# PRENATAL DELTAMETHRIN LOW DOSE EFFECTS ON PHYSICAL DEVELOPMENT OF RATS

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The present study investigated the relationship between deltamethrin (DTM) maternal exposure and the occurrence of prenatal and postnatal physical alterations when this pesticide is ingested during the organogenic period in rats. Female rats were treated once a day from gestation day (GD) 6 to GD 15 with 0.08 mg/kg of DTM or DTM vehicle (1 mL/kg, 1:50 solution w/v). Half of female rats of both groups were submitted to cesarean before birth and pups skeletal and visceral studies were performed. The other dams were lead pregnancy at a term and their pups development examined. Results showed no skeletal and visceral interferences induced by the pyrethroid prenatal treatment. However, eye opening retardation and vaginal opening improvement were observed in pups development. Since both parameters depends on epidermal growth factor (EGF) and DTM is reported as an enzymatic EGF inducer, it is possible that this mechanism is underlying the developmental effects of prenatal exposure to the low DTM dose employed.

*KEY-WORDS: PRENATAL; DELTAMETHRIN; ABNORMALITYS; PHYSICAL DEVELOPMENT; EPIDERMAL GROWTH FACTOR.*

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(22-24°C) with a 12:12 light/dark period (lights on at 6h), and free access to food (Purina) and water. The animals used in this study were maintained in according to the guidelines of the Committee on Care and Use of Laboratory Animal Resources (NATIONAL RESEARCH COUNCIL, 1996).

## 2.2 EXPERIMENTAL DESIGN

Deltamethrin (Decamethrin – S-alpha-cyano-3-phenoxybenzyl-(R)-cis-3-(2,2-dibromovinyl)-2,2-cimethylcyclopropane carboxylate) from Quimio-Ind. Química S/A (São Paulo/SP), was orally administered (gavage). DTM vehicle (formulation not revealed, 1:50, w/v) was used as control solution.

### 2.2.1 Prenatal Studies

Twenty-four nulliparous female rats were distributed into groups of two animals each and placed overnight with one young male rat, previously determined to be fertile. The onset of pregnancy was confirmed by the presence of spermatozoa in vaginal smears on the following morning, designated as gestation day 0 (GD0). On GD6, the dams were distributed into two groups. One group (experimental group, N = 12) was treated once a day from GD6 to GD15 with 0.08 mg/kg of DTM. The second group (vehicle group, N = 12) received the same treatment, only with DTM vehicle (1 mL/kg, 1:50 solution, w/v). These pregnant rats were weighed at GD1, GD6 to GD15, and GD21.

On GD 21 all dams were anesthetized and their uterine horns removed; the number of *corpora lutei*, implants, resorptions, live and dead fetuses were recorded. The placenta and the fetuses were weighed and examined for macroscopic external malformations. Half of each litter was fixed in BOUIN´s solution for subsequent visceral examination according to Wilson´s method (WILSON, 1965), and the other half was stained with Alizarin red according to the technique of STAPLES AND SCHNELL (1964) to reveal alterations of the skeleton. The degree of ossification was evaluated using the parameters proposed by ALIVERTI et al. (1979).

In according to WILSON (1965), anomaly and malformation are listed as distinct entities. Anomaly (irregularity): when it is out of the mean; structurally uncommon, irregular or contradictory something to the normal model. Malformation: insufficient primary structure that it results of a localized mistake of the morphogenesis (for example: cleft palate). All data were analyzed considering the litter as the smallest unit.

### 2.2.2 Offspring studies

Seventeen nulliparous female rats were placed overnight (three females/one male) with one young male rat previously determined to be fertile. The onset of pregnancy was confirmed by the presence of spermatozoa in vaginal smears on the following morning, designated as gestation day 0 (GD0). On GD6, the dams were divided into two groups. One group (experimental group, N = 9) was treated once a day from GD6 to GD15 with 0.08 mg/kg of DTM. The other group (control group, N = 8) received the same treatment, only with DTM vehicle (1 mL/kg, 1:50 solution, w/v). These pregnant rats were weighed at GD1, GD5, GD6, GD15 and GD21.

