Potravinarstvo, vol. 8, 2014, no. 1, p. 107-113
doi:10.5219/343
Received: 14 February 2014. Accepted: 25 February 2014.
Available online: 7 May 2014 at www.potravinarstvo.com

ISSN 1337-0960 (online)

BONE ADAPTATION TO SIMULTANEOUS CADMIUM AND DIAZINON TOXICITY IN ADULT MALE RATS

Hana Chovancová, Radoslav Omelka, Ivana Boboňová, Grzegorz Formicki, Róbert Toman, Monika Martiniaková

ABSTRACT

Food contamination from natural or anthropogenic sources poses severe risks to health of human and animals. Bone is a metabolically active organ, which can be affected by various toxic substances, such as cadmium (Cd) and diazinon (DZN), leading to disruption in bone metabolic processes. The present study was designed to investigate the effect of simultaneous peroral administration to Cd and DZN on femoral compact bone structure in adult male rats. A total of twenty 1-month-old male Wistar rats were randomized into two experimental groups. In the first group (EG), young males were dosed with a combination of 30 mg $CdCl_2$/L and 40 mg DZN/L in drinking water, for 90 days. Ten 1-month-old males without Cd-DZN intoxication served as a control group (CG). After 90 days of daily peroral exposure, evaluations of femoral bone macro- and micro-structure were performed in each group. We found no significant differences in body weight, femoral weight, femoral length and cortical bone thickness between both groups (EG and CG). However, rats from the group EG displayed different microstructure in the middle part of the *substantia compacta* where primary vascular radial bone tissue appeared. In some cases, vascular expansion was so enormous that canals were also present near the periost. On the other hand, they occurred only near endosteal surfaces in rats from the control group. Moreover, a smaller number of primary and secondary osteons was identified in Cd-DZN-exposed rats. This fact signalizes reduced mechanical properties of their bones. Anyway, our results suggest an adaptive response of compact bone tissue to Cd-DZN-induced toxicity in adult male rats in order to prevent osteonecrosis.

Keywords: bone; osteotoxicology; cadmium; diazinon; rats

INTRODUCTION

Most foods contain natural or synthetic chemicals that could represent a toxic hazard for the consumers (**Nassredine and Parent-Massin, 2002**).

Cadmium (Cd) is a heavy metal that is widely present in the environment as pollutant **(Moulis and Thévenod, 2010)**. It still attracts the attention of researchers and the public because its level in food products often exceeds the maximum allowable limits **(Toman et al., 2011)**. According to **Järup and Akesson (2009)**, the diet is the major source (~ 99%) of Cd exposure in the general non-smoking population. Upon absorbed, Cd irreversibly accumulates in the human body, in particularly in kidneys and other vital organs such as lungs and liver **(Bernard, 2008)**. In addition to its cumulative properties, it is also highly toxic metal which can disrupt a number of biological systems, usually at doses that are much lower than most toxic metals **(Järup et al., 1998)**. One of the target organs for Cd toxicity is also bone **(WHO, 1992)**. Exposure to Cd has been linked to bone loss, low bone mass and osteoporosis, and even to an increased incidence of bone fractures **(Wilson et al., 1996; Wang et al., 2003)**. The results obtained by **Brzóska and Moniuszko-Jakoniuk (2005a)** have shown that chronic, even low-level exposure to Cd disturbs bone metabolism during skeletal development and maturity by affecting bone turnover most probably through a direct influence on bone formation and resorption, and indirectly via disorders in Ca metabolism. Besides interfering with Ca, Cd also alters the metabolism of other metals essential for bone health, mainly zinc (Zn), iron (Fe) and copper (Cu); **Noël (et al., 2004)**.

Diazinon (DZN) is a contact organophosphate (OP) pesticide, which is extensively used in agriculture **(Salehi et al., 2009)**. Like other organophosphates (OPs), the main toxic action of DZN is inhibition of acetylcholinesterase (AChE) activity, which results in accumulation of acetylcholine (ACh) and associated neurotoxicity **(Oruc and Usta, 2007)**. Diazinon toxicity varies widely within and among species, and is modified by organism age, sex, body size, climatic conditions, pesticide formulation, chemistry of the environment, and other factors **(Montz, 1983)**. According to **Garg et al. (2004)**, a potential target of pesticide toxicity is the skeletal system. Marked impairment in the development of the backbone in ducklings due to OPs toxicity has been observed in the study by **Ludle et al. (1979)**. Higher amounts of DZN caused additional defects in quail and chicken including folding of the spinal cord, shortening of the neck **(Wyttenbach and Hwang, 1984)**, fusing and twisting of vertebrae, abnormal development of ribs and breastbone **(Meneely and Wyttenbach, 1989)**, curled claws, reduced

growth of leg and wing bones **(Cho and Lee, 1990)**, and reduced bone calcification **(Cho and Lee, 1991)**. In addition, OPs cause a significant reduction in bone mass and density in individuals following chronic low-level intoxication **(Compston et al., 1999)**. Results by **Lari et al. (2011)** indicate that DZN exposure is associated with decrease in trabecular and cortical bone density and might be one of the causes for worldwide increasing prevalence of osteoporosis.

