtained from SIP-CAM (Milan, Italy).

Animals and Housing Conditions

All the experiments have been carried out in compliance with the ethical provisions enforced by the European Union and authorized by the National Committee of the Italian Ministry of Health on *in vivo* experimentation. Adult specimens of *P. sicula* (14–16 g body weight) of both sexes were captured in the neighborhood of Isclero River (Benevento, Italy) in late November. After capture, lizards were housed in large soil-filled terraria (width 30 × length 60 × height 50 cm), all containing 5 kg of heather, and exposed indoor to natural temperature (8–12°C) and photoperiod (11 h daylight). Water dishes were present in the terraria, and the animals were fed on live fly larvae daily. Before beginning experimental procedures, captivity lasted twenty days to reverse capture-related stress (Manzo *et al.* 1994).

Experimental Procedure

The specimens were divided into four groups, each consisting of 20 animals (10 males and 10 females) as follows:

Group A: control animals housed for 15 days in nonpolluted terraria treated twice a week with 100 ml of water by spraying.

Group B: animals housed for 15 days in terraria in which heather, water, and larvae were polluted by spraying twice a week with 100 ml of 1.5% MT (1.5 g of MT in 100 ml of water).

Group C: animals housed for 30 days in terraria in which heather, water, and larvae were polluted twice a week with 100 ml of 1.5% MT (1.5 g of MT in 100 ml of water).

Group D: control animals housed for 30 days in nonpolluted terraria treated twice a week with 100 ml of water by spraying.

For each group, five terraria containing two male and two female lizards were prepared.

Hormone Assay

Blood samples, collected by intracardiac puncture into heparinized capillaries, were centrifuged at 1500 rpm for 10 min to obtain plasma. Corticosterone was measured in 100 µl of plasma using a sensitive and highly specific radioimmunoassay kit (ICN Biomedical, Inc., Costa Mesa, CA). Before assay, plasma samples were diluted one-third with the kit diluents and heated at 80°C for 10 min in order to inactivate corticosterone-binding proteins. The minimum concentration per tube distinguishable from zero was 0.176 $pg.mL^{-1}$ as determined by the kit manufacturer. Cross-reactivities of the corticosterone antiserum with other steroids were 6.1% for deoxycorticosterone; <1% for progesterone and cortisol; <0.1% for aldosterone, 20-α-dehydroprogesterone, testosterone, and 11-deoxycortisol; and <0.01% for all the other steroids examined. Inter- and intra-assay coefficients of variation were 5.8 and 3.4%, respectively. Cross-reactivities of the ACTH antiserum as determined by the kit manufacturer were 0.03% for α-MSH, 0.01% for β-MSH, and 0.02 for β-endorphin. ACTH concentrations were measured in 100 µl of plasma by a two-site immunoradiometric assay using mouse monoclonal antibodies (Diagnostic Products Corp.) as previously described by De Falco *et al.* (2004). Sensitivity was 0.1 $pg.mL^{-1}$ as determined by the kit manufacturer, and the inter- and intra-assay coefficients of variation were 10% and 6%, respectively. Catecholamines were extracted from plasma by the alumina adsorption method, and plasma adrenaline and noradrenaline levels were measured by high-performance liquid chromatography (HPLC) with electrochemical detection (Woodward 1982). The alumina-extracted samples (200 µl) were passed through an Ultratechsphere ODS-C18 5 µm column (HPLC Technology Ltd.), using a catecholamine and metanephrine mobile phase (Chromsystem Munich, Germany). The separated amines were integrated by the Star Chromatography software program (version 4.0, Varian). Concentrations were calculated relative to appropriate standards and with 3,4-dihydroxybenzylalamine hydrobromide as an internal standard in all determinations, with a detection limit of 0.1 nmol L^{-1}. Plasma catecholamine concentrations were expressed as $pg.mL^{-1}$.