All the pregnant rats were allowed to give birth and nurture their offspring normally. No cross-fostering procedure was used. Parturition day was determined to be PN0. On PN1 all litters were examined externally, sexed and weighed. Litters were organized in groups of 8 pups, 4 males and 4 females, and the remaining pups were discarded. Litters were weighed at PN1, PN7, PN14 and PN21. The following physical parameters of development were observed daily: pinna detachment (beginning Day 2), incisor eruption (beginning Day 6), testis descent (beginning Day 15), and eye (beginning Day 10) and vaginal

openings (beginning Day 30) (ALDER & ZBINDER, 1977). Pups were observed daily, between 09h and 11h a.m., separated from the mothers at the moment of observation and immediately returned to their home cages. Mean day of appearance for each of the parameters above was calculated. All data were analyzed considering the litter as the smallest unit. On PN21, the offspring were weaned and the littermates were housed together, separated by sex and treatment.

### 2.3 STATISTICAL ANALYSIS

Results were expressed as litter means ± SD as the maternal unit to avoid litter effects. For the embriotoxic studies (external, skeletal, and central nervous system malformations and anomalies), data were analyzed by Goodman´s test (GOODMAN, 1964). Student t test was employed to analyze number of ossification centers and the reproductive performance of pregnant rats. The offspring developmental parameters studies were analyzed by two ways ANOVA (factors: sex and treatments). As post-hoc tests, an ANOVA followed by the Tukey test (pinna detachment and incisor eruption) or the Mann-Whitney U test (eye opening) were used. Data without interaction were analyzed by the t test (testes descent) or by the Mann-Whitney U test (vaginal opening) when data were heterocedastic. In all experiments, $P < 0.05$ was the critical criterion for statistical significance.

## 3 RESULTS

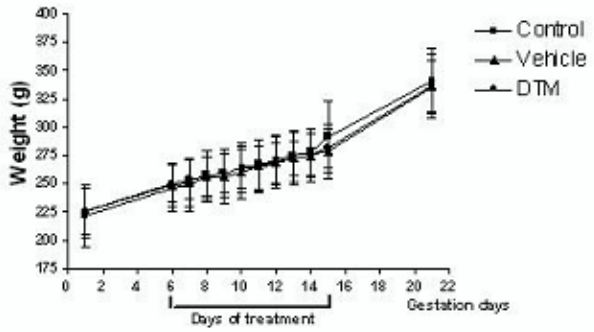
Data from reproductive performance of dams showed that the treatment did not cause statistical differences among all parameters observed (Table 1). Also, maternal weight gain did not differ among groups and offspring weight (Figure 1).

**TABLE 1 – EFFECTS OF DTM ADMINISTRATION AND ITS VEHICLE GIVEN BY GAVAGE, DURING THE ORGANOGENIC PERIOD, ON REPRODUCTIVE PERFORMANCE OF PREGNANT WISTAR RATS**

| | GROUPS | |
|---|---|---|
| | VEHICLE | DTM |
| Number of female Mated | 12 | 12 |
| Pregnant at term | 10 | 11 |
| Number of corpora lutea (mean ± SD) | 14.1 ± 2.23 | 13.1 ± 2.81 |
| Number of implantations (mean ± SD) | 11.8 ± 2.57 | 11.9 ± 2.30 |
| Resorption (mean ± SD) | 0.9 ± 1.19 | 1.0 ± 1.26 |
| Number of life fetuses (mean ± SD) | 10.8 ± 3.58 | 10.9 ± 3.14 |
| Number of dead fetuses (total nº ) | 0 | 2 |
| Postimplantation loss (%) | 10.5 | 9.8 |
| Fetal weight (g) (mean ± SD) | 4.1 ± 0.25 | 4.2 ± 0.07 |
| Placental weight (g) (mean ± SD) | 0.49 ± 0.12 | 0.48 ± 0.11 |

Dunn Test.

**FIGURE 1 - MATERNAL WEIGHT GAIN DURING GESTATIONAL PERIOD OF RATS EXPOSED, BY GAVAGE, TO DTM (0.08 mg/kg), OR ITS VEHICLE, FROM DAY 6 TO 15 OF PREGNANCY**




Data are present as mean ± SEM. Vehicle group: N = 12; experimental group: N = 12.

External malformations and anomalies were not found in the experimental groups. The same fact was observed on central nervous system malformations and anomalies.

Skeletal analysis showed no malformations in the experimental groups. Both control and experimental groups did not presented significant differences between skeletal anomalies (Table 2) and on the number of ossification centers (Table 3).

