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REGULAR ARTICLE

THE INVESTIGATION OF CO-ADMINISTRATION TO CADMIUM, DIAZINON AND SELENIUM ON GROWTH CHARACTERISTICS OF ADULT MALE RATS

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ABSTRACT

In this study, differences in growth characteristics (body weight, femoral weight and femoral length) of adult male rats after single cadmium (Cd), as well as, simultaneous exposure to Cd and diazinon (DZN), and after DZN, Cd and selenium (Se) co-administration were investigated. One-month-old male Wistar rats were randomly divided into four groups of 10 animals each. In the first group (A), rats received a drinking water containing 30 mg of $CdCl_2$/l for 90 days. In the second group (B), males were orally dosed with combination of 40 mg of DZN/l and 30 mg of $CdCl_2$/l in drinking water for 90 days. In the third group C, rats were administered by 40 mg of DZN/l in combination with 5 mg of Na_2SeO_3/l and 30 mg of $CdCl_2$/l in drinking water for the same treatment period. The fourth group of males without additive toxicants, served as a control group (D). The statistical analysis of obtained data showed a beneficial effect of Cd sole dose on femoral weight in adult male rats. Significant changes in femoral weight were observed between rats from the groups A and B, and A and C. On the other hand, there were no significant differences in body weight and femoral length between all experimental (A, B, C) and control (D) groups. Considerable differences were identified only for femoral length among the groups A and C, and the groups B and C. Our

results suggest a protective influence of Cd against Se-induced reduction in body weight and femoral length in rats simultaneously exposed to DZN, Se and Cd in their drinking water. However, positive influence of Cd on rat´s femoral weight is likely suppressed by the toxicity of DZN and/or DZN in combination with Se.

Keywords: rats, femoral bone, diazinon, selenium, cadmium

INTRODUCTION

Cadmium (Cd) is a toxic metal which 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**). Cadmium has been found to produce a wide range of biochemical and physiological dysfunctions in humans and laboratory animals (**Santos *et al*., 2004**). In respect to bone, which is one of the important organs for Cd toxicity (**WHO, 1992**), exposure to this element has been linked to bone loss, low bone mass, and osteoporosis (**Wilson et al., 1996; Wang *et al*., 2003**). The results obtained by **Brzóska and Moniuszko-Jakoniuk (2005)** have shown that chronic, even low-level exposure to Cd disturbs bone metabolism during skeletal development and maturity by affecting bone turnover.

Diazinon (DZN) is a contact organophosphate (OP) pesticide which is extensively used in agriculture (**Salehi *et al*., 2009**). Despite its low persistence in the environment, it is a nonspecific insecticide and highly toxic to animals and humans (**Colovic *et al*., 2010**). According to **Garg *et al*. (2004)**, a potential target of pesticide toxicity is also 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 chord, shortening of the neck **(Wyttenbach and Hwang, 1984)**, fusing and twisting of vertebrae, abnormal development of ribs and breastbone **(Meneely and Wyttenbach, 1989)**, reduced calcification of bones **(Cho and Lee, 1991)**, curled claws, and reduced growth of leg and wing bones **(Cho and Lee, 1990)**. It has also been found that OPs inhibit differentiation of chondrogenic cells in the culture **(Matos *et al*., 1985)**.

Selenium (Se) is an essential dietary trace element. Through selenoproteins it plays an important role in a number of biological processes in humans and other species **(Ognjanovic *et al*., 2008)**. According to **Ebert and Jakob (2007)**, several selenoproteins are expressed in

bone tissue and are important in bone metabolism. However, it is very important to point out that Se beneficial effect is expressed in a very narrow dosage range: the high and low doses of this element are connected with pathological manifestations **(Ošťádalová, 2012)**. Selenium deficiency has been associated with growth retardation, impaired bone metabolism, reduced bone mineral density (i.e. osteopenia; **Moreno-Reyes *et al*., 2006**; **Preedy *et al*., 2009**) and Kashin-Beck disease (osteoarthropathy) which is characterized by atrophy, degeneration and necrosis of cartilage tissue (**Moreno-Reyes *et al*., 1998**). On the other hand, excess intake of Se causes abnormal bone and cartilage development (**Greenberg, 2003**), induces apoptosis in mature osteoclasts (**Chung *et al*., 2006**), osteoblasts (**Chae *et al*., 2000**), and osteoblast-like cells (**Milgram *et al*., 2008**).

