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Body Weight and Malformations in Relation to Pesticide Embryotoxicity

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Abstract

Background: Bendiocarbamate is a carbamate insecticide, which belongs among the cholinesterase inhibitors. Cholinesterase inhibitors is a group of chemical compounds, which inhibits acetylcholine hydrolysis by acetylcholinesterase, thereby acetylcholine accumulates in the reactive locations of a living organism. Objective: The aim of the study was to investigate toxicity dose of bendiocarbamate to chicken embryo. Bendiocarbamate was administered (8-1600 μg/egg) to chicken embryos on day 2 of incubation. Results: The results obtained that toxicity of bendiocarbamate is rather low even if the weight of chicken embryos was low with higher concentration of bendiocarbamate. Only 4 % (144/4) chicken embryos with malformation was observed. Conclusion: We used the chick embryos of Leghorn breed (variant sprayed) and it is possible that another breed could be more sensitive to bendiocarbamate effect. The results obtained that toxicity of bendiocarbamate is rather low even if the weight of chicken embryos was low with higher concentration of bendiocarbamate. Only 4 % chicken embryos with malformation was observed.

Keywords— Bendiocarbamate, chicken, embryo, malformations, toxicity

INTRODUCTION

The pesticides currently used in agriculture include a wide variety of compounds belonging to different chemical classes [1]. Like other carbamate insecticides, bendiocarb (BC) is reversible inhibitor of acetylcholinesterase (AChE). The blockage of AChE caused by BC persists for approximately 24 hours and, subsequently, the situation returns to normal because the insecticide does not accumulate in mammalian tissues [2]. In agriculture it is used against a variety of insects, especially those in soil and to control disease vectors (mosquitoes, flies, household and agricultural pests; [3]). Chick embryo is a popular model for developmental pharmacological and toxicological studies. It is readily available, cost-efficient, and presents an alternative approach to treatment of pregnant mammals. The concordance of data from CHEST [4] and mammals is excellent, and it avoids potentially confounding effect of different maternal metabolism between species by allowing for separate testing of human-relevant metabolites. Given the absence of maternal metabolism, it requires considerably smaller amounts of administered substances per embryo, which is particularly useful for testing rare or expensive compounds, or when maternal toxicity is of concern [5].

MATERIAL AND METHODS

Fertile chicken eggs of Leghorn hybrid, variant spotted, were obtained from animal farm Koleč, Institute of molecular genetics AS CR, Praha, Czech republic (144 eggs). They were incubated in a thermostat with forced circulation of air and temperature maintained at 37.5 ± 0.5 °C, and relative humidity of 60 %. The eggs were opened by the modified „window technique“[6]. Embryotoxicity was observed after application of BC on ED 2 (20^{th} stage of development; [7]). Bendiocarb was dissolved in acetone and diluted with sterile water for tissue cultures to obtain the required concentrations. After cutting out the opening in eggshell the dose of bendiocarb (8-1600 μg/egg) was applied to the surface (on *membrana papyracea*). After application the openings were covered with adhesive tape [8] and the chicken embryos were transferred to the thermostat and incubated under standard incubation conditions. The chicken embryos were dissected out of the membranes on ED 9, and we observed weight, malformations and mortality after bendiocarb administration on ED 2. Statistical comparison (Student T-test) of mortality, body weight and malformations was performed using GraphPad Prism 5.

RESULTS

The toxicity of bendiocarb was fairly low, although mortality increased with advancing bendiocarb dose except experimental dose 800 μg/egg. The body weight was higher at bendiocarb doses 8-200 μg/egg and lower at doses 400-1600 μg/egg in comparison with control group. There was a mild but statistically significant dose-dependent reduction in body weight, most pronounced in treatment with dose 800 μg/egg (12 %) in comparison with control group (Graph. 1, 2). The malformations in

surviving embryos were observed rarely (<3%) and occurred in both control and experimental groups. The lethal dose of bendiocarb on ED 2 is the amount 973 μg/egg (Tab. 1, Fig. 1).