**TABLE 2 - EFFECTS OF DTM ADMINISTRATION AND ITS VEHICLE GIVEN BY GAVAGE, DURING THE ORGANOGENIC PERIOD OF PREGNANT WISTAR RATS ON THE INCIDENCE OF SKELETAL MALFORMATIONS AND ANOMALIES OF THE FETUSES**

| | GROUPS | |
|---|---|---|
| | VEHICLE | DTM |
| Skeletal malformations | | |
| Affected fetuses | 0/55 | 0/55 |
| Affected litters | 0/10 | 0/11 |
| Skeletal abnormalitys | | |
| Affected fetuses | 28/55 | 51/55 |
| Affected litters | 10/10 | 10/11 |
| Sternal abnormalitys | 47/55 | 49/55 |
| Vertebral anomalies | 19/55 | 18/55 |
| Presence of 14th rib | 7/55 | 14/55 |
| Reduced cranial ossification | 3/55 | 1/55 |

N = 12 dams by group.
Goodman Test.

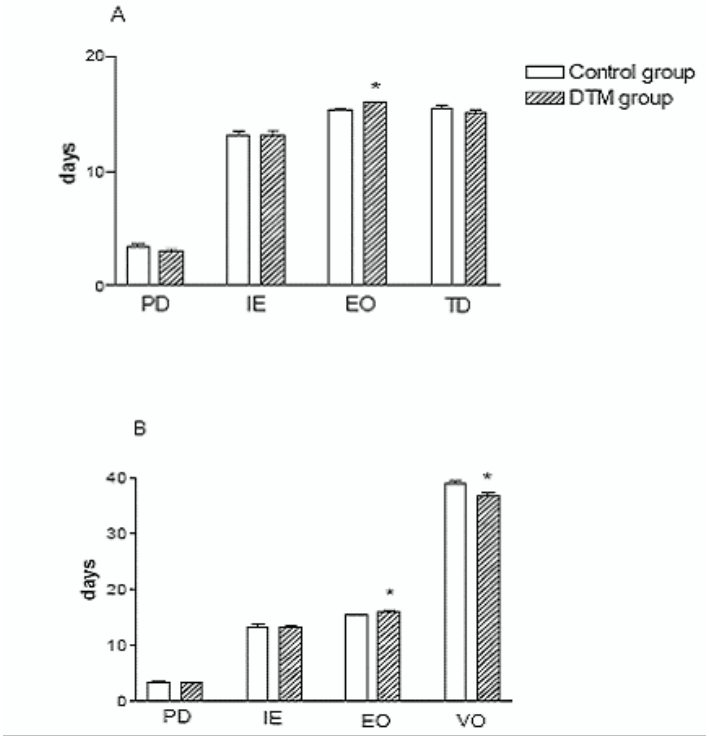
**TABLE 3 - NUMBER OF OSSIFICATION CENTERS OF RATS OFFSPRING EXPOSED, BY GAVAGE, TO DTM (0,08 m/kg), FROM 6TH TO 15TH DAYS OF PREGNANCY**

| | GROUPS | |
|---|---|---|
| | VEHICLE (10) | DTM (11) |
| Anterior phalange | 0.84 ± 0.78 | 0.72 ± 0.69 |
| Metacarpus | 3.40 ± 1.09 | 3.86 ± 0.18 |
| Stern | 5.97 ± 0.03 | 5.89 ± 0.15 |
| Post phalange | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Metatarsus | 4.00 ± 0.00 | 4.00 ± 0.00 |
| Caudal vertebra | 3.02 ± 0.41 | 3.12 ± 0.32 |
| **Total** | **25.40 ± 3.64** | **26.09 ± 1.80** |

Data are present as mean ± sd. () = numbers of litters.
* P < 0,05 in relation to control group – Dunn Test.

In the DTM exposed female pups, the day for eye opening was increased compared to the control [treatment F(1:30) = 16.47, p = 0.0003; Sex F(1:30) = 0.62, p = 0.44; interaction F(1:30) = 0.0, p = 0.99], indicating a delay in this parameter. The day for vaginal opening of the DTM group was decreased in relation to the control group, meaning an acceleration in this physical landmark (Figure 2). Two way ANOVA showed no differences in the days of pinna detachment, incisor eruption and testes descent of control and experimental male and female offspring.

