## EFFECT OF IMMERSION FLUID TEMPERATURE ON THE CHICKEN EMBRYO IN TERATOGENICITY TESTS: SHORT COMMUNICATION

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The influence of immersion fluid temperature on the development of the chicken embryo was studied on the day most commonly used for treating incubated eggs in teratological trials. Embryonated eggs were immersed in tap water for 30 min on the 12th day of incubation at 22–25 °C or at incubation temperature without a waiting time or after 30 min. The incubation was then continued and the eggs were processed on day 19 of the incubation period. Treatment of eggs at 22–25 °C caused a significant increase in embryonic mortality, while the 30-min waiting time did not exert an influence on embryogenesis.

Key words: Embryotoxicity, temperature, immersion, chicken

Fertile eggs are often applied in the teratogenicity testing of xenobiotics (Karnofsky, 1965; Várnagy, 1981*b*; Hoffman and Albers, 1984). The treatment of eggs is carried out in different phases of embryonic development (Somlyay and Várnagy, 1988; Várnagy et al., 1996). One of the models used for simulating the natural contamination of eggs is immersion into a solution of the experimental agent (Várnagy, 1981*a*; Várnagy et al., 1988; Németh et al., 1999).

Tap water is the most frequently used solvent, the temperature of which can affect the embryogenesis. The length of exposure most commonly used in the international practice is 30 min.

Deviation of immersion fluid temperature from the incubation temperature and the length of waiting time before treatment may also have an effect on the further development of embryos.

The aim of this study was to demonstrate in a model experiment the effect of immersion fluid temperature on treatment and the influence of the waiting time before the treatment of eggs on chicken embryogenesis.

Tap water was used as general solvent. The chemical and microbiological quality of water was checked by special laboratories.

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Shaver Starbro broiler (Goldavis, Poultry Incubator Station, Keszthely, Hungary) eggs were used. The eggs were incubated in a Ragus type incubator. Treatment was performed on day 12 of the incubation period by immersion technique (volume: 5 l/group) after or without a 30-min waiting time. The following groups were used: Group I: not immersed eggs; Group II: eggs immersed immediately at 22–25 °C; Group III: eggs immersed immediately at incubation temperature; Group IV: eggs immersed after a 30-min waiting time at 22–25 °C; Group V: eggs immersed after a 30-min waiting time at incubation temperature.

The eggs were processed on day 19 of incubation. Macroscopic evaluation was performed and the embryos were weighed. The numerical results obtained during the experiment are summarised in Table 1. The body mass data did not show significant differences between the untreated and the treated groups. Immersion of the incubated eggs at 22–25 °C caused a remarkable increase in embryotoxicity. The waiting time before the treatments had no influence on the rate of embryonic mortality.

Table 1
Summary of the effects exerted by treatment temperature

Groups	Waiting time before treatment (min)	Water temperature (°C)	Body mass of the embryo (g) Average $\pm$ SD	Live embryos/	Embryonic death, total/after treatment (%)	Embryos showing developmental anomalies <sup>a</sup>	
						No.	%
I	_	_	$33.01 \pm 2.86$	18/20	10/5	1 <sup>b</sup>	5.6
II	0.0	22-25	$32.82 \pm 1.52$	10/20	50/40	0	0.0
III	0.0	36–38	$32.60 \pm 2.19$	16/20	20/10	0	0.0
IV	30.0	22-25	$30.98 \pm 2.69$	11/19	57.9/31.6	1 <sup>b</sup>	9.1
V	30.0	36–38	$32.18 \pm 3.29$	19/19	0/0	0	0.0

<sup>&</sup>lt;sup>a</sup> = Referring to live embryos; <sup>b</sup> = Cyllosis

Embryonic alterations occurred sporadically, only cyllosis was observed in two cases.

The results show that the temperature of the immersion fluid used in the teratogenicity (ecotoxicological) test of xenobiotics on avian embryos should be identical with the temperature of incubation. Otherwise, under the influence of cold, in this case due to the low temperature of the immersion fluid, embryonic mortality will increase, which disturbs the assessment of the toxic effects of xenobiotics.

## References

- Hoffman, D. J. and Albers, P. H. (1984): Evaluation of potential embryotoxicity and teratogenicity of 42 herbicides, insecticides, and petroleum contaminants to mallard eggs. Arch. Environm. Contam. Toxicol. **13**, 15–17.
- Karnofsky, D. A. (1965): The chick embryo in drug screening; survey of teratological effects observed in the 4-day chick embryo. In: Wilson, J. G. and Warkany, J. (eds) Teratology. Principles and Techniques. The Univ. Chicago Press. Chicago and London. pp. 194–213.
- Németh, Á., Hlubik, I., Kertész, V., Palkovics, A., Varga, T., Várnagy, L. and Vu Kim Cau (1999): The effect of a liquid nitrogen fertilizer on the reproduction of birds (in German, with English abstract). Z. Jagdwis. **45**, 35–44.
- Somlyay, I. and Várnagy, L. (1988): Effects of various pesticides on plasma biochemistry of chicken embryos. Microbios Letters **39**, 7–11.
- Várnagy, L. (1981*a*): Teratogenicity testing of Parathion 20 WP, Methylparathion 18 WP and Wofatox 50 EC in Japanese quail embryos by egg immersion technique. Acta Vet. Acad. Sci. Hung. **29**, 85–90.
- Várnagy, L. (1981b): Teratological examination of agricultural pesticides on Japanese quail (Coturnix coturnix japonica) eggs. Acta Vet. Acad. Sci. Hung. 29, 77–83.
- Várnagy, L., Déli, É. and Baumann, M. (1988): Scanning electron microscopic examination of cartilage in chicken embryos treated with the insecticide Wofatox 50 EC. Acta Vet. Hung. 36, 117–121.
- Várnagy, L., Varga, T., Hlubik, I., Budai, P. and Molnár, E. (1996): Toxicity of the herbicides Flubalex, Fusilade S and Maloran 50 WP to chicken embryos after administration as single compounds or in combination. Acta Vet. Hung. 44, 363–376.