**Dimethoate effects on thyroid function in suckling rats**

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**INTRODUCTION**

Organophosphorus dimethoate is widely used in agriculture and plant protection. The application and frequent use of this insecticide and other organophosphorus compounds have resulted in their widespread distribution in the environment and have been shown to exert deleterious effects on the biological system [10].

Organophosphorus exposure can produce long term changes in brain function, affect the human immune system, cardiac and reproductive functions.

Changes may occur in the metabolism and the endocrine activity after organophosphates exposure [2, 17].

In previous studies, the thyroid inhibitory nature of organophosphate pesticides was reported [20, 21, 32, 33]. However, the reports were made on non mammalian-models (fish and cockerel).

Despite well-documented information on the neurotoxicity of dimethoate in laboratory animals, reports concerning the effects of this insecticide on endocrine activity in mice and rats remain scarce in adults [22, 32, 35] or completely absent in young.

The purpose of our study is to investigate thyroid function in suckling rats and their mothers given dimethoate.

**MATERIALS AND METHODS**

**Animals**

Wistar strain rats (Central Pharmacy, Tunisia), weighing about 180g, were...
housed at 22±3 °C, with light-dark periods of 12 hours (light between 8 a.m and 20 p.m), relative humidity of 40%, free access to water and a commercial diet (SICO, Sfax Tunisia) containing 0.720±0.012 µg of iodine/g of diet.

After acclimatization to laboratory conditions for one week, female rats were kept overnight in separate cages containing five females with one male. Mating dates were established from the appearance of vaginal plugs. The presence of spermatozoa in the vaginal smear was taken as an indicator of day zero of pregnancy.

**Experimental procedures**

Twenty pregnant rats were allowed to deliver spontaneously three weeks after coitus. At birth, the litters were reduced to eight pups each and the day of birth was considered as postnatal day zero.

Lactating rats were divided into two groups of ten each. The first group represented control group. The second group was treated with dimethoate (40 mg/kg body weight, equivalent to 0.2 g/L) administered in their drinking water from the day of birth until the 10th day after delivery. This dose represented one quarter of DL50.

Since the birth, daily food, drink consumption and dimethoate quantities ingested by lactating rats were measured during ten days (*table I*).

Suckling pups (n=160) and their mothers were studied on day 10 after parturition. Each lactating rat treated by dimethoate ingested 5.601 mg of this organophosphorus compound and 18 µg of iodine daily (*table I*).

They were anaesthetized with chloral hydrate by intra-abdominal way. Body weights of all animals were measured. Blood samples were collected by the brachial artery of pups and by the aortic puncture of their mothers. Plasma was obtained by centrifugation at 2200 g of all blood samples and stored at –20 °C until FT₃, FT₄ and TSH analysis by radio-immunnoassay, using kits from Immunotech for FT₃ and FT₄ (references: 1579 and 1363 respectively) and kit from Biocode-Hycel for TSH (rat TSH, reference: AH R001).

Some thyroid glands were taken from pups and dams. They were weighed and preserved at –20° C until their acid mineralization and analysis of their iodine contents by Sandell and Kolthoff method [30]. Others were taken with a piece of trachea, fixed in Bouin solution, embedded in paraffin and serially sectioned at 5 micrometer. The sections were stained with hematoxylin-eosine [13].

**Statistical analysis**

Significance among groups was determined by using Student’s t-test or Man and Whitney test [31] when the means of two groups were compared.

**RESULTS**

Compared with the control group, the ten-day-old rats whose mothers had been treated with dimethoate (40 mg/kg of body weight) had a 48% decrease in body weight (p ≤ 0.001) (*fig. 1*), a 18% decrease in femur length, a 34% decrease in femur weight (p ≤ 0.001) (*table II*) and a decrease in all organs weights, among which thyroid glands were reduced by 45%. Their weights were

<table>
<thead>
<tr>
<th>Parameters and treatments</th>
<th>Food consumption (g/day/mother)</th>
<th>Drink consumption (ml/day/mother)</th>
<th>Quantities of dimethoate ingested (mg/day/mother)</th>
<th>Quantities of iodine ingested (µg/day/mother)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>33.214 ± 3.068</td>
<td>34.600 ± 2.524</td>
<td>—</td>
<td>23.914 ± 2.208</td>
</tr>
<tr>
<td>(n = 14)</td>
<td>(n = 15)</td>
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<td></td>
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<tr>
<td>Treated with dimethoate</td>
<td>25.000 ± 2.662</td>
<td>28.055 ± 2.622</td>
<td>5.601 ± 1.049</td>
<td>18.000 ± 1.917</td>
</tr>
<tr>
<td>(n = 12)</td>
<td>(n = 18)</td>
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significantly different from those of control groups (fig. 1). We have also obtained a reduction in plasma free thyroid hormone rates (FT$_3$ and FT$_4$). In fact, plasma free triiodothyronine levels (FT$_3$) decreased by 40% and free thyroxinemia (FT$_4$) was reduced by 56% (fig. 2). Thyroid iodine contents of pups were affected by dimethoate treatment (~75%) (fig. 3), while in their mothers treated with dimethoate, plasma thyroid hormone levels (FT$_3$, FT$_4$) and thyroid iodine contents were less reduced than in those of pups respectively by ~15, ~27 and ~24% (fig. 2 and 3). Their body and thyroid weights also decreased by 25 and 44% respectively (fig. 1). Plasma TSH levels were multiplied in dimethoate-treated group by factors of 2.31 in dams and 1.96 in their offspring (fig. 4). The increase was statistically significant (p ≤ 0.001).