The aim of the current study was to investigate the effect of co-administration to Cd and DZN as food contaminants on femoral bone structure in adult male rats.

MATERIAL AND METHODOLOGY

Our experiment was conducted on twenty 1-month-old male Wistar rats obtained from the accredited experimental laboratory (number SK PC 50004) of the Slovak University of Agriculture in Nitra. The animals were housed individually in plastic cages under constant temperature (20-22 °C), humidity (55 ±10%), and 12/12 h cycle of light and darkness with the provision of food (feed mixture M3, Machal, Bonargo, Czech Republic) and water *ad libitum*. Clinically healthy rats (free of typical rodent pathogens) were randomly divided into two groups, of 10 animals each. In the first group (EG), young males were dosed with a daily intake of 30 mg $CdCl_2$/L in combination with 40 mg DZN/L in drinking water for 90 days. The second group without Cd and DZN supplementation served as a control (group CG). The water consumption was daily monitored during the whole experiment. The xenobiotics used in our experiment were chosen on the basis of their possible occurrence in the human and animal food (**Toman et al., 2011**). Indeed, correlation coefficients found between Cd and DZN in men (0.70) and women (0.69) indicate high probability of exposure to both compounds (**Toman et al., 2012**). The doses of Cd and DZN (chosen based on studied literature and our previous experiments with tested dose-response effects) were high enough to reach a toxicity level but also safe enough to prevent animal mortality (non-lethal doses). All procedures were approved by the Animal Experimental Committee of the Slovak Republic. At the end of the experiment, all animals were killed, weighed, and both their femurs were used for macroscopic and microscopic analyses. After cleaning all soft tissues, right femurs were weighed on analytical scales with an accuracy of 0.01g and the length was measured with a sliding instrument. The unpaired Student′ s T-test was used for establishing statistical significance between both experimental groups. The significance level was accepted at $P < 0.05$. For histological investigation, each right femur was sectioned at the midshaft of its diaphysis. The obtained segments were placed in HistoChoice fixative (Amresco, USA). Specimens were then dehydrated in ascending grades of ethanol and embedded in epoxy resin Biodur (Günter von Hagens, Heidelberg, Germany) according to **Martiniaková et al. (2007)**. Transverse thin sections (70-80 μm) were prepared with a sawing microtome (Leitz 1600, Leica, Wetzlar, Germany) and affixed to glass slides by Eukitt (Merck, Darmstadt, Germany) as previously described (**Martiniaková et al., 2008**). The qualitative histological characteristics of the compact bone were determined according to the internationally accepted classification systems of **Enlow and Brown (1956)** and **Ricqlés et al. (1991)**, who classified bone into three main categories: primary vascular tissue, non-vascular tissue and Haversian bone tissue. Various patterns of vascularization can occur in primary vascular bone: longitudinal, radial, reticular, plexiform, laminar, lepidosteoid, acellular, fibriform and protohaversian. There are three subcategories identified in Haversian bone tissue: irregular, endosteal and dense.

RESULTS

Body weight, femoral weight, femoral length and cortical bone thickness did not differ significantly between both experimental groups (Tab. 1).

Femoral diaphyses of rats from the control group had the following microstructure in common. The internal layer surrounding the medullary cavity (i.e. endosteal border) was formed by non-vascular bone tissue in all views of the thin sections. The bone tissue contained cellular lamellae and osteocytes. Primary and/or secondary osteons were not present. Additionally, there were also identified some areas of primary vascular radial bone tissue in anterior, posterior and lateral views. This type of bone tissue was created by branching or non-branching vascular canals radiating from the bone marrow cavity. Some primary and secondary osteons were also found especially in the anterior and posterior views near the endosteal surfaces.

Table 1 Body weight, femoral weight, femoral length and cortical bone thickness in adult male rats co-administered to 30 mg $CdCl_2$/L and 40 mg DZN/L in drinking water (group EG) and control rats (group CG)

Rat's group	N	Body weight (g)	Femoral weight (g)	Femoral length (cm)	Cortical bone thickness (mm)
EG	10	427.78±19.22	1.03±0.07	3.98±0.09	0.573±0.066
CG	10	405±52.65	1.05±0.17	3.94±0.09	0.575±0.048
T-test		NS	NS	NS	NS

N: number of rats, NS: non-significant changes

In the middle part of the compact bone, a few primary and secondary osteons were identified.

However, dense Haversian bone tissue characterized by dense concentration of secondary osteons was not observed. Finally, the periosteal border of analysed bones was again composed of non-vascular bone tissue, mainly in the anterior and posterior views (Fig. 1).