In most environmental situations, exposures to only a single exogenous agent are fairly rare. Therefore, the aim of current study was to investigate the effect of single Cd, as well as, common peroral application of DZN and Cd, and simultaneous exposure to DZN, Se and Cd on body weight, femoral weight and femoral length of adult male rats and also to assess possible interactions among these three xenobiotics.

MATERIAL AND METHODS

Our study was carried out on fourty 1-month-old male Wistar rats. The animals were housed individually in plastic cages under the same laboratory conditions of temperature (20-24 °C) and relative humidity (55 ± 10 %) with access to food (feed mixture M3, Bonagro, Czech Republic) and drinking water *ad libitum*. All experiments were provided in accordance with accepted standards of animal care in accredited laboratory (SK PC 50004) of the Slovak University of Agriculture in Nitra.

Clinically healthy rats were randomly divided into four groups, of 10 animals each. In the first group (A), young males were dosed with a daily intake of 30 mg of $CdCl_2$ in drinking water for 90 days. In the second group (B), rats were administered 40 mg of DZN/l in combination with 30 mg of $CdCl_2$/l in drinking water for 90 days. The third group (C) was simultaneously exposed to mixture of 40 mg of DZN/l, 5 mg of Na_2SeO_3/l and 30 mg of $CdCl_2$/l in their drinking water for the same period. The fourth group (D) served as a control and the rats received no toxic substances. 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**). All procedures were approved by the Animal Experimental Committee of the Slovak Republic.

At the end of the experiment (90 days), all animals were killed, weighed and their femora were used for macroscopic analysis. After cleaning all soft tissues, all left femora were weighed by analytical scales and their length was measured by a sliding instrument. Values for macroscopic analysis were expressed as mean ± standard deviation. Comparisons between experimental and control groups were assessed by the one-way analysis of variance (ANOVA) and Post Hoc Tukey's test. The significance level was accepted at $p<0.05$.

RESULTS AND DISCUSSION

Our results from macroscopic evaluation demonstrate no significant effects of single peroral application of Cd (group A), as well as, simultaneous exposure to DZN-Cd (group B), and DZN-Se-Cd (group C) on body weight and femoral length in adult male rats. Statistically significant differences were found only for femoral length between the groups of A and C, and B and C (respectively). On the other hand, femoral weight was considerable increased in rats intoxicated with sole dose of Cd. For this macroscopic parameter, significant alterations were identified among the A and B, and A and C groups of rats (Table 1).

Table 1 Average body weight, femoral weight and femoral length in the control (D) and experimental groups (A, B, C) of rats

Rat´s group		n	Body weight (g)	Femoral weight (g)	Femoral length (cm)
A	(1)	10	422.5±27.21	1.27±0.14	3.99±0.14
B	(2)	9	427.78±19.22	1.03±0.07	3.98±0.09
C	(3)	10	406.5±34.65	1.04±0.09	3.82±0.09
D	(4)	10	405±52.65	1.05±0.17	3.94±0.09
Tukey's test			NS	$1:2^{+}$; $1:3^{+}$; $1:4^{+}$	$1:3^{+}$; $2:3^{+}$

N – number of rats; NS – non-significant changes; P<0,05 (+)

Our study revealed a non-significant effect of single Cd intoxication, as well as, DZN-Cd peroral co-administration on body weight in adult male rats. Similar to this, no considerable changes in body weight were also found in rats subchronic administered by sole dose of DZN (the same level as it was used in our study; **Cabaj, 2012**). On the basis of all mentioned findings it can be concluded that peroral exposure to Cd and DZN (separately and in combination) do not affect body weight in adult male rats.

