



2. KONGRES  
SLOVENSKEGA TOKSIKOLOŠKEGA DRUŠTVA

**KEMIJSKI POVZROČITELJI HORMONSKIH MOTENJ  
OD MOLEKULE DO ČLOVEKA**

*ENDOCRINE DISRUPTING CHEMICALS - FROM MOLECULE  
TO MAN*

Ljubljana, 23. in 24. april 2015



Univerza v Ljubljani

Univerza v Ljubljani  
*Medicinska* fakulteta



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Endocrine disrupting chemicals - from molecule to man

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## Pozdravni nagovor

Slovensko toksikološko društvo je bilo ustanovljeno leta 2000. Ponosni in zadovoljni smo, da našo petnajstletnico obeležujemo s kongresom: Kemijski povzročitelji hormonskih motenj – od molekule do človeka. Na kongresu bodo predstavljeni predvsem domači izsledki vplivov kemijskih povzročiteljev hormonskih motenj (KPHM) na zdravje ljudi in drugih organizmov, izsledki temeljnih raziskav, testne metode, zakonodaja in strategija.

Pretekli dve desetletji so KPHM pomembno zaznamovali področje kemijske varnosti tako na strokovni in upravni ravni kot tudi v širši javnosti. V Sloveniji so raziskave o vplivu kemikalij, ki spadajo med KPHM, npr. polikloriranih bifenilov, svineca in živega srebra, na okolje in zdravje potekale že vrsto let pred uvedbo termina KPHM. V teh raziskavah so sodelovali številni strokovnjaki vključno s člani našega društva. Vsaj poldrugo desetletje se s KPHM sistematično ukvarjajo na številnih klinikah in fakultetah, na raziskovalnih inštitutih, kot so Inštitut Jožef Stefan, Kemijski inštitut in Nacionalni inštitut za biologijo. Na Nacionalnem inštitutu za javno zdravje spremljamo KPHM v okviru toksikovigilance, medtem ko se upravne institucije, kot so Uprava za varno hrano, veterinarstvo in varstvo rastlin, Urad Republike Slovenije za kemikalije, Agencija Republike Slovenije za okolje in inšpektorati pristojni za področja zdravstva, okolja, kmetijstva, hrane in trgovine vključujejo v nadzor KPHM v skladu z veljavno zakonodajo. Večina vabljenih predavateljev in udeležencev tega kongresa prihaja iz navedenih inštitucij. Pestrost udeležencev različnih strok dokazuje, da zmoremo splesti učinkovite vezi in konstruktivno sodelovati z namenom prispevati k varovanju zdravja ljudi in okolja, k razvoju stroke in k dvigu ravni ozaveščenosti javnosti.

Zahvaljujemo se vabljenim predavateljem za njihov prijazen odziv, Nacionalnemu inštitutu za javno zdravje, ki nam je omogočil uporabo predavalnice z razgledom in Javnemu podjetju Voda-Kanalizacija d.o.o. za materialno podporo.

V imenu predsednice našega društva, Ester Lovšin Barle, ki zaradi službenih obveznosti na kongresu ne more prisostvovati, in v imenu organizacijskega odbora kongresa, Vam želim navdihujoče in prijetno srečanje.

Lucija Perharič

Podpredsednica Slovenskega toksikološkega društva

## Welcome address

The Slovenian Society of Toxicology was founded in 2000. We are proud and pleased to mark our fifteenth anniversary with a congress: Endocrine disrupting chemicals – from molecule to man. During this congress, predominantly the results of national investigations on the endocrine disrupting chemicals' (EDCs) effects on humans and other organisms, the results of basic research, the testing methods, the legislation and the strategy will be presented.

In the last two decades, the EDCs have characterized various spheres of chemical safety including research and regulation as well as the general public. In Slovenia, research studies on the effects of chemicals, which are nowadays belonging to the EDCs ( i.e. polychlorinated biphenyls, lead and mercury), have been carried out many years prior to the introduction of the term EDCs. A number of professionals including some of our members participated in these studies. For at least a decade and a half the EDCs and their effects have been systematically studied at many teaching clinics and the academia, at research institutes, such as, the Josef Stefan Institute, the National Institute of Chemistry and the National Institute of Biology. The National Institute of Public Health has been surveying the EDCs within its toxicovigilance activities, while the administrative institutions, such as the Administration of the Republic of Slovenia for Food Safety, Veterinary and Plant Protection, the Chemistry Office of the Republic of Slovenia, Slovenian Environment Agency, and the Inspectorates responsible for the matters of health, environment, agriculture, food and trade participate in regulation according to the current legislation. Majority of the invited speakers come from the above stated institutions. The variety of participants from different professions proves that we are capable of effective networking and constructive co-operation aiming to contribute to the protection of human health and the environment, to advance the professional development and to increase the level of public awareness.

We are grateful to the invited speakers for their kind response, to the National Institute of Public Health for the availability of its lecture theatre with a view and “Javno podjetje Voda-Kanalizacija d.o.o”. for the material support.

On behalf of the president of our society, Ester Lovšin Barle, who is not able to attend this congress due to her working commitments, and on behalf of the organizing committee I wish you an inspiring and pleasant meeting.

Lucija Perharič

Vice-president of The Slovenian Society of Toxicology

## In memoriam



Foto: Osebni arhiv

Branko Družina (1950–2014)

Lani poleti je preminil dolgoletni član našega društva, kemik in ekonomist, dr. Branko Družina. Branko je v društvu deloval kot predavatelj; uspešno je tudi pridobil sponzorska sredstva. Nesebično je delil svoje znanje in izkušnje, pridobljene v raziskovalnih inštitutih, v industriji, na Ministrstvu za okolje in prostor, na nekdanjem Inštitutu za varovanje zdravja in na ljubljanski univerzi. Njegova bogata bibliografija obsega skoraj 600 vnosov. Bil je avtor in soavtor številnih raziskovalnih in strokovnih člankov, monografij, učbenikov, recenzij ter urednik in član uredniških odborov več strokovnih revij. Ohranili ga bomo v trajnem spominu kot svetovljana, neposrednega, humornega in dobrosrčnega sodelavca ter prijatelja, ki je za vsakogar našel dobro in spodbudno besedo.

Last summer, a long standing member of our society, dr. Branko Družina, a chemist and an economist, passed away. Branko had participated in our society as a lecturer; he was also successful at winning sponsoring funds. He selflessly shared his knowledge and experience acquired in research institutions and industry, at the Ministry of the environment and spatial planning, the then Institute of Public Health and the University of Ljubljana. His rich bibliography includes nearly 600 entries including numerous research and professional articles, monographs, textbooks, reviews; he also served as an editor and editorial board member of many professional journals. We will keep him in our longstanding memory as a cosmopolite, a direct, humorous and goodhearted fellow-worker and a friend, who was able to find a good word of encouragement for everyone.

Lucija Perharič

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## PROGRAM 2. KONGRESA SLOVENSKEGA TOKSIKOLOŠKEGA DRUŠTVA

### Kemijski povzročitelji hormonskih motenj – od molekule do človeka

23. 4. 2015

8.00-9.00 Registracija

**Simpozij 1: Kemijski povzročitelji hormonskih motenj in vplivi na ljudi**

**Predsedujoče:** K. Geršak, V. Zadnik, M. Horvat, L. Perharič

9:00-9:20 Lucija Perharič (Nacionalni inštitut za javno zdravje)

*Uvodni pozdrav*

*Kemijski povzročitelji hormonskih motenj - zgodovinski pregled*

9:20-9:40 Ksenija Geršak (Ginekološka klinika, Univerzitetni klinični center Ljubljana)

*Kemijski povzročitelji hormonskih motenj in reproduktivno zdravje žensk*

9:40-10:00 Branko Zorn (Ginekološka klinika, Univerzitetni klinični center Ljubljana)

*Kemijski povzročitelji hormonskih motenj in reproduktivno zdravje moških v Sloveniji – Pregled literature, epidemiološki nacionalni podatki*

10:00-10:20 Vesna Zadnik (Onkološki inštitut)

*Epidemiološki trendi hormonsko odvisnih rakov*

10:20-10:40 Simona Gaberšček in Katja Zaletel (Klinika za nuklearno medicino, UKC Lj.)

*Epidemiološki trendi bolezni ščitnice pri odraslih*

10:40-11:10 Odmor

11:10-11:30 Janja Snoj Tratnik (Inštitut Jožef Stefan) in sod.

*Dolgoročna epidemiološka študija izpostavljenosti nizkim koncentracijam živega srebra pri občutljivi populaciji*

11:30-11:50 Darja Mazej (Inštitut Jožef Stefan) in sod.

*Esencialni in neesencialni elementi pri slovenski populaciji: rezultati humanega biomonitoringa*

11:50-12:10 Milena Horvat (Inštitut Jožef Stefan) in sod.

*Evropski humani biomonitoring DEMOCOPHES – Slovenija*

12:10-12:30 Razprava

12:30-13:30 Kosilo

**Simpozij 2:           Kemijski povzročitelji hormonskih motenj in vplivi na druge  
                                  organizme**

**Predsedujoča:**       T. Tišler, J. Kužner

- 13:30-13:50           Milka Vrecl in Vlasta Jenčič (Veterinarska fakulteta, Univerza v Ljubljani)  
*Ugotavljanje interseksualnosti pri sladkovodnih ribah v nekaterih izbranih vodotokih*
- 13:50-14:10           Tatjana Tišler (Kemijski inštitut, Ljubljana) in sod.  
*Ekotoksikološki vidiki bisfenola A in njegovih analogov*
- 14:10-14:30           Boštjan Erjavec (Kemijski inštitut, Ljubljana) in sod.  
*Fotokatalitsko odstranjevanje bisfenola A, F in AF iz vodnih vzorcev*
- 14:30-14:50           Lucija Kolar (COMPLEMENTARIUM, Inštitut za raziskovanje narave in razvoj okoljskih  
tehnologij)  
*Hormonski motilci in njihovi učinki na različne (živalske) vrste*
- 14:50-15:00           Anita Klančar (Fakulteta za farmacijo, Univerza v Ljubljani) in sod.  
*Pojavljanje farmacevtikov v odpadnih vodah v Sloveniji*
- 15:00-15:20           Odmor
- 15:20-15:40           Marjana Regvar (Biotehniška fakulteta, Univerza v Ljubljani)  
*Rastlinski hormoni: več kot regulatorji rastlinskih celic*
- 15:40-16:20           Anita Jemec in Gordana Glavan (Biotehniška fakulteta, Univerza v Ljubljani)  
*Vpliv hormonskih motilcev na nevretenčarje–poudarek na mehanizmih delovanja*
- 16:20-16:30           Jana Brankovič (Veterinarska fakulteta, Univerza v Ljubljana)  
*Vplivi na geometrijo, biomehaniko in vsebnost mineralov v stegenicah podganjih  
mladičev izpostavljenih v obdobju laktacije neplanarnemu PCB-155 ali/in planarnemu  
PCB-169*
- 16:30-16:40           Damjan Balabanič (Fakulteta za industrijski inženiring, Novo mesto) in sod.  
*Ugotavljanje citotoksične in genotoksične aktivnosti kemijskih povzročiteljev hormonskih  
motenj*
- 16:40-17:00           Razprava
- 17:30-19:00           **Redni občni zbor članov Slovenskega toksikološkega društva**

**24. 4. 2015**

8.00-9.00 Registracija

**Simpozij 3: Temeljne (mehanistične) raziskave: *in vivo*, *in vitro*, *in silico***

**Predsedujoči:** M. Sollner Dolenc, J. Mavri, M. Vračko

9:00-9:20 Marija Sollner Dolenc (Fakulteta za farmacijo, Univerza v Ljubljani) in sod.  
*Vpliv izbranih endokrinih motilcev na adhezijske procese celic*

9:20-9:40 Ivana Klopčič (Fakulteta za farmacijo, UL in Kemijski inštitut, Ljubljana ) in sod.  
*Proučevanje glukokortikoidnega delovanja izbranih parabenov, ftalatov in insekticidov ter njihovih mešanic*

9:40-10:00 Maja Plahuta (Kemijski inštitut, Ljubljana) in sod.  
*Študij učinkov hormonskih motilcev na *Asellus aquaticus**

10:00-10:20 Matjaž Jeras (Fakulteta za farmacijo, Univerza v Ljubljani in Zavod Republike Slovenije za transfuzijsko medicino) in sod.  
*Ugotavljanje vplivov bisfenolov BPA, BPF in BPAF na diferenciacijo, dozorevanje in funkcijske lastnosti iz človeških monocitov pripravljenih dendritičnih celic (DC) *in vitro**

10:20-10:40 Katerina Čeh (Veterinarska fakulteta, Univerza v Ljubljani)  
*Vpliv nizkih koncentracij kumafosa na razvoj in delovanje možgan pri miših*

10:40-11:10 Odmor

11:10-11:30 Janez Mavri (Kemijski inštitut, Ljubljana)  
*Narava medmolekularnih sil in prepoznavanje v bioloških makromolekulah*

11:30-11:50 Marjan Vračko (Kemijski inštitut, Ljubljana)  
*Računalniške metode za oceno endokrinih motilcev in razvojne toksičnosti*

11:50-12:10 Katra Kolšek (Heidelberg Institute for Theoretical Studies) in sod.  
*In silico identifikacija hormonskih motilcev - Endocrine Disruptome*

12:10-12:30 Alja Plošnik (Kemijski inštitut, Ljubljana)  
*Endokrini potencial snovi v kozmetičnih izdelkih*

12:30-13:00 Razprava

13:00-14:00 Kosilo

#### **Simpozij 4: Testne smernice in metode, zakonodaja, strategija**

**Predsedujoči:** K. Černe, N. Bratina, J. Drogenik, S. Fajfar

- 14:00-14:30 Katarina Černe (Medicinska fakulteta, Univerza v Ljubljani)  
*Kemijski povzročitelji hormonskih motenj - OECD testne metode*
- 14:30-14:50 Jernej Drogenik (Uprava za varno hrano, veterinarstvo in varstvo rastlin)  
*Kemijski povzročitelji hormonskih motenj in zakonodaja za fitofarmacevtska sredstva*
- 14:50-15:10 Simona Fajfar (Urad republike Slovenije za kemikalije)  
*Kemijski povzročitelji hormonskih motenj - zakonodaja REACH*
- 15:10-15:30 Nataša Bratina (Pediatrična klinika, UKC Lj.) in sod.  
*Pogostnost nekaterih endokrinoloških motenj med otroci in mladostniki v Sloveniji*
- 15:30-15:40 Lucija Perharič (Nacionalni inštitut za javno zdravje)  
*Kemijski povzročitelji hormonskih motenj - strategija Evropske unije*
- 15:40-16:00 Razprava
- 16:00 Zaključek

#### **Plakati**

Med odmori in med kosilom ogled plakatov. Po želji imajo avtorji možnost ustne predstavitve plakatov, 5 min/plakat.

Živa M. Geršak (Medicinska fakulteta, Univerza v Ljubljani) in sod.

*Pojavnost prezgodnje menopavze v Sloveniji v obdobju 2003-2013*

Rebeka Jurca (Veterinarska fakulteta, Univerza v Ljubljani) in sod.

*Določanje nivoja hormonov v živilih živalskega izvora*

# Izvlečki



# **SIMPOZIJ 1:**

## **Kemijski povzročitelji hormonskih motenj in vplivi na ljudi**



Foto: Lucija Perharič

# KEMIJSKI POVZROČITELJI HORMONSKIH MOTENJ – ZGODOVINSKI PREGLED

**Lucija Perharič**

Nacionalni inštitut za javno zdravje, Zaloška cesta 29, 1000 Ljubljana, Slovenija

[lucija.perharic@nijz.si](mailto:lucija.perharic@nijz.si)

Po definiciji Svetovne zdravstvene organizacije iz leta 2002 je kemijski povzročitelj hormonskih motenj (KPHM) od zunaj vnesena kemična snov oziroma mešanica snovi, ki preko sprememb v delovanju hormonov povzroča neželene učinke na zdravje posameznega organizma ali njegovega potomstva oziroma (sub)populacije.

KPHM so zadnji dve desetletji pogosta tema razprav tako v naravoslovnih kot družboslovnih, v strokovnih in laičnih krogih. V epidemioloških študijah je bilo ugotovljeno naraščanje pogostosti bolezni reproduktivnega trakta in zmanjšane plodnosti pri obeh spolih, motenj delovanja ščitnice, nevrološkega razvoja, hormonsko odvisnih rakov, osteoporoze, sladkorne bolezni tipa II, debelosti. V nekaterih študijah so našli povezavo med naštetimi pojavi in izpostavljenostjo kemikalijam iz okolja, kar je spodbudilo številne nadaljnje raziskave.

KPHM lahko z vezavo na receptorje posnemajo ali zavirajo endogene hormone, vplivajo na njihovo sintezo, transport oziroma metabolizem. Med KPHM spadajo poleg nekaterih zdravil številne naravne in umetne kemikalije: bisfenol A, dioksini, etilni alkohol, fitoestrogeni, ftalati, furani, kofein, kovine, nonilfenoli, nekateri pesticidi, poliaromatski ogljikovodiki, polibromirani difenil etri, poliklorirani bifenili, perfluoro-oktanoična kislina, tributil stanati.

Nesporni so škodljivi vplivi KPHM pri visokih odmerkih, medtem ko ostajajo vplivi nizkih odmerkov, to je odmerkov, ki smo jim izpostavljeni v vsakdanjem življenju, kontroverzno področje. Endogeni hormoni delujejo pri zelo nizkih odmerkih, vendar je njihova moč bistveno večja od KPHM. Zato je logično pričakovati, da so za učinke KPHM potrebni večji odmerki kot za učinke endogenih hormonov. Druge ključne negotovosti in pomanjkljivosti, ki nikakor niso ekskluzivne za KPHM, so: obstoj praga učinka, pomen ne-monotonega odnosa med odmerkom in učinkom, učinki mešanic, izpostavljenost v kritičnih obdobjih občutljivosti, to je v času razvoja in programiranja hormonskega sistema zarodka med nosečnostjo, v otroštvu in adolescenci, neustreznost testnih metod za ugotavljanje izidov pri nizkih odmerkih in v kritičnih razvojnih obdobjih ter pomanjkanje ustreznih modelov za nekatere učinke. Posledično ostajajo KPHM pomemben javnozdravstveni, raziskovalni in regulatorni izziv.

Ključne besede: kemijski povzročitelji hormonskih motenj, značilnosti, negotovosti

# ENDOCRINE DISRUPTING CHEMICALS – A HISTORICAL OVERVIEW

**Lucija Perharič**

National institute of public health, Zaloška cesta 29, 1000 Ljubljana, Slovenia

[lucija.perharic@nijz.si](mailto:lucija.perharic@nijz.si)

An endocrine disrupter (ED) is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations according to the 2002 World Health Organization definition.

In the last two decades, the EDs were commonly debated in lay and professional circles in natural and sociological sciences. Certain epidemiological studies established increasing occurrence of reproductive tract diseases and decreased fertility in both sexes, thyroid dysfunction, neurological development, hormonally dependent cancers, osteoporosis, type II diabetes mellitus, obesity. The associations with exposure to environmental chemicals triggered further research.

By binding to the receptors, the EDs may mimic or inhibit the endogenous hormones, affect their synthesis, transport or metabolism. Besides certain medicines, EDs include numerous natural and synthetic chemicals: bisphenol A, dioxins, ethyl alcohol, phyto-oestrogens, phthalates, furans, caffeine, some metals, nonylphenols, some pesticides, polyaromatic hydrocarbons, polybrominated diphenyl ethers, polychlorinated biphenyls, perfluorooctanoic acid, tributyl stannates.

The EDs may undoubtedly be harmful in large doses, while the effects at low doses, found in everyday life, remain controversial. While the endogenous hormones act at very low doses, their potency is significantly higher than that of the EDs. It is therefore logically expected, that higher doses of EDs are required for the same effect. Further key uncertainties and deficiencies, by all means not exclusive to EDs, include: the existence of the effect threshold, the non-monotonous dose-effect relationship, effects of mixtures, exposure during the critical windows of susceptibility, i.e. during the development and programming of the endocrine system in pregnancy, childhood and adolescence, inadequacy of testing methods for the identification of outcomes at low doses and at the relevant developmental stages, and the lack of appropriate models for certain effects. Consequently, the EDs remain an important public health, research and regulatory challenge.

Key words: endocrine disrupting chemicals, characteristics, uncertainties



# KEMIJSKI POVZROČITELJI HORMONSKIH MOTENJ (KPHM) IN REPRODUKTIVNO ZDRAVJE ŽENSK

**Ksenija Geršak**

Ginekološka klinika, Univerzitetni klinični center Ljubljana in Katedra za ginekologijo in porodništvo, Medicinska fakulteta Univerze v Ljubljani

[ksenija.gersak@mf.uni-lj.si](mailto:ksenija.gersak@mf.uni-lj.si)

Uvod in namen: Učinki delovanja KPHM so povezani z reproduktivnim zdravjem žensk. Številna tuja poročila dokazujejo njihov vpliv na menstruacijski cikel, plodnost ter na nastanek hormonsko pogojenih malignih neoplazem dojke in ženskih spolnih organov. Namen naše raziskave je bil ugotoviti pojavnosti nekaterih bolezni, ki so povezane z zmanjšano plodnostjo v slovenskih statističnih regijah v časovnem obdobju od 2003 do 2013.

Materiali in metode: Uporabili smo podatke zunajbolnišnične zdravstvene statistike Nacionalnega inštituta za javno zdravje in demografske podatke Statističnega urada RS. Opazovana populacija so bile ženske, stare od 15 do 49 let, ki so bile obravnavane v ambulantah primarne ravni zaradi ene od treh izbranih diagnoz: sindroma policističnih jajčnikov (PCOS), endometrioze ali leiomiomov. Analizirali smo njihovo pojavnost v 11-letnem obdobju v Sloveniji in jo primerjali s pojavnostjo v posameznih statističnih regijah.

Rezultati: V Sloveniji je bila incidenca PCOS 16/10.000 žensk v starostni skupini od 15-49 let, izstopali sta notranjsko-kraška (41/10.000) in zasavska regija (28/10.000). Incidenca endometrioze je bila enaka kot incidenca PCOS (16/10.000), vendar razlike med regijami niso bile tako izrazite kot pri PCOS, največjo incidence sta imeli gorenjska (23/10.000) in zasavska regija (21/10.000). Incidenca leiomiomov je bila v isti populaciji žensk 5-krat večja (87/10.000) v primerjavi z PCOS in endometriozo, izrazito je izstopala notranjsko-kraška regija (190/10.000), sledile pa so ji pomurska (123/10.000), jugovzhodna (117/10.000) in spodnjeposavska regija (103/10.000).

Zaključki: PCOS, endometrioza in leiomiomi so multifaktorsko pogojene bolezni in v njihovo patogenezo se vpleta tudi okolje s KPHM. Z epidemiološkimi študijami je bil dokazan vpliv bisfenola A na PCOS in endometriozo, klorirani pesticidi, poliklorirani bifenili, ftalati in fitoestrogeni učinkujejo na endometriozo in leiomiome, na leiomiome pa tudi polibromirani bifenili. Predpostavljamo, da je okolje lahko vpleteno v nastanek razlik med incidencami opazovanih treh bolezni v slovenskih statističnih regijah, kjer izstopata notranjsko-kraška in zasavska regija. Za natančnejšo opredelitev vplivov okolja so potrebne nadaljnje raziskave.