**FIGURE 2 - MEAN (± SEM) POSTNATAL DAY VALUES OF THE PHYSICAL PARAMETERS OBSERVED IN MALE (A) AND FEMALE (B) RATS PRENATALLY EXPOSED TO DELTAMETHRIN (DTM) OR ITS VEHICLE**




PD = pinna detachment; IE = incisor eruption; EO = eye opening; TD = testes descent; VO = vaginal opening. Number of litters-vehicle group – n = 8; experimental group – n = 9. * P < 0.05 compared to the vehicle group. Mann Whitney U Test.

## 4 DISCUSSION

According to previous data (LAZARINI et al., 2001) the 0.08 mg/kg DTM exposure was not able to induce maternal toxicity, since during pregnancy weight gain of experimental rats was not modified. In addition, no gross embriotoxic effects were detected.

Presently, the prenatal maternal exposure to DTM (0.08 mg/kg/day) did not interfere with the fetal and placental weights (Table1), indicating that at this dose level no embriotoxicity was induced by the pesticide. The percentage of postimplantation loss (an index that determines the correlation among the number of embryos that were implanted and the ones that usually grew) was not modified suggesting that DTM treatment did not interfere with the embryos development implanted.

The retardation of intrauterus development is usually based on the fetal weight. Besides previously showed a lack of differences between pups body weight at birth control and experimental groups (LAZARINI et al., 2001); this parameter by itself is not conclusive, because it can be related with the size of the brood. With the objective of supplying an additional index for evaluation of retard of the development in mice, ALIVERTI et al. (1979) proposed a method of scores with base in the degree of fetal ossification (esternebrys, proximal and distal phalanges, metacarpi, metatarsi and flows vertebras) at GD21. DTM treated group did not presented alteration in any ossification center as well as in the total ossification centers. The data observed after prenatal exposure of DTM indicates that, at this dose, the drug was unable to induce embriotoxic effects in rats. The absence of placental or embryotoxic alterations observed in the reproductive performance data (Table 1) are in agreement with this assertion. Thus, the treatment did not interfer both, with reproductive parameters of dams and in utero fetuses physical development.

The main finding of this work was observed in the postnatal studies. Several studies showed that pyrethroid insecticides alters offspring development and have some delayed toxicity. Prenatal effects of exposure to the insecticide cyhalothrin, a type II pyrethroid, throughout pregnancy delayed the age of testicle descent but did not modify the age of vaginal opening. In adulthood, both male and female rat sexual behaviors were not different from vehicle-treated animals. In other study, it was verified that cyhalothrin delays the development of fur, the ear and eye openings and descent of the testes in the offspring. No maternal or neonatal signs of toxicity were detected. At 90 days of age, a decreased exploratory behavior was detected, but no differences were observed in inhibitory avoidance and open field behaviors. Alterations in physical parameters and possible effects of the pesticide on epidermal growth factor (EGF) and at hormonal levels were considered (GOMES, BERNARDI & SPINOSA, 1991a; GOMES, BERNARDI & SPINOSA, 1991b). The same pesticide, when administered during all lactation period, did not change either the maternal behavior of the dams or pups physical and motor development (MONIZ et al., 1990). However, the treatment disrupted rat behavior in adulthood when assessed by using an inhibitory avoidance learning task, demonstrating neural toxicity of cyhalothrin.

In the present investigation, a delay in the day of eyes opening for male and female offspring exposed to DTM was observed. In addition, the females presented early vaginal channel opening. The other parameters of the physical development were not modified in relation to those of the control group.

COHEN (1964) verified that certain fractions of the extract of the mice submaxilar gland, when injected to newly born mice daily, produced precocious opening of the eyes and advancement in the eruption of the incisive teeth. Those morphologic alterations were interpreted as a result of the growth and queratinization of the epidermis formation (COHEN & ELLIITTI, 1963) being denominated EGF (COHEN, 1962). The administration of EGF to newly born mice accelerates the eruption of the incisive teeth and the eyes opening as well as it delays the ear

and vaginal opening (SMART et al., 1989). Thus, it is possible that DTM prenatal exposure inhibit the expression of EGF. In addition, the delay in eye opening without other evidence for a general developmental delay suggests a specific effect of the pesticide in this physical landmark. In this matter, the period of development in rats to reach adult age is very short in relation to other species, including humans being. In fact, the male puberty is attained around 16 days of age, during lactation period, and female puberty around 38 days of age. Thus, a slight delay or acceleration in physical landmarks might be of a biological significance. The normal timing for eye opening occurs around 14,5 days of age with a small variability (maximal 0,5).