According to our results, rats fed by a mixture of DZN-Se-Cd in their drinking water (group B) had also unchanged body weight as compared to the control. On the other hand, studies obtained by **Boboňová *et al*. (2011, 2012)** demonstrate a considerable decreased body weights in rats perorally exposed to Se, and DZN-Se (respectively) in drinking water. Indeed, many studies have documented reduced body weight gain in young animals treated with Se compounds and abnormal weight loss in older animals (**Gronbaek *et al*., 1995; Panter *et al*., 1996**). According to **ATSDR (2003)**, growth retardation in intoxicated animals is often accompanied by reduced food and water consumption; however, it also may be caused by the interactions of Se (its compounds) with endocrine hormones that regulate normal growth and body weight of living organisms. In general, it is well known that Se accumulates in the anterior pituitary in Se-exposed animals resulting in decreased secretion of the GH (**Thorlacius-Ussing *et al*., 1987; Thorlacius-Ussing, 1990**). In addition, GH acts directly to control the secretion of insulin-like growth factor (IGF-I), i.e. somatomedin C that provides the proliferative effects of GH on mesenchymal tissue (including bones and muscles). Therefore, this hormone (IGF-I) is also involved in control of organism growth (**Ništiar *et al*., 2006**). The results by **Thorlacius-Ussing *et al.* (1987)** revealed decreased levels of the GH and somatomedin C in Se-treated rats receiving 15 mg of Na_2SeO_3/l in drinking water. Similarly to Se, decreased secretion of IGF-I by ovarian granulosa cells were also induced by effect of cobalt (**Kolesárová *et al*., 2010**) and iron (**Kolesárová *et al*., 2011**) despite their essentiality for living organisms.

In our experiment, however, values of body weight of rats from the group B (DZN-Cd-Se-treated rats) did not differ significantly from those of the control (C). On the basis of results by **Boboňová *et al*. (2011; 2012)** we suppose that Cd and DZN in combination with Se could eliminate the negative effect of Se on rat's body weight. In non-toxic amounts, Se seems to be a relatively protective factor or antagonism element against toxicity of several xenobiotics probably by forming the selenide complexes (**Ani *et al*., 2007**). In respect to Cd, this protection includes the capability of Se to alter the distribution of Cd in tissues and induces binding of the Cd-Se complexes to proteins, which are similar to metallothioneins (**Ognjanovic *et al*., 2008**). On the other hand, Cd has been shown to decrease the toxicity of Se in laboratory rats. Thus, this heavy metal has been used to alleviate Se poisoning. Experimental evidences suggest that Cd exerts a beneficial effect due to reactions with Se in the intestinal tract to form insoluble Se compounds (**Selinus *et al*., 2005**). Protective role of Se was also confirmed against negative impact of DZN in rats (**Kashanian *et al*., 2008; Cabaj, 2012**). However, results by **Boboňová *et al*. (2012)** showed a significant reduction in

body weight not only in rats receiving drinking water with Se but also after simultaneous DZN-Se supplementation. According to **Szarek *et al.* (1997)**, Se either increases or decreases toxicity of various xenobiotics, including pesticides, depending on its amounts introduced into an organisms. In higher concentration, Se is probably able to affect the atom of S in the molecule of DZN and amplificates the DZN toxicity (**Šiška *et al.*, 2008**). Because of the absence of significant differences in body weight among rats exposed to Se and DZN-Se, respectively, (data not published) we excluded a positive effect of DZN against Se toxicity on body weight in DZN-Se-Cd-treated rats. On the contrary, Tukey's test revealed significant difference in body weight between DZN-Se and DZN-Se-Cd-exposed rats. This finding indicates there is antagonism interaction between Cd and Se in rats simultaneously intoxicated with DZN, Se and Cd. Anyway, our results suggest a protective effect of Cd against Se toxic influence on body weight in adult male rats.

