3. KONGRES SLOVENSKEGA TOKSIKOLOŠKEGA DRUŠTVA

OKOLJSKA ONESNAŽILA IN KOMUNIKACIJA TVEGANJA

ENVIRONMENTAL POLLUTANTS AND RISK COMMUNICATION

Ljubljana, 28.9.2017

Univerza v Ljubljani

KEMIJSKI INŠTITUT

NACIONALNI INŠTITUT ZA BIOLOGIJO

UNIVERZA NA PRIMORSKEM

COMPLEMENTARIUM

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V preteklih dveh desetletjih smo se tudi v Sloveniji začeli zavedati pomena določanja in raziskav o vplivu kemikalij, učinkovin ter biocidov oz fitofarmacevtskih sredstev na okolje in človeka ter s tem razvijati področje kemijske varnosti tako na strokovni kot upravni ravni. V teh raziskavah sodelujejo številni strokovnjaki, med katerimi je veliko število članov Slovenskega toksikološkega društva. S sistematičnim določanjem prisotnosti in vplivov različnih onesnažil na okolje in človeka skušajo raziskovalci na različnih raziskovalnih inštitucijah (Institut Jožef Stefan, Kemijski inštitut, Nacionalni inštitut za biologijo), Nacionalnem inštitutu za javno zdravje in mnogih fakultetah ter klinikah zagotoviti, da živimo v čim bolj varnem okolju. K temu prispevajo tudi upravne inštitucije predvsem Urad Republike Slovenije za kemikalije ter Agencija Republike Slovenije za okolje ter inšpektorati pristojni za področja zdravstva, okolja, kmetijstva, hrane in trgovine, ki nadzorujejo uporabo in izpostavitev kemikalijam v skladu z veljavno zakonodajo.
Zato je bila odločitev Organizacijskega odbora lahka, da na 3. kongresu Slovenskega toksikološkega društva skušamo predstaviti vsaj del raziskav na področju usode in problematike učinkovin, pesticidov ter ostalih onesnažil in njihovih mešanic v okolju.
Hkrati smo želeli v obliki dveh okroglih miz obravnavati najbolj aktualna dogajanja na področju toksikologije v Sloveniji. Odločili smo se za dve temi, prva bo vsem udeležencem gotovo zanimiva, saj bo obravnavala komunikacijo tveganja ob različnih kemijskih nesrečah, pri čemer bodo izpostavljeni pomen ustreznosti ter pravočasnosti dajanja mnenj in informacij ter se bo lahko navezovala na aktualne nesreče, ki so se v preteklih mesecih zgodile v Sloveniji. Druga tema okroglih miz pa bo izmenjava mnenj o nikoli zaključeni »zgodbi« o varni uporabi glifosata, širokospektralnega herbicida, ki se uporablja že od leta 1974.
Upamo, da bo vsak izmed udeležencev našel v programu kongresa tematiko, ki ga še posebej zanima ali se s podobno tudi sam ukvarja. In če se bo pri tem stkala še kakšna dodatna nit, ki bo povezala raziskovalce med seboj in/ali s predstavniki upravnih inštitucij pri njihovem delu za čim večjo varnost uporabe kemikalij in ostalih snovi, ki bodo obravnavane na tem kongresu, bo še eden izmed ciljev Organizacijskega odbora uresničen.
Želimo vam prijetno druženje in čim več zanimivih izmenjav mnenj o tematikah, predstavljenih na kongresu!

Prof. dr. Marija Sollner Dolenc
v imenu Organizacijskega odbora

KAZALO VSEBINE

Program kongresa

8.30-9.00	**Registracija udeležencev**
9.00-9.10	**Pozdrav udeležencev in uvodni nagovor** *Prof. dr. Lucija Peterlin Mašič;Univerza v Ljubljani, Fakulteta za farmacijo* *Prof. dr. Irena Mlinarič Raščan; Dekanja Fakultete za farmacijo, Univerza v Ljubljani*
Usoda in problematika učinkovin, ostalih onesnažil in njihovih mešanic v okolju Moderator: Prof. dr. Marija Sollner Dolenc, Univerza v Ljubljani, Fakulteta za farmacijo	
9.10-9.40	**Occurrence and effects of pharmaceuticals and their mixtures in the environment** *Prof. dr. Elena Fabbri; University of Bologna*
9.40-10.00	**Merjenje koncentracij učinkovin v slovenskih odpadnih vodah ter primer razvoja nove napredne tehnologije za njihovo odstranjevanje** *Doc. dr. Jurij Trontelj; Univerza v Ljubljani, Fakulteta za farmacijo*
10.00-10.20	**Toksični učinki ostankov citostatikov na vodne organizme** *Doc. dr. Bojana Žegura; Nacionalni inštitut za biologijo*
10.20-10.40	**Razgradnja enrofloksacina v iztrebkih piščancev pitancev** *Dr. Marko Slana; Krka d.d.*
10.40-11.10	**Odmor**
11.10-11.30	**Usoda in problematika veterinarskih zdravil v okolju - raziskave avermektinov** *Znan. svet. dr. Vesna Cerkvenik Flajs; Univerza v Ljubljani, Veterinarska fakulteta*
11.30-11.50	**Primerjalna študija vsebnosti kovin v mleku in zelenjavi (fižol in korenje) izvedena na območju Slovenije s podatki iz drugih evropskih držav** *Nika Bizjak[1], dr. Zlatka Bajc[2] in dr. Lucija Kolar[1,3]* *[1]Visoka šola za varstvo okolja Velenje, [2]Univerza v Ljubljani, Veterinarska fakulteta, [3]Complementarium, Inštitut za raziskave narave in razvoj okoljskih tehnologij*
11.50-12.10	**In silico orodja za oceno (eko-)toksikoloških lastnosti spojin – izkušnje iz projekta PROSIL** *Dr. Viktor Drgan; Kemijski inštitut*
12.10-12.30	**Učinki mešanic bisfenolov na vodne organizme** *Dr. Tatjana Tišler; Kemijski inštitut*
12.30-12.50	**Pojavnost in kroženje bisfenolov med čiščenjem odpadnih vod** *Dr. Ester Heath; Institut Jožef Stefan*
12.50-13.50	**Odmor – kosilo**
Komunikacija tveganja- pomen ustreznosti in pravočasnosti dajanja mnenj in informacij Moderator: Prof. dr. Lucija Peterlin Mašič; Univerza v Ljubljani, Fakulteta za farmacijo	
13.50-14.20	**The importance of communication -internal and external- during emergency situations** *Dr. Ingrid Håstad; SWECO Environment AB, Stockholm, Sweden*
14.20-15.20	**Okrogla miza 1** Moderator: Prof. dr. Mihael Jožef Toman; Univerza v Ljubljani, Biotehniška fakulteta *Dr. Ingrid Håstad (SWECO Environment),* *Simona Fajfar (Urad RS za kemikalije),* *Mag. Matej Ivartnik (Nacionalni inštitut za javno zdravje),* *Bernarda Kropf (Civilna iniciativa Sinja Gorica)*
Glifosat pod drobnogledom Moderator: Prof. dr. Lucija Peterlin Mašič; Univerza v Ljubljani, Fakulteta za farmacijo	
15.20-15.40	**Bi morali uporabo glifosata prepovedati? Vprašanje, ki razdvaja Evropo!** *Prof. dr. Metka Filipič; Nacionalni inštitut za biologijo*
15.40-16.00	**Potek ocenjevanja glifosata na ravni Evropske unije** *Dr. Lucija Perharič; Nacionalni Inštitut za javno zdravje*
16.00-16.30	**Okrogla miza 2** Moderator: Dr. Lucija Šarc; Univerzitetni klinični center Ljubljana *Prof. dr. Metka Filipič (Nacionalni inštitut za biologijo),* *Dr. Lucija Perharič (Nacionalni inštitut za javno zdravje),* *Dr. Jernej Drofenik (Uprava RS za varno hrano, veterinarstvo in varstvo rastlin),* *Dr. Tanja Fatur (Nacionalni inštitut za javno zdravje),* *Izr. prof. dr. Vesna Zadnik (Onkološki inštitut)*
16.30	**Zaključne misli**

