# HISTOLOGICAL ASSESSMENT OF THE TERATOGENIC POTENTIAL OF THE PESTICIDE FOLIDOL TO INDUCE THE APPEARANCE OF CLEFT PALATE IN *WISTAR* RATS

E.S. Camargo[1*], M.S. Vianna[2], A.M. Bolognese[3], Hiroshi Maruo[1], A.M.T. Grégio[4], O. Guariza Filho[1], J.H.G. Oliveira[1], O. Tanaka [1]

[1] Orthodontics Department, Dental Branch, Pontifical Catholic University of Paraná, Brazil
[2] Dental Branch, Pontifical Catholic University of Paraná, Brazil
[3] Orthodontics Department, Dental Faculty, Federal University of Rio de Janeiro, Brazil
[4] Pharmacology Branch, Pontifical Catholic University of Paraná, Brazil

*Corresponding author: Elisa Souza Camargo, Rua Fernando Simas, nº 327. Curitiba - PR- Brazil. CEP. 80430-190. Tel./Fax: 00(55)413323-5835. E-mail: elisa.camargo@pucpr.br

## Summary

The aim of this study was to assess the teratogenic potential of a pesticide of the organophosphate group (Folidol) to cause the appearance of cleft palate in *Wistar* rats. For this purpose, two groups of females were daily exposed to Folidol (Methyl Parathion): Group 1 received it before gestation and Group 2 during the gestational period. Folidol was used in a 1/250ml dilution, prepared every three days and kept in flasks, in accordance with the manufacturer's guidance. The Folidol was topically applied to the females' four feet. The palates of the progenies of the two groups of females were histologically assessed with Light Microscopy.

No presence of cleft palate was found, the morphogenesis of the palate being normal in all the specimens of the two groups' progenies. In accordance with these results, it could be concluded that the pesticide Folidol did not present teratogenic potential for inducing cleft palate formation in the studied sample.

**Key words:** Teratogenicity; Cleft palate; Pesticide; Folidol; Rat

Although reports in literature point towards the toxicity of some drugs and even vitamins causing cleft palates, attendance centers for patients with congenital facial deformities have associated this malformation to living in the rural area and to agricultural planting activities. It is therefore, suspected that chemical substances used in agriculture may be related to the manifestation of these deformities.

The organophosphates, pesticides that present a chemical composition that is highly toxic to humans, are commonly used in agriculture because they are applicable to a wide variety of agricultural pests. As a result of their high toxicity and the great residual effect, however, their use has been prohibited for a long time in various countries in the world. Notwithstanding this prohibition, they are still used in South America, with the result that families in rural agricultural areas are exposed to this pesticide during the planting and harvesting seasons.

With respect to the teratogenicity of Folidol, an insecticide belonging to the organophosphate group, the literature has shown to be controversial.

Kumar and Devi [1] observed that methyl parathion (MP), the active substance in Folidol, was a teratogen on developing chick embryos, causing retarded growth, which included reduced body weight and reduced body and leg bone lengths, short neck, leg muscle hypoplasia, abdominal hernias and hemorrhagic spots in brain and upper body.

Alvarez, Honrubia and Herraez [2] related that Folidol leads to malformations of the spinal column and the tail**,** as well as bone metabolism alteration during the larval development of *Rana perezi.*

Experiments with another insecticide – Wofatox 50 EC – the active substance of which is also methyl parathion, related various teratogenic effects, generally such as cervical lordosis and scoliosis, cyllosis and sporadic thoraco-gastroschisis [3]; decreased body mass, high incidence of malformation development and deaths with the use of high doses [4] and atrophy of the cervical spinal cord [5]. Tian et al. [6] observed the formation of cleft palates in mice with the application of chlorpyrifos, also of the organophosphate group.

According to Varnagy and Deli [4], however, small doses of the insecticide Wofatox 50 EC, at concentrations used in agriculture, do not present a teratogenic or lethal effect on the embryos of hens and pheasants, and according to the Extension Toxicology Network [7] the available evidences indicate that methyl parathion does not have a teratogenic effect.

Considering the lack of information with respect to the effects of Folidol on human development, more specifically in the face, and taking into consideration its teratogenicity, mainly on bone structures, the present study proposed to assess the influence of Folidol on the formation of the palate in *Wistar* rat fetuses.