Weights of femoral bones in rats perorally receiving mixture of DZN-Cd (group B) and DZN-Se-Cd (group C; respectively) were also similar to those from the control. On the other hand, Cd administered in sole dose (group A) showed a positive considerable impact on femoral weight in these animals. According to **Zglinicki *et al.* (1992)**, very low Cd concentrations (down to 100 pM) stimulate cell growth and DNA synthesis; whilst at concentrations above 1 mM, they inhibit cell growth and DNA synthesis in a wide variety of cell types. We used the intermediate dose of Cd (30 mg $CdCl_2$/l) in our experiment. It is important to point out that Cd in drinking water contributes only to less than a few percent of the total Cd intake (**Järup *et al.*, 1998**). Therefore, prolonged daily intake of 30 mg of Cd/l in drinking water could have a stimulating effect on bone weight in our rats. However, beneficial impact of Cd on rat's femoral weight was not observed in combination with DZN, as well as, in mixture of DZN and Se. We suppose that it was suppressed by interaction with DZN and/or with DZN-Se combination.

Last parameter evaluated in our study from toxicological effects point of view was length of femoral bones in adult male rats. We established that femoral lengths of rats perorally exposed to Cd (group A), as well as, DZN-Cd (group B), and DZN-Cd-Se (group C) were unchanged compared to the control. Additionally, there were reported significant differences in femoral length among groups A and C, as well as, groups B and C. This finding could indicate a negative effect of Se on length of bones in rats. Our assumption is confirmed by our previous report (**Boboňová *et al.*, 2012**). In general, growth involves a complex set of metabolic events, which are genetically, hormonally and environmentally controlled (**Momenah, 2012**). According to mentioned author, plasma GH, as well as, IGF-I levels are

affected by some physiological disorders that could happen by the exposure to toxic metals and pesticides. Indeed, significant reduction in tibia length due to considerable decrease in plasma IGF-I level in rats subchronic exposed to Se in their drinking water was demonstrated in the study by **Gronbaek *et al.* (1995)**. Moreover, the results by **Boboňová *et al.* (2012)** confirmed toxic impact of combined exposure to DZN and Se on femoral length in adult rats. According to **Sarkar et al. (2000)**, pesticide-induced inhibition of AChE leads to considerable accumulation of ACh in hypophysis and hypothalamus, and subsequently to disorders in secretion of adenohypophysial hormones. Results of **Momenah (2012)** revealed lower plasma IGF-I in DZN-treated rabbits compared to the control group. Results of many studies confirmed OP pesticide-induced growth inhibition of many bones (femur, tibia, metatarsals, and digits) in chicks (**Misawa *et al.*, 1982**), as well as, severe shortening and contortion of body vertebral axis and tibiotarsal, rib, and sternum growth defects in embryos of bobwhite quail (**Meneely and Wyttenbach, 1989**). Important role in bone metabolism play also thyroid hormones and glucocorticoids (**Yen, 2001; Garg *et al.*, 2004**). Investigations of all these authors (**Yen, 2001; Garg *et al.*, 2004**) signalize a possible significant retardation of appositional bone growth as a result of disturbances in synthesis and secretion of GH, thyroid hormone and glucocorticoids in OP pesticide-treated broiler chicks. Similar to this, significant reduction in T4 and T3 levels was also demonstrated in rats intoxicated with OPs (**Hasheesh *et al.*, 2002**). Also, negative effects of Se on thyroid hormones were confirmed (**Eder *et al.*, 1995**). Regarding to glucocorticoids, **Garg *et al.* (2004)** pointed to adverse impact of increased levels of these hormones on bone growth. Higher level of adrenocorticotropic hormone (ACTH), stimulating the secretion of glucocorticoids by the adrenal cortex was reported in rats exposed to DZN (**Johari *et al.*, 2010**). Higher plasma cortisol concentration was also identified in Se-exposed fishes (**Miller *et al.*, 2007**). Due to absence of the data of sole dose of DZN in rats, we could not exclude the possible effect of this insecticide on rat´s femoral length. On the other hand, since statistical analysis did not find any considerable differences in femoral length between Se- and DZN-Se-exposed rats, as well as, among DZN-Cd-intoxicated rats and those from the control group, we did not suppose that DZN could affect the length of femoral bone in these animals. Therefore, we hypothesize that differences in femoral length in Se- and DZN-Se-poisoned rats could be associated with disturbances in hormonal regulation of bone growth due to Se intoxication. No significant effect of simultaneous DZN-Se-Cd application on femoral length in our rats could be attributed to the same protective mechanism of Cd on Se toxicity which might have been induced and it was described for body weight in DZN-Se-Cd-exposed rats.