POVZETKI VABLJENIH PREDAVANJ

Occurrence and effects of pharmaceuticals and their mixtures in the environment

Elena Fabbri

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Human pharmaceuticals and veterinary medicines are ubiquitous contaminants in the environment, from freshwater ecosystems, to coastal and marine waters, soil, ground-waters, and even potable waters. Large quantities of pharmaceuticals are produced and sold annually to be consumed worldwide. Subsequently, they are partially excreted as parental compounds or metabolites in an active form, which are spread into the environment in different ways, mainly through STP effluents, and also water run-off from agricultural lands, or other means. Typically, wastewater treatment plants are designed to efficiently remove nutrients, solids and pathogens, and the removal of pharmaceuticals is only a secondary benefit. Removal rates for pharmaceuticals during wastewater treatment range from 10 to 100%, depending from the compound and the treatment. Since pharmaceuticals are continuously discharged and not effectively depurated, exposure of aquatic organisms is pseudo-persistent and increases with a decreased dilution. Pharmaceuticals significantly differ from conventional contaminants, since they are conceived to have biological effects at low doses and be resistant to inactivation before exerting therapeutic effects. These same properties are paradoxically responsible for bioaccumulation and detrimental effects in the environment. Pharmaceuticals with evolutionary conserved molecular targets are more likely to cause adverse effects in non-target species. A well-known case regards 17α-ethynilestradiol, which causes feminization in fish in the ng/L range, however many other examples are documented demonstrating that pharmaceuticals may pose a substantial risk to the ecosystem. Toxicity studies showed that mixture of pharmaceuticals at environmentally relevant concentrations may exhibit additive or even synergistic effects. However, experiments on mixtures are scarce, and far from conclusion. Overall, we must be aware that pharmaceuticals, which are intended to improve the quality of human health, may cause unpredictable risks to the ecosystem and produce boomerang effects on public health. Individual actions as well as policies are needed to reduce pharmaceuticals in the environment.

Merjenje koncentracij učinkovin v slovenskih odpadnih vodah ter primer razvoja nove napredne tehnologije za njihovo odstranjevanje

Jurij Trontelj[1] , Anita Klančar[1], Maja Zupančič Justin[2], Robert Roškar[1]

[1] Fakulteta za farmacijo,Univerza v Ljubljani
[2] Arhel d.o.o.

Uporaba zdravil v svetu narašča; izločene zdravilne učinkovine se pojavijo v odpadnih in posledično v površinskih, talnih in pitnih vodah. V ne-tarčnih organizmih in tudi pri človeku lahko povzročajo neželene učinke. Naš cilj je bil razviti metodo za ekstrakcijo in občutljivo kvantifikacijo širokega spektra zdravilnih učinkovin za njihov monitoring v odpadnih vodah in tudi kot orodje za spremljanje učinkovitosti razvoja novih tehnologij z napredno elektrokemijsko oksidacijo onesnažil, ki jih razvija slovensko podjetje Arhel in s katerim smo sodelovali pri projektu LIFE PharmDegrade (LIFE13 ENV/SI/000466).

Vključenih je bilo več kot 100 analitov iz različnih terapevtskih skupin. Vzorci odpadnih vod (250 mL) zbrani kot kompozitni 24 urni vzorci iztokov različnih čistilnih naprav (ČN) po Sloveniji so bili obdelani z ekstrakcijskim sistemom SPE-DEX Horizon 4790. Eluati so bili nadalje skoncentrirani in analizirani z LC-MS/MS sistemom Agilent 1290+6460. Analizna metoda je bila uspešno validirana. Elektrokemijsko čiščenje odpadnih vod je bilo izvedeno z borom dopirano diamantno elektrodo v patentirani celici in napajalnikom, vse razvito v podjetju Arhel.

Rezultati so pokazali prisotnost večine analitov v iztokih ČN v območju od 1 ng/L do 2 µg/L pa vse do 10 µg/L. Nova tehnologija elektrokemijskega čiščenja se je izkazala za zmerno do zelo učinkovito, odvisno od kemizma spojine.

Naši rezultati kažejo na obremenjenost slovenskih odpadnih voda z velikim naborom učinkovin, ki so sicer v relativno nizkih koncentracijah, a bo vseeno potrebno sistematično spremljanje in skrbno načrtovanje njihovega odstranjevanja za zagotavljanje čistih površinskih in talnih vod v prihodnosti. Ena od možnih tehnologij za dosego tega cilja je tudi napredna elektrokemijska razgradnja.

Ključne besede: zdravilne učinkovine, odpadne vode, SPE, LC-MS/MS, elektrokemična razgradnja

The measurement of pharmaceuticals concentration in Slovenian sewage and the development of a novel advanced technology for their degradation

Jurij Trontelj[1] , Anita Klančar[1], Maja Zupančič Justin[2], Robert Roškar[1]

[1] Faculty of Pharmacy, University of Ljubljana
[2] Arhel d.o.o.

The consumption of pharmaceuticals is rising around the globe; the excreted active substances can occur in wastewater and consequently in surface, ground and drinking water. In non-target organisms and also in humans, they can cause unwanted effects. Our goal was to develop a method for quantification of a wide range of pharmaceuticals for their monitoring in wastewater and also to support the development of new treatment technology using the advanced electrochemical oxidation being developed by the Slovenian company Arhel, our collaborator on the project LIFE PharmDegrade (LIFE13 ENV/SI/000466).