The rats simultaneously exposed to Cd and DZN displayed a similar microstructure to rats from the control group, except for the middle part of the compact bone where primary vascular radial bone tissue was observed. Vascular canals expanded into the central area of the bones from endosteal surfaces. The canal expansion was in some cases so enormous that the canals also occurred near periosteal surfaces. As a result of this process, a smaller number of primary and secondary osteons was identified in the Cd-DZN-intoxicated rats (Fig. 2). But no clinical manifestations of osteoporosis (i.e. resorption lacunae or osteoporotic fractures) were revealed in these rats.

DISCUSSION

Our results showed non-significant effect of simultaneous peroral application of Cd and DZN on body weight, femoral weight and femoral length in adult male rats. Correspondingly, no demonstrable changes in body weight **(Brzóska and Moniuszko-Jakoniuk, 2005b; 2008; Martiniaková et al., 2013)** and femoral length **(Brzóska et al., 2007; 2008; 2010; Martiniaková et al., 2013)** have also been reported in adult male rats after their peroral exposure to 5, 30 or 50 mg Cd/L in drinking water. Also, subchronic intoxication with a sole dose of DZN (the same level as it was used in our study) did not induce significant alterations in body weight of rats **(Cabaj, 2012)**. Weight of femoral bone in rats perorally receiving mixture of Cd and DZN were similar to those from the control group; however, in our previous study **(Martiniaková et al., 2013)** we have found that Cd administered in single dose had a positive impact on femoral weight in adult male rats. On the basis of these findings we can conclude that beneficial effect of Cd on femoral weight in adult male rats is in interaction with DZN suppressed.

The thickness of cortical bone is generally accepted as an important parameter in the assessment of cortical bone quality and strength. According to **Garn et al. (1991)**, cortical thickness of femoral shaft is a good measuring site for evaluation of bone mass. Our research demonstrates no significant differences in cortical bone thickness between rats co-administered to Cd and DZN, and those of the control group. In the study by **Comelekoglu et al. (2007)**, cortical thickness in the femoral diaphysis was also unchanged in adult female rats after common low intraperitoneal administration of Cd for 18 weeks. On the other hand, cortical bone thickness in rats from our control group was higher in comparison with the value published by **Comelekoglu et al. (2007)** for 4 month-old female rats (0.45 ±0.0057 mm). This discrepancy may be influenced by the different gender and strain of the animals in the two experiments. It is well known that earlier completion of longitudinal growth and earlier inhibition of periosteal apposition produces a smaller bone in females **(Seeman, 2008)**.

The results of the qualitative histological analysis of femurs from the control rats correspond with previous works **(Enlow and Brown, 1958; Martiniaková et al., 2005; Reim et al., 2008; Martiniaková et al., 2009)**. The basic structural pattern of compact bone tissue was non-vascular. In addition, there were some areas of primary vascular radial and/or irregular Haversian bone tissues. However, there was no evidence of true Haversian intracortical bone remodeling. It is generally known that aged rats and mice lack true Haversian cortical bone remodeling but not cancellous bone remodeling **(Erben et**

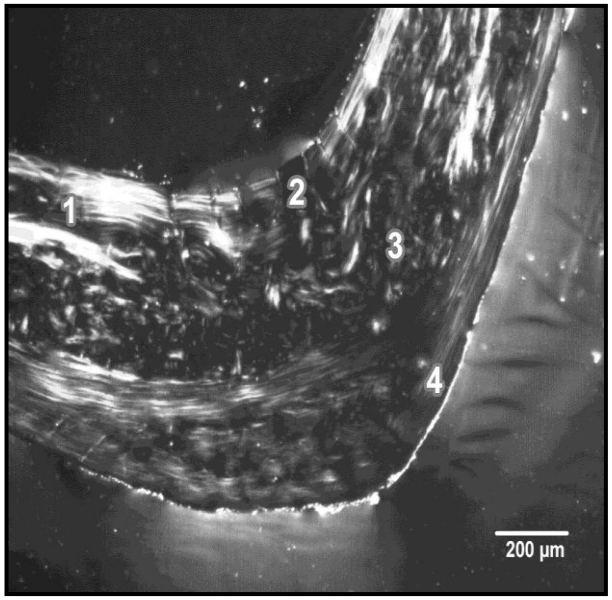

Fig. 1 Microscopic structure of compact bone in rats from the control group:
1 non-vascular bone tissue; 2 vascular canals radiating from marrow cavity; 3 primary and secondary osteons in middle part of compact bone; 4 non-vascular bone tissue.