CONCLUSION

Our results revealed a significant effect of subchronic peroral intoxication with Cd on femoral weight in adult male rats. On the other hand, growth of rats related to body weight, femoral weight and femoral length was non-significantly affected by simultaneous exposure to DZN-Cd, as well as, DZN-Cd-Se in drinking water for 90 days. This study indicates a protective influence of Cd against Se-induced toxicity on body weight and femoral length in male rats. On the other hand, positive impact of Cd on rat's femoral weight is likely eliminated by the toxicity of DZN and/or DZN in combination with Se.

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REFERENCES

ANI, M. - MOSHTAGHIE, A. A. – AGHADAVOOD, M. 2007. Protective effects of selenium and zinc on the brain acetylcholinesterase activity in lead intoxified rat. In *Pharmaceutical Science*., vol. 2, 2007, p 80-84.

ATSDR (Agency for Toxic Substances and Disease Registry) 2003.Toxicological profile for selenium, 2003, p. 418.

BOBOŇOVÁ, I. - CHOVANCOVÁ, H. - MARTINIAKOVÁ, M. - OMELKA, R. - TOMAN, R. 2011. Effect of subchronic peroral application of cadmium and selenium on femoral structure in adult rats. In *Animal Physiology* 2011: proceedings of scientific publication, Nitra: SPU 2011, p. 6-12.

BOBOŇOVÁ, I. - CHOVANCOVÁ, H. - MARTINIAKOVÁ, M. - OMELKA, R. - TOMAN, R. 2012. Effect of subchronic peroral administration of selenium and diazinon on macrostructure of the femoral bone in adult male rats. In *Scientia iuvenis*: Book of Scientific Papers. Nitra: UKF, 2012, p. 420-425.

BRZÓSKA, M. M. - MONIUSZKO-JAKONIUK, J. 2005. Disorders in bone metabolism of female rats chronically exposed to cadmium. In *Toxicology and Applied Pharmacology*, vol. 202, 2005, p. 68–83.

CABAJ, M. 2012. Effect of diazinon and selenium on structure and function of testes and epididymis in rats (in Slovak). Dissertation Thesis. Nitra: SPU, 2012, p. 167.

CHAE, H.-J. – CHAE, S.-W. – KANG, J.-S. – YUN, D.-H. – BANG, B.-G. – KANG, M.-R. – KIM, H.-M. – KIM, H.-R. 2000. Transition metal induces apoptosis in MC3T3E1 osteoblast: Evidence of free radical release. In *The Korean Journal of Physiology and Pharmacology*, vol. 4, 2000, p. 47-54.

CHO, J. - LEE, C. 1990. Effects of diazinon on the anatomical and embryological changes in the developing chick embryo. In *Research Reports RDA(V)*, vol. 32, 1990, p. 35-47.

CHO, J. - LEE, C. 1991. Studies on diazinon induced inhibition of skeletal mineralization in chick embryo. In *Research Reports RDA(V)*, vol. 33, 1991, p. 41-60.