Light Microscopy

The animals were anesthetized by hypothermia, and then killed by decapitation, and their adrenals fixed in a mixture of 2.5% potassium dichromate, 1% anhydrous sodium sulphate (buffered at pH 4.1 with 5 M acetate buffer), and 10% formaldehyde as previously described (De Falco *et al.* 2003). They were then embedded in paraplast, cut into 7-µm sections, affixed to albuminized slides, and stained with each one of the following solutions: 1) a mixture of eosine aniline blue, buffered at pH 4 with 5 M acetate buffer (Wood 1963); 2) Giemsa solution, modified according to Pearse (Pearse 1960); and 3) Mallory trichromic stain.

Morphometry

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. In 20 specimens from each experimental group, processed for L.M., adrenaline cell, steroidogenic cord diameter, and lymphocyte-macrophage

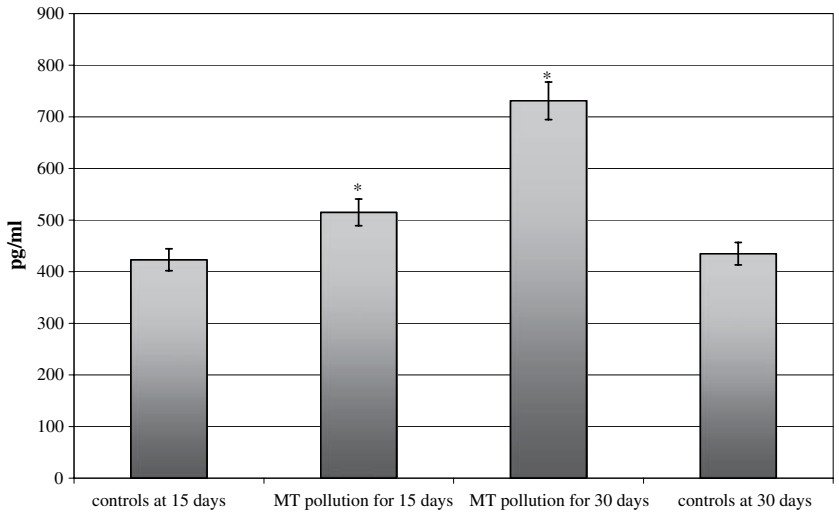

Fig. 1. Corticosterone plasma levels of *Podarcis sicula* lizards. Variations of corticosterone plasma levels (pg/ml) between controls and treated animals. Vertical lines show S.E.M. Values in the treated groups significantly ($p < 0.05$) differ from the control values

cell count were calculated in every tenth longitudinal section from the whole gland of each specimen. All the morphometric evaluations were performed by five observers separately. The level of concordance, expressed as the percentage of agreement between the observers, was 96%. In the remaining specimens the opinions of the three investigators in agreement were taken into consideration. The controls and experimental data of all the groups were tested together for significance using Student's *t* test.

Statistical Analysis

The data obtained from each specimen were average prior to calculating experimental group mean and the standard error of the mean. As revealed by the χ^2 test, the data were not different from normal distribution. The control and experimental data of all the groups were tested together for significance using one-way analysis of variance, 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

Plasma Levels of Lizard Adrenal Hormones After Methyl Thiophanate In Vivo Treatment