## Methods

**Sample:**
The sample consisted of 42 *Wistar* rats aged 60 days, of which 14 were males and 28 females. The males comprised a single group and remained in the study until the mating stage, while the females were divided into two groups:

- Group 1 (n=14): Specimens daily exposed to Folidol before gestation, chronic treatment of 30 days.
- Group 2 (n=14): Specimens daily exposed to Folidol during the gestation period, treatment of 20 days.

All of the experiments followed the guidelines for the Scientific Practice of Vivisection in animals, as well as the Ethical Principals for Animal Experimentation, in accordance with Statute 6.638, of May 8th, 1979 [8]. This study was approved by the Research Ethics Committee of Tuiuti University of Paraná, under the registration number CEP-UTP – 20/2003.

**Pharmacological product preparation**

Folidol (anticholinesterasic insecticide) was used. This agent was used in a 1/250ml dilution, in accordance with the manufacturer's guidelines, and applied topically to the dorsal portion of the females' feet, twice a day , at 9:00h and at 14:00h, mimicking the condition of poisoning that occurs through aerosols and contact with the mucosa in humans.

**Method**

Mating lasted for two days, in the proportion of one male to two females (polygamous mating), which were divided into the two above-mentioned groups.

While the females in Group 2 were exposed to Folidol (Bayer – Brazil), daily during the gestation period, those in Group 1, in this same period, remained in new cages without any exposure whatever to the tested substance.

Although the normal gestation period is 22 to 23 days, on the 20th day of gestation, all the females were duly anesthetized with sodium thiopental (100 mg/Kg) and then sacrificed. Next, laparotomy was performed in each of the pregnant animals, to collect the fetuses [9], which were stored in 10% Formalin. The specimens were taken to the Pontifical Catholic University of Paraná Experimental Pathology Laboratory and histologically processed. The cephalic extremity was separated from the remainder of the body, and then mandible exeresis was performed.

For microscopic assessment of the palate, histologic cuts were made of each tissue in the frontal direction through the entire depth, from the most anterior to the most posterior portion of the palate, in order to observe whether or not there was

presence of a cleft palate. Slides were read by a light microscope (Leitz Wetzlar – dfv, model HM-LUX).

**Results**

Tables 1 and 2 present the data obtained in the observations. Table 1 is with reference to Group 1, whose progenies totaled 110 fetuses. No presence of cleft palate was found in any of the specimens. In the same way, in Group 2 none of the 103 fetuses examined presented any malformation (Table 2).

TABLE 1 – NUMBER OF FEMALES, OF PROGENY INDIVIDUALS AND OF AFFECTED INDIVIDUALS, IN GROUP 1, IN 2003.

| FEMALES | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No.Progeny Individuals | 9 | 9 | 8 | 10 | 9 | * | 10 | 10 | 10 | 9 | * | 9 | 9 | 9 |
| No. Affected Individuals | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | | 0 | 0 | 0 |
| Affected Individuals (%) | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | | 0 | 0 | 0 |

SOURCE: PUCPR Vivarium.
NOTE: * Females not pregnant.

TABLE 2 – NUMBER OF FEMALES, OF PROGENY INDIVIDUALS AND OF
AFFECTED INDIVIDUALS, IN GROUP 2, IN 2003.

| FEMALES | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No.Progeny Individuals | 8 | 10 | * | * | * | 9 | 10 | 9 | 9 | 10 | 10 | 8 | 10 | 10 |
| No. Affected Individuals | 0 | 0 | | | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Affected Individuals (%) | 0 | 0 | | | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

SOURCE: PUCPR Vivarium.
NOTE: * Females not pregnant.

## Discussion

Cleft palate may be produced in experimental animals, with the use of chemical agents or other drugs capable of affecting the embryo. Usually, these agents retard or prevent the elevation of palatine processes. In other cases, however, the growth of palatine processes is retarded in such a way that although elevation occurs, the crests would be too small to establish contact. The formation of a cleft may also result from a failure in the programmed cell death of the covering epithelium, thus preventing coalescence of the mesenchyme which, with the growth of the fetus, would lead to a rupture in the median line [10].