More than 100 analytes from different therapeutic groups were included. Samples of wastewater (250 mL) collected as composite 24 hour samples of effluents from various treatment plants across Slovenia were extracted by the SPE-DEX Horizon 4790 extraction system. The eluates were further concentrated and analyzed using the LC-MS/MS system Agilent 1290+6460. The analytical method was successfully validated. Electrochemical treatment of wastewater samples was carried out by a boron-doped diamond electrode in a patented cell and power supply, both developed by Arhel.

The results showed the presence of majority of monitored analytes in the concentration range from 1 ng/L to 2 μg/L and up to 10 μg/L. The new electrochemical purification technology has proven to be moderate to very effective, depending on the chemical composition of each specific compound.

Our results show a noticeable burden of Slovenian wastewaters with a large range of active substances. Even though they were found in relatively low concentrations, they should nevertheless require systematic monitoring and careful planning of their removal for the preservation of clean surface, ground and drinking water in the future. One of the possible technologies for achieving this goal is the advanced electrochemical degradation.

Key words: pharmaceuticals, wastewater, SPE, LC-MS / MS, electrochemical degradation

Toksični učinki ostankov citostatikov na vodne organizme

Bojana Žegura[1], Matjaž Novak[1], Tina Eleršek[1], Marina Isidori[2], Alfredo Parrella[2], Akos Horvath[3]; Robert Kovacz[3], Vera Garaj Vrhovac[4], Goran Gajski[4], Marko Gerić[4], Metka Filipič[1]

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Ostanki citostatskih zdravil predstavljajo nova onesnažila vodnega okolja. To je poleg njihove naraščajoče uporabe posledica tudi mehanizmov njihovega delovanja, saj so razvrščeni kot mutageni, rakotvorni, teratogeni in/ali strupeni za reproduktivne sisteme. Prav zaradi tega spadajo v skupino posebno nevarnih snovi. Domnevamo lahko, da zaradi svojih lastnosti povzročajo škodljive učinke tudi pri izpostavljenih neciljnih vodnih organizmih.
Namen naših študij je bil oceniti eko-/geno-toksičnost štirih zelo pogosto uporabljenih citostatskih zdravil: 5-fluorouracila (5-FU), cisplatina (CDDP), etoposida (ET) in imatinib-mezilata (IM). Glede na mehanizem njihovega kemoterapevtskega delovanja jih razvrščamo med antimetabolite, alkilirajočim agensom podobna zdravila, inhibitorje topoizomeraz in med zaviralce tirozinskih kinaz.
Akutno in kronično strupenost smo določali na algi (*Pseudokirchneriella subcapitata*), cianobakteriji (*Synechococcus leopoliensis*), rakih (*Daphnia magna* in *Ceriodaphnia dubia*) ter ribi cebrici (*Danio rerio*). Pri rakih in cebricah smo genotoksičnost določali s testom komet. V dvo-generacijski študiji na cebricah smo za 5-FU poleg standardnih toksikoloških učinkov določali njegov genotoksičen potencial s testoma komet in mikrojedra, izvedli pa smo tudi analizo transkriptoma na vzorcih jeter cebric F1 generacije.
Ugotovili smo, da za rake in cebrice citostatiki niso akutno toksični, medtem ko so relativno visoko reproduktivno toksični za alge, cianobakterije in rake. V dvogeneracijski študiji na cebricah 5-FU ni vplival na preživetje, rast in reprodukcijo rib. V jetrih in ledvicah je povzročil histopatološke spremembe skupaj z genotoksičnimi učinki. V vzorcih jeter F1 generacije smo opazili diferencialno izražanje genov, ki so vključeni v odziv na DNA poškodbe in onkogenezo.
Rezultati raziskav so pokazali, da so ostanki določenih citostatskih zdravila nevarni za vodne organizme, zaradi česar bi morala biti ocena tveganja za okolje obvezna in bi morala temeljiti na podatkih iz poskusov kronične izpostvljenosti, vključno z določanjem genotoksičnih lastnosti. Za oceno tveganja za vodno okolje so potrebne nadaljnje ekotoksikološke raziskave še drugih citostatskih zdravil, kot tudi sistematske raziskave pojavljanja in usode njihovih ostankov v vodnem okolju.

Ključne besede: protirakava zdravila, ekotoksičnost, genotoksičnsot, nevarnost za okolje

Zahvala
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Toxic effects of residues of cytostatic drugs on aquatic organisms

Bojana Žegura[1], Matjaž Novak[1], Tina Eleršek[1], Marina Isidori[2], Alfredo Parrella[2], Akos Horvath[3]; Robert Kovacz[3], Vera Garaj Vrhovac[4], Goran Gajski[4], Marko Gerić[4], Metka Filipič[1]

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The residues of cytostatic drugs are emerging pollutants in aquatic environments. This is in addition to their increasing use also due to their mechanisms of action since they are classified as mutagenic, carcinogenic, teratogenic and/or toxic to reproductive systems. Therefore they belong to a group of particularly dangerous compounds. It can be assumed that they elicit adverse effects also in exposed, non-target, aquatic organisms.
The aim of our studies was to evaluate eco-/geno-toxicity of four highly consumed cytostatic drugs: 5- fluorouracil (5-FU), cisplatin (CDDP), etoposide (ET) and imatinib mesylate (IM). According to the mechanism of chemotherapeutic action they represent different classes of anticancer drugs: antimetabolites, alkylating like agents, topoisomerase inhibitors and tyrosine kinase inhibitors, respectively.
Acute and chronic toxicity was determined in algae (*Pseudokirchneriella subcapitata*), cyanobacteria (*Synechococcus leopoliensis*), crustacea (*Daphnia magna* and *Ceriodaphnia dubia*) and zebrafish (*Danio rerio*). In crustacea and zebrafish genotoxic potential was determined with the comet assay. In two generation study of 5-FU in zebrafish in addition to standard toxicological endpoints genotoxic potential was determined with comet and micronucleus assays and whole genome transcriptomic analysis of liver samples of F1 generation of fish was performed.
In crustacean and in zebrafish cytostatic drugs showed low acute toxicity. Relatively high toxicity was observed in the reproduction assays with algae, cyanobacteria and crustacean. In zebrafish, in a two-generation study, the exposure to 5-FU did not affect fish survival, growth and reproduction. Histopathological changes were observed in the liver and kidney along with genotoxic effects. In zebrafish liver samples of F1 generation differential expression of genes involved in DNA-damage-response and oncogenesis was observed.
Altogether the results demonstrated that the residues of certain anticancer drugs are hazardous for aquatic organisms; therefore, for anticancer drugs environmental risk assessment should be mandatory and should be based on data from chronic exposure tests including genotoxicity endpoints.