We showed in treated lizards a time-dependent increase of corticosterone plasma levels that varied from a value of 423 ± 21 pg/ml in the control specimens at 15 days to a value of 515 ± 26 pg/ml ($p < 0.05$) after 15 days of treatment to a value of 731 ± 37 pg/ml ($p < 0.05$) after 30 days of treatment with MT respect to a value of 435 ± 30 pg/ml in the control specimens at 30 days (Figure 1). In order to better understand the significance of corticosterone plasma level increase, we also measured ACTH plasma levels during MT treatment to better determine whether MT could directly or indirectly act on the adrenal glands. Contrary to corticosterone response to MT, we observed a strong decrease of ACTH plasma levels varying from a value of 3.65 ± 0.2 pg/ml in the control specimens at 15 days to a value of 2.45 ± 0.12 pg/ml ($p < 0.05$) in the specimens sacrificed after 15 days to a value of 1.34 ± 0.10 pg/ml ($p < 0.05$) after 30 days of exposure with MT against a value of 3.74 ± 0.3 pg/ml of the control specimens at 30 days (Figure 2). At the same time, in order to have a more deep view of lizard adrenal gland function after treatment with MT, we monitored plasma levels of catecholamines, adrenaline, and noradrenaline. Specifically, we observed variations of both catecholamine plasma concentrations showing a directly proportional increase of adrenaline plasma levels that varied from a value of 205 ± 10 pg/ml in the control specimens at 15 days to a value of 321 ± 16 pg/ml ($p < 0.05$) after 15 days until a value of 456 ± 22 pg/ml ($p < 0.05$) after 30 days of treatment with respect to a value of 188 ± 10 pg/ml in the control specimens after 30 days (Figure 3). A trend opposite to that of adrenaline was observed for noradrenaline plasma levels because they decreased from a value of 315 ± 15 pg/ml of the controls at 15 days to a value of 276 ± 13 pg/ml ($p < 0.05$) after 15 days, reaching a minimum value of 174 ± 9 pg/ml ($p < 0.05$) after 30 days of treatment with MT against a value of 336 ± 16 pg/ml of the controls at 30 days (Figure 3). No sex-related difference was observed in each experimental group.

Histology of the Lizard Adrenal Glands After In Vivo Treatment with Methyl Thiophanate

To further elucidate our biochemical data, we investigated the effects of MT on lizard adrenal gland morphology. The reptilian adrenal glands are formed by both steroidogenic and chromaffin tissues closely connected to each other (Laforgia *et al.* 1991). Particularly, chromaffin tissue, producing both adrenaline and noradrenaline, forms a dorsal ribbon surrounding steroidogenic parenchyma in which chromaffin cells insert their interdigitations (Figure 4a). Adrenaline cells also form islets scattered in steroidogenic cords. In the control specimens, the A/NA cell ratio is 0.7 (Table 2). We showed that MT treatment induced several morphological modifications directly proportional to the exposure time. Specifically, we observed that after 15 days of treatment, the 85% of polluted adrenal glands showed steroidogenic cords increased in diameter

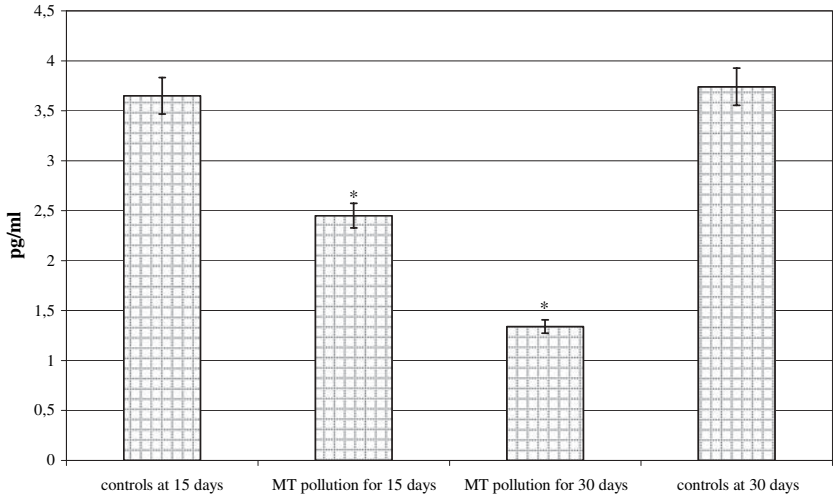

Fig. 2. ACTH plasma levels of *Podarcis sicula* lizards. Variations of ACTH plasma levels (pg/ml) between controls and treated animals. Vertical lines show S.E.M. Values in the treated groups significantly ($p < 0.05$) differ from the control values

