

EFFECT OF AN ORGANOPHOSPHORATE INSECTICIDE ON THE TESTIS, EPIDIDYMIS AND PREIMPLANTATIONAL DEVELOPMENT AND PREGNANCY OUTCOME IN MICE.

Héctor R. CONTRERAS y Eduardo BUSTOS-OBREGON

Department of Cellular Biology and Genetics, Faculty of Medicine, University of Chile, Santiago, Chile.

Among the drugs more utilized in the control of plagues in the agriculture highlights Malathion. This organophosphorate pesticide acts by non reversible inhibition of the acetylcholinesterase in the nervous system and has a low toxicity due to its rapid enzymatic degradation. Therefore, it is a frequently used pesticide.

Both Malathion and its active metabolite, Maloxon, produce chromosomal damage "in vivo" and "in vitro". Given that the seminiferous epithelium has a high mitotic index and that the alteration in the genetic material of germ cells is potentially inheritable, the effect of Malathion on the male fertility was studied.

The objectives of the work are : a) to determine the ability of Malathion to induce alterations in the morphology of the spermatid cells and of the germinal epithelium, b) to determine the relative sensibility of four different germs cell populations to the pesticide; c) to determine the fertilizing ability of male mice treated with Malathion and d) to separate the general toxic effect from the gonadotoxic effect .

The study was carried out in male mice with a single intraperitoneal dose of Malathion (96,6% purity, Cheminova, Denmark) . The control mice were injected with the vehicle (corn oil). Experimental and control mice were sacrificed at 4, 14, 18 and 26 days post injection. Timing was selected with the help of the computer program STAGES to evaluate the following germ cell populations : elongated spermatids (day 4); round spermatids (days 14); pachytene primary spermatocytes (days 18) and from preleptotene spermatocytes (days 26). Epididymal spermatozoa were obtained. Counts were done using a Neubauer hemocytometric chamber. Hematoxylin-eosin stained sperm morphology was evaluated in light microscopy (1000x) to establish a teratozoospermic index. Testes fixed in Bouin's alcoholic solution were processed for light microscopy. PASchiff-hematoxylin stained slides were inspected at 400x. Two hundred cross sections of seminiferous tubules per animal were evaluated for normal or depleted spermatogenesis. In order to evaluate the fertilizing ability of the experimental and control males, they were crossed with female superovulated by intraperitoneal injection of 5 UI de Humegon (FSH + LH, Organon, USA) and 48 hours later 5 UI de Pregnil (hCG, Organon, USA). At 48 hours post coitum a group of females was sacrificed and their oviducts washed in order to count and evaluate the oocytes to the stage of two blastomeres at 96 hours post coitum. Another group was sacrificed in order to evaluate blastocytes gotten from the uterus. The criteria of normality utilized were those established by Blandau (1971). The progenie was evaluated according to the number of animals born alive, weight and phenotype. In order to evaluate the general toxicity of the Malathion, the activity of the plasma acetylcholinesterase was determined by a colorimetric method . The histology of some target organs like liver, lung and kidney were analyzed by conventional methodology , in slides stained by H/E and Arteta.

The results show a significant increment of the teratozoospermia in the experimental animals. All the testicular cell populations studied were sensitive to the pesticide, being more resistant the spermatocytes in pachytene. The spermatid abnormality predominant affected the middle piece and was associated with an increase of tail anomalies, sperm count and depleted tubules on days 18 and 26 post injection. Concerning fertilizing ability of these males, our results indicated that the teratozoospermia due Malathion is not reflected in alterations in the preimplantational development and pregnancy outcome since no significant differences were appreciated in the number of fecundated oocytes, blastocytes and animals born alive. The effect of Malathion in the experimental conditions here used could be classified as gonadotoxicity since no obvious general toxic effects were seen.

REFERENCES

- Blandau, R. (1971) *The Biology of Blastocyst*. The University of Chicago Press. Chicago and London.
- Flessel, P.; Quintan, P. y Hooper, K. (1993) Genetic toxicity of Malathion: a review. *Environ Mol Mutagen* 22:7-17
- Salvadori, D.; Ribeiro, L.; Pereira, C. and Becak, W. (1988) Cytogenetic effects of Malathion insecticide on somatic and germ cells of mice. *Mut Res* 204: 283- 287
- Taylor, P. (1988) Agentes anticolinesterasa. En: *Las Bases Farmacológicas de la Terapéutica*. Goodman, G.A.; Goodman L.S.; Rall, T.W. & Murad, F. (Eds) 7ª Edición. Editorial Medica Panamericana. Buenos Aires, Argentina. pp 121-138.
- Vigil, P. y Bustos-Obregón, E. (1985) Alkylating agents and mouse spermatogenesis : Effects of a single dose of cyclophosphamide. *Andrología* 17:276-282