It was also demonstrated that maternal exposure to fenvalerate, another type II pyrethroid pesticide, during the prenatal and postnatal periods of sexual brain differentiation of male offspring did not change the age of testis descent or testis weight, nor were there changes in monoamine levels or stereotyped behavior. However, there were significant reductions in ductus deferens and seminal vesicle weights and plasma testosterone concentrations. Treated offspring showed a decreased male sexual behavior and increased immobility in the open field at adult age (MONIZ, 1999). These effects can be attributed to a disruption of the maternal hormonal during fetal development since it was demonstrated an anxiogenic effect of this pyrethroid insecticide (SPINOSA et al., 1999).

Recently, MONIZ et al. (2005) showed that maternal exposure to fenvalerate in the prenatal and postnatal periods of sexual brain organization had behavioral, physical and neuroendocrine effects in female offspring. In this way, results showed that sexual maturation was delayed, body weight was unchanged until adulthood, there was a reduction in sexual behavior, abnormal estrous cycle, and the uterine weight at different phases of the estrous cycle was modified. Gonadal hormone levels in the plasma were not affected, neither was stereotypy nor open-field behaviors. These results were attributed to an anti-estrogenic effect of perinatal exposure to the pesticide during the critical periods of female brain sexual organization.

Presently, prenatal exposure to DTM accelerated the day of vaginal opening and did not alter the descent of the testicles. The puberty of the female rat results of complex central mechanisms, which induce changes in the gonadotrofins, resulting in increased secretion of sexual hormones (URBANASKI & OJEDA, 1987). The external sign of puberty is the vaginal canalization with vaginal channel opening. This usually occurs in the day after the first surge of gonadotrofin release (TERASAWA & FERNANDEZ, 2001).

The cytochrome P450 aromatase is responsible for conversion of androgens to estrogens (SIMPSON, 2003). Considerable emphasis has been placed on the role of aromatization in specific brain areas, which includes the medial preoptic/anterior hypothalamus, the medial basal hypothalamus and amygdala (SIMPSON, 2003). Thus, the total amount of estrogen synthesized in these areas may be small, the local tissue concentrations achieved are probably quite high and exert significant biological influence locally, and principally, in a paracrine or intracrine fashion (LABRIE et al., 1997). Sex steroids dependence and asymmetries of aromatase activity have been reported during ontogeny of rats (LAUBER, SARASIN & LICHENSTEIGER, 1997). In both males and females rats, the time of puberty was associated with a decrease in hypothalamic aromatase activity. In females, this decrease was found to occur between the days of first proestrus and estrus (LEPHART & OJEDA, 1990). While in clinic, specific inhibitors of aromatase could improve the precocious puberty, which may decrease the volume of ovarian cysts, frequency of menses, rates of growth and bone maturation (FEUILLAN et al., 1999). Though there are affirmative evidences for involvements of aromatase in regulating central activation of sexual behavior (MEINHARDT & MULLIS, 2002) and sexual differentiation (ROSELLI & KLOSTERMAN, 1998), hitherto the local brain estrogen plays largely unrecognized, physiological and pathophysiological roles. Local estrogen in specific brain areas is the principle determinant of

gonadotropin-releasing hormone (GnRH) neuron functioning and, acting as a homeostatic feedback molecule among compartments of GnRH network, is critical in enabling GnRH cells to exhibit fluctuating patterns of biosynthetic and secretory activity (HERBISON, 1998). Since estrogen has potent effects on the controlling of GnRH discharge (FINK et al., 1991; STAUB & BEER, 1997), the changes of brain aromatization in the advanced onset of puberty is a pivotal question, and it has rare been literate.