CHUNG, Y. W. – KIM, T. S. – LEE, S. Y. – LEE, S. H. – CHOI, Y. – KIM, N. – MIN, B. M. – JEONG, D. W. – KIM, I. Y. 2006. Selenite-induced apoptosis of osteoclasts mediated by the mitochondrial pathway. In *Toxicology Letters*, vol. 160, 2006, p. 143-150.

COLOVIC, M. - KRSTIC, D. - PETROVIC, S. - LESKOVAC, A. - JOKSIC, G. - SAVIC, J. - FRANKO, M. - TREBSE, P. - VASIC, V. 2010. Toxic effects of diazinon and its photodegradation products. In *Toxicology Letters*, vol. 193, 2010, p. 9-18.

EBERT, R. – JAKOB, F. 2007. Selenium deficiency as a putative risk factor for osteoporosis. In *International Congress Series*, vol. 1297, 2007, p. 158-164.

EDER, K. – KRALIK, A. – KIRCHGEBNER, M. 1995. Influence of deficient to subtoxic selenium intake on metabolism of thyroid hormones (in German). In *Zeitschrift für Ernahrungswiss*, vol. 34, 1995, p. 277-283.

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. In *Toxicological Pathology*, vol. 32, 2004, p. 364–369.

GREENBERG, M. I. 2003. Occupational toxicology: Police and lawen for cement personnel. In: Occupational, industrial and environmental toxicology. (Eds. Greenberg, M. I., Hamilton, R. J., Phillips, S. D., Mccluskey, G. J.), Pennsylvania: Mosby, 2003, p. 312-325. ISBN 0-323-01340-6.

GRONBAEK, H. – FRYSTYK, J. – ORSKOV, H. – FLYVBJERG, A. 1995. Effect of sodium selenite on growth, insulin-like growth factor-binding proteins and insulin-like growth factor-I in rats. In *Journal of Endocrinology*, vol. 145, 1995, p. 105-112.

HASHEESH, W. S. – ASSEM, M. M. S. – FAKHRY, F. M. – MOHAMED, E. A. A. 2002. Effect of the organophosphorus pesticide triazophos on the thyroidal and gonadal hormones in male albino rats. In *Journal of Egyptian-German Society of Zoology*, vol. 37, 2002, p. 425-442.

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. In *Scandinavian Journal of Work, Environment & Health*, vol. 24, 1998, p. 52.

JOHARI, H. - SHARIATI, M. – ABBASI, S. - SHARIFI, E. - ASKARI, H. R. 2010. The effects of diazinon on pituitary–gonad axis and ovarian histological changes in rats. In *Iranian Journal of Reproductive Medicine*, vol. 8, 2010, p. 125-130.

KASHANIAN, S. - GHOLIVAND, M. B. - AHMADI, F. - RAVAN, H. 2008. Interaction of diazinon with DNA and the protective role of selenium in DNA damage. In *DNA Cell Biol*., vol. 27, 2008, p. 325-332.

KOLESÁROVÁ, A. – CAPCAROVÁ, M. – SIROTKIN, A. – MEDVEĎOVÁ, M. – KOVÁČIK, J. 2010. Cobalt-induced changes in the IGF-I and progesteron release, expression of proliferation- and apoptosis-related peptides in porcine ovarian granulosa cells in vitro. In *Journal of Environmental Science Health. Part A, Toxic/Hazardous Substances & Environmental Engineering*, vol. 45, 2010, p. 810-817.

KOLESÁROVÁ, A. - CAPCAROVÁ, M. - MEDVEĎOVÁ, M. - SIROTKIN, A. V. – KOVÁČIK, J. 2011. In vitro assessment of iron effect on porcine ovarian granulose cells: secretory activity, markers of proliferation and apoptosis. In *Physiological Research*, vol. 60, 2011, p. 503-510.

LUDLE, J. L. - MEHRLE, M. P. - FOSTER, L. M. - EARLKAISER, T. 1979. Bone development in black ducks as affected by dietarytoxophene. In *Pesticide Biochemistry and Physiology*, vol. 10, 1979, p. 168–173.