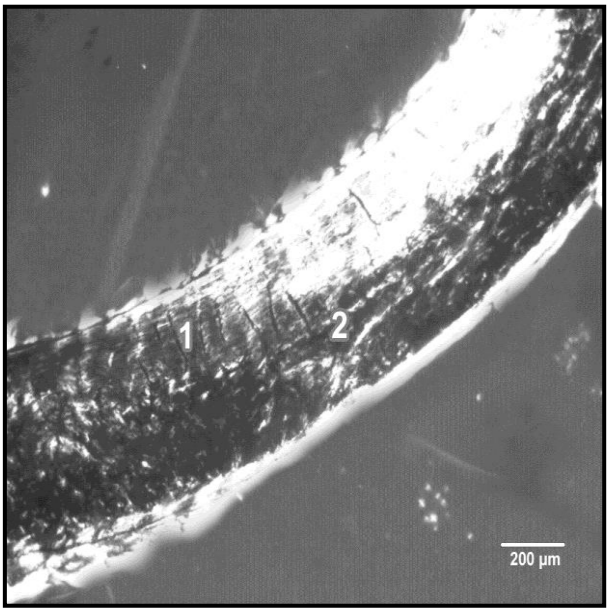

Fig. 2 Microscopic structure of compact bone in Cd-DZN-exposed rats:
1 enormous vascular canals radiating from marrow cavity; 2 smaller number of primary and secondary osteons in middle part of compact bone.

al., 1996; **Reim et al., 2008)**. Therefore, some secondary osteons can be observed in the long bones (near the endosteal border). In our study, the newly formed remodeling units within compact bone originated from the endocortical surface and extended deep into the underlying compact bone. The same findings have also been documented in the study of **Reim et al. (2008)** in 13 month-old male rats.

Prolonged intake of Cd and DZN mixture resulted in induction of demonstrable changes in the middle part of compact bone where vascular canals expanded from endosteal border and led to the formation of primary vascular radial bone tissue. In some cases, vascular canals were also present near periosteal surfaces. The final result of this process was a smaller number of primary and secondary osteons indicating the reduced bone mechanical properties. In general, bone is dynamic tissue that is continuously remodeled to remove microfractures, to adapt to changing mechanical strains and metabolic demands **(Hofstetter, 2007; Chen et al., 2009)**. Disappearance of the Haversian canal system, which was replaced by a large quantity of degenerated, necrotic, and restorative tissues have been demonstrated in the study by **Li et al. (1997)** for ovariectomized rats after a long-term Cd administration. Also, Cd-induced apoptosis of bone cells was documented in many studies **(Coonse et al., 2007; Lévesque et al., 2008; Chen et al., 2009; Smith et al., 2009; Arbon et al., 2012; Brama et al., 2012)**. Furthermore, decreased number of active osteons in broiler chicks was found after exposure to OP pesticides **(Garg et al., 2004)**. In general, DZN exerts its toxicity through inhibition of AChE. According to **Genever et al. (1999)** and **Inkson et al. (2004)**, this enzyme is also expressed by osteoblasts suggesting a role for AChE (i.e. bone matrix protein) in bone tissue. Thus, the expression of high levels of AChE in bone-forming osteoblasts and their progenitors supports a toxic effect of AChE inhibitors (including DZN) on these cells **(Genever et al., 1999; Grisaru et al., 1999; Inkson et al., 2004; Hoogduijn et al., 2006)**. Based on all mentioned aspects we propose that the formation of primary vascular bone tissue, mainly in the central area of the femur, could be explained as an adaptive response of bone to Cd-DZN toxicity, in order to protect the tissue against death of cells and subsequent necrosis. Interestingly, changes in qualitative histological characteristics of compact bone tissue were in these rats less pronounced than in those exposed to only Cd in sole dose **(Martiniaková et al., 2013)**. Indeed, Cd-intoxicated rats displayed a presence of resorption lacunae near endosteal surfaces indicating an early stage of osteoporosis while in rats co-intoxicated with Cd and DZN these structures were absent. This finding suggests that the significant effect of Cd on rat bone microstructure is in combination with DZN partially eliminated. We speculate that the fact could be associated with a molecule of AChE expressing also in bone tissue. According to **Compston et al. (1999)**, this molecule may have a role in the regulation of cell-matrix interactions and in the coupling of bone resorption to bone formation. In addition, the demonstration of Cbfa - 1 and other osteogenic factor-binding motifs on the AChE gene promoter offers further support for a physiological role of AChE in bone formation **(Grisaru et al., 1999)**. Hence, the presence of AChE in bone matrix provides a possible mechanism for OP-induced effects in bone **(Genever et al, 1999)**. The fact was confirmed in the study by **Compston et al. (1999)**, who have found that chronic OP exposure significantly decreased bone formation in agricultural workers. Reduced bone mineral density (BMD) was also observed in rats after application of 15 and 30 mg DZN /kg for 4 weeks **(Lari et al., 2011)**. In respect to all mentioned findings and available scientific papers we propose that absence of resorption lacunae in Cd-DZN-intoxicated rats can be attributed to an opposite impact of Cd and DZN on AChE activity. Indeed, there is evidence that while DZN inhibits AChE, Cd is able to stimulate it. The experiment by **Carageorgiou et al. (2004)** has shown that effect of Cd on brain AChE activity in rats is dose- and exposure duration-dependent. Results revealed that short-term treatment of rats with Cd induces a dose-dependent decrease of brain AChE activity, while long-term Cd administration stimulates it. In accordance with this finding, **Toman et al. (2012)** observed decreasing in AChE activity in rats 36 hours exposed to Cd. On the other hand, Cd administered in drinking water for 3 weeks led to considerable increased the enzyme function in rats **(Srinivasan and Ramprasath, 2011)**. Therefore, we consider that opposite (competitive) effect of Cd and DZN on AChE activity resulted in elimination of DZN adverse impact on function of AChE in bone metabolism that was in microstructural level reflected by absence of resorption lacunae in Cd-DZN treated rats.