Ključne besede: kemijski povzročitelji hormonskih motenj, sindrom policističnih jajčnikov, endometrioza, leiomiomi, incidenca

# ENDOCRINE DISRUPTERS AND FEMALE REPRODUCTIVE HEALTH

**Ksenija Geršak**

Department of Obstetrics and Gynaecology, University Medical Centre Ljubljana and Faculty of Medicine, University of Ljubljana

[ksenija.gersak@mf.uni-lj.si](mailto:ksenija.gersak@mf.uni-lj.si)

**Introduction and aim:** The effects of endocrine disrupters are connected to female reproductive health. Numerous reports prove their influence on the menstrual cycle, fertility and causality in the development of hormone-dependent malignant neoplasms of the breast and female reproductive organs. The aim of our study was to identify the incidence of certain diseases associated with decreased fertility in statistical regions of the Republic of Slovenia within an 11-year timeframe (2003-2013).

**Materials and methods:** We used the healthcare statistical data gathered by the non-hospital based National Institute of Public Health, along with the demographic data from the databases of the Statistical Office of the Republic of Slovenia. The observed population consisted of women, aged 15-49 years, which had been treated at the primary healthcare level due to one of the following three diagnoses: polycystic ovary syndrome (PCOS), endometriosis and uterine fibroids. We analysed the incidence of the three conditions in an 11-year timeframe and compared its significance in different statistical regions of the Republic of Slovenia.

**Results:** In Slovenia, the incidence of PCOS was 16/10,000 women aged 15-49 years; Notranjsko-Kraška (41/10,000) and Zasavje (12/10,000) regions stood out. The national incidence of endometriosis was the same as that for PCOS (16/10,000); the differences between the statistical regions were not as notable though - the highest incidence was in Gorenjska (23/10,000) and Zasavje (21/10,000) regions. The incidence of uterine fibroids was 5-times greater in the same population compared to the incidence of PCOS and endometriosis; greatly distinctive was the Notranjsko-Kraška region (190/10,000), followed by Pomurska (123/10,000), South-eastern (117/10,000) and Spodnjeoposavska (103/10,000) regions.

**Conclusions:** PCOS, endometriosis and uterine fibroids are of multifactorial origins; their pathogenesis is, among other influences, also precipitated by endocrine disrupters present in the environment. Epidemiological studies have proven the influence of bisphenol A on PCOS and endometriosis; chlorinated pesticides, polychlorinated biphenyls, phthalates, and phyto-oestrogens are involved in the pathogenesis of endometriosis and uterine fibroids; the incidence of uterine fibroids is linked to polybrominated biphenyls. We presume that the environment can contribute to the regional differences in incidence of the three observed medical conditions, where Notranjsko-Kraška and Zasavje regions stand out. Further studies are required in order to define the influence of environmental factors in greater detail.

**Key words:** endocrine disrupters, polycystic ovary syndrome, endometriosis, uterine fibroids, incidence

# KEMIJSKI POVZROČITELJI HORMONSKIH MOTENJ IN REPRODUKTIVNO ZDRAVJE MOŠKIH V SLOVENIJI –PREGLED LITERATURE, EPIDEMIOLOŠKI NACIONALNI PODATKI

**Branko Zorn**

Oddelek za andrologijo, Ginekološka klinika, Univerzitetni klinični center Ljubljana, Šljajmerjeva 3, 1000, Ljubljana,

[branko.zorn@kclj.si](mailto:branko.zorn@kclj.si), 00 386 1 522 60 72.

Uvod in namen: Poimenovanje *endocrine disruptors* so začeli prvič uporabljati v začetku devetdesetih letih. Kemijski povzročitelji hormonskih motenj (KPHM) se vežejo na različne hormonske receptorje (predvsem estrogenske in androgenske) in spodbujajo (estrogene snovi, agonisti estrogenov) ali zavirajo (antiandrogene snovi, antagonisti androgenov) delovanje teh receptorjev. Moški reproduktivni sistem je najbolj dovzeten za estrogenske snovi v fetalnem in neonatalnem obdobju. Prekomerna izpostavljenost ženskim hormonom (tudi v obliki KPHM) moti normalen razvoj reproduktivnega sistema, kar lahko privede do poznejših motenj moške plodnosti, ki jih imenujemo sindrom disgeneze moda (SDM).

SDM zajema hipospadijo in slabo spuščeno modo, moško neplodnost in raka moda.

Materiali in metode: Predstavljeni epidemiološki podatki izvirajo iz baze podatkov Nacionalnega inštituta za javno zdravje, iz člankov raziskav, ki smo jih izvedli na Ginekološki kliniki v Ljubljani in Registra raka RS.

Rezultati: Retrospektivno smo med 1983 in 2010 sledili kakovost semenčic in incidenco neplodnih moških. Koncentracija je ostala nespremenjena, medtem ko sta se gibljivost in morfologija znižali. Incidenca neplodnih moških in moških z azoospermijo je ostala nespremenjena. Na Koroškem in v Pomurju je bilo seme slabše. V zadnjem četrstoletju 20. stoletja se je incidenca raka moda močno zvišala. Mednarodna agencija za raziskovanje raka Svetovne zdravstvene organizacije (IARC) predvideva, da se bo incidenca raka moda še višala do l. 2025, takrat bo Slovenija na samem vrhu v Evropi glede na pogostnost. Pogostnost slabo spuščene moda se v zadnjih 13 letih ni povečala.

Zaključki: Državni organi so premalo osveščeni o nevarnosti izpostavljenosti KPHM in o stroških, povezanih z njimi. Svetovalne strukture pa so preobremenjene: IARC je ravnokar označil herbicid glifosat kot verjetno kancerogeno snov in insekticida malation in diazinon kot možni kancerogeni snovi. Moteno delovanje reproduktivnega trakta moškega naj bo pod nadzorom in raziskave na tem področju stimulirane tudi v Sloveniji.

Ključne besede: Kemijski povzročitelji hormonskih motenj, rak moda, kakovost semena, moška neplodnost, slabo spuščeno modo.

# ENDOCRINE DISRUPTORS AND THE REPRODUCTIVE HEALTH OF MEN IN SLOVENIA –REVIEW OF LITERATURE, NATIONAL EPIDEMIOLOGICAL DATA

**Branko Zorn**

Andrology Unit, Division of Obstetrics and Gynaecology, University Medical Centre Ljubljana, Šljajmerjeva 3, 1000, Ljubljana, Slovenia

[branko.zorn@kclj.si](mailto:branko.zorn@kclj.si), 00 386 1 522 60 72.

**Introduction and purpose:** The term *endocrine disruptors (EDs)* was first used in the early nineties. EDs bind to hormone receptors (particularly estrogenic and androgenic) and either promote (estrogenic substances, estrogenic agonists) or inhibit (antiandrogens, androgen antagonists) their activity. The male reproductive system is most susceptible to estrogenic substances in the foetal and neonatal period. Excessive exposure to female hormones (also in the form of EDs) interferes with the normal development of the reproductive system, which may lead to subsequent male fertility disorders, which are collectively described as testicular dysgenesis syndrome (TDS). TDS includes hypospadias and mal-descended testis, male infertility and testicular cancer.

**Materials and methods:** Epidemiological data were obtained from the database of the National Institute for Public Health of the Republic of Slovenia, articles of the research conducted at the Division of Obstetrics and Gynaecology in Ljubljana and the Cancer Registry of the Republic of Slovenia.

**Results:** We retrospectively monitored sperm quality and incidence of infertile males for the period between 1983 and 2010. The concentration remained unchanged, while the motility and morphology had reduced. The incidence of infertile males and azoospermic men remained unchanged. The worst quality semen was found in the regions of Koroška and Pomurje. In the last quarter of the 20<sup>th</sup> century the incidence of testicular cancer strongly increased. IARC (International Agency on Research of Cancer) anticipates that the incidence of testicular cancer will be on the rise up to the year 2025, when Slovenia will be at the Europe's top in terms of its frequency. The frequency of mal-descended testis had not increased in the last 13 years.

**Conclusions:** National authorities have a lack of awareness about the dangers of exposure to ED and the costs associated with them. Consulting structures are overloaded: IARC has just ranked the herbicide glyphosate among the likely carcinogens and the insecticides malathion and diazinon as potential carcinogens. Impaired function of the male reproductive tract should be supervised and research in this field stimulated also in Slovenia.

**Key words:** endocrine disruptors, testicular cancer, sperm quality, male infertility, mal-descended testis.

# EPIDEMIOLOŠKI TRENDI BOLEZNI ŠČITNICE PRI ODRASLIH

**Simona Gaberšček, Katja Zaletel**

Klinika za nuklearno medicino, Univerzitetni klinični center Ljubljana

[simona.geberscek@kclj.si](mailto:simona.geberscek@kclj.si)

Epidemiologija bolezni ščitnice je pomembno povezana z jodno preskrbo. V Sloveniji smo leta 1999 zvečali vsebnost joda v kuhinjski soli s prejšnjih 10 mg kalijevega jodida(KI) na kg soli na 25 mg KI na kg soli. Glede na kriterije Svetovne zdravstvene organizacije smo se iz države z blagim pomanjkanjem joda spremenili v državo z ustreznim vnosom joda.

Ugotovili smo, da se je v desetih letih po zvečanju jodne preskrbe pomembno zmanjšala pojavnost difuzne golše in avtonomnega tkiva v ščitnici. Bolniki z difuzno golšo in avtonomnim tkivom so sedaj starejši kot pred zvečanjem jodne preskrbe. Poleg tega so bolniki z avtonomnim tkivom redkeje hipertirotični kot pred desetimi leti. Pri bolnikih s hipertirozo, ki je posledica čezmernega vnosa joda, je bolezen manj izražena kot pred zvečanjem jodne preskrbe. Povečala se je pojavnost nodozne golše, kar pripisujemo pogostejši uporabi ultrazvoka vratu v zadnjem desetletju. Zmanjšala se je pojavnost najbolj maligne oblike ščitničnega karcinoma. Pojavnost basedovke se ni pomembno spremenila. Po zvečanju preskrbe z jodom se je povečala pojavnost Hashimotovega tiroiditisa v vseh starostnih skupinah, verjetno pri genetsko predisponiranih posameznikih.

V zadnjem desetletju so v številnih raziskavah na živalih in v raziskavah *in vitro* ugotavljali vpliv kemijskih povzročiteljev hormonskih motenj (KPHM) na različne vidike ščitnične funkcije. Najpogosteje testirani KPHM so bili poliklorirani bifenili (PCB) in dioksini, bromirani zaviralci gorenja, ftalati, bisfenol A in perfluorirane kemikalije. Vendar pa so raziskave o učinkih KPHM na delovanje ščitnice pri ljudeh, zlasti dolgoročne raziskave, zelo redke. Zdi se, da zlasti PCB in dioksini vplivajo na transport ščitničnih hormonov in negativno učinkujejo na delovanje ščitnice.

Številni avtorji menijo, da pomanjkanje joda poveča dovzetnost ščitnice za škodljive učinke KPHM. Torej bi lahko ustrezna preskrba z jodom delovala zaščitno.

Ključne besede: kemijski povzročitelji hormonskih motenj, bolezni ščitnice, epidemiologija, preskrba z jodom

# EPIDEMIOLOGICAL TRENDS OF THYROID DISORDERS IN ADULTS

**Simona Gaberšček, Katja Zaletel**

Clinic for nuclear medicine, University Medical Centre Ljubljana, Slovenia

[simona.geberscek@kclj.si](mailto:simona.geberscek@kclj.si)

Epidemiology of thyroid disorders is significantly associated with iodine supply. In 1999, Slovenia increased iodine content in kitchen salt from previous 10 mg of potassium iodide (KI) per kg of salt to 25 mg of KI per kg of salt. According to World Health Organization criteria, Slovenia changed from mild iodine deficient country to a country with adequate iodine supply.

Ten years after the increase in iodine supply, we have established a significant decrease in the incidence of diffuse goiter and thyroid autonomy. Patients with diffuse goiter and thyroid autonomy are now older than before the increase in iodine supply. Additionally, patients with thyroid autonomy are less frequently hyperthyroid than ten years ago. In patients with iodine-induced hyperthyroidism, the disease is less severe than before the increase in iodine supply. The incidence of nodular goiter increased, most likely as a consequence of more frequent use of neck ultrasound in the last decade. The incidence of highly malignant thyroid carcinoma decreased. Incidence of Graves' disease did not change significantly. After the increase in iodine supply, the incidence of Hashimoto's thyroiditis increased, most probably in genetically predisposed individuals.

In the last decade, many animal and *in vitro* studies evaluated the influence of various endocrine disrupting chemicals (EDCs) on various aspects of thyroid function. Most studied EDCs were polychlorinated biphenyls (PCBs) and dioxins, brominated flame retardants, phthalates, bisphenol A, and perfluorinated chemicals. However, human studies - especially studies with a long-term design - on effects of EDCs on thyroid function are very scarce. It seems that especially PCBs and dioxins interfere with the transport of thyroid hormones and negatively affect thyroid function.

Many authors agree that iodine deficiency predisposes the thyroid gland to harmful effects of endocrine disrupting chemicals (EDCs). Therefore, adequate iodine supply could be protective in that respect.

Ključne besede: endocrine disrupting chemicals, thyroid disorders, epidemiology, iodine supply

## **EPIDEMIOLOŠKI TRENDI HORMONSKO ODVISNIH RAKOV**

**Vesna Zadnik**

Epidemiologija in register raka, Onkološki inštitut Ljubljana

[vzadnik@onko-i.si](mailto:vzadnik@onko-i.si)

Spolni hormoni so znan in pomemben nevarnostni dejavnik rakov na dojki, jajčniku in endometriju, medtem ko je njihova vloga pri rakah prostate in mod verjetna, a manj raziskana. Vpletenost hormonov v kancerogenezo se nakazuje tudi pri raku ščitnice. Predvidevamo, da se po enakih mehanizmih kot telesu lastni hormoni v nastanek raka vpletajo tudi hormonski motilci.

Rake hormonsko odzivnih tkiv najpogosteje povezujemo z izpostavljenostjo PCB-jem in dioksinom, DDT/DDE ter nekaterim težkim kovinam in pesticidom. Incidenca hormonsko odvisnih rakov je največja v državah razvitega sveta. V Sloveniji predstavljajo hormonsko odvisni raki četrtno vseh rakov; njihovo število se z izjemo raka jajčnikov vsak leto veča. Tveganje raka dojk je že več desetletji večje na zahodu države, medtem ko pri ostalih lokacijah med območij ne opažamo pomembnih razlik.

Hormonskim motilcem pripisljivega deleža rakov trenutno ni mogoče določiti, saj imamo verodostojnih raziskav pri izpostavljenih, predvsem v kritičnih razvojnih obdobjih, premalo.

Ključne besede: hormonsko odvisen rak, hormonski motilec, breme raka, pripisljiv delež

## **EPIDEMIOLOGICAL TRENDS OF HORMON RELATED-CANCERS**

**Vesna Zadnik**

Epidemiology and cancer register, Institute of Oncology Ljubljana, Slovenia

[vzadnik@onko-i.si](mailto:vzadnik@onko-i.si)

Sex hormones are a recognised risk factor in breast, ovary and endometrium cancers, while their role in prostate and testicular cancers is less well defined. Furthermore, the hormones possibly take part in thyroid cancer cancerogenesis. Chemicals with endocrine disrupting properties that are most commonly associated with cancer are: PCBs and dioxins, DDT/DDE, some heavy metals and pesticides. The incidence rates of hormone-related cancers tend to be higher in the developed world. In Slovenia, six hormone-related cancers represent a quarter of all cancers; their number (with the exception of ovarian cancer) is increasing each year. The risk of breast cancer is higher in the western part of the country, but no geographical patterns can be recognised in other hormone-related cancers. The fraction of cancers attributable to endocrine disruptors' exposure is currently not known as there are not many reliable exposure studies available.

Key words: hormone-related cancer, endocrine disruptor, cancer burden, attributable fraction

# POGOSTNOST NEKATERIH ENDOKRINOLOŠKIH MOTENJ MED OTROCI IN MLADOSTNIKI V SLOVENIJI

**Nataša Bratina, Mojca Žerjav Tanšek, Tadej Battelino**

Klinični oddelek za endokrinologijo, diabetes in bolezni presnove, Pediatrična klinika, Bohoričeva 20  
Ljubljana

[natasa.bratina@kks-kamnik.si](mailto:natasa.bratina@kks-kamnik.si)

**Uvod:** Slovenija sodi med manjše srednjeevropske države z le dvema milijoni prebivalcev. Zato je smiselno bolnike z redkimi boleznimi ali motnjami obravnavati v večjih specializiranih centrih. Centralna umeščenost Ljubljane je bila vodilna pri odločitvi Razširjenega strokovnega kolegija za pediatrijo, da se otroci z endokrinimi motnjami vodijo v terciarnem pediatričnem centru – Kliničnem oddelku za endokrinologijo, diabetes in bolezni presnove, Pediatrična klinika. Tako preprečujemo razdrobljenost obravnave pacientov z redkimi motnjami žlez z notranjim izločanjem ali vrojenimi motnjami presnove in zagotavljamo njihovo enotno obravnavo.

**Materiali in metode:** Na KOEDBP vodimo tri centralne registre v Sloveniji, od katerih imata dva vlogo nacionalnih registrov: Register sladkorne bolezni tipa 1 in Register vrojenih motenj presnove, ob teh pa vodimo še register otrok zdravljenih z rastnim hormonom. Redne letne statistične analize na podlagi teh registrov omogočajo natančno spremljanje pogostnosti posameznih obolenj. Starši pred vpisom v register podajo pisno privolitev, obrazec je odobrila Etična komisija Slovenije, registri so bili predstavljeni tudi informacijski pooblaščenki Slovenije.

**Rezultati:** Na podlagi Registra za sladkorno bolezen tipa 1 redno spremljamo incidenco te bolezni, po zadnjih podatkih znaša 14,6/100.000 otrok mlajših od 15 let, letno pojavnost te bolezni narašča za 4,3%, kar je primerljivo z drugimi državami. Podatki iz tega registra vključujejo tudi podatke o akutnih in kroničnih zapletih sladkorne bolezni in presnovni urejenosti bolnikov in so vključeni v primerljive registre v Evropi (Eubirod, Eurodiab, Sweet), kar zagotavlja neodvisno analizo podatkov. V Register vrojenih motenj presnove so vključeni vsi otroci z motnjami presnove, kjer največji delež predstavljajo otroci s fenilketonurijo, homocistinurijo, motnjami cikla sečnine. Pri najpogostejšo od teh motenj – fenilketonuriji podatki iz registra kažejo, da je pojavnost klasične fenilketonurije v Sloveniji 1/10.000, pogostost prenašalcev klasične fenilketonurije v splošni populaciji pa 1/50. Skupna pojavnost vseh oblik fenilketonurije (klasična, zmerna, blaga) je približno 1/6.000; pojavnost blage hiperfenilalaninemije pa 1/3.500.

**Zaključki:** Vključevanje podatkov o redkih endokrinoloških in metabolnih boleznih v slovenski populaciji otrok in mladostnikov v nacionalne, oziroma centralno vodene registre je ključnega pomena za načrtovanje zdravstvene oskrbe te populacije. Prav tako so pomembne primerjave z državami Evrope in sveta, ki vključuje tako podatke o genetskem ozadju, kot tudi podatke o pojavnosti. Zato mora ostati skrb za delovanje teh registrov ena od prioritet Slovenije.

Ključne besede: otroci, mladostniki, endokrinološke motnje, pogostnost



# INCIDENCE OF SOME ENDOCRINE DISORDERS AMONG CHILDREN AND ADOLESCENTS IN SLOVENIA

Nataša Bratina, Mojca Žerjav Tanšek, Tadej Battelino

Department of endocrinology, diabetes and metabolic diseases, Paediatric clinic, Bohoričeva 20 Ljubljana

[natasa.bratina@kks-kamnik.si](mailto:natasa.bratina@kks-kamnik.si)

**Introduction:** Since Slovenia is a small country with 2,000,000 inhabitants, children with many chronic diseases and conditions receive a centralised health care. Ljubljana's central position was important for the decision of the Expert Collegiate Body that children with endocrine disorders receive central care in the Department of endocrinology, diabetes and metabolic diseases (DEDMD), University children's hospital in Ljubljana. Such a decision gives to all children with the same rare disorder the same possibility to receive a multidisciplinary medical team care, education and support.

**Methods:** In DEDMD three central registries are held, two out of three have the role of national registers – Type 1 diabetes register and Metabolic disorder register, next to them Register of children treated with growth hormone has a growing importance as well. Regular statistical analyses give the possibility to follow the incidence and other specific data of these endocrine disorders. Parents give a written permission for the register; the form was approved by the National Medical Ethics Committee. Data collected in the registries were also presented to the Slovenian Information Commissioner.

**Results:** According to the data in the National type 1 diabetes register standardised incidence is 14.6/100,000 children below the age of 15 years, with a yearly increase of 4.3%, which is comparable to our neighbour countries. In register data on acute, chronic complications and metabolic control are collected as well and compared with similar international databases (Eubirod, Eurodiab, and Sweet). Metabolic disorder register collects data about children with metabolic disorders, the most frequent disorder are phenylketonuria, homocystinuria and others. For phenylketonuria data are showing that the incidence of the classic form of phenylketonuria is 1/10,000, and for carriers of this classic form 1/50. All together the incidence for all forms (classic, moderate and mild) is 1/6,000; for the mild form 1/3,500.

**Conclusions:** Data on rare metabolic and endocrine disorders are collected in national registries and are of importance for the health care planning. Since Slovenia is a small country comparison with other countries in Europe and world brings important information on genetic background and incidence of these disorders.

Key words: children, adolescents, endocrine disorders, incidence

# DOLGOROČNA EPIDEMIOLOŠKA ŠTUDIJA IZPOSTAVLJENOSTI NIZKIM KONCENTRACIJAM ŽIVEGA SREBRA PRI OBČUTLJIVI POPULACIJI

**Janja Snoj Tratnik<sup>1</sup>, Darja Mazej<sup>1</sup>, Ana Miklavčič<sup>1</sup>, Mladen Krsnik<sup>2</sup>, Joško Osredkar<sup>2</sup>, Alfred B. Kobal<sup>1</sup>, Janja Kodrič<sup>2</sup>, David Neubauer<sup>2</sup>, Janja Marc<sup>3</sup>, Milena Horvat<sup>1</sup>**

<sup>1</sup> Institut Jožef Stefan, Ljubljana, <sup>2</sup> Univerzitetni klinični center Ljubljana, <sup>3</sup> Fakulteta za Farmacijo, Univerza v Ljubljani, Slovenija

[janja.tratnik@ijs.si](mailto:janja.tratnik@ijs.si)

**Uvod in namen:** V sklopu največje evropske študije, ki je preučevala vpliv živega srebra zaradi izpostavljenosti preko hrane (projekt PHIME), smo raziskali prenatalno izpostavljenost metil živemu srebru (MeHg), učinke in dovzetnost pri splošni populaciji v Sloveniji. Poleg slovenske populacije so bile v študijo vključene še hrvaška, italijanska in grška populacija.