In this study, in accordance with the microscopic assessment, the palatine processes were shown to be united in the median plane, without remaining epithelial cells at the palatine process lines fusion with the nasal sept (Figure 1). This characteristic is a normal development of the studied fetuses (20 days old), in view of the Bittencourt and Bolognese [11] report that at 16 days, in normal palatogenesis in rats, the palatine projections were already found to be united, or at least in contact, without any space between them.

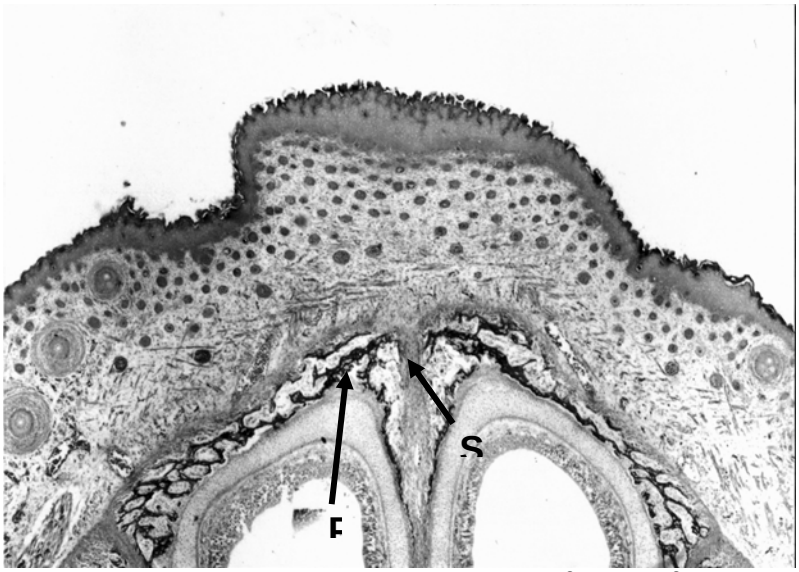


Figure 1 - Photomicrograph illustrating the fusion of palatine processes on the 20$^{th}$ day of fetal development. HE. X100.

Trabeculated bone of variable size and thickness, containing osteoblasts on its surface, was also observed growing in the direction of the median line. The palate bone was mature, with an organized appearance of the trabeculae, disposed horizontally and perpendicular to the median palatine suture in formation.

The fact that no cleft palate was observed in any individual of the progenies, cannot wholly exclude the teratogenic potentiality of the drug. According to Scott [12] the mechanism of interaction between genetic and environmental factors in the production of development anomalies is complex. Furthermore, a single genetic constitution, that is, a single animal species is not sufficient to assess the teratogenicity of a drug, and testing the component in various species is indicated, before making affirmations about its teratogenic potential.
The effects of cortisone, for example, are very variable, depending on the genetic constitution of the specimen the sample is comprised of [13]. Similarly, Biddle and Fraser [14] observed difference in tolerance to cortisone between different species of mice, resulting in a large variation in the frequency of cleft palate. The authors suggested that this difference is related to the small number of genes.

Another factor to consider as regards the results found is that the males were not exposed to the potential teratogenic agent. One of the factors related to the appearance of cleft palate is related to the presence of a genetic mutation, and this could occur in one of the progenitors or in both of them, although available evidences suggest that methyl parathion is non-mutagenic [7].

In accordance with the Extension Toxicology Network [7] various clinical parameters of methyl parathion were assessed in order to verify its systemic and fetal toxicity. Through experiments with animals, it was found that Folidol acts on reproduction, altering fetal growth and bone formation, the effect being potentially fatal in dependant-doses. But the administration of 4 to 6 mg/kg on the $9^{th}$ or $15^{th}$ day of gestation did not cause teratogenicity.

These findings corroborate those of the present study, in which Folidol via topical application administered before and during gestation, did not cause cleft palate.

Topical Folidol application must be considered, as it is an organophosphate agent, it presents high liposolubility, and is easily absorbed by the skin, lungs and gastrointestinal tract. This facility of absorption by all the pathways is responsible for the intoxication of human manipulators, and for its efficiency as an insecticide [15]. This suggests that the administration pathway and the time of exposure to the drug (G1 - 30 days and G2 - 20 days) generates a condition of intoxication, mainly because of being composed of liposolubles that are slowly eliminated, have a high reabsorption content, thus increasing its plasmatic concentration.