CONCLUSION

Our study demonstrates that simultaneous peroral exposure to Cd and DZN did not influence macroscopic structure of femoral bone in adult male rats. However, it has significant impact on bone microstructure in these animals. Co-administration to Cd and DZN affected mainly the middle part of rats' bones where primary vascular radial bone tissue was identified as a result of adaptive response to xenobiotic-induced osteonecrosis. On the other hand, the vascular canal expansion into central area of *substantia compacta* led to a smaller number of primary and secondary osteons signalizing weakened mechanical properties of the bones. Moreover, our study showed that Cd in combination with DZN had less expressive effect on bone microstructure in male rats than Cd in a sole dose.

Food chain contamination is one of the important pathways for the entry of toxic pollutants such as Cd and DZN into the human or animal body. Therefore, monitoring of xenobiotic presence in foods is the first step to prevent their toxic effects on human health including bones.

REFERENCES

Arbon, K. S., Christensen, C. M., Harvey, W. A., Heggland, S. J. 2012. Cadmium exposure activates the ERK signaling pathway leading to altered osteoblast gene expression and apoptotic death in Saos-2 cells. *Food Chem. Toxicol*., vol. 50, p. 198-205. http://dx.doi.org/10.1016/j.fct.2011.10.031 PMid:22019892

Bernard, A. 2008. Cadmium & its adverse effects on human health. *Indian J. Med. Res.*, vol. 128, p. 557-564. PMid:19106447

Brama, M., Politi, L., Santini, P., Migliaccio, S., Scandurra, R. 2012. Cadmium-induced apoptosis and necrosis in human osteoblasts: role of caspases and mitogen-activated protein kinases pathways. *J. Endocrinol. Invest.*, vol. 35, no. 2, p. 198-208. http://dx.doi.org/10.3275/7801 PMid:21697648

Brzóska, M. M., Moniuszko-Jakoniuk, J. 2005a. Disorders in bone metabolism of female rats chronically exposed to cadmium. *Toxicol. Appl. Pharmacol.*, vol. 202, no. 1, p. 68-83. http://dx.doi.org/10.1016/j.taap.2004.06.007 PMid:15589978

Brzóska, M. M., Moniuszko-Jakoniuk, J. 2005b. Bone metabolism of male rats chronically exposed to cadmium. *Toxicol. Appl. Pharmacol.*, vol. 207, no. 3, p. 195-211. http://dx.doi.org/10.1016/j.taap.2005.01.003 PMid:16129113

Brzóska, M. M., Rogalska, J., Galazyn-Sidorczuk, M., Jurczuk, M., Roszczenko, A., Kulikowska-Karpińska, E., Moniuszko-Jakoniuk, J. 2007. Effect of zinc supplementation on bone metabolism in male rats chronically exposed to cadmium. *Toxicology*, vol. 237, no. 1-3, p. 89-103. http://dx.doi.org/10.1016/j.tox.2007.05.001 PMid:17560002

Brzóska, M. M., Galazyn-Sidorczuk, M., Rogalska, J., Roszczenko, A., Jurczuk, M., Majewska, K., Moniuszko-Jakoniuk, J. 2008. Beneficial effect of zinc supplementation on biomechanical properties of femoral distal end and femoral diaphysis of male rats chronically exposed to cadmium. *Chem-Biol. Interact.*, vol. 171, no. 3, p. 312-324. http://dx.doi.org/10.1016/j.cbi.2007.11.007 PMid:18164699

Brzóska, M. M., Majewska, K., Kupraszewicz, E. 2010. Effects of low, moderate and relatively high chronic exposure to cadmium on long bones susceptibility to fractures in male rats. *Environ. Toxicol. Pharmacol.*, vol. 29, no. 3, p. 235-245. http://dx.doi.org/10.1016/j.etap.2010.01.005 PMid:21787608

Cabaj, M. 2012. *The effect of diazinon and selenium on structure and function of testes and epididymis in rats* (in Slovak): dissertation thesis. Nitra: SAU, 180 p.

Carageorgiou, H., Tzotzes, V., Pantos, C., Mourouzis, C., Zarros, A., Tsakiris, S. 2004. In *vivo* and *in vitro* effects of cadmium on adult rat brain total antioxidant status, acetylcholinesterase, (Na+, K+)-ATPase and Mg2+-ATPase activities: protection by L-cysteine. *Basic Clin. Pharmacol. Toxicol.*, vol. 94, no. 3, p. 112-118. PMid:15049340

Chen, X., Zhu, G., Gu, S., Jin, T., Shao, C. 2009. Effects of cadmium on osteoblasts and osteoclasts *in vitro*. *Environ. Toxicol. Pharmacol.*, vol. 28, p. 232-236. http://dx.doi.org/10.1016/j.etap.2009.04.010 PMid:21784008

Cho, J., Lee, C. 1990. Effects of diazinon on the anatomical and embryological changes in the developing chick embryo. *Res. Rep. RDA(V)*, vol. 32, p. 35-47.