**Materiali in metode:** V vseh štirih državah je bilo skupaj vključenih pribl. 1700 mater in njihovih otrok, 500 iz Slovenije. Vsebnost živega srebra smo določali v materinih laseh, popkovni krvi, popkovnem tkivu, mekoniju in materinem mleku. Razvoj otrok smo testirali pri starosti 18 mesecev (Bayley III test). Na pod vzorcu mater in otrok smo genotipizirali gene glutationskega sistema proteinov.

**Rezultati:** V primerjavi z drugimi državami, vključenimi v študijo, smo pri slovenski populaciji ugotovili nizko izpostavljenost živemu srebru. Na splošno, izpostavljenost živemu srebru ni vplivala na izid Bayley testiranja, ugotovili pa smo zmerno pozitiven vpliv uživanja rib v nosečnosti na kognitivni in govorni razvoj otrok. Preliminarni rezultati genotipizacije so pokazali, da glutation-S transferaza vpliva na kopičenje MeHg pri materah. Poleg tega so Llop in sod (1) v študiji, ki je vključevala PHIME populaciji iz Italije in Grčije, pokazali, da imajo ABC transporterji vlogo pri transportu MeHg preko placent in kopičenju MeHg v zgodnjem razvoju. Ker omenjeni geni vplivajo na dozo MeHg v telesu, lahko na ta način vplivajo na pojav neurotoksičnih učinkov MeHg.

**Zaključki:** Rezultati dosedanjih nevro-epidemioloških študij kažejo, da bo potrebno v prihodnjih študijah poseben poudarek nameniti občutljivim skupinam populacije, in sicer na genetski ravni, saj bomo le tako lahko bolj realistično ocenili potencialno tveganje izpostavljenosti nevrotoksičnim onesnaževalom pri nizkih koncentracijah. Obstoječe PHIME populacije bodo v prihodnje vključene v projektih 7. okvirnega programa in LIFE+ (HEALS in CROME), kjer bomo skušali identificirati alelne različice genov, ki so odgovorne za občutljivost posameznikov na izpostavljenost določenim onesnaževalom.

LLOP, Sabrine, SNOJ TRATNIK, Janja, MAZEJ, Darja, HORVAT, Milena, et al. Polymorphisms in ABC transporter genes and concentrations of mercury in newborns - evidence from two Mediterranean birth cohorts. *PLoS one*, 2014, vol. 9/5, e97172-1-e97172-9.

**Ključne besede:** Živo srebro, prenatalna izpostavljenost, uživanje rib, razvoj osrednjega živčnega sistema, glutation sistem, ABC transporterji

# LONGITUDINAL EPIDEMIOLOGICAL STUDY OF LOW-LEVEL MERCURY EXPOSURE IN SUSCEPTIBLE POPULATION

**Janja Snoj Tratnik<sup>1</sup>, Darja Mazej<sup>1</sup>, Ana Miklavčič<sup>1</sup>, Mladen Krsnik<sup>2</sup>, Joško Osredkar<sup>2</sup>, Alfred B. Kobal<sup>1</sup>, Janja Kodrič<sup>2</sup>, David Neubauer<sup>2</sup>, Janja Marc<sup>3</sup>, Milena Horvat<sup>1</sup>**

<sup>1</sup> Jožef Stefan Institute, Ljubljana, <sup>2</sup> University Medical Centre Ljubljana, Ljubljana, <sup>3</sup> Faculty of Pharmacy, University of Ljubljana, Ljubljana, Slovenia

[janja.tratnik@ijs.si](mailto:janja.tratnik@ijs.si)

**Introduction and objectives:** Within the PHIME project - the largest study ever conducted in the general European population on the impact of mercury through food consumption- methyl mercury (MeHg) exposure, effects, and susceptibility in the general population in early life were investigated. The study included populations from Slovenia, Croatia, Italy and Greece.

**Materials and methods:** In total, approx. 1700 mother-child pairs were recruited from the participating countries, 500 from Slovenia. Mercury levels have been determined in the mother's hair, cord blood, cord tissue, meconium and breast milk. Children were tested for neurodevelopment (Bayley III test) at 18 months of age. Glutathione related genes were studied in a subset of participating mother-child pairs as a susceptibility markers.

**Results:** In comparison to other countries involved, Slovenia showed low mercury exposure. In general, mercury exposure did not predict Bayley scores but a moderate beneficial effect of fish consumption in pregnancy was observed in cognitive and verbal domain. Preliminary genotyping results demonstrated that glutathione-S-transferase seems to influence retention of MeHg in the mothers. Moreover, a study including PHIME birth cohorts from Italy and Greece showed that the ABC transporters appear to play a major role in the transport of MeHg across the placenta and accumulation of MeHg during early development (1). As these genes appear to influence MeHg internal dose they might offset MeHg neurotoxicity.

**Conclusions:** The studies performed so far showed that the neuro-epidemiological studies need to include a new focus on genetically susceptible groups in order to assess a more realistic potential risk of neurotoxicant exposures at low levels. In the future work, the existing Mediterranean cohorts will be included in the on-going 7<sup>th</sup> FP project HEALS and LIFE+ project CROME to identify gene variants responsible for individual's susceptibility to selected contaminants.

LLOP, Sabrine, SNOJ TRATNIK, Janja, MAZEJ, Darja, HORVAT, Milena, et al. Polymorphisms in ABC transporter genes and concentrations of mercury in newborns - evidence from two Mediterranean birth cohorts. *PLoS one*, 2014, vol. 9/5, e97172-1-e97172-9.

**Keywords:** Mercury, prenatal exposure, fish consumption, neurodevelopment, glutathione metabolism, ABC transporters

# ESENCIALNI IN NEESENCIALNI ELEMENTI PRI SLOVENSKEI POPULACIJI: REZULTATI HUMANEGA BIOMONITORINGA

**Darja Mazej<sup>1</sup>, Janja Snoj Tratnik<sup>1</sup>, Zdenka Šlejkovec<sup>1</sup>, Marta Jagodic<sup>1</sup>, Vesna Fajon<sup>1</sup>, Majda Pavlin<sup>1</sup>, Anja Stajnik<sup>1</sup>, Mladen Kršnik<sup>2</sup>, Alenka Sešek Briški<sup>2</sup>, Marija Prezelj<sup>2</sup>, Milan Skitek<sup>2</sup>, Alfred B. Kobal<sup>1</sup>, Lijana Kononenko<sup>3</sup>, Milena Horvat<sup>1</sup>**

<sup>1</sup> Institut Jožef Stefan, Ljubljana, <sup>2</sup> Univerzitetni klinični center Ljubljana, <sup>3</sup> Ministrstvo za zdravje, Urad Republike Slovenije za kemikalije, Ljubljana, Slovenia

[darja.mazej@ijs.si](mailto:darja.mazej@ijs.si)

**Uvod in namen:** Namen humanega biomonitoringa je pridobiti podatke o obremenitvah ljudi v Republiki Sloveniji z določenimi kemikalijami (onesnaževali), ki se nahajajo v okolju in pogledati prostorske razlike v izpostavljenosti.

**Materiali in metode:** Ciljna populacija so bile matere prvega otroka, ki dojijo, z otrokom starim 2 do 8 tednov, stare od 20 - 40 let ter moški v enakem starostnem obdobju, ki so ustrezali vključitvenim kriterijem. Geografsko smo vzorčenje osredotočili na tri različne tipe območij v Sloveniji: podeželsko okolje, mestno okolje in okolje, ki je potencialno obremenjeno zaradi pretekle človekove dejavnosti. Skupno je bilo vzorčenih 1096 oseb (535 žensk in 561 moških)\*. V prvi fazi analiz smo spremljali koncentracije strupenih elementov (kadmija, svineca, živega srebra, arzena) in esencialnih elementov (selen, baker in cink) v krvi, materinem mleku, urinu in/ali laseh ter biokemijske markerje poškodb ledvic v urinu. V drugi fazi analiz pa bomo določali obstojna organska onesnaževala (dioksine, furane, organoklorne pesticide, poliklorirane bifenile in polibromirane zaviralce gorenja).

**Rezultati:** Izpostavljenost splošnega prebivalstva strupenim elementom, kot so svinec, živo srebro, kadmij in arzen, je nizka in v splošnem ne predstavljajo tveganja za preiskovano populacijo. Rezultati opravljenih analiz so primerljivi z rezultati podobnih raziskav v drugih državah. Za esencialne elemente (selen, baker in cink) so vsebnosti pri preiskovani populaciji znotraj meja primerjalnih vrednostih, ki jih podajajo nekatere mednarodne organizacije in so primerljive s študijami drugod po svetu, kar pomeni, da ni bilo opaznega pomanjkanja oz. presežka teh elementov pri preiskovancih razen pri nekaterih posameznikih.

**Zaključki:** Pridobljeni podatki bodo omogočili oceno tveganja za zdravje ljudi v Sloveniji ter nadalje pripravo in uvedbo ukrepov za zmanjšanje tveganja.

\*Pri naboru in vzorčenju so sodelovali bivši regionalni zavodi za zdravstveno varstvo, ki so sedaj vključeni v Nacionalni inštitut za javno zdravje ter regionalne bolnišnice in zdravstveni domovi.

**Ključne besede:** Humani biomonitoring, živo srebro, kadmij, svinec, arzen, esencialni elementi

# ESSENTIAL AND NON-ESSENTIAL ELEMENTS AT SLOVENIAN POPULATION: RESULTS OF HUMAN BIOMONITORING

**Darja Mazej<sup>1</sup>, Janja Snoj Tratnik<sup>1</sup>, Zdenka Šlejkovec<sup>1</sup>, Marta Jagodic<sup>1</sup>, Vesna Fajon<sup>1</sup>, Majda Pavlin<sup>1</sup>, Anja Stajniko<sup>1</sup>, Mladen Kršnik<sup>2</sup>, Alenka Sešek Briški<sup>2</sup>, Marija Prezelj<sup>2</sup>, Milan Skitek<sup>2</sup>, Alfred B. Kobal<sup>1</sup>, Lijana Kononenko<sup>3</sup>, Milena Horvat<sup>1</sup>**

<sup>1</sup> Jožef Stefan Institute, Ljubljana, <sup>2</sup> University Medical Centre Ljubljana, <sup>3</sup> Ministry of Health, Chemical Office of the Republic of Slovenia, Ljubljana, Slovenia

[darja.mazej@ijs.si](mailto:darja.mazej@ijs.si)

**Introduction/Objectives:** The main objective of the human biomonitoring programme is to provide data on exposure of the inhabitants of Slovenia to environmental chemicals (pollutants) and to look at spatial differences in exposure.

**Materials and methods:** The study population includes lactating women who have given birth for the first time and men from the same area in the age from 20-40 years who met the inclusion criteria. Twelve areas in Slovenia covering urban, rural and potentially contaminated area due to past human activities were chosen. A total of 1096 persons were sampled (535 women and 561 men)\*. In the first stage of analysis, we monitored the concentration of toxic elements (cadmium, lead, mercury, arsenic) and essential elements (selenium, copper and zinc) in blood, breast milk, urine and/or hair and biochemical markers of kidney damage in urine. In the second phase of analysis determination of POPs (dioxins, furans, organochlorine pesticides, polychlorinated biphenyls, and polybrominated flame retardants) will be done.

**Results:** Exposure of general population to toxic elements such as lead, mercury, cadmium and arsenic is low and generally do not pose a risk to the population under investigation. The results of analysis are comparable with the results of similar studies in other countries. Results of the analysis for essential elements (selenium, copper and zinc) are within the ranges given by international organizations and are comparable with other studies elsewhere in the world, which means that there was no noticeable lack or excess of these elements in participating subjects except in some individuals.

**Conclusions:** The data obtained will allow a health risk assessment of Slovenian population as well as further development and implementation of risk reduction measures.

\* Recruitment and sampling phase was implemented by the help of former regional Institutes of Public Health, which are now joined within National Institute of Public Health, and regional hospitals and health centres.

**Key words:** Human biomonitoring, mercury, cadmium, lead, arsenic, essential elements

# PILOTNI EVROPSKI HUMANI BIOMONITORING DEMOCOPHES – SLOVENIJA

**Milena Horvat, Darja Mazej, Janja Snoj Tratnik, Ester Heath, Tina Kosjek**

Odsek za znanosti o okolju, Institut “Jožef Stefan”, Ljubljana

[milena.horvat@ijs.si](mailto:milena.horvat@ijs.si)

Uvod/cilj: Namen EU projektov COPHES/DEMOCOPHES je bil prikazati izvedljivost programa harmoniziranega humanega biomonitoringa na nivoju Evrope.

Material in metode: Vzorce urina in las smo zbrali pri 156 parih mati-otrok in 69 moških (očetje/partnerji). Družine smo povabili preko 3 urbanih in 2 podeželskih osnovnih šol. V lasih smo določili živo srebro (Hg), v urinu pa kadmij (Cd), arzen (As), kotinin, ftalate (MEP, MEHP, DEHP=5oxoMEHP+5OH-MEHP, MBzP and MnBP), BPA (prosti in konjugirani), triklosan (TCL) metabolite parabenov (MeP, EtP, PrP and BuP). Rezultate smo normalizirali na kreatinin. V krvi pa smo določili Cd, Hg, svinec (Pb), As in selen (Se).

Rezultati: V vseh biomarkerjih smo opazili pomembne razlike v koncentracijah. Koncentracije Cd, TLC in metabolitov parabenov v urinu so bile najvišje pri materah ( $p < 0.001$ ). Očetje so imeli višje koncentracije Hg in kotinina, pri otrocih pa so bile najvišje koncentracije BPA in ftalatov ( $p < 0.001$ ). Koncentracije Hg v lasih so bile višje v urbanem okolju ( $p < 0.001$ ), prav tako tudi ftalatov MnBP, TCL in parabenov ( $p < 0.05$ ). MEHP in DEHP pa so bili pomembno višji pri podeželski populaciji ( $p < 0.001$ ). Pogostnost uživanja rib je bila povezana s koncentracijo Hg v lasih, medtem kot sta bila Cd in kotinin povezana s kajenjem. Pasivna izpostavljenost kajenju je pokazala povišane koncentracije kotinina pri otrocih, Cd pa ni bil povišan. Povišane koncentracije Hg v urinu smo opazili pri družinah kjer so poročali o razbitju živosrebrovega termometra. Metaboliti ftalatov so bili v pozitivni korelaciji z uživanjem lešnikovega namaza (MEP, MnBP), sladoleda (MEP) in uporabo žvečilnih gumijev (MEP, DEHP). Opremljenost stanovanj z PVC je bila povezana s koncentracijo MBzP. Le-ta je bil opazen tudi pri pogosti rabi sredstev za osebno nego otrok. Paraben EtP je bil povezan z uporabo sredstev za osebno nego, ter MeP z uporabo losijonov in krem. Vsebnost BPA v urinu in uživanja hrane iz konzerv sta bila v pozitivni korelaciji vendar šele ob upoštevanju uživanja kontracepcijskih tablet pri ženski populaciji.

Zaključek: Izpostavljenost preučevanim kemikalijam pri slovenski populaciji je primerljiva ali nižja od evropskega povprečja.

*Zahvala:* Delo je bilo financirano preko 7OP EU COPHES projekta, Life+ DEMOCOPHES projekta, ARRS programa P1-0143 in IJS.

Ključne besede: Kotinin, kadmij, živo srebro, svinec, arzen, ftalati, BPA, parabeni, triklosan, kri, urin, lasje

# PILOT EUROPEAN HUMAN BIOMONITORING STUDY DEMOCOPHES – CASE STUDY SLOVENIA

**Milena Horvat, Darja Mazej, Janja Snoj Tratnik, Ester Heath, Tina Kosjek**

Department of Environmental Sciences, Jožef Stefan Institute, Ljubljana, Slovenia

[milena.horvat@ijs.si](mailto:milena.horvat@ijs.si)

**Introduction/Objectives:** The aim of two European projects COPHES/DEMOCOPHES was to demonstrate the feasibility of the harmonized HBM protocol to be implemented at the European scale.

**Materials and methods:** Urine and hair samples were collected from 156 mother–child pairs and 69 men (fathers/ partners). Families were recruited through schools, 3 from urban and 2 from rural area. Mercury (Hg) was determined in hair, cadmium (Cd), arsenic (As), cotinine, phthalate metabolites (MEP, MEHP, DEHP=5oxoMEHP+5OH-MEHP, MBzP and MnBP), bisphenol A (BPA, free and conjugated), triclosane (TCL) and parabene metabolites (MeP, EtP, PrP and BuP) in urine samples. All concentrations were adjusted for creatinine. Hg, Cd, lead (Pb), As and selenium (Se) were determined in blood.

**Results:** In all biomarkers, significant differences between population groups were obtained. Concentration of Cd in urine, TCL and parabene metabolites levels were significantly higher in mothers ( $p<0.001$ ). Fathers had the highest Hg and cotinine levels, while children the highest BPA and phthalate levels ( $p<0.001$ ). Hair Hg was observed to be significantly higher in urban than in rural area ( $p<0.001$ ), the same was observed for phthalate metabolite MnBP, TCL and parabens ( $p<0.05$ ). MEHP and DEHP were significantly higher in rural than in urban area ( $p<0.001$ ). As expected, hair Hg was influenced by the frequency of fish consumption, while Cd and cotinine by smoking of mothers and fathers. Passive smoking in children influenced cotinine but not Cd levels. Higher Hg was observed also in families, where broken Hg thermometer were reported. In addition to smoking, frequency of game consumption influenced Cd in urine. For phthalate metabolites significant positive associations were observed with hazelnut spread (MEP, MnBP), ice cream (MEP), chewing gum (MEP, DEHP), and convenience food consumption (MEHP). Presence of PVC floor or walls in family's house influenced MBzP significantly, but not other phthalate metabolites. MBzP was observed to be associated with the use of personal care products in children. Among parabene and TCL metabolites, only EtP was significantly associated with the use of personal care products in general, and MeP with body lotion and crème usage. Prominent source of BPA exposure was not observed, however mothers taking contraception pills had higher BPA in urine (total and conjugated), and when adjusted for pills, significant positive association between BPA and consumption of canned food was observed.

**Conclusions:** Exposure to studied chemicals in Slovenia is comparable or lower than in other European countries.

**Acknowledgement:** Project COPHES was funded through EU 7<sup>th</sup>FP, DEMOCOPHES by EU Life+, ARRS P1-0143 programme and JSI.

**Key words:** Cotinine, cadmium, lead, mercury, arsenic, phthalates, BPA, parabens, triclosane, blood, urine, hair

# POJAVNOST PREZGODNJE MENOPAVZE V SLOVENIJI V OBDOBJU 2003-2013

**Živa M. Geršak<sup>1</sup>, Blaž M. Geršak<sup>1</sup>, Ksenija Geršak<sup>1,2</sup>**

<sup>1</sup>Medicinska fakulteta, Univerza v Ljubljani, <sup>2</sup>Ginekološka klinika, Univerzitetni klinični center Ljubljana

[ziva.gersak@gmail.com](mailto:ziva.gersak@gmail.com), [blaz.gersak@maat.si](mailto:blaz.gersak@maat.si), [ksenija.gersak@mf.uni-lj.si](mailto:ksenija.gersak@mf.uni-lj.si)

Uvod in namen: Prezgodnja menopavza ali prezgodnja ovarijska insuficienca (POI) je opredeljena kot prenehanje spontanega menstruacijskega ciklusa pred 40. letom starosti. Diagnozo potrdimo z dvema določitvama serumskih koncentracij folikle stimulirajočega hormona nad 40 IE/L, v obdobju 2 do 3 mesecev. Vzroki za prezgodnjo menopavzo so različni (genetski, avtoimunski, iatrogeni, kajenje), v večini primerov pa so multifaktorski ali ostanejo nepojasneni (idiopatka POI). Novejša poročila dokazujejo tudi pomembno vzročno povezanost s KPHM.

Namen naše raziskave je ugotavljanje pojavnosti prezgodnje menopaze v slovenskih statističnih regijah v časovnem obdobju od 2003 do 2013.

Materiali in metode: Uporabili smo podatke zunajbolnišnične zdravstvene statistike Nacionalnega inštituta za javno zdravje in demografske podatke Statističnega urada RS. Kot osnovno diagnozo smo izbrali primarno ovarijsko motnjo: prezgodnjo menopavzo in sindrom rezistentnega ovarija. Bolnice z neopredeljeno amenorejo ali sekundarno amenorejo so bile izključene iz raziskave. Izbrane diagnoze smo upoštevali samo, če so bile razlog za prvi obisk v ambulantah primarne ravni. Za izbrano časovno obdobje smo analizirali pojavnost POI v Sloveniji in jo primerjali s pojavnostjo po statističnih regijah.

Rezultati: V 11-letnem obdobju je bilo v Sloveniji odkritih 255 novih bolnic s POI, povprečno 23 na leto (incidenca 5,07/100.000 žensk starih od 0-39 let). Izstopa leto 2012, ko je bilo odkritih 55 novih bolnic. Incidenca je bila največja v zasavski regiji (12,43/100.000 žensk starih od 0-39 let), sledijo so ji savinjska, notranjsko-kraška in obalno-kraška regija (11,06/100.000, 9,68/100.000 in 9,12/100.000 žensk starih od 0-39 let). V ostalih regijah je bila letna incidenca manjša od slovenske za isto opazovano obdobje.

Zaključki: Razlago za razlike v pojavnosti POI v slovenskih statističnih regijah verjetno lahko iščemo v multifaktorskem vzročnem modelu, ki vključuje tudi škodljive učinke iz okolja. Znano je, da nekateri KPHM povečajo apoptozo ali atrezijo primarnih foliklov in tako vplivajo na zmanjšanje jajčnikove rezerve (bisfenol A, parabeni). Za identifikacijo njihove prisotnosti v okolju in razumevanje mehanizmov škodljivih učinkov so potrebne nadaljnje raziskave.

Ključne besede: primarna ovarijska motnja, prezgodnja menopavza



# THE INCIDENCE OF PREMATURE OVARIAN FAILURE IN SLOVENIA IN THE PERIOD FROM 2003 TO 2013

Živa M. Geršak<sup>1</sup>, Blaž M. Geršak<sup>1</sup>, Ksenija Geršak<sup>1,2</sup>

<sup>1</sup>Faculty of Medicine, University of Ljubljana, <sup>2</sup>Department of Obstetrics and Gynecology, University Medical Centre Ljubljana, Slovenia

[ziva.gersak@gmail.com](mailto:ziva.gersak@gmail.com), [blaz.gersak@maat.si](mailto:blaz.gersak@maat.si), [ksenija.gersak@mf.uni-lj.si](mailto:ksenija.gersak@mf.uni-lj.si)

**Introduction and aim:** Premature menopause, also known as premature ovarian insufficiency (POI), is defined as a cessation of spontaneous menstrual cycle before the age of 40 years. The diagnosis is confirmed with two independent measurements of follicle-stimulating hormone serum concentrations equalling more than 40 IE/L within the period of 2 to 3 months. The causes for premature menopause are diverse (genetic, autoimmune, iatrogenous, smoking), in most cases; however, they are multifactorial or rather remain unknown (idiopathic POI). Newer reports also prove a significant causal relationship with endocrine disrupters.