Table 1: Embryotoxic effect of bendiocarbamate on ED 2

Dose (μg)	**Number of embryos**	**Dead embryos**	**Dead embryos (%)**	**Malformations**	**Mean od the body weight (g)**	**p-value (body weight)**
0	35	5	14	3	1,355	
8	20	0	0	0	1,546	0,011*
80	22	1	5	1	1,399	0,627
200	13	5	38	0	1,363	0,938
400	11	5	46	0	1,305	0,659
800	21	7	33	0	1,196	0,259
1600	22	16	73	0	1,304	0,675
total	**144**	**39**		**4**		

* $p < 0,05$ (statistical significance)

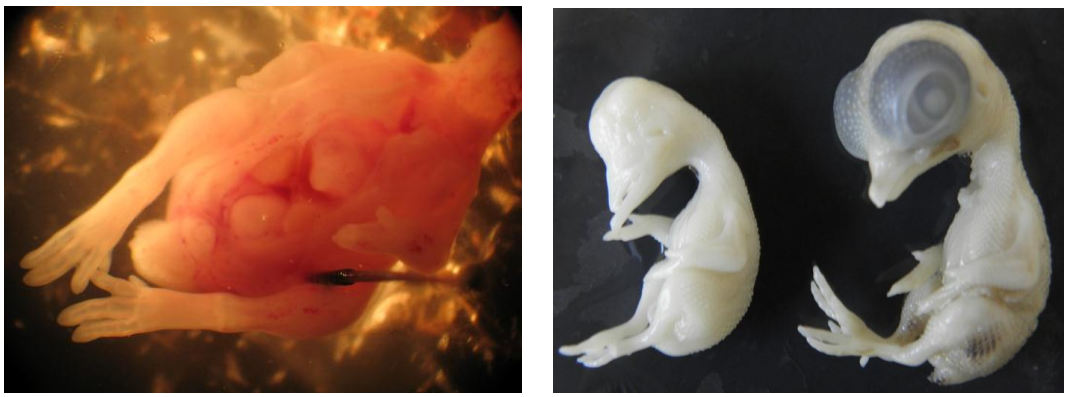
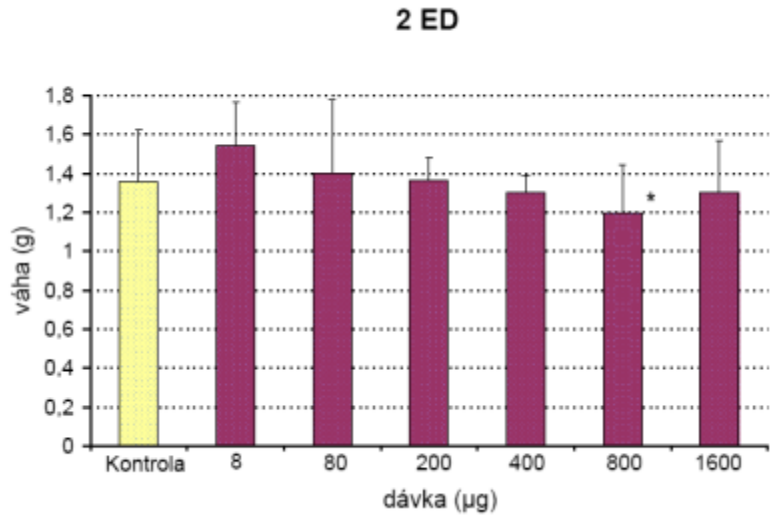
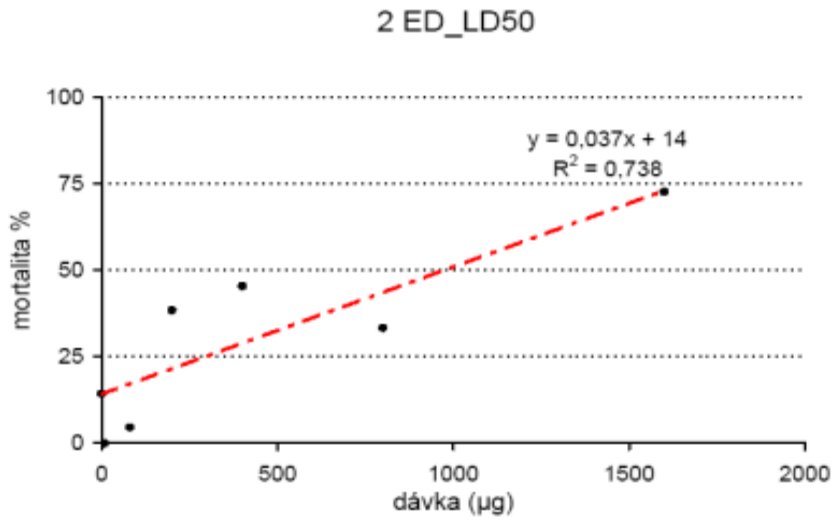


Fig. 1: Opened body cavity (left) and retardation of the growth after bendiocarbamate administration (ED9)