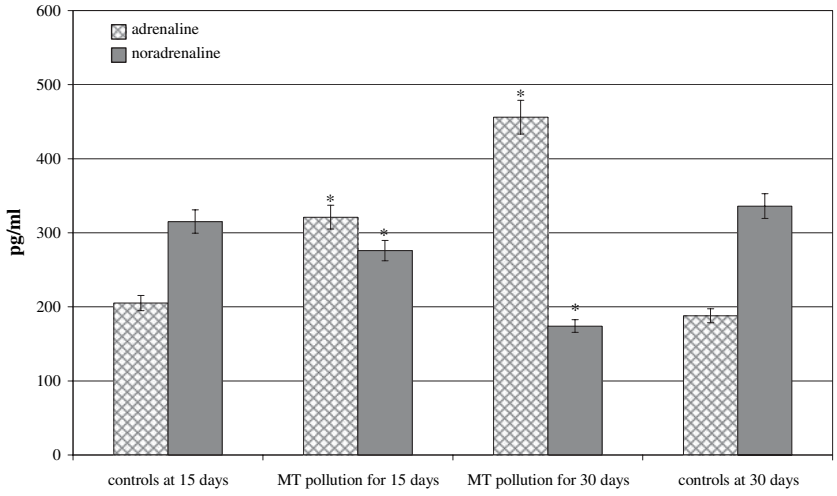

Fig. 3. Adrenaline and noradrenaline plasma levels of *Podarcis sicula* lizards. Variations of adrenaline and noradrenaline plasma levels (pg/ml) between controls and treated animals. Vertical lines show S.E.M. Values in the treated groups significantly ($p < 0.05$) differ from the control values

(Table 1) with respect to the control specimens at 15 days, and there were more enlarged blood capillaries (Figure 4b and c). Moreover, we observed in the 88% of polluted adrenal glands an increase of adrenaline cells (Table 1) that occupied the outer layers of the dorsal chromaffin ribbon normally formed by noradrenaline cells (Figure 4d). In fact, after 15 days of exposure, the A/NA cell ratio increased to 1.9 (Table 2). After 30 days of treatment, the 93% of polluted adrenal glands showed hypertrophic steroidogenic cords (Tables 1 and 2), formed by swollen cells (Figure 4e) with respect to the control specimens at 30 days. Moreover, we observed a greater enlargement of blood capillaries that occupied the central portion of steroidogenic parenchyma (Figure 4f) and a strong increase of adrenaline cells compared to a decrease of noradrenaline cells in the 95% polluted adrenal glands (Table 1). Specifically, after 30 days of exposure, the A/NA cell ratio became 2.3 (Table 2). In addition, adrenaline cells showed a weaker staining because of degranulation and subsequent hormone secretion. In addition, some chromaffin cells appeared to contain both noradrenaline and adrenaline (Figure 4f). Intriguingly, we observed that MT treatment induced already after 15 days a lymphocyte infiltration more evident between chromaffin layers (Figure 5a and b) in the 78% of polluted adrenal glands (Table 1). Moreover, after 30 days of treatment it was possible to observe a great number of macrophages scattered between chromaffin cells and near blood vessels (Figure 5c and d) in the 95% of polluted adrenal glands (Table 1). No lymphocyte or macrophage infiltrations were observed in control specimens at 15 and 30 days. No sex-related difference was observed in each experimental group.