Cho, J., Lee, C. 1991. Studies on diazinon induced inhibition of skeletal mineralization in chick embryo. *Res. Rep. RDA(V)*, vol. 33, p. 41-60.

Comelekoglu, U., Yalin, S., Bagis, S., Ogenler, O., Sahin, N. O., Yildiz, A., Coskun, B., Hatungil, R., Turac, A. 2007. Low-exposure cadmium is more toxic on osteoporotic rat femoral bone: mechanical, biochemical, and histopathological evaluation. *Ecotox. Environ. Safe.*, vol. 66, no. 2, p. 267-271. http://dx.doi.org/10.1016/j.ecoenv.2006.01.006 PMid:16530835

Compston, J. E., Vedi, S., Stephen, A. B., Bord, S., Lyons, A. R., Hodges, S. J., Scammell, B. E. 1999. Reduced bone formation after exposure to organophosphates. *Lancet.*, vol. 354, p. 1791-1792. http://dx.doi.org/10.1016/S0140-6736(99)04466-9 PMid:21697648

Coonse, K. G., Coonts, A. J., Morrison, E. V., Heggland, S. J. 2007. Cadmium induces apoptosis in the human osteoblast-like cell line Saos-2. *J. Toxicol. Environ. Health A.*, vol. 70, no. 7, p. 575-581. PMid:17365611

Enlow, D. H., Brown, S. O. 1956. *A comparative histological study of fossil and recent bone tissues*. Part I. Texas J. Sci., vol. 8, p. 405-412.

Enlow, D. H., Brown, S. O. 1958. *A comparative histological study of fossil and recent bone tissues*. Part III. Texas J. Sci., vol. 10, p. 187-230.

Erben, R. G. 1996. Trabecular and endocortical bone surfaces in the rat: modeling or remodeling? *Anat Rec.*, vol. 246, p. 39-46. http://dx.doi.org/10.1002/(SICI)1097-0185(199609)246:1<39::AID-AR5>3.0.CO;2-A PMid:8876822

Garg, U. K., Pal, A. K., Jha, G. J., Jadhao, S. B. 2004. Pathophysiological effects of chronic toxicity with synthetic pyrethroid, organophosphate and chlorinated pesticides on bone health of broiler chicks. *Toxicologic. Pathol.*, vol. 32, no. 3, p. 364-369. http://dx.doi.org/10.1080/01926230490431745 PMid:15204980

Garn, S. M., Rohmann, C. G., Nolan, P. 1991. The developmental nature of bone changes during aging. *Nutr. Rev.*, vol. 49, no. 6, p. 176-178. http://dx.doi.org/10.1111/j.1753-4887.1991.tb03014.x

Genever, P. G., Birch, M. A., Brown, E., Skerry, T. M. 1999. Osteoblast-derived acetylcholinesterase: a novel mediator of cell-matrix interactions in bone? *Bone*, vol. 24, no. 4, p. 297-304. http://dx.doi.org/10.1016/S8756-3282(98)00187-2 PMid:10221541

Grisaru, D., Lev-Lehman, E., Schapira, M., Chaikin, E., Lessing, J. B., Eldor, A., Eckstein, F., Soreq, H. 1999. Human osteogenesis involves differentiation-dependent increases in the morphogenically active 39 alternative splicing variant of acetylcholinesterase. *Mol. Cell Biol.*, vol. 19, no. 1, p. 788-795. PMid:9858601

Hofstetter, W. 2007. Bone remodeling. *Eur. Cell Mater.*, vol. 14, p. 31.

Hoogduijn, M. J., Rakonczay, Z., Genever, P. G. 2006. The effects of anticholinergic insecticides on human mesenchymal stem cells. *Toxicol. Sci.*, vol. 94, no. 2, p. 342-350. http://dx.doi.org/10.1093/toxsci/kfl101 PMid:16960032

Inkson, C. A., Brabbs, A. C., Grewal, T. S., Skerry, T. M., Genever, P. G. 2004. Characterization of acetylcholinesterase expression and secretion during osteoblast differentiation. *Bone*, vol. 35, no. 2, p. 819-827. http://dx.doi.org/10.1016/j.bone.2004.05.026 PMid:21697648

Järup, L., Berglund, M., Elinder, C. G., Nordberg, G., Vahter, M. 1998. Health effects of cadmium exposure - a review of the literature and a risk estimate. *Scand. J. Work Environ. Health*, vol. 24 (Suppl 1), p. 1-51. PMid:9569444

Järup, L., Ákesson, A. 2009. Current status of cadmium as an environmental health problem. *Toxicol. Appl. Pharmacol.*, vol. 238, p. 201-208. http://dx.doi.org/10.1016/j.taap.2009.04.020 PMid:19409405

Lari, R., Elahi, M. H., Lari, P. 2012. Diazinon exposure reduces trabecular and cortical bone mineral density. *J. Med. Toxicol.*, vol. 8, p. 231.