The aim of our study was to determine the incidence of premature menopause in the statistical regions of the Republic of Slovenia within an 11-year period (2003-2013).

**Materials and methods:** We used the healthcare statistical data gathered by the non-hospital based National Institute of Public Health, along with the demographic data from the databases of the Statistical Office of the Republic of Slovenia. We selected primary ovarian dysfunction as the base diagnosis: premature menopause with resistant ovary syndrome, including only those that led to the first medical visit at the primary healthcare level. Patients with an idiopathic or secondary amenorrhoea were excluded from the study. We analyzed the incidence of POI and compared its significance in different statistical regions of the Republic of Slovenia for the chosen 11-year timeframe.

**Results:** In an 11-year timeframe, there were 255 newly discovered cases of POI in Slovenia, 23 per year on average (incidence 5.07/100,000 women aged 0-39 years). The year 2012 stands out, in which 55 new cases were discovered. Incidence was highest in the Zasavje region (12.43/100,000 women aged 0-39 years), followed by Savinjska, Notranjsko-Kraška and Obalno-Kraška regions (11.06/100,000; 9.68/100,000; 9.12/100,000 women aged 0-39 years). In other statistical regions, the yearly incidence was lower than the national one.

**Conclusions:** The explanation for the differences in POI incidence in different statistical regions can be sought in a multifactorial sample model, which also includes harmful effects pertaining to the environment. It is known that some endocrine disrupters increase apoptosis or atresia of primary follicles, and so reduce the ovarian reserve (bisphenol A, parabens). Further studies are required to identify their presence in the environment and to understand the mechanisms of their adverse effects.

**Key words:** premature ovarian failure, premature menopause

## **SIMPOZIJ 2**

# **Kemijski povzročitelji hormonskih motenj in vplivi na druge organizme**



Foto: Anita Jemec

# UGOTAVLJANJE INTERSEKSUALNOSTI PRI SLADKOVODNIH RIBAH V IZBRANIH SLOVENSКИH VODOTOKIH

**Milka Vrecl, Vlasta Jenčič\***

Veterinarska fakulteta Univerze v Ljubljani, Gerbičeva 60, 1000 Ljubljana

[milka.vrecl@uni-lj.si](mailto:milka.vrecl@uni-lj.si), [vlasta.jencic@vf.uni-lj.si](mailto:vlasta.jencic@vf.uni-lj.si)

\*Predavateljica

Celinske vode in morja so izpostavljeni različnim onesnaževalcem. Globalni problem predstavlja onesnaževanje s snovmi oz. kemikalijami, ki delujejo kot motilci endokrinega sistema. Sladkovodne in morske ribe so zelo primerne za spremljanje učinkov hormonskih motilcev, ker so jim izpostavljene dlje časa, njihov endokrini sistem je kljub številnim razlikam podoben višjim vretenčarjem, hitro dosežejo spolno zrelost in imajo v kratkem času številno potomstvo. Že v osemdesetih letih prejšnjega stoletja so v Veliki Britaniji prvič poročali o pojavu interseksualnih rib (ribe s spolnimi žlezami, ki imajo moške in ženske spolne celice), kar je spodbudilo več nacionalnih eko-epidemioloških študij. V naši raziskavi smo v izbranih slovenskih rekah ugotavljali pojav feminizacije samcev. V prvi del raziskave so bile vključene ribe različnih vrst, izlovljene na različnih lokacijah iz reke Ljubljanice, od njenega izvira do izliva. V drugem delu raziskave smo se osredotočili samo na same šarenke (*Oncorhynchus mykiss*) in potočne postrvi (*Salmo trutta m. fario*) iz izbranih rek in potokov po vsej Sloveniji. Prisotnost in pogostnost jajčnih celic v testisih ter stopnjo interseksualnosti smo ugotavljali s histološko analizo. V dveh primerih smo ugotovili prisotnost jajčnih celic v tkivu testisov. Poleg tega smo v posameznih primerih ugotovili še cistam podobne tvorbe, ki so domnevno degenerirane jajčne celice. Z opravljeno študijo smo dobili prvi vpogled na pojav interseksualnosti pri ribah, za natančnejšo oceno tega problema v slovenskih vodah, pa bi bila potreba obsežnejša študija.

Ključne besede: hormonski motilci, interseksualnost, ribe

# INTERSEXUALITY RESEARCH IN SOME FERAL FRESHWATER FISH IN THE SELECTED SLOVENIAN RIVERS

**Milka Vrecl, Vlasta Jenčič\***

*Veterinary faculty University of Ljubljana, Gerbičeva 60, SI-1000 Ljubljana*

[milka.vrecl@uni-lj.si](mailto:milka.vrecl@uni-lj.si), [vlasta.jencic@vf.uni-lj.si](mailto:vlasta.jencic@vf.uni-lj.si)

\*Presenting author

Continental waters and sea are exposed to different pollutants. Pollution with environmental chemicals that have a potential to cause endocrine disruption has become a global concern. Freshwater and marine fish are well suited for monitoring the effects of hormonal disrupters because they are exposed to environmental concentrations of pollutants for a long time, their endocrine system is, despite many differences, similar to the higher vertebrates, they quickly reach sexual maturity and produce large numbers of progeny over short time intervals. First reports of intersex fish (fish with gonads containing male and female tissue, and/or feminized reproductive ducts) from UK in early 1980s, prompted several national eco-epidemiological studies. In our study we evaluated the possibility of male fish feminization in selected Slovenian rivers. In the first part we evaluated all fish species obtained from Ljubljanica River from its source to the estuary. The second part of the survey was focused on the rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salmo trutta m. fario*) males from selected rivers and streams from different parts of Slovenia. The male gonads were histologically assessed for the presence of oocytes and to determine the frequency and degree of intersex. Oocytes occurrence in the testes tissue was found in two cases. Besides, several cyst-like structures that are presumptive degenerative oocytes were also presents in some trout testes. This study gave a first insight on the intersex occurrence in some feral freshwater fish; however, a more widespread study is required in order to gain a more accurate picture of this problem in Slovenian rivers.

Key words: endocrine disruptors, intersexuality, fish

# EKOTOKSIKOLOŠKI VIDIKI BISFENOLA A IN NJEGOVIH ANALOGOV

Tatjana Tišler\*, Boštjan Erjavec\*, Marija Sollner Dolenc\*\*, Albin Pintar\*

\* Laboratorij za okoljske vede in inženirstvo, Kemijski inštitut, Hajdrihova 19, SI-1000 Ljubljana, Slovenija

\*\* Fakulteta za farmacijo, Univerza v Ljubljani, Aškerčeva 7, SI-1000 Ljubljana, Slovenija

E-mail: [tatjana.tisler@ki.si](mailto:tatjana.tisler@ki.si)

Bisfenol A (BPA) je pomembna industrijska kemikalija, ki se proizvaja v velikih količinah in se najpogosteje uporablja pri proizvodnji polikarbonatne plastike in epoksi smol. Številne raziskave potrjujejo, da je BPA zmerno strupen za nevretenčarje in ribe ter estrogeno aktiven, zato so se pojavile zahteve po njegovi zamenjavi z okolju manj škodljivimi analogi. Vendar je poznavanje usode teh analogov v vodnem okolju, kot tudi poznavanje strupenosti in estrogene aktivnosti, pomanjkljivo. V študiji smo preučevali letalne in subletalne učinke BPA ter analogov bisfenola F (BPF) in bisfenola AF (BPAF) na bakterije (*Vibrio fischeri*), alge (*Desmodesmus subspicatus*), vodne bolhe (*Daphnia magna*) in ribe zebrice (*Danio rerio*) v kratkotrajnih akutnih in dolgotrajnih kroničnih testih strupenosti. Estrogeno aktivnost BPA in analogov smo ugotavljali z YES biološkim testom z uporabo gensko spremenjenih kvasovk *Saccharomyces cerevisiae*. Dobljeni rezultati so pokazali, da je bil najbolj strupen BPAF, saj smo določili EC50 vrednosti pri koncentracijah pod 10 µmol/l za alge, vodne bolhe in zarodke zebric. BPAF je bil zelo strupen tudi za vodne bolhe po 21 dnevni izpostavitvi, saj smo škodljive učinke na razmnoževanje opazili že pri koncentraciji 1,34 µmol/l BPAF. V primerjavi z BPA je bil BPF manj strupen za *D. magna* v akutnem testu strupenosti, *D. rerio* in *D. subspicatus*, medtem ko se je vpliv na razmnoževanje vodnih bolh pokazal pri nižjih koncentracijah (21d LOEC = 8,39 µmol/l) kot v primeru BPA (21d LOEC = 43,8 µmol/l). Vsi testirani bisfenoli so bili estrogeno aktivni; BPA in BPF sta pokazala podobno aktivnost, medtem ko je bil BPAF najbolj estrogeno aktiven. Rezultati raziskave so pokazali, da so BPA in njegova analoga strupeni za vodne organizme in estrogeno aktivni, posebno BPAF. Zaradi povečane uporabe BPF and BPAF v industrijskih procesih in posledično dokazane prisotnosti v mnogih segmentih okolja lahko predstavljajo tveganje za vodno okolje.

**Ključne besede:** estrogeno delovanje, strupenost, vodni organizmi, bisfenol A, bisfenol F, bisfenol AF

# ECOTOXICOLOGICAL ASPECTS OF BISPHENOL A AND ITS ANALOGUES

Tatjana Tišler\*, Boštjan Erjavec\*, Marija Sollner Dolenc\*\*, Albin Pintar\*

\* *Laboratory for Environmental Sciences and Engineering, National Institute of Chemistry, Hajdrihova 19, SI-1000 Ljubljana, Slovenia*

\*\* *Faculty of Pharmacy, University of Ljubljana, Aškerčeva 7, SI-1000 Ljubljana, Slovenia*

Bisphenol A (BPA) is an important industrial high-production-volume chemical, commonly used in the production of polycarbonate plastics and epoxy resins. Many studies confirmed that BPA is moderately toxic to invertebrates and fish and revealed estrogenic activity. For these reasons a demand for replacement of BPA with ecologically less harmful analogues as substitutes has grown. However, data about fate of analogues in the aquatic environment as well as their toxic and estrogenic potential are scarce. In this study, lethal and sublethal effects of BPA, BPF and BPAF were studied on bacteria (*Vibrio fischeri*), algae (*Desmodesmus subspicatus*), water fleas (*Daphnia magna*), and zebrafish (*Danio rerio*) in short-term acute and long-term chronic toxicity tests. Estrogenic activity of BPA and analogues was studied with YES assay using genetically modified yeast strain *Saccharomyces cerevisiae*. The obtained results showed that BPAF was the most toxic compound among tested analogues. Furthermore, high chronic toxicity was determined in the case of water fleas as effects on reproduction were determined at even 1.34  $\mu\text{mol/L}$  of BPAF after 21 days of exposure. In comparison to BPA, BPF revealed lower toxicity to *D. magna* in acute toxicity test, *D. rerio* and *D. subspicatus*. However, reproduction of water fleas exposed to BPF in long-term experiment was impaired at lower concentrations (21d LOEC = 8.39  $\mu\text{mol/L}$ ) than in the case of BPA (21d LOEC = 43.8  $\mu\text{mol/L}$ ). All tested BPs were estrogenically active; BPA and BPF showed similar estrogenicity, while the highest estrogenic activity was found in the case of BPAF. The obtained results showed that BPA and both analogues are acutely and chronically toxic and revealed estrogenic activity, especially BPAF. Due to their increased use in industrial processes and consequently the presence in many environmental samples we assume that BPA analogues could pose a risk to aquatic environment.

**Keywords:** estrogenic activity, toxicity, aquatic organisms, bisphenol A, bisphenol F, bisphenol AF

# FOTOKATALITSKO ODSTRANJEVANJE BISFENOLA A, F IN AF IZ VODNIH VZORCEV

**Boštjan Erjavec<sup>1</sup>, Petra Hudoklin<sup>2</sup>, Katja Perc<sup>2</sup>, Tatjana Tišler<sup>1</sup>, Marija Sollner Dolenc<sup>2</sup>, Albin Pintar<sup>1</sup>**

<sup>1</sup> *Laboratorij za okoljske vede in inženirstvo, Kemijski inštitut, Hajdrihova 19, 1001 Ljubljana, Slovenija*

<sup>2</sup> *Fakulteta za farmacijo, Univerza v Ljubljani, Aškerčeva 7, 1000 Ljubljana, Slovenija*

*e-mail:* [bostjan.erjavec@ki.si](mailto:bostjan.erjavec@ki.si)

Bisfenol A (BPA) in njegova analoga (BPF in BPAF) uvrščamo v skupino kemikalij, ki dokazano spodbujajo motnje v delovanju hormonskega sistema (Song in sod. 2010). Čiščenje vodnih virov, vsebujoč hormonske motilce, je zato nujno potrebno, saj mnoge študije poročajo o škodljivih učinkih bisfenolov, četudi so v sledeh. Heterogena fotokatalitska oksidacija je ocenjena kot ena najbolj preprečljivih metod za odstranjevanje tovrstnih spojin, saj zagotavlja visoko stopnjo mineralizacije, nizko generacijo strupenih spojin in pretvarja onesnažila do ogljikovega dioksida, vode ter anorganskih ionov (Wang in sod. 2009).

V pričujoči študiji smo predstavili nov tip imobiliziranega TiO<sub>2</sub> fotokatalizatorja, katerega smo temeljito okarakterizirali in nato testirali v tri-faznem šaržnem (BR) in pretočnem mešalnem (CSTR) reaktorju, osvetljenem z UV žarki, v katerem smo odstranjevali strupenost in estrogenost v vodi raztopljenih bisfenolov (A, F in AF). Biotesti na očiščenih vodnih vzorcih so pokazali, da so le-ti estrogensko neaktivni in nestrupeni do morskih bakterij *Vibrio fischeri* in vodnih bolh *Daphnia magna*. Ti rezultati so bili skladni z visoko stopnjo mineralizacije bisfenolov in njihovih derivatov v vzorcih, ki so bili zajeti po 4 h osvetljevanja. Namreč, TOC analize so pokazale, da smo v tem času dosegli skoraj popolno mineralizacijo BPA in BPF, medtem ko smo pri BPAF dosegli nekoliko nižjo stopnjo mineralizacije, tj., 70 %. Fotolitski poskusi v šaržnem reaktorju so pokazali, da je BPF najmanj foto-stabilen analog izmed testiranih. Nasprotno se obnaša BPAF, ki z dvema CF<sub>3</sub> skupinama na centralnem C atomu kaže najmanj foto-labilen značaj. Kljub temu, fotokatalitski oksidacijski testi niso pokazali bistvenih razlik med molekulami, saj smo v vseh treh primerih dosegli vsaj 99 % pretvorbo izhodiščnega onesnažila v času 3,5 h.

**Ključne besede:** fotokatalitska oksidacija, TiO<sub>2</sub>, bisfenoli, estrogenost, strupenost, čiščenje vode

# PHOTOCATALYTIC REMOVAL OF BISPHENOL A, F AND AF FROM WATER SAMPLES

**Boštjan Erjavec<sup>1</sup>, Petra Hudoklin<sup>2</sup>, Katja Perc<sup>2</sup>, Tatjana Tišler<sup>1</sup>, Marija Sollner Dolenc<sup>2</sup>, Albin Pintar<sup>1</sup>**

<sup>1</sup> *Laboratory for Environmental Sciences and Engineering, National Institute of Chemistry, Hajdrihova 19, SI-1001 Ljubljana, Slovenia*

<sup>2</sup> *Faculty of Pharmacy, University of Ljubljana, Aškerčeva 7, SI-1000 Ljubljana, Slovenia*

*e-mail:* [bostjan.erjavec@ki.si](mailto:bostjan.erjavec@ki.si)

Bisphenol A (BPA) and its analogues (BPF and BPAF) are a class of chemicals that are proven to elicit endocrine disrupting effects (Song et al. 2010). Purification of water samples containing these endocrine disrupting chemicals (EDCs) is necessary, since many studies have reported harmful effects of bisphenols at very low concentrations. Heterogeneous photocatalytic oxidation is one of the most promising methods for degradation of such chemicals, due to its high mineralization efficiency, low toxicity, ideally producing carbon dioxide, water and inorganic mineral ions as end products (Wang et al. 2009).

In the present study, immobilized TiO<sub>2</sub> photocatalyst was synthesized by a novel preparation procedure, thoroughly characterized and subsequently applied in a UV irradiated three-phase batch (BR) and continuous stirred-tank reactor (CSTR) for removal of toxicity and estrogenicity of water dissolved BPs (bisphenol A, F and AF). Bioassays of treated aqueous samples showed no estrogenic activity and complete removal of toxicity to marine bacteria *Vibrio fischeri* and water fleas *Daphnia magna*. These results were in accordance with high mineralization efficiency of bisphenols and their derivatives attained after 4 h of illumination. Namely, TOC analyses demonstrated that almost complete mineralization of BPF and BPA was achieved after 4 h of irradiation, while mineralization of BPAF was less efficient, but still almost 70 %. Photolytic experiments in batch reactor (BR) demonstrated that BPF possessed the least photo-resistant character among examined BF analogues. On contrary, BPAF with two CF<sub>3</sub> groups attached to the central C atom was the most stable under UV light irradiation. However, the photocatalytic oxidation runs showed very similar behavior; after 3.5 h more than 99 % of initial pollutant concentration was degraded.

**Keywords:** photocatalytic oxidation, TiO<sub>2</sub>, bisphenols, estrogenicity, toxicity, water treatment



# HORMONSKI MOTILCI IN NJIHOVI UČINKI NA RAZLIČNE (ŽIVALSKÉ) VRSTE

**Dr. Lucija Kolar**

Complementarium, Inštitut za raziskovanje narave in razvoj okoljskih tehnologij, Lopata 60, 3000 Celje

[lucija@complementarium.si](mailto:lucija@complementarium.si)

*Ključne besede: hormonski motilci, različne živalske vrste, posledice, predvidevanje, preprečevanje*

Hormonski motilci so v znanosti poznani že kar nekaj časa. Malo manj vedenja je o njihovih potrjenih učinkih na različne vrste in hkrati tudi ali so ti (skriti) učinki dejansko posledica njihovega delovanja ali pa gre za genske predispozicije ali zgolj za biološke lastnosti-občutljivosti nekega organizma. Danes se v znanosti že zelo dobro zavedamo tega problema, a vendar pravih rešitev še nimamo. V prispevku bomo predstavili nekaj primerov delovanja HM na različne vrste živeče v ekosistemu, posledice in predvideni potek sanacije. Na koncu bomo predstavili pilotni primer, ki se ga lotevamo in kjer bomo skušali z uporabo ocene tveganja najprej predvideti potem pa dejansko preprečiti onesnaževanje z uporabo znanstvenih metod, a ekonomsko učinkovitim in trajnostnim pristopom.

# ENDOCRINE DISRUPTORS AND THEIR IMPACTS ON VARIOUS (ANIMAL) SPECIES

**Dr. Lucija Kolar**

Complementarium, Institute for research of nature and development of environmental technologies, Lopata 60, SI-3000 Celje

[lucija@complementarium.si](mailto:lucija@complementarium.si)

*Key words: endocrine disruptors, various animal species, consequences, prediction, prevention*

Endocrine disruptors are known in science for quite some time. However, there is little less knowledge about scientifically proven effects of their action on various species and also if these (hidden) effects are really a consequence of their action. It is an ongoing debate, especially with epidemiological studies, whether toxic effects show only genetic preposition of an individual or even just sensitivity to a certain impulse and are not connected to EDs. In today's science EDs have much of recognition, but we are still struggling with thorough solutions, especially environmentally connected. This paper will show some examples of detrimental impact of EDs on various species living in the ecosystem, the consequences and possible solutions. We will also discuss a pilot experiment we are working on, where we use an environmental risk assessment tool for prediction in order to prevent actual contamination. The model uses scientific methods but is also sustainable and cost efficient.

# POJAVLJANJE FARMACEVTIKOV V ODPADNIH VODAH V SLOVENIJI

Anita Klančar <sup>a</sup>, Jurij Trontelj <sup>a</sup>, Albin Kristl <sup>a</sup>, Andrej Meglič <sup>b</sup>, Tinkara Rozina <sup>c</sup>,  
Maja Zupančič Justin <sup>b</sup>, Robert Roškar <sup>a</sup>

[anita.klancar@ffa.uni-lj.si](mailto:anita.klancar@ffa.uni-lj.si)

<sup>a</sup> Fakulteta za farmacijo, Univerza v Ljubljani, Slovenija

<sup>b</sup> Arhel d.o.o., Slovenija

<sup>c</sup> Envit d.o.o., Slovenija

Ključne besede: odpadne vode, farmacevtiki, izpostavljenost, tekočinska kromatografija, tandemska masna spektrometrija

## Uvod

Stalna rast starejšega prebivalstva in povečana poraba zdravil v starosti predstavljata resno grožnjo okolju in hkrati tveganje za celotno populacijo zaradi onesnaževanja vodnih virov z izločenimi farmacevtiki. Predstavljenih je bilo kar nekaj študij, ki dokazujejo prisotnost farmacevtikov v odpadnih vodah in površinskih vodah, ter prav tako v podtalni in pitni vodi.

Glavni namen raziskave je bilo določiti najpogosteje predpisovane učinkovine, ki bi potencialno lahko predstavljale grožnjo okolju in razviti občutljivo analitsko metodo za določanje analitov v odpadnih vodah v Sloveniji.

## Metode

Izbrani farmacevtiki so bili izolirani iz kompleksih vzorcev odpadnih voda z ekstrakcijo na trdnem nosilcu in bili nato analizirani s tekočinsko kromatografijo sklopljeno s tandemsko masno spektrometrijo.

## Material

Standardi: bisoprolol, karbamazepin, ciprofloksacin, klofibrična kislina, diklofenak, fluoksetin, imatinib, metoprolol so bili pridobljeni pri Sigma-Aldrich (Nemčija). Odpadna voda je bila vzorčena v čistilnih napravah in odplakah domov za ostarele.

## Rezultati

Analitska metoda je bila validirana in dokazano natančna, točna in linearna za vse analite z limito kvantifikacije v območju ng/L. Najbolj pogosto zaznani farmacevtiki so bili v srednje visokem nanogramskem (beta-blokatorja bisoprolol, metoprolol in antikonvulziv karbamazepin) in v nizkem mikrogramskem (analgetik diklofenak in antibiotik ciprofloksacin) koncentracijskem območju.

## Zaključek

Predstavljena analitska metoda je bila uspešno validirana in preizkušena na vzorcih odpadnih voda. Pridobljeni podatki so ključni pri strateškem načrtovanju obdelave tovrstnih vod, z namenom preprečevanja negativnega vpliva tako na vodne organizme kot tudi na splošno zdravje ljudi, saj je le malo znanega o tveganju zaradi dolgoročne izpostavljenosti neciljnih organizmov tovrstnim spojinam.