The toxicity of the organophosphates has been under constant investigation. Chlorpyrifos caused cleft palate in mice, but did not result in maternal toxicity [6]. It is worth pointing out that a single dose of 80 mg/kg was administered intraperitoneally, which suggests a dose dependant effect. This suggests that the dose and administration pathway are determinant factors for setting of a condition of teratogenicity.

When compared with the present findings, the tested organophosphate did not cause cleft lip or maternal toxicity, possibly because it did not attain adequate seric levels with relation to the drug dose, as there is a certain limit beyond which the teratogenic effects appear. This limit of harmful values characterizes the teratogenic zone [16].

Therefore, the relation between the dose, administration pathway, nature and pharmacologic effect interfere in the teratogenic action of drugs; furthermore, certain groupings have an affinity for certain tissues, thus explaining the certain constancy of organic effects. Other pharmaceutical substances, with multiple embryonic organotropism, may cause the appearance of multiple defects [17].

Thus, the histologic assessment allowed one to conclude that the pesticide Folidol did not present teratogenic potential for inducing cleft palate formation in the specimens of the studied sample.

**Acknowledgements**

The authors thank the Federal University of Rio de Janeiro, Pontifical Catholic University of Paraná, CAPES and CNPq.

**References**

1. Kumar KB, Devi KS. Teratogenic effects of methyl parathion in developing chick embryos. Vet Hum Toxicol 1992; 34:408-10.

2. Alvarez R, Honrubia MP, Herraez MP. Skeletal malformations induced by the insecticides ZZ-Aphox and Folidol during larval development of Rana perezi. Arch Environ Contam Toxicol 1995; 28:349-56.

3. Deli E, Varnagy L. Teratological examination of Wofatox 50 EC (50% methylparathion) on pheasant embryos. Anat Anz 1985; 158:237-40.

4. Varnagy L, Deli E. Comparative teratological study of insecticide Wofatox 50 EC (50% methyl-parathion) on chicken and pheasant fetuses. Anat Anz 158:1-3.

5. Varnagy L, Korzenszky M, Fancsi T. Teratological examination of the insecticide methylparathion (Wofatox 50 EC) on pheasant embryos. 1. Morphological study. Vet Res Commun 1984; 8:131-9.

6. Tian Y, Ishikawa H, Yamaguchi T, Yamaguchi T, Yokoyama K. Teratogenicity and developmental toxicity of chlorpyrifos. Maternal exposure during organogenesis in mice. Reprod Toxicol. 2005; 20:267-270.

7. Extension Toxicology Network. Pesticide Information Profiles. Methyl-parathion. 1996. Available: http://extoxnet.orst.edu/pips/methylpa.htm [accessed 26 October 2004].

8. Goldim JR. Pesquisa em Saúde: leis, normas, e diretrizes. 2 ed. HCPA: Porto Alegre, 1995.

9. Bivin WS, Crawford MP, Brewer NR. Morphophysiology. In: Baker HJ, Lindsey JR, Weisbroth SH, editor. The laboratory rat: biology and diseases. New York: Academic Press, 1979: 73-103.

10. Moore KL. The developing human: clinically oriented embryology. WB Saunders, Philadelphia, 1982.

11. Bittencourt MAV, Bolognese AM. Epithelial alterations of secondary palate formation. Braz Dent J 2000; 11:117-126.

12. Scott JH. The embryology of cleft palate and hare lip. Brit Dent J 1966;120:17-20.

13. Walker B. 1971. Induction of cleft palate in rats with anti-inflammatory drugs. Teratolology 1985; 4:39-42.

14. Biddle FG, Fraser FC. Cortisone-induced cleft palate in the mouse: a search for the genetic control of the embryonic response trait. Genetics 1977; 85:289–302.

15. Leblanc FN, Benson BE, Gilg AD. A severe organophosphate poisoning requiring the use of an atropine drip. Clin. Toxicol 1986; 24:69.

16. Beeley L. Adverse effects of drug in later pregnancy. Clin Obstet Gynecol 1981; 8:275-289.

17. Farrar HC, Blumer JL. Fetal effects of maternal drug exposure. Annu Rev Pharmacol 1991; 31:525-547.