Graph. 1 Embryotoxic effect of bendiocarbamate on ED 2 and its influence on weight of chicken embryo



Graph. 2 Embryotoxic effect of bendiocarbamate on ED 2 and its influence on mortality of chicken embryo (LD_{50})

DISCUSSION

Carbaryl was applicated on ED 5 and ED 15in a dose 45 mg/kg of the egg. Carbaryl was extremely toxic on ED 5 compared to application on ED 15. It caused deformations of legs [9]. Prenatal a postnatal dose (40 mg/kg) caused reduction of fetal body weight and their viability in rat females [10]. Effect of carbamate pesticide on the morphogenesis and development of the chick embryo was observed after

injection of carbaryl in the allantoic sac on ED 5 and ED 6. In comparison with a control group, some morphological alterations have been found in the embryos treated, at the expanse of the skeleton apparatus, particularly in the most distal portions, as tibial and metatarsal angulations with their curtailment [11]. Effect of bendiocarbamate was observed in relation to its dose and embryonic stage of chick embryo development [12]. It is unlikely that such doses or concentration would be achieved during environmental exposure; however, it does not necessarily mean that even lower concentrations could not cause harm to more sensitive individuals.

CONCLUSION

We thus conclude that bendiocarbamate does not possess a significant teratogenic potential, at least in the avian embryo. Nevertheless, large doses that would impair maternal metabolism could cause secondary problems to the developing embryo or fetus in mammals. We used the chick embryos of Leghorn breed (variant sprayed) and it is possible that another breed could be more sensitive to bendiocarbamate effect. The results obtained that toxicity of bendiocarbamate is rather low even if the weight of chicken embryos was low with higher concentration of bendiocarbamate. Only 4 % chicken embryos with malformation was observed.

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REFERENCES

[1] Bjorling-Poulsen, M, Andersen, HR and P, Grandjean., 2008. Potential developmental neurotoxicity of pesticides used in Europe. Environ Health, 7: 50

[2] Kristoff, G, Guerrero, NV, Pechén de D´Angelo, AM and AC, Cochón, 2006. Inhibition of cholinesterase aktivity by azinphos-methyl in two freshwater invertebrates: Biomphalaria glabrata and Lumbriculus variegatus. Toxicology, 3: 185-194

[3] Petrovová, E, Sedmera, D, Lešník, F and L, Luptáková, 2009. Bendiocarb effect on liver and central nervous system in the chick embryo. Journal of Environmental Science and Health, Part B, 4: 383-388

[4] R, Jelinek, 1977: The chick embryotoxicity screening test (CHEST). In: Methods in prenatal toxicology, eds. Neubert, D., Merker, J., Kwasigroch, T. E., pp. 381-386, G. Thieme, Stuttgart.

[5] Petrovová, E, Sedmera, D, Míšek, I, Lešník, F and L, Luptáková,, 2009: Bendiocarbamate toxicity in the chick embryo. Folia Biologica, 55: 61-65.

[6] R,Jelínek,1977. The chick embryotoxicity screening test (CHEST). In: Methods in prenatal toxicology (Neubert, D., Merker, J., Kwasigroch, T. E., Eds.), G. Thieme, Stuttgart, 381-386

[7] Hamburger,V and HL, Hamilton, 1951. A series of normal stages in the development of chick embryos. Journal of Morphology, 88: 49-92

[8] Sedmera, D, Hu, N, Weiss, KM, Keller, BB, Denslow, S and RP, Thompson, 2002. Cellular changes in experimental left heart hypoplasia. Anatomical Record, 267: 137-145

[9] M, Farage-Elawar, 1990. Effects of in ovo injection of carbamates on chick embryo hatchability, esterase enzyme activity and locomotion of chicks. Journal of Applied Toxicology, 10: 197-201

[10] Hayes, WJ and ER, Lawes, 1990. Classes of pesticides. (Ed.)Handbook of pesticide toxicology. Academic Press, New York

[11] L´Abbate, N, Virgintino, D, Ribatti, D, Roncali, L, Miccoli, MG and L, Ambrosi, 1986. Effects of carbaryl on the morphogenesis of the extremities in chick embryos. Giornale italiano di medicina del lavoro Journal, 8: 123-126

[12] Sedmera, D and B, Novotná, 1994. Bromodeoxyuridine-induced shift of malformation spectrum in the chick embryo: stage effect, dose effect and role of cell death. European Archives of Biology, 105: 41-49