Keywords: cytostatic drugs, ecotoxicity, genotoxicity, , environmental hazard

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Razgradnja enrofloksacina v iztrebkih piščancev pitancev

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Enrofloksacin je fluorokinolon, ki se najpogosteje uporablja za zdravljenje respiratornih ter prebavnih obolenj živali, namenjenih za prehrano ljudi. Izločki tretiranih ali netretiranih živali se raztresejo po poljih, zato je koncentracija veterinarskih zdravil, ki prehajajo v okolje, odvisna predvsem od načina in količine apliciranega zdravila, vendar tudi od dolžine terapije. Kako dolgo bo posamezna učinkovina prisotna v okolju in kakšni bodo njeni ekotoksikološki vplivi, je odvisno predvsem od fizikalno-kemijskih, razgradnih ter toksikoloških lastnosti učinkovine. Enrofloksacin je razmeroma stabilna učinkovina, ki se delno metabolizira v tarčnih živalih in se izloča predvsem v nespremenjeni obliki ali v obliki glavnega metabolita ciprofloksacina, ki prav tako deluje protimikrobno. Ker se enrofloksacin v okolje izloči pretežno v nespremenjeni obliki, je njegova razgradnja precej odvisna od dejavnikov, prisotnih v okolju. V raziskavi smo spremljali usodo enrofloksacina od aplikacije piščancem pa vse do raztrosa piščančjih iztrebkov po zemlji. Tako smo proučili metabolno-ekskrecijski mehanizem enrofloksacina in njegovo razgradnjo v hlevu in v naravi in jo primerjali z razgradnjo pod različnimi laboratorijskimi pogoji. Raziskava je pokazala, da se v piščančjih izločkih pojavlja 72,3 % nespremenjenega enrofloksacina, 25,5 % ciprofloksacina, 0,9 % dezetilen-enrofloksacina in 0,3 % dezetilen-ciprofloksacina. Spremljanje razgradnje metabolitov v hlevu, v fazi rejnega cikla piščancev, je pokazalo, da se enrofloksacin in ciprofloksacin deloma razgrajujeta v iztrebkih že v hlevu pred prenosom iztrebkov v naravo. Prav tako je v hlevski fazi narastla koncentracija dezetilen-enrofloksacina, ki je metabolni in razgradni produkt enrofloksacina. Spremljanje koncentracije analitov v kompostiranih iztrebkih v naravi je pokazalo, da se razgradnja enrofloksacina in ciprofloksacina ter tvorba dezetilen-enrofloksacina v naravi nadaljuje. Enrofloksacin in vsi njegovi metaboliti so bili prisotni v kompostu v celotnem 60-dnevnem opazovalnem obdobju. V kompostiranih iztrebkih smo zasledili tudi nov razgradni produkt; hidroksiliran enrofloksacin. Vzporedno spremljanje razgradnje enrofloksacina pod različnimi laboratorijskimi pogoji je pokazalo, da je razgradnja erofloksacina pod laboratorijskimi pogoji počasnejša v primerjavi z naravnimi pogoji. Kljub temu, da so iztrebki bili izpostavljeni različnim laboratorijskim pogojem, smo zaznali en sam razgradni produkt dezetilen-enrofloksacin, ki je v 120 dnevih opazovanja dosegel do 15 % začetne koncentracije.

Ključne besede: razgradna kinetika enrofloksacina, aerobna in anaerobna inkubacija, inkubacija v temi/svetlobi, primerjava laboratorijskih pogojev s pogoji v naravi, razgradna produkta dezetilen-enrofloksacin in OH-enrofloksacin.

Degradation of enrofloxacin in chicken broiler excreta

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Enrofloxacin is a fluoroquinolone that is most commonly used to treat respiratory and digestive diseases of animals intended for human consumption. The secretions of treated or untreated animals are spread on the arable- or grassland, therefore the concentration of the veterinary compounds released into the environment mainly depends on the type and quantity of medications, as well as the length of the therapy. The presence of each compound in the environment and its ecological impacts mainly depend on the physico-chemical, degradable and toxicological properties of the active compound. Enrofloxacin is a relatively stable substance that is not very susceptible for metabolic processes in the target animals and is excreted mostly in an unchanged form or in the form of its major metabolite ciprofloxacin, which has also antimicrobial activity. As enrofloxacin is exposed into the environment predominantly in an unchanged form, therefore its degradation depends primarily on the factors present in the environment. In the presented study we monitored the fate of enrofloxacin starting from the application to chicken all the way until the chicken excreta was applied onto the earth's surface. Thus, we examined the metabolic - excretory mechanism of enrofloxacin and its degradation pathway inside the rearing facility as well after the transfer of the excreta into the nature was performed. Additionally, the degradation data obtained in the nature was compared with the degradation data obtained under different laboratory conditions. The survey showed that enrofloxacin, ciprofloxacin, desethylene-enrofloxacin and desethylene-ciprofloxacin were presented in 72.3 %, 25.5 %, 0.9 % and 0.3 % in chicken excreta, repectively. Monitoring the degradation of metabolites during the rearing cycle phase inside the rearing facility showed that enrofloxacin and ciprofloxacin were partly degraded in the excreta already before the transfer of the excreta into nature occurred. Additionally, a concentration increase of desethylene-enrofloxacin concentration was determined, which is a metabolic and degradation product of enrofloxacin. Furthermore, the concentration of the analytes in the nature composted excreta showed that the degradation of enrofloxacin and ciprofloxacin and the formation of desethylene-enrofloxacin continued. According to this, enrofloxacin and all the metabolites were therefore present during the 60-day observation period. In the composted excreta also a new degradation product - hydroxylated enrofloxacin was determined. In parallel, the enrofloxacin degradation study under various laboratory conditions has shown that enrofloxacin degradation in the laboratory is slower comparing to the conditions presented in the nature. Irrespective of the different laboratory conditions, a single metabolite desethylene enrofloxacin was formed, which represented not more than 15 % of the initial enrofloxacin application after 120 days of observation.

Keywords: enrofloxacin degradation kinetics, aerobic in anaerobic incubation, incubation in under light/dark regime, comparison of the laboratory with the environmental conditions, degradation products desethylene-enrofloxacin in OH-enrofloxacin.

Usoda in problematika veterinarskih zdravil v okolju - raziskave avermektinov

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Uporaba veterinarskih zdravil je močno narasla zaradi intenzivne živinorejske proizvodnje in naraščajočega števila ljubiteljskih živali. Posledično vstopajo farmacevtiki v vodno in kopensko okolje z neželenimi stranskimi učinki na številne neciljne vrste. Vhodne poti vstopa veterinarskih zdravil v okolje so bolj zapletene v primerjavi s humanimi zdravili. Predstavljene raziskave so proučevale različne okoljske vidike uporabe antiparazitarnih avermektinskih zdravil, ki se pogosto uporabljajo v veterinarski medicini.