MATOS, E. - LORIA, D. - DAEDO, A. - LUSTING, E. S. 1985. Effects of pesticides and 12-0-tetra dicanoylphorbol-13-acetate on chondrogenesis. In *In vitro Biology*, vol. 4, 1985, p. 175–184.

MENEELY, G. A. – WYTTENBACH, C. R. 1989. Effects of the organophosphate insecticides diazinon and parathion on bobwhite quail embryos: Skeletal defects and acetylcholinesterase embryos: Skeletal defects and acetylcholinesterase activity. In *Journal of Experimental Zoology*, vol. 252, 1989, p. 60-70.

MILGRAM, S. – CARRIERE, M. – SIMON, A. – GOUGET, B. 2008. Toxicity of lead and selenium on cultured cells from kidney and bone. In *Metal Ions in Biology and Medicine*, vol. 10, (Eds. Collery, Ph., Maymard, I., Theophanides, T., Khassanova, L., Collery, T.), Paris: John Libbey Eurotext, 2008, p. 291-296.

MILLER, L. L. – WANG, F. – PALACE, V. P. – HONTELA, A. 2007. Effects of acute and subchronic exposures to waterborne selenite on the physiological stress response and

oxidative stress indicators in juvenile rainbow trout. In *Aquatic Toxicology*, vol. 83, 2007, p. 263-271.

MISAWA, M. – DOULL, J. – UYEKI, E. M. 1982. Teratogenic effects of cholinergic insecticides in chick embryos. III. Development of cartilage and bone. In *Journal of Toxicological Environmental Health*, vol. 10, 1982, p. 551-563.

MOMENAH, M. A. 2012. Immunological and productive responses in male growing white New Zealand rabbits exposed to Diazinon®. In *African Journal of Pharmacy and Pharmacology*, vol. 6, 2012, p. 824-828.

MORENO-REYES, R. – SUETENS, C. – MATHIEU, F. – BEGAUX, F. – ZHU, D. – RIVERA, M. T. – BOELAERT, M. – NEVE, J. – PERLMUTTER, N. – VANDERPAS, J. 1998. Kashin-Beck osteoarthropathy in rural Tibet in relation to selenium and iodine status. In *New England Journal of Medicine*, vol. 339, 1998, p. 1112-1120.

MORENO-REYES, R. – EGRISE, D. – BOELAERT, M. – GOLDMAN, S. – MEURIS, S. 2006. Iodine deficiency mitigates growth retardation and osteopenia in selenium-deficient rats. In *Journal of Nutrition*, vol. 136, 2006, p. 595-600.

NIŠTIAR, F. – BEŇAČKA, R. – RÁCZ, O. 2006. Insulin family genes: regulators of metabolism, growth and reproduction (in Slovak). In *Laboratórna diagnostika*, vol. 11, 2006, p. 20-25.

OGNJANOVIC, B. I. - MARKOVIC, S. D. - PAVLOVIC, S. Z. - ŽIKIC, R. V. - ŠTAJN, A. Š. – SAIČIC, Z. S. 2008. Effect of chronic cadmium exposure on antioxidant defense system in some tissues of rats: protective effect of selenium. In *Physiological Research*, vol. 57, 2008, p. 403-411.

OŠŤÁDALOVÁ, I. 2012. Biological effects of selenium compounds with a particular attention to the ontogenetic development. In *Physiological Research*, vol. 61 (Suppl. 1), 2012, p. S19-S34.

PANTER, K. E. – HARTLEY, W. J. – JAMES, L. F. 1996. Comparative toxicity of selenium from seleno-DLmethionine, sodium selenate, and *Astragalus bisulcatus* in pigs. In *Fundamental and Applied Toxicology*, vol. 32, 1996, p. 217-223.

PREEDY, V. R. – BURROW, G. N. – WATSON, R. R. 2009. Comprehensive book of iodine: Nutritional, biochemical, pathological and therapeutic aspects. London: Elsevier, 2009, 1312 p. ISBN 978-0-12-374135-6.