## Zahvala

Raziskovalno delo podpira projekt LIFE+: LIFE13ENV/SI/466.

# PRESENCE OF PHARMACEUTICALS IN WASTEWATER IN SLOVENIA

Anita Klančar <sup>a</sup>, Jurij Trontelj <sup>a</sup>, Albin Kristl <sup>a</sup>, Andrej Meglič <sup>b</sup>, Tinkara Rozina <sup>c</sup>,  
Maja Zupančič Justin <sup>b</sup>, Robert Roškar <sup>a</sup>

[anita.klancar@ffa.uni-lj.si](mailto:anita.klancar@ffa.uni-lj.si)

<sup>a</sup> Faculty of Pharmacy, University of Ljubljana, Slovenia

<sup>b</sup> Arhel d.o.o., Slovenia

<sup>c</sup> Envit d.o.o., Slovenia

## Introduction

The growing population of elderly people and their increasing pharmacotherapy presents a significant burden for the environment and may cause health risks for the general population by contaminating water sources with excreted pharmaceuticals. Recently, several studies have been published about the presence of pharmaceuticals in wastewater, surface, and ground and even in drinking water.

The aim of this study was to choose the compounds according to the frequency of their use and their toxicity to the environment and to develop sensitive analytical procedures for the determination of pharmaceuticals in wastewater in Slovenia.

## Methods

The selected pharmaceutical compounds were isolated from the samples by solid phase extraction and subjected to LC-MS/MS analysis.

## Material

All selected standards: bisoprolol, carbamazepine, ciprofloxacin, clofibric acid, diclofenac, fluoxetine, imatinib, metoprolol were purchased from Sigma-Aldrich (Germany). Wastewater samples were obtained from wastewater-treatment-plants and sewage outflow from retirement homes in Slovenia.

## Results

The analytical method was validated and confirmed to be precise, accurate and linear for all analytes with limits of quantification in ng/L range. The most frequently detected pharmaceuticals were in middle ng/L (beta-blockers bisoprolol, metoprolol and anticonvulsant carbamazepine) and low µg/L (analgesic diclofenac and antibiotic ciprofloxacin) concentration levels.

## Conclusion

The presented analytical method was successfully validated and applied to the determination of the target pharmaceuticals in wastewater samples. This data is crucial for strategic planning of water treatment to prevent the negative impact on aquatic biota and on human health as well since a little is known about the long-term risk to non-target organisms.

## Acknowledgments

This work was supported by the LIFE+ project: LIFE13ENV/SI/466.

Key words: wastewater, pharmaceuticals, exposure, LC-MS/MS

# RASTLINSKI HORMONI: VEČ KOT REGULATORJI RASTLINSKIH CELIC

**Marjana Regvar**

Oddelek za biologijo, Biotehniška fakulteta, Univerza v Ljubljani, Jamnikarjeva 101, SI 1000 Ljubljana

[marjana.regvar@bf.uni-lj.si](mailto:marjana.regvar@bf.uni-lj.si)

Namen prezentacije je pregled rastlinskih hormonov in mehanizmov njihovega delovanja. Rastlinski hormoni so ključni regulatorji celičnih delitev, diferenciacije, rasti in razvoja. So skupina kemijsko nesorodnih organskih molekul, ki vplivajo na fiziološke procese v koncentracijah, ki so precej nižje od koncentracij nutrientov ali vitaminov (Davies, 2004). Poleg klasičnih petih skupin rastlinskih hormonov: avksinov, citokininov, giberelinov, abscizinske kisline in etilena, spoznavamo še nove hormone in signalne molekule, med njimi brasinosteroide, strigolaktone, sistemine, jasmonske in salicilno kislino. Večji del sinteze rastlinskih hormonov poteka lokalno, kasneje pa se transportirajo v tarčne celice, v katerih sprožijo fiziološke odgovore z aktivacijo različnih signalnih verig. So osnovni regulatorji celičnega cikla in rasti rastlinskih celic. Z osveščanjem o pozitivnih učinkih rastlinskih hormonov na rast in razvoj rastlin ter povečevanjem njihove dostopnosti na spletnih straneh, bo potrebno preveriti tudi varnost njihove vsakodnevne uporabe, še posebej v proizvodnji rastlin, ki se uporabljajo v prehrani. Dokazi negativnih učinkov rastlinskih hormonov na netarčne organizme so se začeli kopičiti po odkritju vpliva avksinskih herbicidov na bakterije, žuželke, ribe, in sesalce (Wafa *et al.*, 2012). V zadnjem obdobju je dostopnih tudi vse več znanstvenih dokazov o negativnih vplivih ostalih skupin rastlinskih hormonov na netarčne organizme.

Ključne besede: avksini, citokinini, giberelini, brasinosteroidi, abscizinska kislina, etilen, salicilna kislina, jasmonska kislina

**Davies P. 2004.** *Plant Hormones - Biosynthesis, Signal Transduction, Action!* | Springer. Dordrecht, The Netherlands: Kluwer Academic Publishers.

**Wafa T, Amel N, Ikbal C, Mohamed H. 2012.** Oxidative Stress - Environmental Induction and Dietary Antioxidants. In: Lushchak V, ed. InTech, 115–130.

# PLANT HORMONES: REGULATION IN PLANT CELLS AND BEYOND

**Marjana Regvar**

Department of Biology, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, SI- 1000 Ljubljana

[marjana.regvar@bf.uni-lj.si](mailto:marjana.regvar@bf.uni-lj.si)

The main aim of the presentation is an overview of plant hormones and their mechanisms of action. Plant hormones are key regulators of cell division, differentiation, growth and development. They are a group of chemically unrelated organic molecules, which influence physiological processes at concentrations far below those of nutrients or vitamins (Davies, 2004). In addition to the classical five groups of plant hormones: auxins, cytokinins, gibberellins, abscisic acid and ethylene, new groups of hormones and signalling molecules are recognised, among them brassinosteroids, strigolactones, systemin, jasmonic and salicylic acid. It is generally believed that the synthesis of plant hormones is rather localized and they are transported to distant target cells, in which they induce physiological responses by activating diverse signalling cascades. They are primary regulators of plant cell cycle and cell growth. With the increasing awareness of the positive effects of plant hormones on growth and development and their worldwide availability on web pages, safety of their use on a daily basis, in particular in the production of plant foods, should be critically evaluated. Evidence on the detrimental consequences of plant hormones on non-target species have started to accumulate after the discovery of impact of auxin herbicides on bacteria, insects, fish and mammals (Wafa *et al.*, 2012). Reports on negative impact of other hormone groups on non-target organisms are slowly starting to accumulate.

Keywords: Auxin, cytokinin, gibberellin, brassinosteroid, abscisic acid, ethylene, salicylic acid, jasmonic acid

**Davies P. 2004.** *Plant Hormones - Biosynthesis, Signal Transduction, Action!* | Springer. Dodrecht, The Netherlands: Kluwer Academic Publishers.

**Wafa T, Amel N, Ikbal C, Mohamed H. 2012.** Oxidative Stress - Environmental Induction and Dietary Antioxidants. In: Lushchak V, ed. InTech, 115–130.

# VPLIV HORMONSKIH MOTILCEV NA NEVREtenČARJE- POUdAREK NA MEHANIZMIH DELOVANJA

**Jemec Anita in Gordana Glavan\***

Univerza v Ljubljani, Biotehniška fakulteta, Oddelek za Biologijo, Večna pot 111, 1000 Ljubljana; [anita.jemec@bf.uni-lj.si](mailto:anita.jemec@bf.uni-lj.si); [gordana.glavan@bf.uni-lj.si](mailto:gordana.glavan@bf.uni-lj.si)

\*delo bosta predstavili obe avtorici

Kemijski povzročitelji hormonskih motenj oz. hormonski motilci pri vretenčarjih motijo druge nevro-endokrine sisteme kot pri nevretenčarjih (Mu et al., 2005; LeBlanc, 2007). Nevretenčarji, med njimi žuželke in raki, so pogosto v neposrednem stiku s hormonskimi motilci. Nekateri insekticidi namreč tarčno delujejo na hormonski sistem insektov (Dhadialla et al., 1998). Raki so tem kemikalijam izpostavljeni posredno preko onesnaževanja. Poleg insekticidov na omenjeni skupini organizmov dokumentirano delujejo tudi ostale kemikalije, ki imajo učinek na hormonalni sistem pri vretenčarjih, npr. bisfenol A (BPA) (LeBlanc, 2007).

Cilj prispevka je pripraviti kratko predstavitev endokrine toksikologije insektov in rakov s ciljem predstaviti mehanizem delovanja hormonskih motilcev na te organizme. V ospredje bomo postavili ekdisteroidne in terpenoidne signalne poti, ki so najbolj pogosto motene. Ekdisteroidi so steroidni hormoni, ki nadzirajo razvoj, levitev, rast in razmnoževanje členonožcev. Hormonski motilci delujejo na samo sintezo teh hormonov kot tudi na delovanje njihovih receptorjev. Med terpenoide uvrščamo *trans*-retinojsko kislino, juvenilne hormone (pri žuželkah) in metil farnesoat pri rakah. Predstavili bomo svoje raziskave vpliva BPA na zelo pogoste testne organizme v vodni toksikologiji rake vodne bolhe *Daphnia magna*. Pokazali bomo učinke BPA na rast, razmnoževanje in antioksidativni sistem po kratkotrajni (48 ur) in kronični (21 dnevni) izpostavitvi. Predstavili pa bomo tudi učinke nekaterih hormonskih motilcev na medonosne čebele *Apis mellifera*.

**Ključne besede:** raki, insekti, ekdisteroidi, terpenoidi, levitev, juvenilni hormoni (JH), pesticid

## **Viri:**

Mu et al., 2005. Environ. Toxicol. Chem. 24: 146-152.

LeBlanc, 2007. Ecotoxicology 16:61-81.

Dhadialla et al., 1998. Annu.Rev.Entomol. 43: 545-69.

# EFFECTS OF ENDOCRINE DISTRUPTORS ON INVERTEBRATES- FOCUS ON THE MODE OF ACTION

**Jemec Anita and Gordana Glavan\***

University of Ljubljana, Biotechnical faculty, Department of Biology, Večna pot 111, 1000 Ljubljana,  
[anita.jemec@bf.uni-lj.si](mailto:anita.jemec@bf.uni-lj.si); [gordana.glavan@bf.uni-lj.si](mailto:gordana.glavan@bf.uni-lj.si)

\*both authors will present this work

Endocrine signalling disorders have mainly been studied in vertebrates, where different neuro-endocrine signalling cascades are present than in invertebrates (Mu et al., 2005; LeBlanc, 2007). However invertebrates, among these insects and crustacean, are frequent target organisms in close contact with endocrine disruptors. Namely, there are some specific classes of insecticides that are specifically targeted to alter some specific hormone systems in insects (Dhadialla et al., 1998). Crustaceans are indirectly exposed to these pesticides through environmental pollution. In addition, some other generally well known endocrine disruptors, such as bisphenol A (BPA), also affect the invertebrate hormonal signalling (LeBlanc, 2007).

The aim of this contribution is to present a short overview of general endocrine toxicology of insects and crustaceans with the aim to shed a light on the mode of endocrine disruptor action in invertebrates. The focus will be on ecdysteroid and terpenoid signalling, which are most commonly affected. Ecdysteroids represent a class of steroid hormones that regulate development, moult, growth and reproduction. Endocrine disruptors act directly on ecdysteroid receptor or through disturbances in their synthesis. Terpenoids consist of *trans*-retinoic acid, juvenile hormone (insects), and methyl farnesoate (crustaceans), which are involved in reproductive maturation. We will present our research on the effects of BPA on a very commonly aquatic toxicity test organism water flea *Daphnia magna*. The effects after acute (48 h), and chronic (21d) exposure on growth, reproduction and antioxidant system of these organisms will be presented. In addition, examples on the effects of endocrine disruptors on honey bees *Apis mellifera* will be also presented.

**Keywords:** crustaceans, insects, ecdysteroid and terpenoid signalling, moult, juvenile hormones (JH), pesticide

## **Ref.**

- Mu et al., 2005. Environ. Toxicol. Chem. 24: 146-152.  
LeBlanc, 2007. Ecotoxicology 16:61-81.  
Dhadialla et al., 1998. Annu.Rev.Entomol. 43: 545-69.

## SIMPOZIJ 3

### Temeljne (mehanistične) raziskave: *in vivo, in vitro, in silico*



Foto: Anita Jemec



# VPLIV IZBRANIH ENDOKRINIH MOTILCEV NA ADHEZIJSKE PROCESSE CELIC

**Katra Kolšek<sup>1</sup> in Marija Sollner Dolenc<sup>2</sup>**

<sup>1</sup>Molecular Biomechanics Group, Heidelberg Institute for Theoretical Studies, Schloss-Wolfsbrunnenweg 35, 69118 Heidelberg, Germany

<sup>2</sup>Fakulteta za farmacijo, Univerza v Ljubljani, Aškerčeva 7, 1000 Ljubljana, Slovenija

Ključne besede: endokrini motilci, triklosan, triklokarban, adhezija

Za hormonske motilce so dovzetni vsi fiziološki sistemi, ki so hormonsko občutljivi in vplivajo na plodnost moških in žensk, razvoj raka dojk, raka prostate, ščitnico, presnovo, debelost itd. Nekatere hormone (npr. tiroksin, dihidrotestosteron) povezujejo z vplivom na adhezijo celic, saj se vežejo na vitronektinski receptor ( $\alpha_v\beta_3$  integrin) in ga aktivirajo (1). Integrini so adhezijski proteini, preko katerih poteka adhezija celic na ekstracelularni matriks. Integrini z  $\alpha_v$ - podenoto so vključeni v celično adhezijo in celično signaliziranje ter nekateri izmed njih so povezani z napredovanjem in širjenjem tumorja. Pri tem je potrebno izpostaviti predvsem integrin  $\alpha_v\beta_3$  ali vitronektinski receptor, ki je pomemben za angiogenezo ter metastaziranje in je pogosto ekspresiran v tumorskih celicah (2).

Na osnovi teh podatkov smo želeli določiti ali tako kot nekateri hormoni tudi triklokarban in triklosan vplivata na adhezijo celic HUVEC na vitronektin. S triklokarbanom in triklosanom se srečujemo vsakodnevno z uporabo različnih izdelkov za osebno nego (mila, geli za tuširanje, zobne paste, deodorantski stiki itd.). Z njima so povezani mnogi neželeni učinki, med drugim sta uvrščena tudi med hormonske motilce.

Test adhezije smo izvajali s sistemom xCELLigence, RTCA. Rezultate smo dobili v obliki grafa celičnega indeksa v odvisnosti od časa, iz katerega smo izračunali povprečne naklone krivulje v izbranem intervalu (3). Celični indeks je parameter, ki odraža priterjenost celic na čipu in je tako premosorazmeren s celično adhezijo.

Določili smo, da triklokarban in triklosan povečata adhezijo HUVEC celic na vitronektin v submikromolarnih koncentracijah.

1. Davis et al., 2013, Horm. Cancer 4, 335-342
2. Perdih and Sollner Dolenc, 2010, Curr. Med. Chem. 17, 2371-92
3. Vlahovič, 2015, magistrska naloga

# INFLUENCE OF SELECTED ENDOCRINE DISRUPTORS ON CELL ADHESION PROCESSES

Katra Kolšek<sup>1,2</sup> and Marija Sollner Dolenc<sup>2</sup>

<sup>1</sup>Molecular Biomechanics Group, Heidelberg Institute for Theoretical Studies, Schloss-Wolfsbrunnenweg 35, 69118 Heidelberg, Germany

<sup>2</sup>Faculty of Pharmacy, University of Ljubljana

Keywords: endocrine disruptors, triclosan, triclocarban, adhesion processes

All physiological systems which are hormone sensitive are susceptible to endocrine disruptors. In addition, they affect men's and women's fertility, progress of breast and prostate cancer, thyroid, metabolism, obesity, etc. Some of the hormones (thyroxine, dihydrotestosterone, etc.) are connected with effects on cell adhesion, because they bind to vitronectin receptor ( $\alpha_v\beta_3$  integrin) (1). Integrins are adhesion proteins which enable adhesion of cells to extracellular matrix. Integrins with  $\alpha_v$ -subunit are involved in cell adhesion and cell signalling. Furthermore, some of them are related to the promotion and dissemination of tumor. Especially, integrin  $\alpha_v\beta_3$  or vitronectin receptor is important for angiogenesis and metastasis and it is often expressed in tumor cells (2).

Based on these data, we wanted to determine whether, as some hormones also triclocarban and triclosan affect the adhesion of HUVEC on vitronectin. Triclocarban and triclosan are encountered daily by using different consumer's products for personal care (solid and liquid soaps, shower gels, toothpaste, deodorant's sticks, etc.). They are connected with many side effects, among other things they are classified as endocrine disrupting chemicals.

Test adhesion was carried out with the system xCELLigence RTCA. Results were obtained in the form of a graph of the cell index as a function of time, from which we calculated the average slope of the curve in the selected interval (3). Using obtained results adhesion of cells was determined.

We determined that triclocarban and triclosan increase the adhesion of HUVEC cells on vitronectin in submicromolar concentrations.

1. Davis et al., 2013, *Horm. Cancer* 4, 335-342
2. Perdih and Sollner Dolenc, 2010, *Curr. Med. Chem.* 17, 2371-92
3. Vlahovič, 2015, master thesis, Faculty of Pharmacy

# **IN VITRO PROUČEVANJE GLUKOKORTIKOIDNEGA DELOVANJA IZBRANIH PARABENOV, FTALATOV IN INSEKTICIDOV TER NJIHOVIH MEŠANIC**

**Ivana Klopčič<sup>1,2</sup>, Katra Kolšek<sup>1</sup>, Marija Sollner Dolenc<sup>1</sup>**

[ivana.blagojevic@ffa.uni-lj.si](mailto:ivana.blagojevic@ffa.uni-lj.si)

<sup>1</sup>Fakulteta za Farmacijo, Univerza v Ljubljani, Aškerčeva 7, 1000 Ljubljana, Slovenija

<sup>2</sup>Laboratorij za računalniške bioznanosti in bioinformatiko, Kemijski inštitut, Hajdrihova 19, 1000 Ljubljana, Slovenija

**Ključne besede:** *In vitro* proučevanje, glukokortikoidni receptor, parabeni, ftalati, piretroidni insekticidi, učinki mešanic

Za nekatere parabene, mehčalce in pesticide je dokazano, da imajo vliv na endokrini sistem kot endokrini motilci. Tem spojinam smo ljudje izpostavljeni preko kože, z zaužitjem ter pri vdihavanju. Modulacija glukokortikoidnega receptorja je postala aktualna tema raziskovanja na področju iskanja potencialnih endokrinih motilcev. Čeprav smo ljudje običajno izpostavljeni številnim endokrinim motilcem hkrati pa so današnje raziskave kljub temu bolj fokusirane na učinke posameznih spojin. Proučevanje učinka mešanic na moduliranje glukokortikoidnega receptorja je nujno za popolno oceno tveganja pri izpostavljenosti tem spojinam. Naše *in vitro* študije temeljijo na MDA-kb2 celični liniji, ki izraža endogeni glukokortikoidni receptor in stabilen transficirani luciferazni reporterski gen, ki predstavlja konstrukt za kvantifikacijo glukokortikoidne aktivnosti. Izbrane testirane spojine pripadajo različnim kemijskim razredom: dve spojini iz razreda parabenov, propilparaben (PP) in butilparaben (BP), mehčalec dietilheksil ftalat (DEHP) in pesticid tetrametrin (TM). Spojine smo testirali pri koncentracijah 1  $\mu$ M in 10 nM, saj se pri teh koncentracijah pojavljajo v človeški plazmi. Ugotovljene glukokortikoidom podobne aktivnosti za testirane mešanice so bile med 1.25 in 1.51 krat večje pri koncentraciji 1  $\mu$ M v primerjavi s kontrolo topila ter med 1.23 in 1.44 krat večje pri koncentraciji 10 nM v primerjavi s kontrolo topila. Pri testiranju posameznih spojin smo ugotovili za BP, PP in DEHP pri koncentraciji 1  $\mu$ M glukokortikoidom podobno aktivnost, ki je med 1.50 in 1.60 krat večja glede na kontrolo topila. Pri koncentraciji 10 nM je samo BP pokazal glukokortikoidom podobno aktivnost. Raziskava potrjuje, da izpostavitvev nizkim neaktivnim koncentracijam posameznih glukokortikoidom podobnih spojinam niso zanesljiv dokaz, da ne bodo doprinesle k moduliranju glukokortikoidnega receptorja, ko smo le-tem izpostavljeni v različnih mešanicah [1].

[1] Klopčič, I., Kolšek, K. Dolenc, M.S., 2015. Glucocorticoid-like activity of propylparaben, butylparaben, diethylhexyl phthalate and tetramethrin mixtures studied in the MDA-kb2 cell line. *Toxicol. Lett.* 232, 376-383.

# **IN VITRO STUDY OF GLUCOCORTICOID-LIKE ACTIVITY OF PARABENS, PHTHALATES AND PYRETHROID INSECTICIDES AND THEIR MIXTURES**

**Ivana Klopčič<sup>1,2</sup>, Katra Kolšek<sup>1</sup>, Marija Sollner Dolenc<sup>1</sup>**

[ivana.blagojevic@ffa.uni-lj.si](mailto:ivana.blagojevic@ffa.uni-lj.si)

<sup>1</sup>Faculty of Pharmacy, University of Ljubljana, Aškerčeva 7, 1000 Ljubljana, Slovenia

<sup>2</sup>Laboratory for Biocomputing and Bioinformatics, National Institute of Chemistry, Hajdrihova 19, 1000 Ljubljana, Slovenia

**Keywords:** *In vitro* study, glucocorticoid receptor, parabens, phthalates, pyrethroid insecticides, effects of mixtures

Endocrine-disrupting compounds (EDCs) are widely used as parabens, plasticizers and pesticides to which humans can be exposed through dermal contact, ingestion and inhalation. Modulation of the glucocorticoid receptor has become important subject of research. Humans are usually exposed to multiple EDCs simultaneously but nowadays research is more focused on effects of single compound. Identification of mixture effects on glucocorticoid receptor is necessary to fully assess risk of individually compound. Our *in vitro* study is based on the MDA-kb2 cell line, which expresses endogenous glucocorticoid receptor and a stably transfected luciferase reporter gene construct, to quantify the glucocorticoid-like activity. The compounds selected are from different chemical classes: two parabens, propylparaben (PP) and butylparaben (BP), one plasticizer, diethylhexyl phthalate (DEHP), and one pesticide, tetramethrin (TM). Compounds are found in human biological samples at the tested concentration (1  $\mu$ M and 10 nM). Identified glucocorticoid-like activities for their mixtures were between 1.25 and 1.51 fold at the concentration of 1  $\mu$ M and between 1.23 and 1.44 fold at the concentration of 10 nM in comparison with the solvent control. Individually BP, PP, and DEHP had glucocorticoid-like activity between 1.50 and 1.60 fold over the solvent control at the concentration of 1  $\mu$ M. At the concentration of 10 nM only BP showed glucocorticoid-like activity. This study emphasizes that low ineffective levels of individual glucocorticoid-like compounds are not a reliable indicator for dismissing risk when we include mixed exposures [1].