Lévesque, M., Martineau, C., Jumarie, C., Moreau, R. 2008. Characterization of cadmium uptake and cytotoxicity in human osteoblast-like MG-63 cells. *Toxicol. Appl.*

Pharmacol., vol. 231, no. 3, p. 308-317. http://dx.doi.org/10.1016/j.taap.2008.04.016 PMid:18538363

Li, J. P., Akiba, T., Marumo, F. 1997. Long-term, low-dose, cadmium-induced nephropathy with renal osteopathy in ovariectomized rats. J. Toxicol. Sci., vol. 22, no. 3, p. 185-198. http://dx.doi.org/10.2131/jts.22.3_185 PMid:9279821

Ludle, J. L., Mehrle, M. P., Foster, L. M., Earlkaiser, T. 1979. Bone development in black ducks as affected by dietarytoxophene. *Pestic. Biochem. Physiol.*, vol. 10, p. 168-173. http://dx.doi.org/10.1016/0048-3575(79)90018-X

Martiniaková, M., Grosskopf, B., Vondráková, M., Omelka, R., Fabiš, M. 2005. Observation of the microstructure of rat cortical bone tissue. *Scripta Med*., vol. 78, no. 1, p. 45-50. http://www.med.muni.cz/biomedjournal/pdf/2005/01/45-50.pdf

Martiniaková, M., Grosskopf, B., Omelka, R., Dammers, K., Vondráková, M., Bauerová, M. 2007. Histological study of compact bone tissue in some mammals: a method for species determination. *Int. J. Osteoarch*., vol. 17, no. 1, p. 82-90. http://dx.doi.org/10.1002/oa.856

Martiniaková, M., Omelka, R., Grosskopf, B., Sirotkin, A. V., Chrenek, P. 2008. Sex-related variation in compact bone microstructure of the femoral diaphysis in juvenile rabbits. *Acta Vet. Scand*., vol. 50, p. 15. http://dx.doi.org/10.1186/1751-0147-50-15 PMid:18522730

Martiniaková, M., Omelka, R., Grosskopf, B., Mokošová, Z., Toman, R. 2009. Histological analysis of compact bone tissue in adult laboratory rats. *Slovak J. Anim. Sci*., vol. 42, p. 56-59. http://www.cvzv.sk/slju/sup09/Martiniakova.pdf

Martiniaková, M., Chovancová, H., Omelka, R., Boboňová, I 2013. *Effects of risk substances on bone tissue structure in rats* (in Slovak): scientific monograph. Nitra: UKF, p. 187. ISBN 978-80-558-0295-4.

Meneely, G. A., Wyttenbach, C. R. 1989. Effects of the organophosphate insecticides diazinon and parathion on bobwhite quail embryos: Skeletal defects and acetylcholinesterase activity. *J. Exp. Zool.*, vol. 252, no. 1, p. 60-70. http://dx.doi.org/10.1002/jez.1402520109 PMid:2809535

Montz, W. E. Jr. 1983. *Effects of organophosphate insecticides on aspects of reproduction and survival in small mammals*: dissertation thesis. Blacksburg: Virginia Polytechnic Institute and State University. 176 p.

Moulis, J. -M., Thévenod, F. 2010. New perspectives in cadmium toxicity: an introduction. Biometals, vol. 23, no. 5, p. 763-768. http://dx.doi.org/10.1007/s10534-010-9365-6 PMid:20632201

Nassredine, L., Parent-Massin, D. 2002. Food contamination by metals and pesticides in the European Union. Should we worry? *Toxicol. Lett.*, vol. 127, no. 1-3, p. 29-41. http://www.sciencedirect.com/science/article/pii/S0378427401004805#

Noël, L., Guérin, T., Kolf-Clauw, M. 2004. Subchronic dietary exposure of rats to cadmium alters the metabolism of metals essential to bone health. *Food Chem. Toxicol*., vol. 42, no. 8, p. 1203-1210. http://dx.doi.org/10.1016/j.fct.2004.02.017 PMid:15207369

Oruc, Ö. E., Usta, D. 2007. Evaluation of oxidative stress responses and neurotoxicity potential of diazinon in different tissues of *Cyprinus carpio*. *Environ. Toxicol. Pharmacol*., vol. 23, no. 1, p. 48-55. http://dx.doi.org/10.1016/j.etap.2006.06.005 PMid:21783736

Reim, N. S., Breig, B., Stahr, K., Eberle, J., Hoeflich, A., Wolf, E., Erben, R. G. 2008. Cortical bone loss in androgen-deficient aged male rats is mainly caused by increased endocortical bone remodeling. *J. Bone Miner. Res*., vol. 23, no. 5, p. 694-704. http://dx.doi.org/10.1359/jbmr.080202 PMid:18433303

Ricqlés, A. J. de, Meunier, F. J., Castanet, J., Francillon-Vieillot, H. 1991. Comparative microstructure of bone. Hall, B. K. (ed.): *Bone 3, Bone Matrix and Bone Specific Products*. Boca Raton: CRC Press, p. 1-78. ISBN 0-8493-8823-6.