V letih 2001 do 2008 so bile na Veterinarski fakulteti Univerze v Ljubljani izvedene sistematične raziskave avermektinov v okolju. V laboratorijskih pogojih smo proučevali toksičnost abamektina na ribe šarenke (*Oncorhynchus mykiss*), v hlevnih pogojih in na pašnikih pa smo sledili izločanju avermektinov z iztrebki ovc avtohtone slovenske mlečne pasme Istrska pramenka in njihovi degradaciji. Ovrednotili smo tudi toksičnost teh substanc za zemeljske nevretenčarje ter proučevali potencialno razgradnjo doramektina s kompostiranjem. Uporabili smo inštrumentalne kromatografske tehnike (HPLC), patohistološko analizo, Smernice OECD za preskušanje kemikalij, fluorescenčno mikroskopijo, verižno reakcijo s polimerazo (PCR) in filogenetsko analizo (T-RFLP).

Potrdili smo toksičnost abamektina za šarenke z degenerativnimi spremembami, predvsem v možganih in ledvicah. Po vnosu v ovce smo na pašniku lahko sledili abamektinu v iztrebkih, mešanici zemlje in iztrebkov oz. zemlje do 70 dni, doramektinu pa do 50 dni. Visoke koncentracije obeh antiparazitikov so bile na pašniku prisotne prvih 20 dni po vnosu v ovce. Eprinomektin smo v iztrebkih ovc ugotovili še 32. dne po vnosu. Abamektin v iztrebkih je bil bolj toksičen od doramektina za zemeljske nevretenčarje. Koncentracija doramektina se je s kompostiranjem zmanjšala za 39 %, doramektin pa je vplival na številčnost živih bakterij, ne pa tudi na raznolikost bakterijske združbe.

Rezultati naših raziskav so potrdili visoko obstojnost avermektinov v okolju in njihovo potencialno toksičnost za neciljne organizme ter prikazali možnost njihove razgradnje.

Ključne besede: veterinarska zdravila, ostanki, okolje, makrociklični laktoni, avermektini, ribe, ovce, nevretenčarji, iztrebki, zemlja, kompostiranje

Zahvala

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Fate and problematics of veterinary medicines in the environment - research of avermectins

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The use of veterinary drugs has increased significantly due to intensive animal food industry and growing number of companion animals. Consequently, the pharmaceuticals can enter the aquatic and terrestrial environment with adverse side effects on many non-target species. Pathways of entering veterinary drugs into the environment are more complex compared to human drugs. Presented research investigated various environmental aspects of the use of antiparasitic avermectin drugs, widely used in the veterinary medicine.

In the years 2001 to 2008, systematic studies of avermectins in the environment were carried out at the Veterinary Faculty of the University of Ljubljana. In laboratory conditions the toxicity of abamectin to the rainbow trout (*Oncorhynchus mykiss*) was studied, while in stable conditions, as in pasture, the elimination of avermectins with faeces of the sheep of the autochthonous Slovene milk breed Istrska pramenka and their degradation, were evaluated. We also determined the toxicity of these substances for terrestrial invertebrates and studied the potential degradation of doramectin by composting. Instrumental chromatographic techniques (HPLC), pathohistological analysis, OECD Chemical Testing Guidelines, fluorescence microscopy, polymerase chain reaction (PCR) and phylogenetic analysis (T-RFLP) were used.

Abamectin was toxic for rainbow trout with degenerative changes, especially in the brain and kidneys. On the pasture (faeces, mixture of soil and faeces, soil) abamectin and doramectin could be traced up to 70 and 50 days postadministration, respectively, and high concentrations of both drugs were present on the pasture for the first 20 days. Eprinomectin was detected in sheep faeces up to the 32nd day postadministration. Abamectin in faeces was more toxic than doramectin for terrestrial invertebrates. Doramectin in the compost decreased by 39% and affected the abundance of viable bacteria, but not the diversity of the bacterial community. The results of our research have demonstrated the high persistence of avermectins in the environment, their potential toxicity to non-target organisms, and the possibility of their degradation.

Keywords: veterinary medicines, residues, environment, macrocyclic lactones, avermectins, fish, sheep, invertebrates, faeces, soil, composting

Acknowledgement

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Primerjalna študija vsebnosti kovin v mleku in zelenjavi (fižol in korenje) izvedena na območju Slovenije s podatki iz drugih evropskih držav

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Z raziskavo smo preučili kovine kot hormonske motilce, določili vsebnosti kovin v mleku in zelenjavi ter preverili potencialno škodljivost teh pridelkov. Rezultate smo primerjali med tremi območji – Šaleška kotlina, Celjska kotlina in Spodnja Savinjska dolina, nato pa smo rezultate omenjenih treh območij primerjali še z razmerami po svetu. Poseben poudarek temelji na vsebnosti kovin v mleku, korenju in fižolu, pri čemer smo preučili slovenske, evropske in svetovne raziskave, ki se dotikajo te tematike.
Vzorce mleka, korenja in fižola, smo pridobili iz osemnajstih kmetij, po šest z vsakega območja. Vzorce smo homogenizirali in razklopili s pomočjo mikrovalov ob prisotnosti HNO_3 in H_2O_2. Vsebnost mangana, cinka, kroma, kadmija, svinca, arzena, kobalta, železa in niklja v raztopini smo določili z ICP-MS.
Vzorci mleka so vsebovali največ cinka, sledita baker, nikelj, mangan in kobalt, najmanj pa kadmija, svinca in arzena. Slednji trije so bili v večini vzorcev pod mejo zaznave. Med regijami so majhne razlike. V primerjavi z evropskimi študijami, npr. na Madžarskem (Kodrik, 2011), so bile ugotovljene višje vrednosti, razen v primeru niklja (zelo primerljive z našimi vrednostmi) in cinka (nižja vrednost, a vseeno izstopa, kar pripisujejo problematiki pesticidov). V zelenjavi smo svinec najpogosteje zaznali v vzorcih iz Celjske kotline. Korenje iz Celjske kotline je vsebovalo tudi več kadmija in železa kot korenje iz drugih dveh območij. Vsebnosti mangana, železa in niklja v korenju so bile primerljive z rezultati raziskav drugod po Evropi, in sicer s tistimi, ki so bile opravljene na neonesnaženih področjih. Vsebnosti cinka, bakra, kadmija in svinca v korenju pa se bolj ujemajo z vsebnostmi s srednje onesnaženih področij. Pri fižolu smo opazili najvišjo koncentracijo železa, zopet je izstopala Celjska kotlina. Primerjalno – na globalni ravni, pa so raziskave ugotavljale višje vrednosti svinca, izmerjeni cink v kitajski študiji, pa se je ujemal z vrednostmi naše raziskave (Hang Zhou in sod., 2016; Guerra in sod., 2011).