Discussion

In the last few years, EDCs have received a considerable attention. There are several mechanisms of endocrine disruption (Sanderson *et al.* 1999; Sanderson *et al.* 2000; Sanderson *et al.* 2001; Hilscherova *et al.* 2004; Xu *et al.* 2006) that are often exerted indirectly via effects on common signal transduction pathways, or by acting as direct or indirect stimulators or inhibitors of the enzymes involved in the production, transformation, or elimination of steroid hormones (Baker 2001; Xu *et al.* 2006). In this context, the adrenal cortex is unique in its biosynthetic capabilities and so represents the most common target for toxicity in the endocrine system

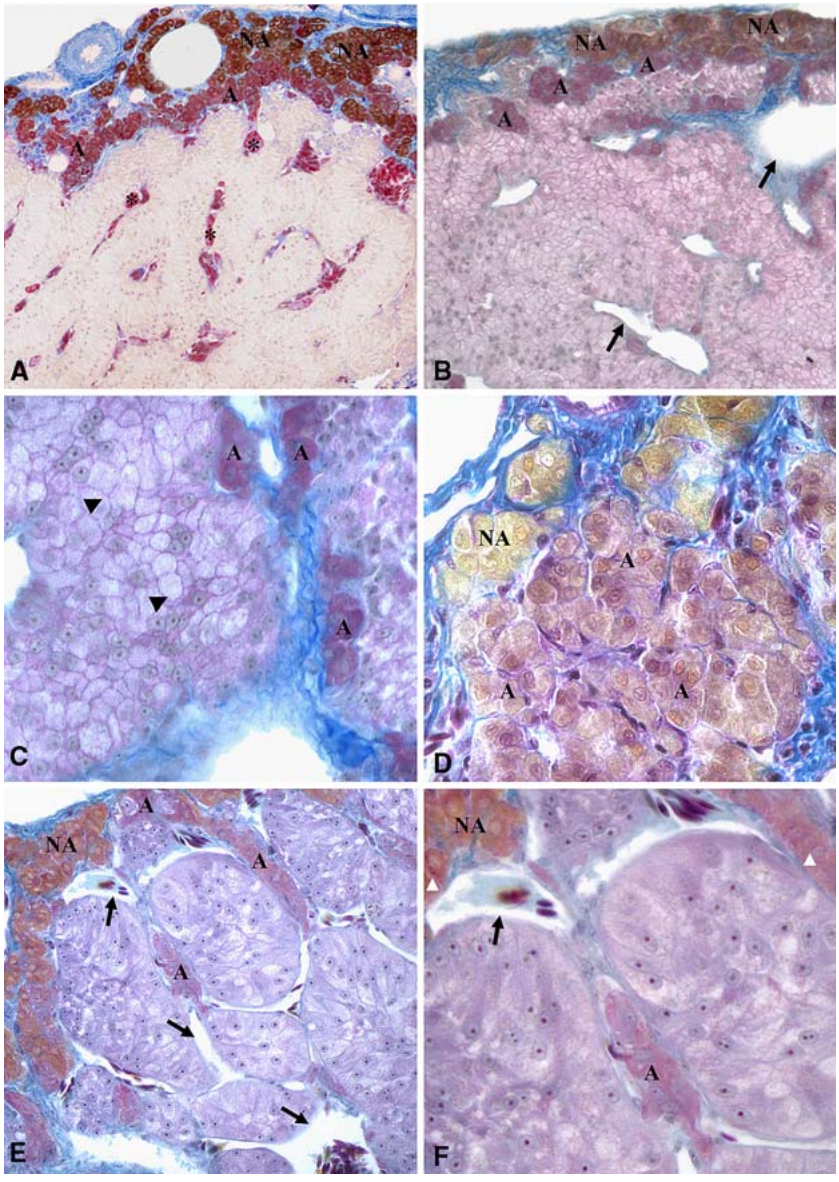

Fig. 4. *Podarcis sicula* adrenal glands exposed with methyl thiophanate (MT). (**a**) Control adrenal gland formed by a chromaffin dorsal ribbon of noradrenaline and adrenaline cells on steroidogenic parenchyma. A cells also form islets in steroidogenic parenchyma (stars). Mallory trichromic stain. (150×); (**b**) Stimulated steroidogenic cords and increased vascularization (arrows) in 15-day exposed glands. Mallory trichromic stain. (150×); (**c**) Greater magnification of 15-day exposed adrenal glands showing swollen steroidogenic cells (arrowheads). Mallory trichromic stain. (640×); (**d**) Increase of adrenaline cells in 15-day exposed glands. Mallory trichromic stain. (640×); (**e**) Enlarged steroidogenic cords and strong vascularization (arrows) of the gland exposed to MT for 30 days. A cells reached outer layer of chromaffin ribbon. Mallory trichromic stain. (300×); (**f**) Higher magnification showing enlargement of steroidogenic cords. Note the slighter staining of A cells to indicate a degranulation and the presence of mixed chromaffin cells containing both adrenaline and noradrenaline (white arrowheads). Arrow indicates vascularization. Mallory trichromic stain. (640×). For all the images: A = adrenaline cells; NA = noradrenaline cells