Salehi, M., Jafari, M., Moqadam, M. S., Salimian, M., Asghari, A. R., Nateghi, M., Abasnejad, M., Haggholamali, M. 2009. The effect of diazinon on rat brain antioxidant system. *Toxicol. Lett*., vol. 189S, p. 123S. http://dx.doi.org/10.1016/j.toxlet.2009.06.424

Seeman, E. 2008. Bone quality: the material and structural basis of bone strength. *J. Bone Miner. Metab*., vol. 26, no. 1, p. 1-8. http://dx.doi.org/10.1007/s00774-007-0793-5 PMid:18095057

Smith, S. S., Reyes, J. R., Arbon, K. S., Harvey, W. A., Hunt, L. M., Heggland, S. J. 2009. Cadmium-induced decrease in RUNX2 mRNA expression and recovery by the antioxidant N-acetylcysteine (NAC) in the human osteoblast-like cell line, Saos-2. Toxicol. Vitro., vol. 23, no. 1, p. 60-66. http://dx.doi.org/10.1016/j.tiv.2008.10.011 PMid:19017541

Srinivasan, R., Ramprasath, C. 2011. Protective role of silibinin in cadmium induced changes of acetylcholinesterase, ATPases and oxidative stress in brain of albino wistar rats. *J. Ecobiotechnol*., vol. 3, p. 34-39.

Toman, R., Adamkovičová, M., Hluchý, S., Cabaj, M., Golian, J. 2011. Quantitative analysis of the rat testes after an acute cadmium and diazinon administration. *Animal Sci. Biotech*., vol. 44, no. 2, p. 188-191. http://spasb.ro/index.php/spasb/article/view/638

Toman, R., Hluchý, S., Golian, J., Cabaj, M., Adamkovičová, M. 2012. Diazinon and cadmium neurotoxicity in rats after an experimental administration. *Scientific Papers: Animal Science and Biotechnologies*, vol. 45, no. 2, p. 137-141. http://spasb.ro/index.php/spasb/article/view/334

Wang H., Zhu, G., Shi, Y., Weng, S., Jin, T., Kong, Q., Nordberg, G. F. 2003. Influence of environmental cadmium exposure on forearm bone density. J. Bone Miner. Res., vol. 18, no. 3, p. 553-560. http://dx.doi.org/10.1359/jbmr.2003.18.3.553 PMid:12619941

WHO: Environmental Health Criteria 134, *Cadmium*. Geneva: IPCS; 1992.

Wilson, A. K., Cerny, E. A., Smith, B. D., Wagh, A., Bhattacharyya, M. H. 1996. Effects of cadmium on osteoclast formation and activity in vitro. *Toxicol. Appl. Pharmacol.*, vol. 140, no. 2, p. 451-460. http://dx.doi.org/10.1006/taap.1996.0242 PMid:8887463

Wyttenbach, C. R., Hwang, J. D. 1984. Relationship between insecticide-induced short and wry neck and cervical defects visible histologically shortly after treatment of chick embryos. *J. Exp. Zool.*, vol. 229, no. 3, p. 437-446. http://dx.doi.org/10.1002/jez.1402290311 PMid:6707597

Acknowledgments:

This study was supported by the grants KEGA 025UKF-4/2012 and KEGA 035UKF-4/2013. This work was co-funded by European Community under project no 26220220180: Building Research Centre „AgroBioTech".

Contact address:
Hana Chovancová, Constantine the Philosopher University, Faculty of Natural Sciences, Department of Zoology and Anthropology, 949 74 Nitra Slovakia, E-mail: hchovancova@ukf.sk.
Radoslav Omelka, Constantine the Philosopher University, Faculty of Natural Sciences, Department of Botany and Genetics, 949 74 Nitra Slovakia, E-mail: romelka@ukf.sk.
Ivana Boboňová, Constantine the Philosopher University, Faculty of Natural Sciences, Department of Zoology and Anthropology, 949 74 Nitra Slovakia, E-mail: ivana.bobonova@ukf.sk.
Grzegorz Formicki, Krakow Pedagogical University, Institute of Biology, Department of Zoology, Krakow 31 054 Poland, E-mail: formicki@ap.krakow.pl
Róbert Toman, Slovak University of Agriculture, Faculty of Agrobiology and Food Resources, Department of Veterinary Sciences, 949 76 Nitra Slovakia, E-mail: robert.toman@uniag.sk.
Monika Martiniaková, Constantine the Philosopher University, Faculty of Natural Sciences, Department of Zoology and Anthropology, 949 74 Nitra Slovakia, E-mail: mmartiniakova@ukf.sk.