

**Zuzana Kňazická – Hana Ďúranová – Veronika Fialková
Jana Bilčíková & Norbert Lukáč**

**Potentially toxic elements
in relation to sexual steroid hormones**

Nitra 2021

Title: Potentially toxic elements in relation to sexual steroid hormones

Authors: **Ing. Zuzana Kňazická, PhD.** (5.22 AQ)
Faculty of Biotechnology and Food Sciences
Slovak University of Agriculture in Nitra
Slovak Republic

RNDr. Hana Ďúranová, PhD. (3.10 AQ)
AgroBioTech Research Centre
Slovak University of Agriculture in Nitra
Slovak Republic

RNDr. Veronika Fialková, PhD. (3.10 AQ)
AgroBioTech Research Centre
Slovak University of Agriculture in Nitra
Slovak Republic

Mgr. Jana Bilčíková (2.74 AQ)
AgroBioTech Research Centre
Slovak University of Agriculture in Nitra
Slovak Republic

prof. Ing. Norbert Lukáč, PhD. (2.74 AQ)
Institute of Applied Biology
Faculty of Biotechnology and Food Sciences
Slovak Republic

Reviewers: **doc. MVDr. Eva Petrovová, PhD.**
Department of Morphological Disciplines
The University of Veterinary Medicine and Pharmacy in Košice
Košice, Slovak Republic

dr. hab. (prof. UP) Łukasz J. Binkowski, Ph.D., D.Sc.
Institute of Biology
Pedagogical University of Krakow
Krakow, Poland

Approved as a scientific monograph on 18. 11. 2021 by the Rector of the Slovak University of Agriculture in Nitra, Slovak Republic.

This publication has been printed on ecological paper.



ISBN 978-80-552-2404-6

CONTENT

Authors	6
Acknowledgements	8
Papers discussed	9
Nomenclature	11
List of figures	18
List of tables	21
Introduction	23
CHAPTER I Review of literature	25
1.1 Endocrine disruptors	25
1.1.1 <i>What are endocrine disruptors and endocrine active substances?</i>	26
1.1.2 <i>Action mechanisms of endocrine disruptors</i>	26
1.1.3 <i>Origins of endocrine disruptors</i>	29
1.1.4 <i>The routes of exposure to endocrine disruptors</i>	34
1.1.5 <i>Endocrine disruptors and their possible impacts on health – reproductive and hormone system</i>	37
1.2 Potentially toxic elements	39
1.2.1 <i>What are heavy metals?</i>	39
1.2.2 <i>MERCURY</i>	40
1.2.2.1 <i>Chemical and physical properties</i>	40
1.2.2.2 <i>Exposure</i>	41
1.2.2.3 <i>Biological properties</i>	45
1.2.2.4 <i>Toxicokinetics</i>	45
1.2.2.5 <i>Effects on the organism – reproductive toxicity</i>	46
1.2.3 <i>CADMIUM</i>	48
1.2.3.1 <i>Chemical and physical properties</i>	48
1.2.3.2 <i>Exposure</i>	50
1.2.3.3 <i>Biological properties</i>	51
1.2.3.4 <i>Toxicokinetics</i>	51
1.2.3.5 <i>Effects on the organism – reproductive toxicity</i>	52
1.2.4 <i>NICKEL</i>	57
1.2.4.1 <i>Chemical and physical properties</i>	57
1.2.4.2 <i>Exposure</i>	58
1.2.4.3 <i>Biological properties</i>	59
1.2.4.4 <i>Toxicokinetics</i>	60
1.2.4.5 <i>Effects on the organism – reproductive toxicity</i>	61
1.2.5 <i>IRON</i>	65
1.2.5.1 <i>Chemical and physical properties</i>	65

1.2.5.2 Exposure	66
1.2.5.3 Biological properties	67
1.2.5.4 Toxicokinetics	68
1.2.5.5 Effects on the organism – reproductive toxicity	74
1.2.6 COPPER	76
1.2.6.1 Chemical and physical properties	76
1.2.6.2 Exposure	77
1.2.6.3 Biological properties	78
1.2.6.4 Toxicokinetics	80
1.2.6.5 Effects on the organism – reproductive toxicity	82
1.3 Human adrenocortical carcinoma (NCI-H295R) cell line	85
1.3.1 Origin, development and establishment of the cell line	85
1.3.2 Growth conditions of the cell line	86
1.3.3 Intracellular characteristic and physiological properties of the cell line	88
1.4 Steroidogenesis	93
1.4.1 How do steroid hormones act?	94
1.4.2 Progesterone	99
1.4.3 Testosterone	101
1.4.4 Estradiol	102
CHAPTER II The aim of the study	105
CHAPTER III Material and methods	106
3.1 Cell culture	106
3.2 <i>In vitro</i> exposure	108
3.3 Cell viability	109
3.4 Hormone measurement	110
3.5 ICP-OES elemental analysis	112
3.6 Transmission electron microscopy	113
3.7 Statistical analysis	114
CHAPTER IV Results	115
4.1 Mercury	115
4.1.1 Cell viability	115
4.1.2 Sexual steroid production	116
4.2 Cadmium	119
4.2.1 Cell viability	119
4.2.2 Sexual steroid production	119
4.3 Nickel	123
4.3.1 Cell viability	123
4.3.2 Sexual steroid production	123