Hepatic biotransformation plays a key role in elimination and detoxification of foreign compounds (xenobiotics). Many foreign compounds are capable of inducing their own metabolism and/or metabolism of other xenobiotics, which is considered an adaptive response to xenobiotic exposure. Induction of hepatic drug metabolism by barbiturates, e.g., by Phenobarbital, was described more than 40 years ago (REMMER, 1958). The spectrum of hepatic enzymes subject to phenobarbital-dependent regulation of expression includes members of the cytochrome P-450 (CYP) subfamilies CYP2A, CYP2B, CYP2C and CYP3A, and other xenobiotic-metabolizing enzymes, such as UDP-Glucuronosyltransferase isoforms (SUEYOSHI & NEGISHI, 2001). Also, numerous xenobiotics are capable of inducing their own metabolism and, by enzyme induction, can also lead to enhanced biotransformation of other xenobiotics. Pyrethroids (permethrin, cypermethrin, and fenvalerate) induce the expression and activity of the phenobarbital inducible CYP2B1 in primary rat hepatocyte cultures (HEDER et al., 2001).

JOHRI et al. (2006) showed that prenatal exposure to low doses (0.25 or 0.5 or 1.0 mg/kg, p.o.) of deltamethrin to pregnant dams from gestation days 5 to 21 (GD5-21) produced dose-dependent alterations in the ontogeny of xenobiotic metabolizing CYP isoforms in brain and liver of the offsprings. RT-PCR analysis revealed dose-dependent increase in the mRNA expression of cerebral and hepatic CYP1A1, 1A2, 2B1, 2B2, and 2E1 isoenzymes in the offsprings exposed prenatally to deltamethrin. Similar increase in the activity of the marker enzymes of these CYP isoforms has indicated that placental transfer of the pyrethroid, a mixed type of CYP inducer, even at these low doses may be sufficient to induce the CYPs in brain and liver of the offsprings. The authors have further revealed persistence in the increase in expression of xenobiotics metabolizing CYPs up to adulthood in brain and liver of the exposed offsprings, suggesting the potential of deltamethrin to imprint the expression of CYPs in brain and liver of the offsprings following its *in utero* exposure. Furthermore, though the levels of CYPs were several fold lower in brain, almost equal magnitude of induction in cerebral and hepatic CYPs has further suggested that brain CYPs are responsive to the induction by environmental chemicals. These data indicating alterations in the expression of xenobiotic metabolizing CYPs during development following prenatal exposure to deltamethrin may be of significance as these CYP enzymes are not only involved in the neurobehavioral toxicity of deltamethrin, but have a role in regulating the levels of ligands that modulate growth, differentiation, and neuroendocrine functions.

## 5 CONCLUSION

The lack of differences in maternal weight as well as in the fetal skeletal and visceral development between vehicle and experimental groups in prenatal exposure indicates that DTM, at this dose (0.08 mg/kg), was unable to induce embriotoxic effects in rats.

About the postnatal experiment, previous studies showed that EGF receptors of hepatocytes from rats treated with phenobarbital are sensitized to down-regulation by phenobarbital in culture. Thus, the delay in eye opening and vaginal opening acceleration here observed should be a consequence of CYPs induction by deltamethrin prenatal exposure on EGF. This hypothesis need to be further investigated.

## RESUMO

### EFEITOS DE BAIXA DOSE PRÉ-NATAL DE DELTAMETRINA NO DESENVOLVIMENTO FÍSICO DE RATOS

No presente estudo investigou-se a relação entre exposição maternal à deltametrina (DTM) e a ocorrência de alterações físicas pré e pós-natais quando esse pesticida é ingerido durante o período da organogênese do rato. Ratas foram tratadas uma vez por dia do 6º ao 15º dia de gestação com 0,08 mg/kg de DTM ou veículo (1 mL/kg, solução 1:50). Metade das fêmeas de cada grupo foram submetidas à cesariana antes do nascimento, verificando-se o desenvolvimento do esqueleto e as possíveis alterações viscerais dos filhotes. A prenhez das outras fêmeas foi a termo, sendo estudado o desenvolvimento físico e reflexológico de suas proles. Os resultados mostraram que o tratamento pré-natal com DTM não interferiu no desenvolvimento esquelético dos animais nem promoveu lesões ou alterações viscerais, mas retardou a abertura do olho e adiantou o dia da abertura vaginal dos filhotes. Como os parâmetros alterados dependem de fator de crescimento epidermal (EGF), e DTM tem sido relatado como indutor enzimático de EGF, é possível que esse mecanismo esteja relacionado com os efeitos da exposição pré-natal à baixa dose de DTM administrada.