Table 1. Incidence of morphological alterations of lizard adrenal glands exposed to methyl thiophanate (MT)

Histological data	Controls at 15 days (n = 20)	MT exposure for 15 days (n = 20)	MT exposure for 30 days (n = 20)	Controls at 30 days (n = 20)
Steroidogenic cord enlargement	0/20 (0%)	17/20 (85%)[a]	18/20 (88%)[a]	0/20 (0%)
Adrenaline cell increase	0/20 (0%)	18/20 (88%)[a]	19/20 (95%)[a]	0/20 (0%)
Lymphocyte/macrophage infiltration	1/20 (0.2%)	15/20 (78%)[a]	19/20 (95%)[a]	3/20 (0.6%)

Number (%) of lizards that presented steroidogenic enlargement, adrenaline cell increase, and infiltration of lymphocyte and macrophages on the total specimens treated for 15 and 30 days with MT and controls sacrificed at 15 and 30 days
[a] Significantly ($p < 0.05$) different from control values

(Ribelin 1984; Colby 1994; Harvey and Everett 2003) because of its large blood supply, its lipophilicity that allows the accumulation of lipophilic compounds, its high concentration of cytochrome P450 that can also bioactivate toxicants, and its capacity to synthesize all major classes of steroids (androgens, estrogens, progesterone, as well as glucocorticoids and mineralcorticoids) (Harvey and Everett 2003; Xu *et al.* 2006).

Despite the importance of adrenocortical function and steroidogenesis to human health, the effects of many EDCs on adrenocortical function are surprisingly still missing in current risk assessment programs (Harvey and Everett 2003; Xu *et al.* 2006). In the present study, we investigated the *in vivo* effects of the fungicide MT on adrenal glands of the lizard *P. sicula* from both the biochemical and histological point of view. Although

Table 2. Histomorphometric examination of adrenal glands of lizards exposed to methyl thiophanate (MT)

Histomorphometric data	Controls at 15 days (n = 20)	MT exposure for 15 days (n = 20)	MT exposure for 30 days (n = 20)	Controls at 30 days (n = 20)
Steroidogenic cord diameter	90μm	165μm[a]	210μm[a]	90μm
A/NA cell ratio	456/651 = 0.7 ± 0.02	765/397 = 1.9 ± 0.08[a]	699/301 = 2.3 ± 0.11[a]	450/643 = 0.7 ± 0.02