4.4 Iron	127
4.4.1 <i>Cell viability</i>	127
4.4.2 <i>Sexual steroid production</i>	127
4.4.3 <i>Quantitative determination of the iron in the NCI-H295R cells</i>	130
4.4.4 <i>Ultrastructure of the NCI-H295R cell line exposed to FeSO₄.7H₂O</i>	130
4.5 Copper	138
4.5.1 <i>Cell viability</i>	138
4.5.2 <i>Sexual steroid production</i>	138
4.5.3 <i>Quantitative determination of the copper in the NCI-H295R cells</i>	141
4.5.4 <i>Ultrastructure of the NCI-H295R cell line exposed to CuSO₄.5H₂O</i>	141
CHAPTER V Discussion	149
5.1 Mercury in relation to sexual steroid hormones (NCI-H295R)	149
5.2 Cadmium in relation to sexual steroid hormones (NCI-H295R)	152
5.3 Nickel in relation to sexual steroid hormones (NCI-H295R)	156
5.4 Iron in relation to sexual steroid hormones (NCI-H295R)	159
5.4.1 <i>Content of iron in the NCI-H295R cell line</i>	162
5.4.2 <i>Ultrastructure of the cells after exposure to FeSO₄.7H₂O</i>	163
5.5 Copper in relation to sexual steroid hormones (NCI-H295R)	165
5.5.1 <i>Content of copper in the NCI-H295R cell line</i>	167
5.5.2 <i>Ultrastructure of the cells after exposure to CuSO₄.5H₂O</i>	168
CHAPTER VI Conclusions and Recommendation for practice	169
CHAPTER VII References	175
Abstract	227

AUTHORS



Zuzana Kňazická / Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Slovak Republic/
is specialist in the area of molecular biology, morphology, physiology and toxicology. Her research focuses on *in vitro* reproductive toxicity testing of toxic and/or biologically active substances on the model cells.



Hana Ďúranová / AgroBiotech Research Centre, Slovak University of Agriculture in Nitra, Slovak Republic/
is specialist in microscopy techniques applied in the structural characterization of diverse biological samples. Her research focuses on evaluation of ultrastructural changes in various cells induced mainly by the actions of toxic and/or biologically compounds using a transmission electron microscopy.



Veronika Fialková / AgroBiotech Research Centre, Slovak University of Agriculture in Nitra, Slovak Republic/
is postdoctoral researcher focused on studying the molecular effects of potentially toxic elements in the selected cancer cell lines and genetic changes alergenesis.



Jana Bilčíková / AgroBiotech Research Centre,
Slovak University of Agriculture in Nitra, Slovak
Republic/
partakes in the research of the effect of endocrine
disruptors and biologically active substances on
molecular changes in the model cell line.



Norbert Lukáč /Institute of Applied Biology,
Faculty of Biotechnology and Food Sciences,
Slovak University of Agriculture in Nitra, Slovak
Republic/.

His research activity focuses on the analysis of
selected environmental risk factors, xenobiotics,
bioactive substances, and their impact on animal
/human health, physiological status and male
reproduction.

ACKNOWLEDGEMENTS

Each named author has made a substantial, direct and intellectual contribution to the work. We hope, the monograph will serve as a forum for presenting contemporary knowledge on basic and applied research, thus making new findings, methods, and techniques easily accessible and applicable.

The authors are thankful to all the colleagues and technical staff from the National Institute of Chemical Safety in Budapest (Hungary), Institute of Applied Biology, the Faculty of Biotechnology and Food Sciences (Slovak University of Agriculture in Nitra, Slovak Republic) and AgroBioTech Research Centre (Slovak University of Agriculture in Nitra, Slovak Republic). The scientific cooperation of the team has been reinforced in bilateral cooperation, mainly with the partner institutions - the National Institute of Chemical Safety in Budapest and the Department of Animal Physiology and Toxicology, the Faculty of Exact and Natural Sciences (Pedagogical University of Krakow, Krakow, Poland). These participating institutions and partner universities were the key pillars of a proactive platform in the field of research.

Special acknowledgement goes to Dr. Zsolt Forgacs, for his useful advice, professional guidance and indispensable assistance in carrying out experimental works.

Last but not the least, the authors would like to thank the reviewers of this monograph, doc. MVDr. Eva Petrovová, PhD. (Department of Morphological Disciplines, The University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovak Republic) and dr. hab. (prof. UP) Łukasz J. Binkowski, Ph.D., D.Sc. (Institute of Biology, Pedagogical University of Krakow, Krakow, Poland), for their critical and constructive reviewing within a short span of time.

This study was financially supported by the Scientific Agency of the Slovak Republic VEGA No. 1/0163/18, the Slovak Research and Development Agency projects APVV Nos. 16-0289 and 20-0218, as well as SUA grants Nos. 33/2019 and 35/2019.

Authors

"Research is to see what everybody else has seen, and to think what nobody else has thought."