Ključne besede: kovine, kemijska analiza, mleko, korenje, fižol

Comparative study of the metal content in milk and vegetables (beans and carrots) in Slovenia with data from other European countries

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This research deals with metals as potential endocrine disruptors and studies their negative impacts on the flora, fauna and people. In this research, we have analysed the content of heavy metals in milk and vegetables, and determined potential harmfulness of these products. We compared the results between the three areas – the basin of Šalek and Celje and the Lower Savinja Valley. Emphasis was given to the (heavy) metal content in milk, carrots and beans, and we reviewed Slovenian, European and global research that touches this issue.
We analysed samples of milk, carrots and beans, which were obtained from eighteen farms, six from each region. Samples were homogenized and digested with microwaves in the presence of HNO_3 and H_2O_2. Concentration of manganese, zinc, copper, cobalt, iron, cadmium, nickel and lead in solution was determined by IPC-MS.
Examined samples of milk contained high levels of zinc, followed by copper, nickel, manganese whereas concentrations of lead, arsen and cadmium were below the detection limit. Hungarian study (Kodrik, 2011), showed higher concentrations at above mentioned elements as in our study, but in the case of nickel and zinc. Carrots from Celje basin contained more cadmium and iron as carrot samples from other two regions. Concentrations of manganese, iron and nickel in carrots are comparable to other European studies, which were performed on uncontaminated sites. Concentrations of zinc, copper, cadmium and lead in carrots align better with traces measured at medium contaminated sites. With beans, we observed highest concentration of iron, especially in Celje basin. Globally other studies determined high lead traces in beans, whereas measured zinc was comparable with concentrations observed in our study (Hang Zhou in sod., 2016; Guerra in sod., 2011).

Key words: metals, chemical analysis, milk, carrots, beans

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In-silico orodja za oceno (eko-)toksikoloških lastnosti spojin – izkušnje iz projekta PROSIL

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Projekt PROSIL -»Promoting the use of in-silico methods in industry« iz okvira LIFE+ projektov, ki jih financira Evropska Komisija, je bil, kot že ime pove, namenjen promoviranju *in-silico* metod v industriji. S projektom smo želeli povezati laboratorije v industriji in v raziskovalnih inštitucijah, ki delujejo na področju ocene tveganja kemikalij. V okviru projekta smo deležnike iz industrije seznanili z obstoječimi *in-silico* orodji, ki se lahko uporabljajo za oceno toksikoloških lastnosti spojin.

Poznavanje toksičnih lastnosti spojin je pomembno pri ukrepih za zmanjševanje negativnih vplivov kemikalij na žive organizme v okolju in na človeka. Zato tudi evropska REACH zakonodaja zahteva registracijo vseh spojin, ki so ali vstopajo na evropski trg. Pri registraciji je potrebno podati tudi oceno toksičnosti, npr. vodne toksičnosti spojin, mutagenosti, rakotvornosti, bio-koncentracije, itd.. Eksperimentalna določitev toksičnosti na živih organizmih vzbuja etična vprašanja glede testiranja spojin na živih organizmih (vretenčarjih), raziskave so povezane z visokimi stroški in so dolgotrajne. V nekaterih primerih pa je takšno testiranje na živalih (vretenčarjih) že prepovedano, kot npr. pri kozmetičnih izdelkih (»Cosmetic Products Regulation, 2009«). *In-silico* orodja lahko znatno prispevajo k zmanjšanju uporabe eksperimentalnih metod na živih organizmih in predstavljajo pomembno alternativno metodo za oceno toksičnosti. Uporabo *in-silico* metod dopušča in podpira tudi REACH zakonodaja.

V okviru projekta smo zgradili nekaj novih modelov za napovedovanje toksičnosti spojin ter razvili nekaj novih *in-silico* orodij v ta namen. V okviru te predstavitve bo prikazanih nekaj teh rezultatov in možnosti, ki jih omogočajo novo-zgrajena orodja pri napovedovanju vodne toksičnosti za ribe, mutagenosti in rakotvornosti kemikalij.

Ključne besede: Ocena toksičnosti, vodna toksičnost, mutagenost, rakotvornost, *in-silico* orodja, registracija kemikalij.

In-silico tools for evaluation of (eco-)toxicological properties of compounds – experiences from PROSIL project

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The PROSIL project - "Promoting the use of in-silico methods in industry" from the framework of LIFE+ projects financed by the European Commission, as the name implies, was intended to promote in-silico methods in industry. With the project, we wanted to connect laboratories in industry and in research institutes working in the field of risk assessment of chemicals. During the project, we introduced existing in-silico tools for toxicity assessment of chemicals to the stakeholders from industry.
Knowing the toxicological properties of the compounds is important when new measures are taken to reduce adverse effects of chemicals to living organisms in the environment and to humans. Therefore, European REACH legislation requires the registration of all compounds that are already on the European market or are entering it. When registering the compounds, toxicity assessment of the compounds must be given (e.g.: aquatic toxicity, mutagenicity, carcinogenicity, bio-concentration, etc.). The experimental determination of toxicity to living organisms raises ethical issues regarding the testing of compounds on living organisms (vertebrates), the research is also associated with high costs and takes lots of time. In some cases, the testing on animals (vertebrates) is already prohibited, e.g. testing of cosmetic products ("Cosmetic Products Regulation, 2009"). In-silico tools can significantly contribute to reducing the use of the testing methods on living organisms and represent an important alternative for toxicity assessment. The use of in-silico tools is also permitted and supported by REACH legislation.

During the project we built new models for prediction of compounds' toxicity and developed some new in-silico tools for that purpose. Within this presentation some of these results will be shown and the possibilities of using these tools for prediction of toxicity of chemicals towards fish, their mutagenicity and/or carcinogenicity.

Keywords: toxicity assessment, aquatic toxicity, mutagenicity, carcinogenicity, in-silico tools, registration of chemicals.