[a] Significantly ($p < 0.05$) different from control values

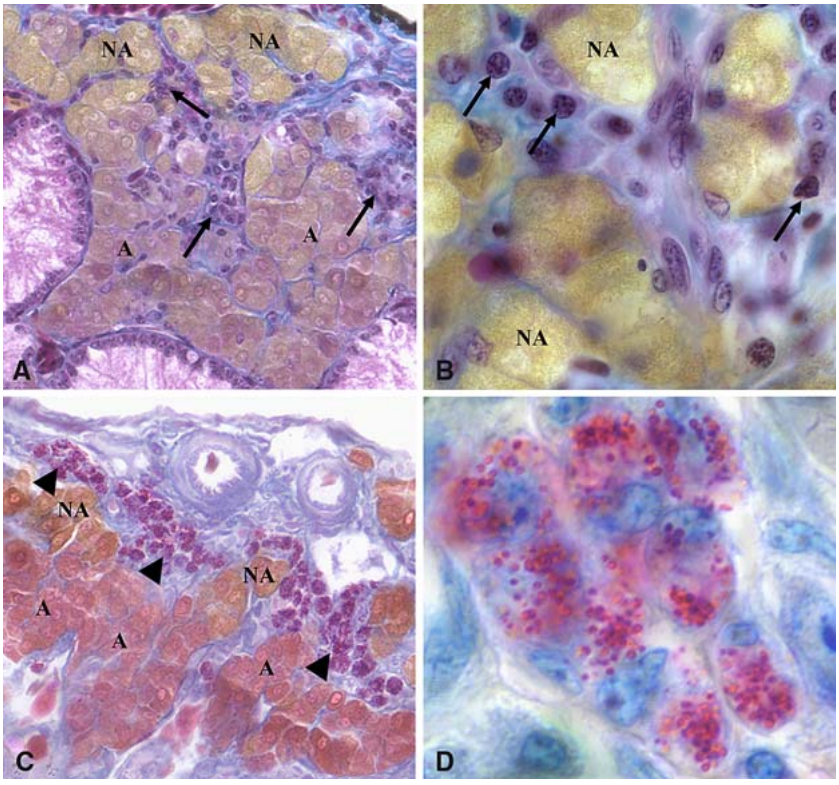

Fig. 5. Signs of cell degeneration in *Podarcis sicula* adrenal glands exposed with methyl thiophanate. (**a**) Lymphocyte infiltration (arrows) evident between chromaffin layers. Mallory trichromic stain (640×); (**b**) Higher magnification showing lymphocyte infiltration (arrows) between chromaffin cells. Mallory trichromic stain (1600×); (**c**) Macrophage infiltration (arrowheads) inside chromaffin dorsal ribbon and closed to blood vessels. Mallory trichromic stain (600×); (**d**) Higher magnification showing a cluster of macrophages marked by numerous intracytoplasmic granulations. Giemsa stain. (1600×). For all the images: A = adrenaline cells; NA = noradrenaline cells

the anatomy is very different among classes of vertebrates, physiologically the adrenal hormones produced are equivalent in vertebrates; in fact, in *P. sicula* lizard, the chromaffin tissue produces both adrenaline and noradrenaline hormones and the steroidogenic tissue produces most of the steroid hormones that are present in mammals (De Falco *et al.* 2004). Thus, the use of animal models such as the lizard *P. sicula* as a suitable bioindicator remains essential to understand the mechanism whereby the endocrine disruptors can induce anomalies or pathologies and to reveal the potential health effects of these compounds on humans and wildlife (Vadenbergh 2004).

Our data demonstrated that MT exposure determined a time-dependent increase of corticosterone plasma levels by 1.2–1.7-fold ($p < 0.05$) with respect to control values measured at 15 and 30 days, suggesting its ability to interfere with glucocorticoid synthesis and secretion *in vivo*. To further clarify whether this corticosterone increase was a direct effect of MT exposure or instead an indirect consequence, we also measured pituitary ACTH plasma levels demonstrating that after exposure with this fungicide, ACTH plasma levels decreased by 1.5–2.8-fold ($p < 0.05$) in a time-dependent manner with respect to the control values measured at both 15 and 30 days. MT effects on steroidogenesis appeared more evident in view of the fact that the experimental procedures were performed in winter when the lizard adrenal glands are still in functional stasis (De Falco *et al.* 2004). These results suggest that MT could act directly on adrenal glands, mimicking ACTH endogenous action and so evoking synthesis and secretion of corticosterone. This hormone in turn could induce a decrease of ACTH through negative feedback on the hypothalamus–pituitary–adrenal axis as also demonstrated in the literature (Vadenborne *et al.* 2005). The net increase of corticosterone production by MT can be considered dangerous in consideration of numerous corticosterone functions as well as metabolic, developmental, and immunosuppressive activities and its role in regulation of the catabolism of carbohydrates, proteins, and lipids. Moreover, the strong increase of corticosterone during winter because of MT exposure stimulates an increase of energy demand that can be deleterious for an ectotherm such as *P. sicula*, which is strongly influenced by climatic variations, temperature, and photoperiod (Borrelli *et al.* 2000; De Falco *et al.* 2004).