Albert Szent-Györgyi (biochemist)

PAPERS DISCUSSED

This work is based on the publications listed below, which will be referred to in the text:

- Knazicka, Z., Duranova, H., Fialkova, V., Miskeje, M., Jambor, T., Makarevich, A. V., Roychoudhury, S., Kovacik, A., Massanyi, P., Lukac, N. (2021a) *In vitro* effect of ferrous sulphate on bovine spermatozoa motility parameters, viability and Annexin V-labeled membrane changes. *PLOS ONE* **16**(9): e0257766.
- Knazicka, Z., Fialkova, V., Duranova, H., Bilcikova, J., Kovacikova, E., Miskeje, M., Valkova, V., Forgacs, Z., Roychoudhury, S., Massanyi, P., Lukac, N. (2021b) Human adrenocortical carcinoma (NCI-H295R) cell line as an *in vitro* cell culture model for assessing the impact of iron on steroidogenesis. *Folia biologica (Praha)* **67**: 76-81.
- Lukac, N., Forgacs, Z., Duranova, H., Jambor, T., Zemanova, J., Massanyi, P., Tombarkiewicz, B., Roychoudhury, S., Knazicka, Z. (2020) *In vitro* assessment of the impact of nickel on the viability and steroidogenesis in the human adrenocortical carcinoma (NCI-H295R) cell line. *Physiol Res* **69**: 1-30.
- Bilcikova, J., Fialkova, V., Duranova, H., Kovacikova, E., Forgacs, Z., Gren, A., Massanyi, P., Lukac, N., Roychoudhury, S., Knazicka, Z. (2020) Copper affects steroidogenesis and viability of human adrenocortical carcinoma (NCI-H295R) cell line *in vitro*. *J Environ Sci Health, Part A* **55**: 1070-1077.
- Duranova, H., Valkova, V., Knazicka, Z., Olexikova, L., Vasicek, J. (2020) Mitochondria: A worthwhile object for ultrastructural qualitative characterization and quantification of cells at physiological and pathophysiological states using conventional transmission electron microscopy. *Acta Histochem* **122**: 151646.
- Jambor, T., Bistakova, J., Lukac, N. (2020) *Identifikácia účinku endokrinných disruptorov v bunkách samčieho reprodukčného systému in vitro*. [in Slovak], 1. ed. Nitra: Slovak University of Agriculture in Nitra, Slovak Republic, 124 p. ISBN 978-80-552-2269-1.
- Knizatova, N., Greifova, H., Tokarova, K., Lukac, N. (2020) Human adrenocarcinoma (H295R) cells as potencial predictors of bisphenols ability to interfere with steroid hormone production: a mini review. *Animal Science and Biotechnologies* **53**: 195-199.
- Bilcikova, J., Fialkova, V., Kovacikova, E., Miskeje, M., Tombarkiewicz, B., Knazicka, Z. (2018) Influence of transition metals on animal and human health: a review. *Contemporary agriculture* **67**: 187-195.

- **Bistakova, J., Forgacs, Z., Bartos, Z., Racz Szivosne, M., Jambor, T., Knazicka, Z., Tvrda, E., Libova, L., Goc, Z., Massanyi, P., Lukac, N.** (2017) Effects of 4-nonylphenol on the steroidogenesis of human adrenocarcinoma cell line (NCI-H295R). *J Environ Sci Health, Part A* **52(3)**: 221-227.
- **Slivkova, J., Knazicka, Z., Massanyi, P.** (2017) *In vitro* cytotoxicita ortuti – celulórne a molekulárne aspekty toxicity ortuti. [in Slovak], 1. ed. Nitra: Slovak University of Agriculture in Nitra, Slovak Republic, 89 p. ISBN 978-80-552-1637-9.
- **Knazicka, Z., Forgacs, Z., Lukacova, J., Roychoudhury, S., Massanyi, P., Lukac, N.** (2015) Endocrine disruptive effects of cadmium on steroidogenesis: Human adrenocortical carcinoma cell line NCI-H295R as a cellular model for reproductive toxicity testing. *J Environ Sci Health, Part A* **50**: 348-356.
- **Knazicka, Z., Lukac, N., Forgacs, Z., Tvrda, E., Lukacova, J., Slivkova, J., Binkowski, L., Massanyi, P.** (2013) Effects of mercury on the steroidogenesis of human adrenocarcinoma (NCI-H295R) cell line. *J Environ Sci Health, Part A* **48**: 348-353.
- **Lukac, N., Lukacova, J., Pinto, B., Knazicka, Z., Tvrda, E., Massanyi, P.** (2013) The effect of nonylphenol on the motility and viability of bovine spermatozoa *in vitro*. *J Environ Sci Health, Part A* **48**: 973-979.

Offprints are published with the permission of the authors concerned.

Authors:

Zuzana Kňazická – Hana Ďúranová – Veronika Fialková
Jana Bilčíková & Norbert Lukáč

Title:

**Potentially toxic elements
in relation to sexual steroid hormones**

Publisher: Slovak University of Agriculture in Nitra

Edition: first

Number of copies: 100

AQ – PQ: 16.90 – 17.20

ISBN 978-80-552-2404-6