Učinki mešanic bisfenolov na vodne organizme

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Raziskave ekotoksičnosti novo proizvedenih kemikalij na vodno okolje temeljijo na testiranju posameznih kemikalij z določanjem letalnih in subletalnih učinkov na vodne organizme iz različnih taksonomskih skupin. Odpadne in površinske vode, iztoki iz čistilnih naprav ter izcedne vode iz deponij so mešanice številnih kemikalij, kar lahko vodi v njihovo povečano toksičnost v primerjavi z vsoto toksičnosti posameznih kemikalij. Vzrok za povečane ali zmanjšane učinke mešanic na vodne organizme je v sinergističnih ali antagonističnih interakcijah med kemikalijami. Tak primer smo ugotovili v raziskavi ekotoksičnosti mešanic bisfenolov na rake in ribe. Pogosto v vodnem okolju kot onesnažilo najdemo bisfenol A (BPA), ki pa se zaradi poznanih škodljivih učinkov na ljudi in živali, predvsem v zvezi z motnjami endokrinega sistema, nadomešča z analogi, kot sta bisfenol F (BPF) in bisfenol AF (BPAF). Akutni in kronični učinki posameznih BPA, BPF in BPAF na nekatere vodne organizme so poznani, odgovorov organizmov, ki so izpostavljeni binarnim in terciarnim mešanicam, pa ne poznamo. V raziskavi smo uporabili uravnotežen način priprave mešanic, kar pomeni, da vsak bisfenol v mešanici prispeva enak delež učinka k celotnemu učinku mešanice bisfenolov. Ugotavljali smo akutno toksičnost mešanic bisfenolov na gibanje vodnih bolh (*Daphnia magna*) in preživetje ter subletalne učinke (odsotnost pigmentacije, neizvaljenost, pojav edema) na zarodke zebric (*Danio rerio*). Ugotovili smo, da je toksičnost različnih mešanic BPA, BPF in BPAF veliko večja, kot je predvidena toksičnost posameznih bisfenolov na osnovi aditivnega učinka tako na vodne bolhe, kot tudi na zarodke rib zebric. To pripisujemo vzajemnemu delovanju bisfenolov na tarčnem mestu v celici. Izrazito sinergistično delovanje bisfenolov na gibanje vodnih bolh smo ugotovili v mešanicah BPA in BPAF, pri čemer je bil BPAF najbolj strupen. Glede na to, da so toksični učinki različnih mešanic bisfenolov na zarodke zebric, predvsem pa na vodne bolhe, opazni že pri koncentracijah, ki so dejansko prisotne v vodnem okolju, je potrebno raziskati tudi kronične učinke na vodne organizme pri dolgotrajnih izpostavitvah mešanicam bisfenolov.

Ključne besede; bisfenol A, bisfenol AF, bisfenol F, *Danio rerio*, *Daphnia magna*, letalni učinki, subletalni učinki, sinergizem.

Effects of mixtures of bisphenols on aquatic organisms

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Ecotoxicity studies of newly produced chemicals are based on testing of individual chemicals by determination of lethal/sublethal effects on aquatic organisms from different taxonomic groups. Waste and surface waters, effluents from waste water treatment plants and landfill leachates are complex mixtures of chemicals. This can result in increased/decreased overall toxicity in comparison to the sum of toxicities of individual chemicals, which is a consequence of synergistic or antagonistic interactions between chemicals. Such a case was found in our ecotoxicity study of mixtures of bisphenols to crustacea and fish. It is well-known that bisphenol A (BPA) induces adverse effects on humans and wildlife, particularly in relation to estrogen-like activity. Therefore, a demand for its replacement with less harmful analogues such as bisphenol F (BPF) and bisphenol AF (BPAF) has grown. The acute and chronic effects of individual BPA, BPF and BPAF on some aquatic organisms are known, but the effects of mixtures are unknown. In this study, a balanced design was used to prepare mixtures since each bisphenol is expected to contribute equally to the overall toxicity of a bisphenol mixture. We studied the acute toxicity of mixtures of bisphenols to mobility of water fleas (*Daphnia magna*) and lethal as well as sublethal effects (depigmentation, hatching success, pericardial edema) to zebrafish embryos (*Danio rerio*). We found out that the toxicity of different BPA, BPF and BPAF mixtures to water fleas and zebrafish embryos was higher in comparison to the expected toxicity of individual bisphenol based on additive effects. This could be attributed to the interactions between bisphenols at the target site in the cell. Synergistic effects were clearly observed in the case of water fleas in the BPA and BPAF mixtures, with being the BPAF the most toxic. Considering that the toxic effects of various mixtures of bisphenols to zebrafish embryos and, in particular, water fleas are already noticeable at concentrations actually present in the aquatic environment, it is also necessary to investigate the chronic effects on aquatic organisms during long-term exposure to the mixtures of bisphenols.

Keywords: bisphenol A, bisphenol AF, bisphenol F, *Danio rerio*, *Daphnia magna*, lethal effects, sublethal effects, synergism.

Pojavnost in kroženje bisfenolov med čiščenjem odpadnih vod

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Študija opisuje pojavnost in kroženje bisfenola A (BPA) in osem njegovih nadomestkov (BP): BPAF, BPAP, BPB, BPC, BPE, BPF, BPS in BPZ v odpadnih vodah (OV). Priprava vzorcev vključuje ekstrakcijo na trdnem nosilcu, derivatizacijo in analizo s plinsko kromatografijo z masno spektrometrijo, v primeru identifikacije transformacijskih produktov pa s tekočinsko kromatografijo s tandemsko masno spektrometrijo. Validirano metodo (LOD v ngL^{-1}) smo uporabili za analizo OV s petih čistilnih naprav (ČN), na področju ČN Domžale-Kamnik (DK) in Ljubljana (LJ) pa smo analizirali tudi glavne vire OV. Kot alternativni postopek čiščenja smo preučevali fotoragradnjo (UV, 254 nm) BPF, BPS in BPZ, kjer smo določali odstotek odstranitve, kinetiko in tvorbo stabilnih transformacijskih produktov.

Po pričakovanjih je bila najvišja vsebnost BPA (do 6 μgL^{-1}), medtem, ko je bila prisotnost BP potrjena v treh virih DK in LJ OV. Visoke kumulativne vrednosti BP smo določili v odpadnih vodah iz prehrambene industrije (LJ: 3,030 ngL^{-1} in DK: 599 ngL^{-1}). Rezultati so tudi pokazali, da sta bila samo BPF in BPS prisotna >LOD v vzorcih dotokov, medtem ko so bili ostale spojine določene tudi v iztokih. Med izbranimi BPji smo določili BPZ v najvišji koncentraciji (403 ng L^{-1}) v OV ČN DK, ki je vsebovala tudi največ BPE (238 ngL^{-1}). Četudi ne moremo uspešnosti odstranitve izbranih spojin neposredno primerjati med različnimi ČN, lahko zaključimo, da so bili BP večinoma odstranjeni >90 %. Poleg tega smo preučevali tudi kroženje in tvorbo stabilnih transformacijskih produktov (TP) ter določili pseudo prvi red razgradnje bisfenolov. Preučevanje tvorbe stabilnih TP pa je pokazalo tvorbo hidroksiliranih in drugih razgradnih produktov, katerih določitev strukture trenutno poteka.

V naši raziskavi smo pokazali prisotnost izbranih bisfenolov v OV, kar nakazuje, da so bisfenoli postali del našega okolja. Njihove potencialne strupene učinke pri koncentracijah, v katerih so zaznani in v mešanicah, v katerih se nahajajo, pa je potrebno še preučiti.