SALEHI, M. - JAFARI, M. - MOQADAM, M. S. - SALIMIAN, M. – ASGHARI, A. - NATEGHI, M. - ABASNEJAD, M. – HAGGHOLAMALI, M. 2009. The effect of diazinon on rat brain antioxidant system. In *Toxicological Letters*, vol. 189S, 2009, p. S123.

SANTOS, F. W. – ORO, T. – ZENI, G. – ROCHA, J. B. T. – DO NASCIMENTO, P. C. – NOGUEIRA, C. W. 2004. Cadmium induced testicular damage and its response to administration of succimer and diphenyl diselenide in mice. In *Toxicological Letters*, vol. 152, 2004, p. 255-263.

SARKAR, R. – MOHANAKUMAR, K. P. – CHOWDHURY, M. 2000. Effects of an organophosphate pesticide, quinalphos, on the hypothalamo–pituitary–gonadal axis in adult male rats. In *Journal of Reproduction and Fertility*, vol. 118, 2000, p. 29–38.

SELINUS, O. – ALLOWAY, B. J. – CENTENO, J. A. – FINKELMAN, R. B. – FUGE, R. – LINDH, U. – SMEDLEY, P. 2005. Essentials of medical geology: Impacts of the natural environment on public health. London: Academic Press, 2005, p. 812.

ŠIŠKA, B. - TOMAN, R. - GOLIAN, J. - KRAČÍROVÁ, A. - HLUCHÝ, S. 2008. Selenium and diazinon interaction and effect on rat serum cholinesteraseactivity after intraperitoneal administration. In *Metal Ions in Biology and Medicine*, vol. 10, 2008, p. 615-620.

SZAREK, J. - PRZYBYLSKA-GORNOWICZ, B. - ZASADOWSKI, A. - FABCZAK, J. 1997. Effects of a mixed administration of selenium and diazinon on the ultrastructure of hepatocytes in rat. In *Scandinavian Journal of Laboratory Animal Science*, vol. 24, 1997, p. 6-10.

THORLACIUS-USSING, O. - FLYVBJERG, A. - ESMANN, J. 1987. Evidence that selenium induces growth retardation through reduced growth hormone and somatomedin C production. In *Endocrinology*, vol. 120, 1987,p. 659-663.

THORLACIUS-USSING, O. 1990. Selenium-induced growth retardation. Histochemical and endocrinological studies on the anterior pituitaries of selenium treated rats. In *Danish Medical Bulletin*, vol. 37, 1990, p. 347-358.

TOMAN, R. – ADAMKOVIČOVÁ, A. – HLUCHÝ, S. – CABAJ, M. – GOLIAN, J. 2011. Quantitative analysis of the rat testes after an acute cadmium and diazinon administration. In *Scientific Papers: Animal Science and Biotechnologies*, vol. 44, 2011, p. 188-191.

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. In *Journal of Bone and Mineral Research*, vol. 18, 2003, p. 553–560.

WHO, 1992. Environmental Health Criteria 134, Cadmium. IPCS, Geneva World Health Organization, 1992. Cadmium. Environ. Health Criteria 134, p. 146–151.

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. In *Toxicology and Applied Pharmacology*, vol. 140, 1996, p. 451–460.

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. In *Journal of Experimental Zoology*, vol. 229, 1984, p. 437-446.

YEN, P. M. 2001. Physiological and molecular basis of thyroid hormone action. In *Physiological Review*, vol. 81, 2001, p. 1097-1142.

ZGLINICKI, T. V. – EDWALL, C. – ÖSTLUND, E. – LIND, B. – NORDBERG, M. – RINGERTZ, N. R. – WROBLEWSKI, J. 1992. Very low cadmium concentrations stimulate DNA synthesis and cell growth. In *Journal of Cell Science*, vol. 103, 1992, p. 1073-1081.