In order to have a whole picture of MT effects on the adrenal gland, we also decided to investigate variations of catecholamine plasma levels after MT exposure demonstrating that MT treatment induced a strong increase of adrenaline

plasma levels by 1.5–2.4-fold ($p < 0.05$) in a time-dependent manner and a concurrent decrease of noradrenaline plasma levels by 1.1–1.9-fold ($p < 0.05$). This overproduction of adrenaline could be explained as a direct action of MT on chromaffin cells or instead as secondary effects due to corticosterone plasma levels increase or both. In fact, it has been demonstrated that in the lizard *P. sicula*, corticosterone is able to influence in paracrine manner adrenaline synthesis and secretion. The latter acts on the activity of phenylethanolamine-*N*-methyltransferase enzyme, which is responsible for the conversion of noradrenaline to adrenaline through methylation (Laforgia and Muoio 1997). Recently, Capaldo *et al.* (2006) have demonstrated that also in the newt *Triturus carnifex*, MT was able to increase synthesis and release of adrenaline from chromaffin cells, suggesting its ability to influence the adrenal glands.

Although biochemical biomarkers such as hormone plasma level variations are very useful to test EDC toxicity, during recent years histomorphometric analysis has being utilized because it may represent a sensitive tool to assess the effects of EDCs on target organ/tissues and to provide more insight about the significance of the observed effects (Maranghi *et al.* 2003). In the present study, we observed that MT induced a progressive hypertrophy of steroidogenic tissue characterized by a greater diameter of steroidogenic cords and formed by swollen cells full of more lipid droplets, all signs of cellular degeneration and edema. In addition, we observed a time-dependent enlargement of blood capillaries, especially those occupying the steroidogenic parenchyma. In confirmation of our biochemical data, we observed a growing increase of adrenaline cells that also appeared degranulated as a result of adrenaline secretion in blood. Moreover, we observed an alteration of A/NA cell ratio that varied from 0.7 of the controls to 2.3 of lizards exposed for 30 days to MT. Intriguingly, other than alterations of both adrenocortical and chromaffin tissues, in our study we also observed further histopathological changes represented by lymphoid cell infiltrations between chromaffin cells and numerous macrophages close to blood capillaries on the border between chromaffin and steroidogenic tissues. These lymphocyte and macrophage infiltrations can be considered signs of cell degeneration and necrosis in response to toxic effects of MT as demonstrated also in mammals (Barlas *et al.* 2002; Maranghi *et al.* 2003).

In consideration of equivalence among lizard and mammal adrenal hormone physiology, our findings on the bioindicator *P. sicula* indicate that this lizard can be considered a powerful animal model useful for in-depth study of intracellular mechanisms and potential toxic and systemic effects of endocrine disruptors. Moreover, it has been recently demonstrated that several pesticides can be detected in fruits and vegetables (Aysal *et al.* 2004; Rawn *et al.* 2006a, b) and so enter the human food chain.

In conclusion, MT is able to influence negatively the normal function of adrenal glands by affecting the balance of hormone levels, mimicking endogenous hormone function and altering histomorphological features of adrenal glands.

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