[1] Klopčič, I., Kolšek, K. Dolenc, M.S., 2015. Glucocorticoid-like activity of propylparaben, butylparaben, diethylhexyl phthalate and tetramethrin mixtures studied in the MDA-kb2 cell line. *Toxicol. Lett.* 232, 376-383.

# ŠTUDIJ UČINKOV HORMONSKIH MOTILCEV NA *ASELLUS AQUATICUS*

**Maja Plahuta<sup>1</sup>, Tatjana Tišler<sup>1</sup>, Mihael Jožef Toman<sup>2</sup> & Albin Pintar<sup>1</sup>**

<sup>1</sup>Laboratory za okoljske vede in inženirstvo, Kemijski inštitut, Hajdrihova 19, SI-1000 Ljubljana, Slovenija

<sup>2</sup>Univerza v Ljubljani, Biotehniška fakulteta, Oddelek za biologijo, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenija  
E-mail: [maja.plahuta@ki.si](mailto:maja.plahuta@ki.si)

Ključne besede: *Asellus aquaticus*, bisfenol A, 17 $\alpha$ -etinilestradiol, hormonski motilci.

Motilci endokrinega sistema (MES), predvsem sintetične, estrogensko aktivne snovi, kot sta 17 $\alpha$ -etinilestradiol in bisfenol A, se stalno odvajajo v vodno okolje, ter tako predstavljajo tveganje za ljudi in druge živali. 17 $\alpha$ -etinilestradiol (EE2) predstavlja pomembno komponento v oralnih kontraceptivih, v okolje se izloča z urinom ter iztrebki, z iztoki iz čistilnih naprav. Bisfenol A (BPA) se obsežno uporablja v proizvodnji polikarbonatne plastike in epoksi smol. V okolje vstopa preko industrijskih odplak, iztokov čistilnih naprav in izcednih vod odlagališč komunalnih odpadkov. Prisotnost MES v okolju škoduje živalskim populacijam s povečanjem smrtnosti, povzročanjem deformacij, motnjah embriogeneze rib, dvoživk, rakov ter drugih vodnih živali. Namen študije predstavlja določanje škodljivih učinkov dveh estrogensko aktivnih snovi na vodnega raka enakonožca *Asellus aquaticus*. Akutne in kronične izpostavitve smo izvajali na juvenilnih in odraslih osebkih, gojenih v laboratoriju. Opazovali smo učinke na smrtnost, stopnjo rasti, stopnjo hranjenja, levitve, pigmentacijo telesa ter mobilnost in izračunali 96 h LC<sub>50</sub> in 21 d LOEC/NOEC. Rezultati akutnih testov so pokazali letalnost testiranih MES, predvsem v višjih koncentracijah. Kronične izpostavitve EE2 so povzročile signifikantno zmanjšanje frekvence levitev, ter podaljšanje obdobja med dvema levitvama. Učinki so bili koncentracijsko odvisni le pri nižjih koncentracijah, medtem ko so višje koncentracije povzročile visoko smrtnost po enkratni levitvi osebkov v 48 urah po izpostavitvi. Kronične izpostavitve enakonožcev BPA so povzročile koncentracijsko odvisno zmanjšanje števila levitev. Opazili smo tudi zmanjšanje pigmentacije telesne površine, mobilnosti, stopnje hranjenja in rasti. Rezultati so pokazali, da estrogensko aktivna EE2 in BPA povzročata inhibicijo frekvence levitev, podaljšanje časa med dvema levitvama ter zmanjšanje pigmentacije telesne površine, izpostavljenih osebkov že pri okoljsko relevantnih koncentracijah. Kronična izpostavljenost BPA pa povzroča škodljive učinke tudi na druge bistvene fiziološke procese.

# STUDY OF THE EFFECTS OF ENDOCRINE DISRUPTING COMPOUNDS ON *ASELLUS AQUATICUS*

Maja Plahuta<sup>1</sup>, Tatjana Tišler<sup>1</sup>, Mihael Jožef Toman<sup>2</sup> & Albin Pintar<sup>1</sup>

<sup>1</sup>Laboratory for Environmental Sciences and Engineering, National Institute of Chemistry, Hajdrihova 19, SI-1000 Ljubljana, Slovenia

<sup>2</sup>University of Ljubljana, Biotechnical Faculty, Department of Biology, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia  
E-mail contact: [maja.plahuta@ki.si](mailto:maja.plahuta@ki.si)

Keywords: *Asellus aquaticus*, bisphenol A, 17 $\alpha$ -ethynylestradiol, endocrine disruption.

Endocrine disrupting compounds (EDC), especially synthetic, estrogenically active compounds, for example 17 $\alpha$ -ethynylestradiol and bisphenol A, which are constantly released into the aquatic environment, present a major concern for humans and wildlife. 17 $\alpha$ -ethynylestradiol (EE2) is an oral contraceptive component, released into the environment from urine and faeces, via the wastewater effluents. Bisphenol A (BPA) is extensively used in the production of polycarbonate plastic and epoxy resins, and entering the environment via the industrial wastewater, wastewater treatment plants and landfill leachate. These EDC's can seriously affect local wildlife populations in reducing survival, causing deformities, disrupting embryogenesis in fish, amphibians, crustacean and other aquatic species. The aim of our study was to determine the adverse effects of two estrogenically active compounds on a freshwater, isopod *Asellus aquaticus*. Experiments were conducted, using laboratory bred, juvenile and adult organisms. Acute and chronic exposures were conducted, where survival, growth rate, feeding rate, molting, body pigmentation and mobility were observed and the 96 h LC<sub>50</sub> and 21 d LOEC/NOEC were calculated. The results of acute exposure tests show mortality at higher concentrations, and a higher sensitivity of juveniles in comparison to adults. Chronic exposures to EE2 caused a significant inhibition of molting frequency, as well as an increase of the intermolt period. The effects were concentration dependent at lower exposure concentrations of EE2; higher concentrations cause mortality, after a rapid molting, during 48 hours of exposure. Chronic exposure of isopods to BPA caused concentration dependent molting inhibitions; in addition a reduction in body pigmentation, mobility, feeding rate and growth rate were observed. In conclusion, our research indicates that estrogenically active EDC's cause a significant inhibition in molting frequency, increased intermolt phase, and reduced body pigmentation, at environmentally relevant concentrations, aside from causing other physiological adverse effects.

# UGOTAVLJANJE VPLIVOV BISFENOLOV BPA, BPF IN BPAF NA DIFERENCIACIJO, DOZOREVANJE IN FUNKCIJSKE LASTNOSTI IZ ČLOVEŠKIH MONOCITOV PRIPRAVLJENIH DENDRITIČNIH CELIC (DC) *IN* *VITRO*

Urban Švajger<sup>2</sup>, Marija Sollner Dolenc<sup>1</sup>, Matjaž Jeras<sup>1</sup>

<sup>1</sup> UL – Fakulteta za farmacijo, Aškerčeva 7, 1000 Ljubljana

<sup>2</sup> Zavod Republike Slovenije za transfuzijsko medicino, Šlajmerjeva 6, 1000 Ljubljana

Ključne besede: Bisfenoli, nezrele in zrele dendritične celice, fenotipske spremembe

Bisfenoli (BP), ki so močno razširjeni onesnaževalci našega okolja, delujejo kot estrogenu podobni endokrini motilci in predstavljajo dolgoročno potencialno grožnjo za človekovo zdravje. Ker so njihove interakcije z imunskim sistemom razmeroma slabo raziskane, nas je zanimalo, kakšne vplive imajo spojine BPA, BPF in BPAF na fenotipske in funkcijske lastnosti nezrelih (nDC) in zrelih dendritičnih celic (iDC), pripravljenih iz človeških monocitov *in vitro*. Monocitom, izoliranim iz periferne krvi smo tako že na začetku postopka njihove diferenciacije v nDC dodali 17 $\beta$ -estradiol (E2) kot kontrolno spojino, prav tako pa tudi vsakega od preiskovanih BP posamezno.

Ugotovili smo, da odmerki, 10 in 50  $\mu$ M BPA in BPF, 10 in 30  $\mu$ M BPAF ter 10 in 50 nM E2, niso imeli vpliva na živost celic. Med preverjanjem sposobnosti endocitoze nDC smo odkrili, da so BPA in BPF, v odmerku 50  $\mu$ M ter BPAF, v koncentracijah 10 in 30  $\mu$ M, statistično značilno zmanjšali to njihovo pomembno funkcijsko lastnost. Tako BPA (50  $\mu$ M) kot BPAF (30  $\mu$ M) sta na nDC zmanjšala izražanje molekul CD1a, povečala pa količino molekul DC-SIGN. Prisotnost E2 je povzročila naslednje fenotipske spremembe zDC: zmerno zmanjšanje izražanja kostimulatornih molekul CD80, CD86 in CD83 ter sočasno povečanje gostote molekul HLA-DR. Med vsemi preskušanimi spojinami je le BPAF statistično značilno vplival na fenotipske lastnosti DC, saj je izrazito zmanjšal obseg izražanja molekul CD80 in CD86 ter povečal število molekul CD83 in HLA-DR na površini zDC. Posledično so le tiste nDC in zDC, ki smo jih pripravili v prisotnosti BPAF in jih uporabili kot dražilne celice, izrazito zmanjšale obseg proliferacije alogenskih limfocitov T *in vitro*.

Koncentracije BP, ki smo jih uporabljali v eksperimentih pa so sicer več kot 1.000-krat višje od tistih, katerim smo, v obliki hrane, pijače in prašnih delcev, izpostavljeni v našem vsakdanjem življenju.

# THE INFLUENCES OF BISPHENOLS BPA, BPF, AND BPAF ON IN VITRO DIFFERENTIATION, MATURATION AND FUNCTIONAL PROPERTIES OF HUMAN MONOCYTE-DERIVED DENDRITIC CELLS

Urban Švajger<sup>2</sup>, Marija Sollner Dolenc<sup>1</sup>, Matjaž Jeras<sup>1</sup>

<sup>1</sup> University of Ljubljana – Faculty of Pharmacy, Aškerčeva 7, 1000 Ljubljana

<sup>2</sup> Blood Transfusion Centre of Slovenia, Šlajmerjeva 6, 1000 Ljubljana

Keywords: bisphenols, immature and mature human monocyte-derived dendritic cells, phenotypic changes

Bisphenols (BPs) are pollutants with estrogen-like endocrine disrupting properties that are widely spread in our environment. They represent a potential long term threat to human health. As their interactions with human immune system are still rather underexplored, we decided to find out what kind of influences BPA, BPF and BPAF have on the phenotypes and functions of both, immature (iDCs) and fully mature (mDCs) human monocyte-derived dendritic cells (DCs). Therefore, monocytes isolated from peripheral blood of healthy individuals were *in vitro* transformed to DCs in the presence of 17 $\beta$ -estradiol (E2), as a control substance, and then also with each individual BP to be tested.

We found that 10 and 50  $\mu$ M of BPA and BPF, as well as 10 and 30  $\mu$ M of BPAF and 10 and 50 nM of E2 did not affect cell viability. However, BPA (50  $\mu$ M), BPF (50  $\mu$ M) and BPAF (10 and 30  $\mu$ M) were able to significantly decrease the endocytotic capacity of iDCs. Additionally, the presence of BPA (50  $\mu$ M) or BPAF (30  $\mu$ M) decreased the expression of CD1a and concomitantly increased the density of DC-SIGN molecules on iDCs. E2 was able to cause the following phenotypic changes of mDCs: moderately decreased expression of CD80, CD86 and CD83 co-stimulatory molecules with simultaneously increased cell surface density of HLA-DR. Of all substances tested, only BPAF showed statistically significant impact on the phenotype of mDCs, as it markedly decreased the expression of CD80 and CD86 and increased that of CD83 and HLA-DR molecules, on the surface of these cells. Consequently, only those nDCs and mDCs that were generated from monocytes in the presence of BPAF and were then used as *in vitro* stimulators of allogeneic T cells, were capable to significantly reduce the extent of their proliferation.

It has to be stressed, however, that the concentrations of all three BPs that were tested in our study were more than 1.000-times higher than those found in food, beverages and dust particles to which we are exposed in our everyday's life.



# VPLIV NIZKIH KONCENTRACIJ KUMAFOSA NA RAZVOJ IN DELOVANJE MOŽGAN PRI MIŠIH

Nataša Hojnik<sup>2\*</sup>, Katerina Čeh<sup>1\*</sup>, Gregor Majdič<sup>1</sup>

Naslov organizacije, e-naslov:

\* Avtorci sta enakovredno prispevali k delu

<sup>1</sup>Center za genomiko živali, Veterinarska fakulteta, Univerza v Ljubljani, Gerbičeva 60, 1000 Ljubljana

[gregor.majdic@vf.uni-lj.si](mailto:gregor.majdic@vf.uni-lj.si)

<sup>2</sup>Odsek za tehnologijo površin in optoelektroniko – F4, Inštitut Jožef Stefan, Jamova 39, 1000 Ljubljana

Ključne besede:

kumafos, možgani, miš

Uvod in namen:

Kumafos je organofosfatni insekticid, ki se uporablja za zatiranje varoe (povzročajo jo *Varroa Jacobsoni*) v čebelarstvu. Organofosfati (OP) v visokih dozah delujejo strupeno tudi na ljudi z zaviranjem encima acetilholin-esteraze (AChE). Morebitne posledice dolgotrajne izpostavljenosti nizkim odmerkom kumafosa bi se lahko odražale v nepravilnem delovanju možganov in pojavu različnih duševnih bolezni.

Materiali in metode:

V raziskavi smo preučevali vpliv dolgotrajne izpostavljenosti nizkim odmerkom kumafosa (1 mg/kg in 0,1 mg/kg) na razvoj možganov miši balb/c. Prvo skupino miši smo kumafosu izpostavili v obdobju pred rojstvom do odstavitve, druga skupina je bila kumafosu izpostavljena v odraslem obdobju. Spremembe v delovanju možganov smo spremljali pri odraslih miših s testi obnašanja, s katerimi ugotavljamo določena stanja, značilna za duševne motnje pri ljudeh. Obnašanje, podobno anksioznosti smo preverjali s testom dvignjenega labirinta (EPM), testom z zakopavanjem frnikol in testom odprtega polja (OFT). Socialno obnašanje in spomin smo spremljali s testom socialnega prepoznavanja. S testom prisiljenega plavanja (FST) smo ugotavljali depresiji podobno obnašanje.

Rezultati:

Ob preučevanju vpliva nizkih odmerkov na razvoj pri nobenem izmed izvedenih testov ni prišlo do statistično značilnih razlik med kumafosu izpostavljenima skupinama in kontrolno skupino. Pri EPM, testu socialnega prepoznavanja in OFT smo ugotovili statistično značilne razlike med spoloma, kar je v skladu s podatki iz literature, da so samice prirojeno bolj nagnjene k anksioznemu obnašanju.

Zaključki:

Naši rezultati kažejo, da kumafos v nizkih odmerkih in ob dolgotrajni izpostavljenosti ne vpliva na razvoj možganov miši.

# LOW CONCENTRATIONS OF COUMAPHOS DO NOT AFFECT BRAIN DEVELOPMENT AND FUNCTION

Nataša Hojnik<sup>2\*</sup>, Katerina Čeh<sup>1\*</sup>, Gregor Majdič<sup>1</sup>

\* These authors contributed equally to this work

Organization address, email:

<sup>1</sup>Center za genomiko živali, Veterinarska fakulteta, Univerza v Ljubljani, Gerbičeva 60, 1000 Ljubljana

[gregor.majdic@vf.uni-lj.si](mailto:gregor.majdic@vf.uni-lj.si)

<sup>2</sup>Department of Surface Engineering and Optoelectronics – F4, Jozef Stefan Institute, Jamova 39, 1000 Ljubljana

Key words: Coumaphos, Brain, Mice

Introduction and purpose:

Coumaphos is an organophosphate insecticide, used for the treatment of varosis (caused by *Varroa Jacobsoni*) in beekeeping. Organophosphates (OP) with their irreversible inhibition of enzyme acetylcholinesterase as an important component of cholinergic part of the nervous system are also very harmful for humans.

Besides acute toxicity of organophosphates, potential harmful effects of prolonged exposure to low concentrations of some OP during development and in adulthood could result in an abnormal activity of brain function and consecutively contribute to the development of various mental disorders.

Materials and methods:

In our study, we have examined the effects of low doses of coumaphos (1 mg/kg and 0,1 mg/kg) on brain development and function in balb/c mouse strain. The first group of mice was exposed to coumaphos through mothers before birth until weaning and the second group was exposed in adulthood. Adult mice were examined by standard behavioural tests as models for different neuropsychiatric disorders in humans. Anxiety related behaviours were tested with elevated plus maze test (EPM), marble burying test and open field test (OFT). Social behaviour and social memory disorders, which are one of the signs for autism by humans, were tested with social recognition test. Depression related behaviours were tested with forced swim test (FST).

Results:

Results did not reveal any statistically significant differences between two groups exposed to coumaphos and control group. In EPM, OFT and social recognition test there were statistically significant differences between sexes, as expected.

Conclusions:

Results of our study therefore suggest that prolonged neonatal exposure or adult exposure to low doses of coumaphos does not have harmful effects on brain development and/or function in mice.

# NARAVA MEDMOLEKULARNIH SIL IN PREPOZNAVANJE V BIOLOŠKIH MAKROMOLEKULAH

**Janez Mavri**

Kemijski inštitut , Hajdrihova 19, SI-1000 Ljubljana, e-mail: [janez.mavri@ki.si](mailto:janez.mavri@ki.si)

Vezava liganda na biološko makromolekulo je pogoj za njen biološki odgovor. V primeru encima je tvorba Michaelisovega kompleksa uvod v encimsko reakcijo. Močna vezava nereaktivnega liganda v encimsko aktivno mesto pomeni inhibicijo encima in s tem se spremeni funkcija encima. Receptorji so proteinske makromolekule, ki so se tekom evolucije razvile izjemno občutljivost za vezavo ligandov, ki imajo glede na naravo liganda različni odgovori. Predstavil bom naravo medmolekularnih sil v hidratiranih fluktuirajočih bioloških makromolekulah [1]. Pokazal bom vodilno vlogo neveznih interakcij in še zlasti daljnosežnih elektrostatskih interakcij [2]. Biološke makromolekule so prekompleksirane za analitično obravnavo in zato so molekularne simulacije prava izbira za teoretsko obravnavo takih sistemov. Predstavil bom nekaj farmakološko relevantnih sistemov, ki jih študiramo v našem laboratoriju [3,4,5]. Podal bom kritično mnenje o pomenu nuklearnih kvantnih efektov v bioloških sistemih [5,6].

[1] H. J.C. Berendsen, *Simulating the Physical World*, Cambridge University Press, 2007

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# NATURE OF INTERMOLECULAR FORCES AND RECOGNITION IN BIOLOGICAL MACROMOLECULES

**Janez Mavri**

National Institute of Chemistry, Slovenia, Hajdrihova 19, SI-1000 Ljubljana, e-mail: [janez.mavri@ki.si](mailto:janez.mavri@ki.si)

Ligand binding to a biological macromolecule is a necessary condition for its biological response. In the case of an enzyme reaction formation of the Michaelis complex leads to the enzyme reaction. Strong nonbonding binding of the inhibitor to the enzyme binding site changes its function. Receptors are protein macromolecules that during the evolution developed sensitivity to ligand binding in the context of biological response. I will talk about the nature of intermolecular forces in the hydrated fluctuating biological macromolecules[1]. I will demonstrate the dominating role of nonbonding interactions, where long-range electrostatics is of prime importance [2]. Since the systems are too complex for analytical treatment molecular simulation is the method of choice for their treatment. I will mention few pharmacologically relevant systems that we currently treat in our laboratory [3,4,5]. I will critically discuss the role of nuclear quantum effects [5,6].

[1] H. J.C. Berendsen, *Simulating the Physical World*, Cambridge University Press, 2007

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# RAČUNALNIŠKE METODE ZA OCENO ENDOKRINIH MOTILCEV IN RAZVOJNE TOKSIČNOSTI

**Marjan Vračko**, Kemijski inštitut Ljubljana, Slovenija

[marjan.vracko@ki.si](mailto:marjan.vracko@ki.si)

**Ključne besede:** QSAR, read across, internetni programi, VEGA modeli, T.E.S.T. modeli, OECD dokumenti

## **Povzetek**

Predstavili bomo koncepta razvojne toksičnosti in reprodukcijske toksičnosti, kot ju predstavlja OECD (Organizacija za Ekonomski Razvoj in Sodelovanje). V tej skupini so omenjeni tudi endokrini motilci, saj predstavlja motnje endokrinega sistema prvo stopnjo v razvojni ali reprodukcijski toksičnosti. Predstavili bomo nekaj računalniških modelov in metod za ocenjevanje toksičnosti. Modeliranje potencialno toksične snovi v okolju hormonskega receptorja ('docking') je predstavljeno v drugih prispevkih, tukaj se bomo omejili na QSAR (Quantitative Structure-Activity Relationship) modeliranje in na grupiranje in kategorizacijo kemikalij ('read across'). Podajamo pregled nekaterih QSAR modelov in računalniških pristopov, ki so dostopni na internetu. Dva modela, CAESAR, ki je dostopen na platformi VEGA in T.E.S.T. CAESAR je bil razvit v okviru evropskih znanstvenih projektov, T.E.S.T. pa je razvila US EPA. Predstavili bomo rezultate in komentarje za nekaj izbranih spojin.

## COMPUTER METHODS FOR THE ASSESSMENT OF ENDOCRINE DISRUPTERS AND DEVELOPMENTAL TOXICITY

**Marjan Vračko**, National Institute of Chemistry, Slovenia

[marjan.vracko@ki.si](mailto:marjan.vracko@ki.si)

**Keywords:** QSAR, read across, internet accessible models, VEGA, T.E.S.T., OECD documents

## **Abstract**

We will present concepts of developmental toxicity and reproductive toxicity in the light of OECD (Organization of economic Cooperation and Development). The disruption of endocrine systems of human (or other organisms) belongs to this framework. In many cases the endocrine disruption represents the initial molecular event, which leads to developmental or reproductive disruptions. We will present some computational methods and models for evaluating of toxicity. The modeling of interaction between a compound and hormon receptors (docking) is introduced in other contributions, therefore we will focus our attention on QSAR (Quantitative Structure-Activity Relationship) and read across (grouping and categorisation of chemicals) approaches. We will present two models: CAESAR (VEGA platform) and T.E.S.T., which are freely available over internet. CAESAR was developed in the framework of EU research projects, TEST was developed in US EPA. For some compounds will be presented and discussed the models outputs.