*PALAVRAS-CHAVE: DELTAMETRINA; ORGANOGÊNESE; PRÉ-NATAL; PIRETRÓIDE; FATOR DE CRESCIMENTO.*

## REFERENCES

1 ALDER, S.; ZBINDER, G. Methods for the evaluation of physical, neuromuscular and behavioral development of rats in early postnatal period. In: NEUBERT, D.; MERKER, H. J.; KWASIGROCH T. E. (eds.). **Methods in prenatal toxicology**. Stuttgart: Thieme, 1977.

2 ALIVERTI, V.; BONANOMI, L.; GIAVINI, E.; LEONE, V.G.; MARIANI, L. Extent of fetal ossification as na index of delayed development in teratogenic studies on the rat. **Teratology**, v. 20, p. 237-242, 1979.

3 COHEN, S. Isolation of a mouse submaxillary gland protein accelerating incisor eruption and eyelid opening in the newborn animal. **J. Biol. Chem**, v. 237, p. 1555-1562, 1962.

4 COHEN, S. Isolation and biological effects of an epidermal growth-stimulating protein. **Nat. Cancer Inst. Monogr.**, v. 13, p. 13-37, 1964.

5 COHEN, S.; ELLIOTT, G.A. The stimulation of epidermal keratinization by a protein isolated from the submaxillary gland of the mouse. **J. Invest. Dermatol**., v. 40, p. 1-5, 1963.

6 FEUILLAN, P.; MERKE, D.; LESCHEK, E. W.; CUTLER, G. B. Use of aromatase inhibitors in precocious puberty. **Endocr. Relat. Cancer**, v. 6, p. 303–306, 1999.

7 FINK, R. M.; ROSIE, R.; SHEWARD, W. J.; THOMSON, E.; WILSON, H. Steroid control of central neuronal interactions and functions. **J. Steroid Biochem. Mol**. Biol.,v. 40, p. 123–132, 1991.

8 GOMES, M. S.; BERNARDI, M. M.; SPINOSA, H. S. Pyrethroid insecticides and pregnancy: effect on physical and behavioral development of rats. **Vet. Hum. Toxicol**., v. 33, 315-317, 1991a.

9 GOMES, M. S.; BERNARDI, M. M.; SPINOSA, H. S. Effects of prenatal pyrethroid insecticide exposure on the sexual development of rats. **Vet. Hum. Toxicol**., v. 33, 427-428, 1991b.

10 GOODMAN, L. A. Simultaneous confidence intervals for contrasts among multinomial population. **Annals Mathematical Statistics**, v. 35, p. 716-725, 1964.

11 HEDER, A. F.; HIRSCH-ERNST, K. I.; BAUER, D.; KAHL, G. F.; DESEL, H. Induction of cytochrome P450 2B1 by pyrethroids in primary hepatocyte cultures. **Biochem. Pharmacol**., v. 62, 1, p. 71-79, 2001.

12 HERBISON, A. E. Multimodal influence of estrogen upon gonadotropin-releasing hormone neurons. **Endocr**. **Rev**., v. 19, p. 302–330, 1998.

13 JOHRI, A.; DHAWAN, A.; LAKHAN, S. R.; PARMAR, D. Effect of prenatal exposure of deltamethrin on the ontogeny of xenobiotic metabolizing cytochrome P450s in the brain and liver of offsprings. **Toxicol. Appl. Pharmacol**., v. 214, n. 3, p. 279-289, 2006.

14 LABRIE, F. A.; BELANGER, A.; CUSAN, L.; CANDAS, B. Physiological changes in dehydroepiandrosterone are not reflected by serum levels of active androgens and oestrogens but by their metabolites: intracrinology. **J.Clin. Endocr. Metab**., v. 82, p. 2403–2409, 1997.

15 LAUBER, M.E.; SARASIN, A.; LICHTENSTEIGER, W. Sex differences and androgen-dependent regulation of aromatase (CYP19) mRNA ex-pression in the developing and adult rat brain. **J. Steroid Biochem.Mol**. **Biol**., v. 61, p. 359–364, 1997.

16 LAZARINI, C.A.; FLORIO, J.C.; LEMONICA, I.P.; BERNARDI, M. M. Effects of prenatal exposure to deltamethrin on forced swimming behavior, motor activity, and striatal dopamine levels in male and female rats. **Neurotoxicol**. **Teratol**., v. 23, p. 665-673, 2001.