These biochemical modifications observed in pups and dams confirmed the histological aspects of hypothyroid animals. In fact, in rats treated with dimethoate during ten days, thyroid follicles of adult rats presented cubical epithelial cells which surrounded empty vesicular cavities (fig. 5b). We noted in treated pups that some vesicular cavities surrounded by flattened epithelium cells contained...
DISCUSSION

Our results showed that the daily ingestion of dimethoate during ten days by lactating mothers reduced thyroid hormone secretion in pups. In fact, plasma $T_3$ and $T_4$ levels decreased significantly comparatively to those of control groups. Our results confirmed anterior data of Akhtar and his collaborators [2] who had found a reduced serum $T_3$ and $T_4$ concentrations after malathion treatment of adult rats during 21 days.

Dimethoate involved also an important decrease in thyroid iodine content, which may be explained by a deep iodine deficiency. The uptake of iodine by the thyroid gland became insufficient and the pool of intrathyroidal iodine decreased. A great reduction in thyroid iodine content in treated pups explained the important fall of free thyroid hormone rates: $FT_3$ and $FT_4$, which are necessary for growth. In fact, treatment with dimethoate led to a reduction in pups’body weight and in all organs, among which thyroid glands.

The decrease in body weight could be explained by a severe reduction in thyroxinemia. In fact, thyroid hormones strengthen GH hormone effects on growth [15, 26, 38].

On the other hand, a reduction in daily food consumption by dimethoate-treated lactating rats could explain the pups’growth perturbations.

The high reduction in thyroid hormone levels and in thyroid iodine contents observed in pups could be explained by milk dimethoate transfer from dams to pups. In fact, transfer of organophosphorus compounds into milk was found by anterior investigations carried out in rats [23, 40] and in Cows [1].

Our results confirmed previous data of Singh and his collaborators [34, 35], where insecticide treatment was shown to induce morphological and biochemical changes in the thyroid gland of rats. The inhibition of thyroid secretory activity was also obtained in fish [25, 33] and monkeys [37] after insecticide exposure for a longer period than that employed in the present study.

The biochemical modifications were in agreement with histological aspects of thyroid glands where we have observed morphological changes in follicular cells. There was a decrease in colloid space, which reflected an increase in the net fluid transport [9]. Some of follicles were empty, without colloid. Histopathological changes seen in the thyroid gland of rats were characterized by follicular cell hyperplasia suggestive for enhanced thyroid activity.

In France, between 70% and 80% of cancers are now due to environmental pollution from chemicals, some heavy metals, nitrates, nitrites and pesticides [4]. Carcinomas were observed by Reuber [28] in the adrenal, thyroid and pituitary glands of Osborne-Mendel adult rats given dimethoate. Neoplasms at all sites, as well as malignant neoplasms, were increased in both low and high doses of dimethoate-treated male rats in the National Cancer Institute study. The malignant neoplasms were both carcinomas and sarcomas. Neoplasms of the endocrine organs, particularly carcinomas, were increased in male and female rats given dimethoate. It was reported that carcinogenicity and tumor formation are mediated, in part, by the perturbation in thyroid activity [16].

Exposure to pesticides has been linked to cancer in farmers and other occupational groups [29]. It is predicted that acute oral LD 50 of dimethoate to humans is about 30 mg/kg [39].

The human health risk due to endocrine disruptors at environmental exposure level has been reviewed by Brucker-Davis [8] and Melnick [24]. Brucker-Davis [8] reported that exposure to background level of environmental synthetic chemicals is unlikely to have adverse effects on thyroid function. Biochemical parameters used for the evaluation of thyroid function tests [13, 19] included: the measurement of TSH, total and free $T_3$ and $T_4$. Among these parameters, TSH is usually considered as a first aid test for screening thyroid function [19]. Zaidi et al [41] reported that TSH level increased by about 28% while $T_3$ and $T_4$ decreased by 13 and 7% respectively in formulators as compared to the control
group. Our data obtained in dimethoate treated rats which indicated thyroid impairment, were in agreement with anterior findings realized in humans exposed to pesticides.