# IN SILICO IDENTIFIKACIJA HORMONSKIH MOTILCEV - ENDOCRINE DISRUPTOME

Katra Kolšek<sup>1</sup>, Janez Mavri<sup>2</sup>, Stanislav Gobec<sup>3</sup>, Marija Sollner Dolenc<sup>3</sup> in Samo Turk<sup>4</sup>

<sup>1</sup>Molecular Biomechanics Group, Heidelberg Institute for Theoretical Studies, Schloss-Wolfsbrunnenweg 35, 69118 Heidelberg, Germany (katra.kolsek@h-its.org), <sup>2</sup>Laboratory for Biocomputing and Bioinformatics, National Institute of Chemistry, Hajdrihova 19, 1000 Ljubljana, Slovenia (janez.mavri@ki.si), <sup>3</sup>Faculty of Pharmacy, University of Ljubljana, Aškerčeva 7, 1000 Ljubljana, Slovenia (stanislav.gobec@ffa.uni-lj.si and marija.sollner@ffa.uni-lj.si), <sup>4</sup>BioMed X, Innovation Center, Im Neuenheimer Feld 583, Heidelberg ([turk@bio.mx](mailto:turk@bio.mx))

Ključne besede: hormonski motilci, *in silico* metode, Endocrine Disruptome

Dandanes ljudi poznamo že več kot 90 milijonov različnih kemikalij. Nekatere od njih t.i. hormonski motilci (endocrine disrupting chemicals, EDCs) motijo hormonski sistem in posledično ogrožajo zdravje, tako da povzročajo ali posredno vplivajo na razvoj različnih bolezenskih stanj [1]. Zaradi tega je njihova identifikacija izredno pomembna [2]. Vendar je zaradi ogromnega števila znanih kemikalij identifikacija EDCs nemogoča samo s standardnimi *in vitro* in *in vivo* metodami. Tako pridejo v poštev računalniške presejalne metode. Tradicionalno se v toksikologiji uporabljajo QSAR metode za napovedovanje toksičnosti. Čeprav so QSAR metode lahko zelo natančne, imajo svoje pomankljivosti, predvsem zaradi njihove omejenosti na strukturno sorodne spojine [3]. Naš cilj je bil razviti metodo, ki je enostavna za uporabo in uporabna za vse kemikalije. Tako smo razvili prostodostopno internetno računalniško aplikacijo imenovano Endocrine Disruptome (<http://endocrinedisruptome.ki.si/>), ki uporabniku omogoča napoved vezave spojine na 12 jedrnih receptorjev [4].

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[4] K. Kolšek et al., *J Chem Inf Model*, 2014, 54 (4), 1254–1267.

# **IN SILICO IDENTIFICATION OF ENDOCRINE DISRUPTING CHEMICALS- ENDOCRINE DISRUPTOME**

**Katra Kolšek<sup>1</sup>, Janez Mavri<sup>2</sup>, Stanislav Gobec<sup>3</sup>, Marija Sollner Dolenc<sup>3</sup> in Samo Turk<sup>4</sup>**

<sup>1</sup>Molecular Biomechanics Group, Heidelberg Institute for Theoretical Studies, Schloss-Wolfsbrunnenweg 35, 69118 Heidelberg, Germany (katra.kolsek@h-its.org), <sup>2</sup>Laboratory for Biocomputing and Bioinformatics, National Institute of Chemistry, Hajdrihova 19, 1000 Ljubljana, Slovenia (janez.mavri@ki.si), <sup>3</sup>Faculty of Pharmacy, University of Ljubljana, Aškerčeva 7, 1000 Ljubljana, Slovenia (stanislav.gobec@ffa.uni-lj.si and marija.sollner@ffa.uni-lj.si), <sup>4</sup>BioMed X, Innovation Center, Im Neuenheimer Feld 583, Heidelberg ([turk@bio.mx](mailto:turk@bio.mx))

Keywords: endocrine disrupting chemicals, *in silico* method, Endocrine Disruptome

Humans have discovered or made more than 90 million different chemicals. Some of them, so called endocrine disrupting chemicals (EDCs), interfere with endocrine system and can be serious health threat by causing and/or contributing to major diseases [1]. Therefore their identification is of greatest importance [2], but this enormous pool of substances makes assessment and identification of EDCs impossible with only standard *in vitro* and *in vivo* methods. Thus computational screening approaches should be used. Traditionally in toxicology QSAR methods are used for predicting toxic endpoints. Even though QSAR can be very accurate it has its weaknesses, especially because of its limitation to structurally related compounds [3]. Our aim was to develop a method that works on any type of chemical and is easy to use also for non-computational scientists. We made a open source internet application called Endocrine Disruptome (<http://endocrinedisruptome.ki.si/>), which allows prediction of binding of user specified substance to 12 nuclear receptors [4].

[1] E. Diamanti-Kandarakis et al., *Endocr Rev*, 2009, 30(4), 293-342 .

[2] Endocrine Disruptor Screening Program for the 21st Century.

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[4] K. Kolšek et al., *J Chem Inf Model*, 2014, 54 (4), 1254–1267.

# ENDOKRINI POTENCIAL SNOVI V KOZMETIČNIH IZDELKIH

Alja Plošnik, Kemijski inštitut Ljubljana, Slovenija

[alja.plosnik@ki.si](mailto:alja.plosnik@ki.si)

**Ključne besede:** hormonski motilci, sestavine kozmetičnih izdelkov, *in silico*

## Povzetek

Kozmetična regulativa, sprejeta leta 2009, je prisilila industrijo in raziskovalne ustanove k iskanju novih alternativnih metod za odkrivanje potencialno toksičnih spojin, saj so standardni *in vivo* testi dragi in zamudni ter etično sporni. Proučevali smo sestavine kozmetičnih izdelkov kot potencialne hormonske motilce z *in silico* metodo molekulskega sidranja. Naključno smo izbrali 558 spojin iz CosIng Inventory podatkovne baze in testirali potencialno zmožnost povzročanja motenj na endokrinem hormonskem sistemu. Upoštevali smo le mehanizem povezan z direktno vezavo na jedrne receptorje z dobro definiranimi kristalnimi strukturami, ki predstavlja prvi iniciacijski dogodek na molekulski ravni, ki lahko privedejo do hormonskih motenj. Napovedi so bile narejene s pomočjo novo razvitega programa Endocrine Disruptome <http://endocrinedisruptome.ki.si/>. Identificirali smo 21 spojin kot potencialne hormonske motilce. Še posebej so bile ugodne interakcije med progesteronskimi, mineralokortikoidnimi, glukokortikoidnimi receptorji in aromatskimi spojinami. Raziskavo smo zaključili s primerjavo rezultatov računalniških napovedi z znanimi eksperimentalnimi rezultati. Na koncu našega raziskovanja smo primerjali rezultate molekulskega sidranja z znanimi eksperimentalnimi podatki iz literature, s čimer lahko potrdimo, da ima uporabljena napovedna metoda dokaj dobro napovedno moč za identificiranje potencialnih hormonskih motilcev.

## ENDOCRINE POTENTIAL OF INGREDIENTS IN COSMETIC PRODUCTS

**Keywords:** Endocrine disruption, cosmetic ingredients, nuclear receptors, *in silico*, molecular docking

## Abstract

Cosmetic regulation, adopted in 2009 has forced industry and research institutions to search for alternative methods to detecting potentially toxic compounds as standard *in vivo* screenings are, besides being ethically questionable, also expensive and time-consuming. We studied the ingredients of cosmetic products as potential endocrine disruptors (ED) by *in silico* methods (molecular docking). We randomly selected 558 compounds from CosIng Inventory database (Cosmetic Ingredients) and examined their endocrine disrupting potential. We considered only the mechanism linked with direct binding to nuclear receptors with well-defined crystal structures, what represents the first event in the chain, which may lead to endocrine disruption. Predictions were performed using the Endocrine Disruptome docking program <http://endocrinedisruptome.ki.si/>. We identified 21 compounds as potential endocrine disruptors. Particularly favourable interactions were between progesterone, mineralocorticoid, glucocorticoid receptors and aromatic compounds. In the end of our research we compared molecular docking results with known experimental data from literature and we can confirm that the applied approach has sufficient predictive power to study endocrine disruption potential.



# VPLIVI NA GEOMETRIJO, BIOMEHANIKO IN VSEBNOST MINERALOV V STEGNENICAH PODGANJIH MLADIČEV IZPOSTAVLJENIH V OBDOBJU LAKTACIJE NEPLANARNEMU PCB-155 ALI/IN PLANARNEMU PCB-169

**Jana Brankovič<sup>1</sup>, Sašo Jovanovski<sup>2,3</sup>, Alexander Hofmeister<sup>4</sup>, Gregor Fazarinc<sup>1</sup>, Azra Pogačnik<sup>1</sup>,  
Janja Jan<sup>5</sup> in Milka Vrecl<sup>1</sup>**

<sup>1</sup>Inštitut za anatomijo, histologijo in embriologijo, Veterinarska fakulteta Univerze v Ljubljani, Gerbičeva 60, Ljubljana, Slovenija, <sup>2</sup> *Post-doc*. Katedra za stomatološko protetiko, Medicinska fakulteta Univerze v Ljubljani, Hrvatski trg 6, Ljubljana, Slovenija, <sup>3</sup>Katedra za stomatološko protetiko, Stomatološka fakulteta, Univerza Sv. Ciril in Metod, Vodnjanska 17, Skopje, Makedonija, <sup>4</sup>Biomedical Research, Medicinska univerza Graz, Roseggerweg 48, Graz, Avstrija, <sup>5</sup>Katedra za zobne bolezni in normalno morfologijo zobnega organa, Medicinska fakulteta Univerze v Ljubljani, Hrvatski trg 6, Ljubljana, Slovenija

[jana.brankovic@vf.uni-lj.si](mailto:jana.brankovic@vf.uni-lj.si)

Poliklorirani bifenili (PCB) so izjemno obstojni onesnaževalci okolja, sposobni bioakumulacije, in imajo raznolike neželene učinke na zdravje, vključno z vplivom na endokrini sistem. Hormoni so nujno potrebni za rast in razvoj, zato je tvegana predvsem izpostavljenost PCB med prenatalnim in/ali zgodnjim postnatalnim obdobjem. Cilj raziskave je bil proučiti učinke neplanarnega in planarnega heksaklorobifenila (PCB-155 in PCB-169), individualno in v kombinaciji, na določene serumske biokemijske parametre, geometrijo, mineralno gostoto in biomehanične lastnosti stegenice mladičev podgan, ki so bile v obdobju laktacije izpostavljene PCB.

Petnajstim podganam seva Wistar smo intraperitonealno aplicirali PCB raztopljene v olivnem olju v različnih časovnih razmakih po kotitvi. Celokupno je prva skupina prejela 3 mg PCB-169/kg telesne teže, druga skupina 12 mg PCB-155/kg telesne teže ter tretja skupina 3 mg PCB-169 in 12 mg PCB-155/kg telesne teže. Kontrolna skupina je prejela le olivno olje. Mladiče smo žrtvovali 22. dan po skotitvi (n=7–8 na skupino), odvzeli kri za biokemično analizo serumskega kalcija, anorganskega fosfata in aktivnosti alkalne fosfataze ter odstranili stegenice. Geometrijo kosti smo izmerili z računalniškim tomografskim (CT) slikanjem z mikro-CT aparatom, biomehanične lastnosti določili s tri-točkovnim upogibnim preizkusom ter vrednosti kalcija in fosforja v anorganski snovi kosti pridobili s tehniko induktivno sklopljene plazme z masno spektrometrijo.

Pri mladičih izpostavljenih PCB-169 smo v primerjavi s PCB-155 in/ali kontrolno skupino ugotovili krajše in tanjše stegenice, zmanjšan endostealni in periostealni obseg kortikalne kostnine srednjega dela diafize, znižane serumske biokemične markerje v krvi ter vsebnost mineralov v kosti in zmanjšan vztrajnostni moment stegenice v prečnem prerezu. Pri kombinaciji obeh PCB so spremembe podobne, vendar manj izrazite, kot pri skupini PCB-169.

Na podlagi pridobljenih rezultatov lahko zaključimo, da laktacijska izpostavitve planarnemu PCB-169 povzroči motnje v razvoju skeleta, kar se kaže predvsem v spremenjeni geometriji stegenic in vrednostih proučevanih biokemičnih parametrov.

Ključne besede: poliklorirani bifenili; stegenica; biokemijski parametri; geometrija, biomehanika, sestava

# CHANGES IN FEMUR GEOMETRY, BIOMECHANICS AND MINERAL COMPOSITION IN JUVENILE RATS LACTATIONALLY EXPOSED TO NON-PLANAR PCB-155 OR/AND PLANAR PCB-169

**Jana Brankovič<sup>1</sup>, Sašo Jovanovski<sup>2,3</sup>, Alexander Hofmeister<sup>4</sup>, Gregor Fazarinc<sup>1</sup>, Azra Pogačnik<sup>1</sup>, Janja Jan<sup>5</sup>, and Milka Vrecl<sup>1</sup>**

<sup>1</sup>Institute of Anatomy, Histology and Embryology, Veterinary Faculty, University of Ljubljana, Gerbičeva 60, 1000 Ljubljana, Slovenia, <sup>2</sup>Post-doc. Department of Prosthodontics, Faculty of Medicine, University of Ljubljana, Hrvatski trg 6, 1000 Ljubljana, Slovenia, <sup>3</sup>Department of Prosthodontics, Faculty of Dental Medicine, University of St. Cyril and Methodius, Vodnjanska 17, 1000 Skopje, Macedonia, <sup>4</sup>Biomedical Research, Medical University of Graz, Roseggerweg 48, 8036 Graz, Austria, <sup>5</sup>Department of Dental Diseases and Normal Dental Morphology, Faculty of Medicine, University of Ljubljana, Hrvatski trg 6, 1000 Ljubljana, Slovenia

[jana.brankovic@vf.uni-lj.si](mailto:jana.brankovic@vf.uni-lj.si)

Polychlorinated biphenyls (PCBs) are highly persistent and bio-accumulative environmental pollutants with a variety of potential health risks, including effects on the endocrine system. Exposure during prenatal and/or early postnatal life can pose the greatest risk as hormones are essential for growth and development. The aim of our study was to examine effects of a non-planar and a planar hexachlorobiphenyl (PCB-155 and PCB-169, respectively), individually and in combination, on selected serum biochemical parameters, femur geometry, mineral density and biomechanical properties in rat offspring lactationally exposed to PCBs.

15 Wistar rats were intraperitoneally administered PCB congeners dissolved in olive oil at different time points after delivery. In total, group 1 received 3 mg of PCB-169 per kg body weight, group 2 received 12 mg of PCB-155 and group 3 received 3 mg of PCB-169 and 12 mg of PCB-155. The control (group 4) was administered olive oil only. Offspring were sacrificed on postnatal day 22 (n=7-8 per group), blood samples collected for biochemical analysis of serum calcium, inorganic phosphate and alkaline phosphatase activity and femora dissected. Computed tomography using microCT scanner and three-point bending test were used to obtain data on femur geometry and biomechanical properties. Calcium and phosphorus levels in inorganic substance of bone were determined by inductively coupled plasma mass spectrometer.

Exposure to PCB-169 resulted in shorter and thinner femur, reduced endosteal and periosteal circumference of the cortical bone, lowered serum bone markers and bone mineral content and lowered cross-sectional moment of inertia when compared to PCB-155/control group. In the combined group, changes were similar to those observed in PCB-169 group, but less pronounced.

In conclusion, our data show that effects in skeletal development in lactationally exposed offspring were mainly induced by the planar PCB-169 seen as alterations in femur geometry and studied biochemical parameters.

Keywords: polychlorinated biphenyls; femur; biochemical parameters; geometry; biomechanics; composition

# UGOTAVLJANJE CITOTOKSIČNE IN GENOTOKSIČNE AKTIVNOSTI KEMIJSKIH POVZROČITELJEV HORMONSKIH MOTENJ

**Damjan Balabanič<sup>1</sup>, Bojana Žegura<sup>2</sup>, Aleksandra Krivograd Klemenčič<sup>3,4</sup>, Metka Filipič<sup>2</sup>**

<sup>1</sup>Fakulteta za industrijski inženiring, Šegova ulica 112, 8000 Novo mesto, [damjan.balabanic@gmail.com](mailto:damjan.balabanic@gmail.com); <sup>2</sup>Nacionalni inštitut za biologijo, Večna pot 111, 1000 Ljubljana, [bojana.zegura@nib.si](mailto:bojana.zegura@nib.si), [metka.filipic@nib.si](mailto:metka.filipic@nib.si); <sup>3</sup>Inštitut za vode Republike Slovenije, Hajdrihova 28c, 1000 Ljubljana, [aleksandra.krivograd@izvrs.si](mailto:aleksandra.krivograd@izvrs.si); <sup>4</sup>Fakulteta za gradbeništvo in geodezijo, Univerza v Ljubljani, Hajdrihova 28, 1000 Ljubljana

Uvod in namen: V zadnji letih se v okolju večja število kemijskih snovi, ki lahko posnemajo delovanje hormonov (kemijski povzročitelji hormonskih motenj, KPHM) in posledično motijo delovanje endokrinega sistema pri živalih in ljudeh. Dokazano je, da imajo KPHM lahko tudi citotoksične in/ali genotoksične lastnosti (1). Namen raziskave je bil ugotoviti, ali lahko izbrani KPHM (dimetil ftalat, dietil ftalat, dibutil ftalat, benzilbutil ftalat, bis(2-etilheksil) ftalat, bisfenol A in nonilfenol) delujejo citotoksično in/ali genotoksično.

Materiali in metode: Citotoksičen in genotoksičen potencial izbranih KPHM smo testirali s testom SOS/*umuC* na gensko spremenjeni bakteriji *Salmonella typhimurium* (sev TA 1535/pSK 1002). Nadalje smo citotoksičnost izbranih KPHM določali s testom MTT, njihovo genotoksično aktivnost pa s testom komet. Kot model smo uporabili jetrno celično linijo človeškega hepatoma (celice HepG2). Vse teste smo izvajali pri koncentracijah KPHM od 1,0 µg/L do 1,0 mg/L.

Rezultati: Rezultati testa SOS/*umuC* so pokazali, da izbrani KPHM v testiranem koncentracijskem območju niso vplivali na rast bakterij in niso delovali genotoksično. S testom MTT smo ugotovili, da izbrani KPHM (1,0 µg/L do 1,0 mg/L) niso vplivali na živost celic HepG2. Nadalje smo s testom komet določali, ali KPHM povzročajo verižne prelome DNK. Rezultati so pokazali, da so vsi KPHM vključeni v študijo, povzročili poškodbe DNK. Dimetil ftalat, dietil ftalat, benzilbutil ftalat in bis(2-etilheksil) ftalat so povzročili verižne prelome DNK jetrnih celic pri koncentraciji 100 µg/L in 1,0 mg/L, medtem ko so dibutil ftalat, bisfenol A in nonilfenol povzročili verižne prelome DNK tudi pri koncentraciji 10 µg/L.

Zaključki: Rezultati naše raziskave so pokazali, da so izbrani KPHM pri ne-citotoksičnih in okoljsko relevantnih koncentracijah (2) povzročili poškodbe DNK celic HepG2, kar pomeni, da so nujne nadaljnje raziskave o mehanizmih njihovega delovanja, še posebej, ker zadnji izsledki nakazujejo, da naj bi imeli KPHM poleg delovanja kot hormonski motilci, tudi pomembno vlogo pri nastanku rakavih obolenj (3).

Literatura: 1) Martinez-Paz P in sod, *Mutat Res*, 2013. 2) Balabanič D in sod, *Fresen Environ Bull*, 2011. 3) Quinn-Hosey K in sod, *J Environ Prot*, 2012.

Ključne besede: kemijski povzročitelji hormonskih motenj, biološki testi, citotoksičnost, genotoksičnost

# DETERMINATION CYTOTOXIC AND GENOTOXIC ACTIVITY OF ENDOCRINE DISRUPTING COMPOUNDS

**Damjan Balabanič<sup>1</sup>, Bojana Žegura<sup>2</sup>, Aleksandra Krivograd Klemenčič<sup>3,4</sup>, Metka Filipič<sup>2</sup>**

<sup>1</sup>Faculty of Industrial Engineering, Šegova ulica 112, 8000 Novo mesto, Slovenia [damjan.balabanic@gmail.com](mailto:damjan.balabanic@gmail.com); <sup>2</sup>National Institute of Biology, Večna pot 111, 1000 Ljubljana, Slovenia, [bojana.zegura@nib.si](mailto:bojana.zegura@nib.si), [metka.filipic@nib.si](mailto:metka.filipic@nib.si); <sup>3</sup>Institute for Water of the Republic of Slovenia, Hajdrihova 28c, 1000 Ljubljana, Slovenia [aleksandra.krivograd@izvrs.si](mailto:aleksandra.krivograd@izvrs.si); <sup>4</sup>Faculty of Civil and Geodetic Engineering, University of Ljubljana, Hajdrihova 28, 1000 Ljubljana, Slovenia

**Introduction and purpose:** In the last few years increased number of chemical substances that can mimic the action of hormones (endocrine disrupting compounds, EDCs) has been detected in the environment. These chemicals can consequently interfere with the functioning of the endocrine system in animals and humans. Recent studies indicate that EDCs show in addition to interfering with the hormonal system also genotoxic properties (1). The aim of the study was to determine potential cytotoxic and genotoxic activities of selected EDCs (dimethyl phthalate, diethyl phthalate, dibutyl phthalate, benzyl butyl phthalate, bis (2-ethylhexyl) phthalate, bisphenol A and nonylphenol).

**Materials and methods:** Cytotoxic and genotoxic potential of selected EDCs was tested with SOS/*umuC* assay on genetically modified bacteria *Salmonella typhimurium* (strain TA 1535/pSK 1002). Furthermore, cytotoxicity of selected EDCs was determined with MTT assay, and their genotoxic activity with the comet assay. As a model, a liver human hepatoma cell line (HepG2 cells) was used. All assays were carried out at EDCs concentrations from 1.0 µg/L to 1.0 mg/L.

**Results:** The results of SOS/*umuC* assay showed that the selected EDCs at tested concentration range did not affect the growth of bacteria and were not genotoxic. The selected EDCs (1.0 µg/L to 1.0 mg/L) did not affect the viability of HepG2 cells determined by the MTT assay. Furthermore, the induction of DNA strand breaks by selected EDCs was evaluated with comet assay. The results showed that all EDCs caused increased DNA damage. Dimethyl phthalate, diethyl phthalate, benzylbutyl phthalate and bis (2-ethylhexyl) phthalate increased the amount of DNA strand breaks at concentrations of 100 µg/L and 1.0 mg/L, while dibutyl phthalate, bisphenol A and nonylphenol caused DNA damage at concentration ≥ 10 µg/L.

**Conclusions:** The results of our study showed that selected EDCs at non-cytotoxic environmentally relevant concentrations (2) caused DNA damage in HepG2 cells. Therefore there is a need for further studies on the mechanisms of genotoxic potential of EDCs, as recently there are several indications showing that these chemicals might play an important role in human cancer development (3).

**References:** 1) Martinez-Paz P et al, *MutatRes*, 2013. 2) Balabanič D et al, *Fresen Environ Bull*, 2011. 3) Quinn-Hosey K et al. *J Environ Prot*, 2012.