17 LEPHART, E.D.; OJEDA, S.R. Hypothalamic aromatase activity in male and female rats during juvenile peripubertal development. **Neuroendocrinology**, v. 51, p. 385–393, 1990.

18 MEINHARDT, U.; MULLIS, P.E. The essential role of the aromatase/p450arom. **Semin. Reprod. Med**., v. 20, p. 277–284, 2002.

19 MONIZ, A. C.; BERNARDI, M. M.; SOUZA-SPINOSA, H.S.; NETO, J. P. Effects of exposure to a pyrethroid insecticide during lactation on the behavior of infant and adult rats. **Braz. J. Med. Biol. Res.**, v. 23, n. 1, p. 45-48, 1990.

20 MONIZ, A. C.; CRUZ-CASALLAS, P.E.; OLIVEIRA, C. A.; LUCISANO, A.; FLORIO, J. C.; NICOLAU, A. A.; SPINOSA, H. S.; BERNARDI, M. M. Perinatal fenvalerate exposure: behavioral and endocrinology changes in male rats. **Neurotoxicol. Teratol**., v. 21, p. 611-618, 1999.

21 MONIZ, A. C.; CRUZ-CASALLAS, P.E.; SALZGEBER, A. S.; VAROLI, F. M.; SPINOSA, H. S.; BERNARDI, M. M. Behavioral and endocrine changes induced by perinatal fenvalerate exposure in female rats. **Neurotoxicol. Teratol**., v. 27, p. 609-614, 2005.

22 NATIONAL RESEARCH COUNCIL. **Guide for the care and use of laboratory animals**. Washington, D.C.: National Academy Press, 1996. 140 p.

23 REMMER, H. The mode of action of hexobarbital & thiopental in short term anesthesia. **Anaesthesist**., v. 7, n. 4, p. 97-100, 1958.

24 ROSELLI, C. E.; KLOSTERMAN, A. S. Sexual differentiation of aromatase activity in the rat brain: effects of perinatal steroid exposure. **Endocrinology**, v. 139, p. 3193–3201, 1998.

25 SIMPSON, E. R. Source of estrogen and their importance. **J. Steroid Biochem**. **Mol. Biol**., v. 86, p. 225–230, 2003.

26 SMART, J. L.; da SILVA, V. A.; MALHEIROS, L. R.; PAUGARTTEN, F. J. R.; MASSEY, R. F. Epidermal growth advances some aspects of development but retards others in both rats and ramsters. **J. Develop. Physiol**., v. 11, p. 153-158, 1989.

27 STAPLES, R. E.; SCHENELL, V. L. Refinements in rapid techniques in the KOH Alizarin red S method for fetal bone. **Stain Technology**, v. 39, p. 61-64, 1964.

28 SPINOSA, H. S.; SILVA, Y. M.; NICOLAU, A. A.; BERNARDI, M. M.; LUCISANO, A. Possible anxiogenic effects of fenvalerate, a type II pyrethroid pesticide, in rats. **Physiol. Behav**., v. 67, n. 4, p. 611-615, 1999.

29 STAUB, N. L.; BEER, M. D. The role of androgens in female vertebrates. **Gen. Comp. Endocrinol**., v. 108, p. 1–24, 1997.

30 SUEYOSHI, T.; NEGISHI, M. Phenobarbital response elements of cytochrome P450 genes and nuclear receptors. **Annu. Rev. Pharmacol. Toxicol**., v. 41, p. 123-143, 2001.

31 TERASAWA, E. I.; FERNANDEZ, D. L. Neurobiological mechanism of the onset of puberty in primates. **Endocr. Rev**., v. 22, p. 111–150, 2001.

32 URBANASKI, H. F; OJEDA, S. R. Activation of luteinizing hormone releasing hormone release advances the onset of female puberty. **Neuroendocrinology**, v. 46, p. 273-276, 1987.

33 VERSCHOYLE, R. D.; ALDRIDGE, W. N. Structure-activity relationships of some pyrethroids in rats. **Arch. Toxicol**., v. 45, p. 325-329, 1980.

34 WILSON, J.G. Methods for administering agents and detecting malformations in experimental animals. In: WILSON, J. G.; WARKANY, J. (eds.) **Teratology**: principles and techniques. Chicago: University of Chicago Press, 1965.