Previous studies suggest that insecticides may decrease iodine binding protein [3] or provoke abnormalities of follicular cells [34]. The reduction in thyroid function could be attributed to deficient iodine trapping, morphological changes in follicular cells or inhibition of enzyme activities such as peroxidase enzyme which is necessary for iodine organification and type I desiodase, an enzyme which converts $T_4$ to $T_3$. The last hypothesis confirmed previous data by Maiti and Kar [22] after dimethoate treatment of mice, who suggested that this organophosphorus compound induced alterations in thyroid function mediated through the changes in extrathyroidal conversion of $T_4$ to $T_3$.

Figure 2: Plasma free triiodothyronine levels ($FT_3$) and free thyroxinemia ($FT_4$) of 10-day-old rats and their mothers: controls and treated with dimethoate (0.2 g/L) from day zero until day ten after delivery. Treated vs controls ***: $p \leq 0.001$.

Figure 2 : Taux plasmatiques en triiodothyronine ($FT_3$) et en thyroxine ($FT_4$) des rats âgés de 10 jours et de leurs mères témoins et traitées au diméthoate (0,2 g/L) de la mise bas jusqu’au 10e jour après la parturition. Traités vs témoins *** : $p \leq 0.001$. 
The combined findings of thyroid histology and thyroid hormone disbalance point to a hypothyroidism, reflected in lowered serum T₄ induced by dimethoate treatment. This organophosphorus compound may interfere with thyroid hormone carriers such as transthyretine (prealbumin), a carrier with binding site for both thyroxine and retinol binding protein (RBP). It is possible that this interaction may also play a role in reducing thyroid hormone levels in rats, suggesting a reduced availability of binding sites on thyroxine-carrying proteins. The decrease in production of thyroid hormones after dimethoate treatment affect plasma thyroid stimulating hormone (TSH) levels which were increased in dams and their offspring treated with dimethoate. Our results paralleled anterior data realized in 

**Figure 3:** Thyroid iodine contents of 10-day-old rats and their mothers: controls and treated with dimethoate (0.2 g/L) from day zero until day ten after delivery.  
Treated Vs controls ***: p ≤ 0.001.

**Figure 3 :** Contenus en iode des thyroïdes de rats âgés de 10 jours et de leurs mères témoins et traitées au diméthoate (0,2 g/L) de la mise bas jusqu’au 10° jour après la parturition.  
Traités Vs témoins *** : p ≤ 0.001.

**Figure 4:** Plasma thyroid stimulating hormone levels (TSH) of 10-day-old rats and their mothers: controls and treated with dimethoate (0.2 g/L) from day zero until day ten after delivery.  
Treated vs controls ***: p<0.001.  
The number of determinations was represented above columns of each figure.

**Figure 4 :** Taux plasmatiques en TSH des rats âgés de 10 jours et de leurs mères témoins et traitées au diméthoate (0,2 g/L) de la mise bas jusqu’au 10° jour après la parturition.  
Traités vs témoins *** : p ≤ 0.001.  
Le nombre de déterminations est représenté au dessus des colonnes de chaque figure.
rats and humans where an increase of TSH levels has been observed in adult rats after malathion treatment [2]; in men [41] and women [27] after exposure to a mixture of some pesticides.

The decrease in thyroid weight obtained after dimethoate treatment in dams and their pups could be interpreted as the result of the hypothalamic pituitary axis dysfunction [11] or a dysfunction of the TSH
receptor induced either by gene mutation [5] or by the presence of autoantibodies blocking this receptor [36]. Our results concerning the increase of plasma TSH levels in dimethoate treated rats confirmed the second hypothesis. The thyroid gland atrophy is known to occur when the effects of TSH on thyroid cells are either reduced or completely lacking. This pathological state is generally characterized by a decrease in thyroid cell volume [9]. On the other hand, the decrease in thyroid cell volume could be due to a reduction of follicular cells’ number. Three main mechanisms could be involved in the reduction of the number of follicular cells during the involution of the gland: inhibition of cell proliferation, cell death by necrosis and cell death by apoptosis. In normal human thyroid tissue, total cell mass is maintained by a balance between cell proliferation and apoptosis. Expression of antiapoptotic bcl2 and bcl1 is very consistent [5], suggesting the existence of an active antiapoptotic regulation. However in thyroid tumors, this equilibrium is disrupted [6].

Cytotoxicity and oxidative stress can also occur after dimethoate intoxication by the generation of free radicals and induce lipid peroxidation in liver (LPO) of chicken [21] and mice [22] and in rat erythrocytes [18].

CONCLUSION

Dimethoate can reduce thyroid secretory activity in suckling rats and may even decrease body growth. Thyroid hormone secretion at normal levels is essential for the growth and central nervous system maturation of suckling pups.

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REFERENCES


24. Melnick RL. introduction-workshop on characterizing the effects of endocrine disruptors on human health at environ...