**Keywords:** endocrine disrupting compounds, biological assays, cytotoxicity, genotoxicity

# **SIMPOZIJ 4**

## **Testne smernice in metode, zakonodaja, strategija**

Foto: Anita Jemec



# OECD TESTNE SMERNICE ZA KEMIJSKE POVZROČITELJE HORMONSKIH MOTENJ

**Katarina Černe**, Inštitut za farmakologijo in eksperimentalno toksikologijo, Medicinska fakulteta, Univerza v Ljubljani, Korytkova 2, 1000 Ljubljana, [katarina.cerne@mf.uni-lj.si](mailto:katarina.cerne@mf.uni-lj.si)

V okviru programa OECD testnih smernic je bila leta 1997 ustanovljen kot posebna dejavnost Delovna skupina za testiranje in ocenjevanje hormonskih motilcev (EDTA) z namenom (1) raziskati regulatorne zahteve in potrebe v državah članicah glede hormonskih motilcev; (2) da bi v državah članicah poskušala razviti usklajeno prakso za ocenjevanje hormonskih motilcev in (3) razvoj smernice za testiranje hormonskih motilcev. Pod nadzorom EDTA sta bili ustanovljeni leta 1999 in 2001 še dve delovni skupini, in sicer Delovna skupina za vodenje validacije pri sesalcih (VMG-sesalci) in Delovna skupina za ekotoksičnost (VMG-eko). V OECD konceptualni okvir so vključene OECD testne smernice in standardne testne metode, ki so že na voljo, v razvoju ali pa so predlagane in katere lahko uporabljamo za oceno kemikalij glede hormonskih motenj. Namen konceptualnega okvira je, da preskrbi smernice o različnih preskusih, ki so na voljo in je stavljen iz petih ravni. Raven 1 - obstoječi podatki in ne-testne informacije. Stopnja 2 - *in vitro* preskusi za zagotavljanje podatkov o endokrinem(-ih) mehanizmu(-ih). Stopnja 3 - *in vivo* preskusi za zagotavljanje podatkov o endokrinem(-ih) mehanizmu(-ih). Nivo 4 - *in vivo* preskusi za zagotavljanje podatkov o neželenih učinkih na relevantne tarče endokrinega sistema. Stopnja 5 - *in vivo* preskusi za zagotavljanje celovitejših podatkov o neželenih učinkih na pomembne endokrine tarče s pomočjo ustreznih študij, ki trajajo daljši življenjski cikel organizma, npr Razširjena študiji eno-generacijske strupenosti za razmnoževanje (OECD test guideline 433). Na ravni 5, ekotoksikologija vključuje preskuse strupenosti za razvoj in razmnoževanje rib, ptic, dvoživk in nevretenčarjev, ki trajajo celotno življenjsko obdobje. Ocena vsake kemikalije glede njenih potencialnih učinkov na hormonsko ravnotežje mora temeljiti na osnovi posameznega primera, pri čemer upoštevamo vse razpoložljive informacije.

Ključne besede: hormonski motilci, OECD testne smernice, *in vivo*, *in vitro*, delovna skupina EDTA

## OECD TEST GUIDELINES FOR ENDOCRINE DISRUPTING CHEMICALS

**Katarina Černe**, Institute of pharmacology and experimental toxicology, Faculty of medicine, University Ljubljana, Korytkova 2, 1000 Ljubljana, [katarina.cerne@mf.uni-lj.si](mailto:katarina.cerne@mf.uni-lj.si)

The Endocrine Disrupter Testing and Assessment task force (EDTA) was established in 1997 as a special activity under OECD test guideline program: (1) to investigate regulatory requirements and needs in member countries for Endocrine Disrupting Chemicals (EDCs); (2) to try to develop harmonized assessment practice in member countries for EDCs; and (3) to develop test guidelines for EDCs. Under the EDTA's supervision, the validation management groups for mammalian (VMG-mammalian) and ecotoxicity (VMG-eco) tests were established in 1999 and 2001, respectively. OECD conceptual framework lists the OECD test guidelines and standardized test methods available, under development or proposed that can be used to evaluate chemicals for endocrine disruption. The conceptual framework is intended to provide a guide to the test available and consist of 5 levels. Level 1 – existing data and non-test information. Level 2 – *in vitro* assays providing data about endocrine mechanism(s) pathway(s). Level 3 – *in vivo* assays providing data about selected mechanism(s) pathway(s). Level 4 – *in vivo* assays providing data on adverse effects on endocrine relevant endpoints. Level 5 – *in vivo* assays providing more comprehensive data on adverse effects on endocrine relevant endpoints over more extensive parts of the life cycle of the organism, e.g. Extended on generation reproductive toxicity study (OECD TG 433). In level 5, ecotoxicology include developmental and reproduction full life cycle assays in fish, birds, amphibians and invertebrate. The assessment of endocrine disrupting potential of each chemical should be based on a case by case basis, taking into account all available information.

Key words: endocrine disrupters, OECD test guidelines, *in vivo*, *in vitro*, EDTA task force

# KEMIJSKI POVZROČITELJI HORMONSKIH MOTENJ IN ZAKONODAJA ZA FITOFARMACEVTSKA SREDSTVA

**Jernej Drofenik**, Uprava za varno hrano, veterinarstvo in varstvo rastlin, Sektor za fitofarmacevtska sredstva, [jernej.drofenik@gov.si](mailto:jernej.drofenik@gov.si)

Kemijski povzročitelji hormonskih motenj (KPHM) terjajo zaradi svojih lastnosti posebno pozornost pri regulaciji njihove dostopnosti in uporabe, tudi pri uporabi v kmetijstvu. Zato so v preteklih letih v zakonske predpise na področju pesticidov, kemikalij (REACH) in biocidov, vključili jasne določbe omejevanja ali celo prepovedi uporabe teh kemikalij. Zakonske določbe so za različna področja kemikalij podobna, s skupno točko, ki v prvi vrsti zahteva jasne kriterije glede opredelitve KPHM.

Na področju fitofarmacevtskih sredstev je bila v letu 2009 sprejeta Uredba (ES) št. 1107/2009 o dajanju fitofarmacevtskih sredstev v promet, ki je vpeljala pojem KPHM. Lastnost vplivanja na hormonsko ravnovesje sodi med osnovne izključitvene kriterije ali se določeni aktivni snovi dovoli uporaba v kmetijski proizvodnji v državah članicah. Tako zakonodaja opredeljuje, da se aktivna snov, varovalo ali sinergist odobri in lahko uporablja v EU le, če se na podlagi rezultatov smernic Skupnosti ali mednarodno priznanih smernic za preskušanje ali drugih razpoložljivih podatkov in informacij, vključno s pregledom strokovne literature, oceni, da nima lastnosti motilcev hormonskega ravnovesja. Uporaba KPHM je dovoljena samo v primeru, če se sredstvo uporablja na tak način, da je izpostavljenost uporabnikov in ljudi preko prehrane zanemarljiva.

Zakonodaja tudi navaja izdelavo smernic za jasno opredelitev snovi, ki so KPHM. EU Komisija je ustanovila skupino strokovnjakov za izdelavo smernic. Glede na veliko zanimanje za to področje in različne interese, postopek izdelave smernic še ni zaključen. Do sprejetja meril se za pesticidne aktivne snovi, ki so razvrščene v skladu z določbami iz Uredbe (ES) št. 1272/2008 kot rakotvorne skupine 2 in strupene za razmnoževanje skupine 2, šteje, da imajo lastnosti motilcev hormonskega ravnovesja. Poleg tega se lahko za snovi, ki so skladu z določbami iste Uredbe razvrščene kot strupene za razmnoževanje skupine 2 in ki imajo toksične učinke na organe z notranjim izločanjem, šteje da so motilci hormonskega ravnovesja.

Slovenija je pri izdelavi smernic aktivno sodelovala in to izključno iz strokovnega vidika ob neupoštevanju interesnih skupin, tako industrije kot tudi uporabnikov. Uprava za varno hrano je zavzela stališče, da smernice za določanje KPHM temeljijo samo na osnovi strokovnih izhodišč. Takšno stališče bomo zavzemali tudi v nadaljnje.

Ključne besede: Uredba 1107/2009, hormonski motilci

# HORMONE DISRUPTING CHEMICALS AND PLANT PROTECTION

## PRODUCTS LEGISLATION

**Jernej Drofenik**, The Administration of the Republic of Slovenia for Food Safety, Veterinary Sector and Plant Protection, [jernej.drofenik@gov.si](mailto:jernej.drofenik@gov.si)

Due to its toxicological properties the special attention is focused on the regulation of accessibility and use of hormone disrupting chemicals (HDC), including agriculture. That is why unambiguous criteria for restriction and banding of use of HDC were introduced into legislation for pesticides, chemicals (REACH) and biocides. Legal provisions are very similar in all legislations, having in common requirements for criteria for definition of HDC.

The Regulation (EC) 1107/2009/EC concerning the placing of plant protection products on the market was adopted in 2009, introducing the term HDC. Chemical's ability to affect hormonal balance in organisms is one of the basic cut-off criteria for the approval of an active substance in the EU Countries. According to the Regulation an active substance, safener or synergist shall only be approved and used in the EU, if, on the basis of assessment of Community or internationally agreed test guidelines or other available data and information, including a review of the scientific literature, it is not considered to have endocrine disrupting properties. The use of HDC shall not be approved, unless the proposed use of plant protection product will result in negligible exposure of users and consumers.

The need for the Guidance on criteria for determination of HDC is expressed in the legislation. EU Commission has therefore established an expert group to prepare it. Because of the high interest in this issue and many different positions, the preparation of guidance is still ongoing. Pending the adoption of the criteria, substances that are classified, in accordance with the provisions of Regulation (EC) No 1272/2008, as carcinogenic category 2 and toxic for reproduction category 2, shall be considered to have endocrine disrupting properties. In addition, substances such as those are or have to be classified, as toxic for reproduction category 2 and which have toxic effects on the endocrine organs, may be considered to have endocrine disrupting properties.

Slovenia was an active member in the process of guidance preparation, considering exclusively the technical position of our experts, without the influence of interest groups, such as industry or consumers. The Opinion of the Administration for Food Safety is that the guidance for determination of HDC must be based on the technical position only. This opinion will be held also in the future.

Keywords: Regulation 1107/2009, hormone disrupting chemicals



# KEMIJSKI POVZROČITELJI HORMONSKIH MOTENJ-ZAKONODAJA

## REACH

**Simona Fajfar**, Urad RS za kemikalije, [simona.fajfar@gov.si](mailto:simona.fajfar@gov.si)

Uredba REACH (**R**egistration, **E**valuation and **A**uthorisation and **R**estriction of **C**hemicals) je glavna evropska kemijska zakonodaja, ki ima primarni cilj varovanje zdravja in okolja. Eden od ključnih stebrov je registracija, kjer zakonodaja določa predložitev toksikoloških in ostalih podatkov odvisno od tonaže v kateri je proizvedena/uvožena določena snov. Najbolj zahtevne študije so zahtevane za najvišje tonažah kot je reproduktivna toksičnost, razvojna toksičnost in študija rakotvornosti. Ključen element je tudi avtorizacija, katere cilj je v končni fazi, da se snovi, ki povzročajo veliko zaskrbljenost, nadomestijo z bolj varnimi in primernimi alternativami. Med snovmi, ki povzročajo veliko zaskrbljenost se štejejo tudi kemijski povzročitelji hormonskih motenj. Pot do avtorizacije se začne s pripravo dosjeja XV in nadaljuje do vključitve v Prilogo XIV- snovi, ki so predmet avtorizacije. Podlaga je člen 57(f) določa, da se snovi, ki imajo lastnost, da povzročajo hormonske motnje, lahko uvrstijo v Prilogo XIV na podlagi postopka iz člena 58 (vključitev snovi v Prilogo XIV).

Ključne besede: snovi, ki povzročajo veliko zaskrbljenost, člen 57(f), avtorizacija, dosje XV

## ENDOCRINE DISRUPTING CHEMICALS –REACH LEGISLATION

**Simona Fajfar**, Chemicals Office of the Republic of Slovenia, [simona.fajfar@gov.si](mailto:simona.fajfar@gov.si)

REACH Regulation (**R**egistration, **E**valuation and **A**uthorisation and **R**estriction of **C**hemicals) is a main European chemicals legislation, which has a primary goal protection of human health and the environment. One of key pillars is registration which is part of legislation that requires a submission of toxicological and other data depending on a tonnage level at which certain chemical substance is produced/ imported into European Union. Most sophisticated studies are requested for highest tonnage level as reproductive toxicity, developmental toxicity and carcinogenicity study. Key element is also authorisation, with its final aim that the substances of very high concern are finally replaced with more safe and suitable alternatives. Among the substances which causes high concern are also hormone disruptors. Path towards authorisation starts with preparing Annex XV dossier and continues until a substance is included in Annex XIV –substances which are subject of authorisation. Legal basis is in article 57(f), which determines, that substances, with hormone disrupting properties, can be included in Annex XIV on a basis of a procedure laid down in Article 58 (inclusion of substances into Annex XIV).

Key words: substances of very high concern, article 57(f), authorisation, annex XV dossier

# KEMIJSKI POVZROČITELJI HORMONSKIH MOTENJ – STRATEGIJA EVROPSKE UNIJE

**Lucija Perharič**, Nacionalni inštitut za javno zdravje, Zaloška cesta 29, 1000 Ljubljana

[lucija.perharic@nijz.si](mailto:lucija.perharic@nijz.si)

Evropski parlament je 1998 pozval Evropsko komisijo (EK) k izboljšanju pravnih podlag za nadzor kemijskih povzročiteljev hormonskih motenj (KPHM), spodbudi raziskav in obveščanju javnosti. EK je v strategiji iz leta 1999 predstavila aktivnosti, ki bi na podlagi previdnostnega principa hitro in učinkovito zmanjšale zaskrbljenost javnosti in okvir nadaljnjih raziskav za osvetlitev vzrokov in posledice ugotovljenih hormonskih motenj. V naslednjem desetletju so potekali številni raziskovalni projekti, EK je redno poročala o aktivnostih na področju KPHM.

Zaradi Uredbe Evropske unije (EU) 1107/2009 o dajanju fitofarmaceutskih sredstev v promet in Uredbe EU 528/2012 o dostopnosti na trgu in uporabi biocidnih proizvodov, ki navajata, da osnovne snovi odobrene za uporabo v EU, same po sebi ne povzročajo motenj hormonskega ravnovesja, naj bi bila do 13. decembra 2013 določena generična znanstvena merila za identifikacijo KPHM. Zato je bila 2010 ustanovljena *Ad-hoc* skupina predstavnikov EK, Evropskih agencij, držav članic, industrije in nevladnih organizacij za pripravo nadaljnje strategije EU za KPHM. Splošni cilji so varovanje zdravja ljudi in okolja ter zagotavljanje delovanja trga; specifični cilji pa zagotovitev pravno jasnih, predvidljivih in skladnih kriterijev za identifikacijo KPHM, zagotovitev znanstvenih kriterijev, ki omogočajo funkcionalen nadzor in generično uporabo kriterijev v zakonskih aktih EU. V podporo odločitvam *Ad-hoc* skupini je bila leta 2011 ustanovljena Ekspertna svetovalna skupina (ESS), ki je 2013 objavila poročili: Ključne znanstvene točke za identifikacijo in karakterizacijo KPHM ter Prag KPHM in sorodne negotovosti. Istočasno je Evropska agencija za varno hrano objavila Znanstveno mnenje o oceni nevarnosti KPHM. Vendar zaradi raznolikosti stališč v *Ad-hoc* skupini ni prišlo do dogovora.

Junija 2014 je EK objavila predloge za določitev kriterijev za identifikacijo KPHM. Sledila je javna razprava z 22411 odgovori. Trenutno poteka presoja zdravstveno-socialno-ekonomskih vplivov predlaganih kriterijev. Dopolnjena strategija se pričakuje 2017.

Ključne besede: Kemijski povzročitelji hormonskih motenj (KPHM), strategija EU

# ENDOCRINE DISRUPTING CHEMICALS – EUROPEAN UNION (EU)

## STRATEGY

**Lucija Perharič**, National Institute of Public Health, Zaloška cesta 29, 1000 Ljubljana

[lucija.perharic@nijz.si](mailto:lucija.perharic@nijz.si)

In 1998, the European Parliament adopted a Resolution calling upon the European Commission (EC) for an improvement in the legislative framework of endocrine disruptors (EDs), reinforcement of research and communication to the public. In 1999, the EC presented the necessary activities for a quick and effective alleviation of public concern on the basis of the precautionary principle, and a research framework for elucidation of causes and consequences of identified endocrine disturbances. In the following decade numerous research projects were carried out; the EC regularly reported on the activities.

In view of the EU plant protection product Regulation (1107/2009), and the EU biocidal product Regulation (528/2012), which state that basic substances approved in the EU should not have an inherent capacity to cause endocrine disruption, generic scientific criteria for the identification of EDs were to be determined by 13<sup>th</sup> December 2013. Consequently, an Ad-hoc group of EC services, EU agencies, member states, industry and non-governmental organizations was established in 2010 to further develop the EU strategy on EDs with general and specific objectives: to protect human health and the environment and to assure the functioning of the market; to provide legally clear, predictable and coherent criteria for the identification of EDs enabling operational regulation and generic applicability in EU legislation. In support of Ad-hoc group an Expert advisory group (EAG) was established in 2011. In 2013, EAG published two reports: Key scientific issues relevant to the identification and characterisation of endocrine disrupting substances and Thresholds for endocrine disruptors and related uncertainties while the European Food Safety Authority published its scientific opinion on hazard assessment of EDs. However, due to a diversity of opinions in the Ad-hoc group a consensus was not reached.

In June 2014, the EC published proposals for determination of criteria for ED identification followed by a public consultation resulting in 22411 responses. A health-socio-economic impact assessment is being carried out for each of the proposed criteria. The accomplished strategy is expected in 2017.

Key words: endocrine disrupting chemicals, EU strategy

## DOLOČANJE NIVOJA HORMONOV V ŽIVILIH ŽIVALSKEGA IZVORA

**Rebeka Jurca, Tomaž Pezdir**, Inštitut za higieno živil in bromatologijo, Veterinarska fakulteta, Univerza v Ljubljani, Gerbičeva 60, 1000 Ljubljana, [tomaz.pezdir@vf.ini-lj.si](mailto:tomaz.pezdir@vf.ini-lj.si)

Uvod in namen: Živinorejska industrija stremi k temu, da bi bila vzreja živali bolj učinkovita. Eden izmed načinov za to je uporaba pospeševalcev rasti, med katerimi so tudi naravni in sintetični hormoni, ki stimulirajo povečanje mišične mase ter reducirajo maščobno tkivo. Uporaba rastnih hormonov v živalski krmni je prepovedana, saj so škodljivi za zdravje ljudi in živali, vplivajo pa tudi na kakovost živil. Zaradi zakonske prepovedi dodajanja hormonov pri vzreji živali je prišlo do pojava črnega trga in je zato potrebna kontrola. Namen študije je optimizacija priprave vzorcev živalskega izvora in iskanje ustreznih pogojev analitske metode za določanje sintetičnih steroidnih hormonov.

Materiali in metode: Kot vzorce smo vzeli živalski urin. Pri pripravi vzorca smo iskali ustrezne pogoje ekstrakcije analitov iz urina in uporabili različnih načinov čiščenja ekstraktov. Uporabili smo ekstrakcijo na trdno fazo z pomočjo Oasis HLB kolone in Amino kolone. Ker so steroidni hormoni pogosto vezani na endogene komponente vzorcev, smo za ločbo preizkusili kislo, bazično in encimatsko hidrolizo z encimom  $\beta$ -glukuronidaza/aril sulfataza pridobljenim iz *E.coli* in iz *Helix pomatia*. Steroidne hormone smo določevali s tekočinsko kromatografijo v povezavi s tandemskim masnim spektrometrom (HPLC-MS/MS). Kot detektor smo uporabili kvadrupolni masni spektrometer. MS meritve pa so potekale tako pri negativni kot pri pozitivni napetosti.

Rezultati: Izbrane steroidne hormone,  $17\beta$ -estradiol, progesteron in testosteron, smo uspeli ločiti od drugih endogenih komponent urina. Povečali smo občutljivost metode, kar je omogočilo kvantifikacijo izbranih steroidov.

Zaključek: Nova metoda omogoča kvantitativno določevanje nivojev  $17\beta$ -estradiol, progesteron in testosteron v živalskem urinu. Za točno določitev vsebnosti sintetičnih hormonov, ki izvirajo iz živalske krme ali injekcij, je potrebna analiza razmerja ogljikovih atomov.

Ključne besede: steroidni hormoni, HPLC-MS/MS, ekstrakcija na trdno fazo, encimatska hidroliza

## DETERMINATION OF HORMONE LEVELS IN FOOD OF ANIMAL ORIGIN

**Rebeka Jurca, Tomaž Pezdir**, Institute of food hygiene and bromatology, Veterinary faculty, University Ljubljana, Gerbičeva 60, 1000 Ljubljana, [tomaz.pezdir@vf.ini-lj.si](mailto:tomaz.pezdir@vf.ini-lj.si)

**Introduction and aim:** The livestock industry tends to indicate, that the breeding of animals as effective as possible. One way to do this is to use growth promoters, which include natural and synthetic hormones that stimulate muscle growth and reducing adipose tissue. The use of growth hormones in animal feed is forbidden, because they are harmful to human and animal health. Growth hormones also affect the quality of the food. Due to the statutory prohibition of adding hormones in animal breeding, has led to the emergence of a black market and it is therefore necessary checks. The purpose of the study is to optimize the preparation of samples of animal origin and search for relevant terms of the analytical method for the determination of synthetic steroid hormones.

**Materials and methods:** As samples, we used animal urine. Sample preparation included searching for appropriate conditions extraction of analytes from urine and the use of different methods of purification of extracts. We used solid phase extraction, using Oasis HLB cartridges and amino cartridges. Since steroid hormones are often linked to the endogenous component of the samples, for separation we tested acidic, alkaline and enzymatic hydrolysis by the enzyme  $\beta$ -glucuronidase/aryl sulfatase derived from *E. coli* and from *Helix pomatia*. Steroid hormones were determined by liquid chromatography, coupled with tandem mass spectrometry (HPLC-MS / MS). As a detector, was used a quadrupole mass spectrometer. MS measurements were carried out in both the negative and the positive voltages.

**Results:** We managed to separate the selected steroid hormones,  $17\beta$ -oestradiol, progesterone and testosterone, from the other endogenous components of urine. We have increased the sensitivity of the method, which has allowed the quantification of selected steroids.

**Conclusion:** The new method allows the quantitative determination of levels of  $17\beta$ -oestradiol, progesterone and testosterone in animal urine. For the accurate determination of the levels of synthetic hormones originating from the animal feed or injection, it is necessary to analyse the ratio of carbon atoms.

**Key words:** steroid hormones, HPLC-MS / MS, solid phase extraction, enzymatic hydrolysis

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**Hvala za prispevke.**

*Slovensko toksikološko društvo  
Gerbičeva 60, 1000 Ljubljana*