Ključne besede: BPA, bisfenoli, nadomestki, odpadne vode, viri, okolje

Occurrence and Fate of Bisphenols during Wastewater Treatment

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This study reports the occurrence of bisphenol A (BPA) and eight bisphenols (BPs): BPAF, BPAP, BPB, BPC, BPE, BPF, BPS and BPZ in wastewaters (WWs). Sample preparation involved pre-concentration (SPE), derivatization and analysis by gas chromatography-mass spectrometry. Transformation products were characterized directly by liquid chromatography tandem mass spectrometry. The validated method (LOD<ngL-1) was applied to five Slovene WWTP inflows and to WW inflows from industrial, commercial and residential sources into the Domžale-Kamnik and Ljubljana sewerage systems. Photodegradation (UV, 254 nm), as an alternative water treatment, was studied to determine removal efficiency, kinetics and identification of stable transformation products of BPF, BPS and BPZ.

BPA was found in the highest concentration in all wastewater samples (≤6 μgL-1). Other bisphenols were found in three DK and LJ inflows. The highest cumulative levels of all BPs were in WW from meat processing facilities (LJ: 3,030 ngL-1 and DK: 599 ngL-1). The analysis of WW from WWTPs revealed that only BPF and BPS were >LODs in the influents, whereas other BPs were present in the effluents. BPZ had the highest concentration (403 ngL-1 in DK catchment). This WW also contained the highest amount of BPE (238 ngL-1). Levels of BPC >LOD are reported for first time. Although BPs removal could not be directly compared between WWTPs, in general large amounts were removed (≥ 90 %). The cycling and the formation of stable TPs of selected BPs with UV were studied. Kinetic profiles and degradation efficiencies were established and showed pseudo first order kinetics. Structural elucidation indicated the formation of hydroxylated TPs and cleavage products. In addition, new TPs were detected and their structure is being elucidated.

In conclusion, the presence of BP residues in wastewaters samples shows how ubiquitous BPs residues are. Despite this, their toxicity and that of their mixtures at detected concentrations are yet to be determined.

Keywords: BPA, bisphenols, alternative, wastewaters, sources, environment

The importance of internal and external communication during emergency situations

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The Swedish experience is that 80 % of the success associated with incident is communication and only 20 % is related to the actual clean-up and remediation. The impacted parties and the entire society must be assured that the incident management staff is doing their job and that the resources are used efficiently.

The key elements of efficient communication are:

1. Identify roles and chain of command. Appoint one person as responsible for the communication (Public Relations person).
2. Call a press conference where the PR representative is presented. Just a brief information regarding the incident.
3. Involve media, the PR representative (with a side-kick) to take care of all practical arrangements. Make sure that all participants understand the importance of not making headlines, but instead assisting the incident staff in conveying important information.
4. If possible arrange a public hearing/meeting, the direct interaction between incident management staff and impacted parties is usually of great value.
5. When an incident occurs such as an oil spill, people often has unrealistic hopes and demands on the speed of the clean-up and the end results. What to expect must be communicated from the very beginning.

Hence, the following components are necessary:

1. Preparedness. When an emergency/incident occurs, no doubt, there will be chaos. To structure the confusion, plans must be made in advance how to meet such a situation.
2. Formation of an internal platform/phone list/digital communications system to make sure that all relevant parties get the same information.
3. Frequent and short meetings for the incident management staff to inform about the current state and avoid unnecessary misunderstandings.
4. Formation of an external platform. E.g. a phone number/home page where the public may call in/submit questions.

Potek ocenjevanja glifosata na ravni Evropske unije

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Glifosat je aktivna snov (a.s.) v fitofarmacevtskih sredstvih (FFS) za zatiranje plevela. Ocenjevanje a.s. poteka v skladu z uredbo 1107/2009 pri Evropski upravi za varnost hrane (EFSA) in v skladu z uredbo 1272/2008 pri Evropskem uradu za kemikalije (ECHA). Strokovnjaki države članice (d.č.) ocenjevalke proučijo vse dostopne študije: o učinkovitosti a.s., njenih fizikalno-kemijskih, toksikoloških in ekotoksikoloških lastnostih, analitskih metodah za določanje, usodi in obnašanju v okolju ter ostankih v hrani in krmi. Toksikološke študije vključujejo izsledke pridobljene na celicah mikroorganizmov, celičnih kulturah, laboratorijskih živalih in v epidemioloških študijah ter informacije spremljanja zdravja delavcev in morebitnih zastrupljencev. Študije se presojajo v luči teže dokazov. Večjo težo imajo študije narejene v skladu z mednarodno sprejetimi testnimi smernicami in dobro laboratorijsko prakso. Zanesljivost študij se ocenjuje s pomočjo Klimischevega točkovanja. Pripravi se osnutek monografije o a.s. Osnutek pregledajo vse d.č. Pripombe in vprašanja obravnavajo strokovni odbori pri EFSA oziroma ECHA. ECHA odloči o razvrstitvi snovi, torej o nevarnosti snovi, ki je do ponovne presoje zavezujoča. EFSA poleg nevarnosti snovi oceni tudi tveganje za potrošnike, delavce, naključne opazovalce in prebivalce, ki živijo v bližini kmetijskih površin, na katerih se uporabljajo FFS. Po zaključeni oceni se a.s. lahko uporablja 10 let, če ne predstavlja tveganja za zdravje ljudi, drugih organizmov in podtalnice. Po tem obdobju se a.s. ponovno oceni. Vlagatelj je dolžan predložiti vse izsledke, ki so se nakopičili od zadnje presoje. V Evropski uniji ostaja glifosat do nadaljnjega razvrščen kot dražilen (povzroča hude poškodbe oči) in strupen za vodne organizme z dolgoročnimi posledicami, a ni razvrščen kot mutagen, rakotvoren, ali strupen za razmnoževanje.

Ne glede na obširnost študij pred registracijo a.s. je nemogoče natančno predvideti vse možne škodljive učinke. Zato je potrebno budno spremljanje (toksikovigilanca) tudi po registraciji. V primeru glifosata je to predvsem toksikovigilanca morebitnih učinkov pri ponavljajoči se izpostavljenosti.

The course of the assessment of glyphosate at the level of the European Union

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Glyphosate is an active substance (a.s.) in plat protection products (PPP) used for weed control. A.s. is assessed according to the Regulation 1107/2009 at the European Food Safety Authority (EFSA) and according to the Regulation 1272/2008 at the European Chemicals Agency (ECHA). The experts from the assessing member state (MS) examine all the available studies: on efficacy, physico-chemical, toxicological and ecotoxicological properties, analytical methods, environmental fate and behaviour, residues in food and feed. Toxicological studies include results gained in microorganisms, cell cultures, laboratory animals, epidemiological studies, workers health surveillance and eventual poisonings data. The studies are assessed applying weight of evidence approach. More weight is given to the studies done in line with the internationally accepted test guidance and good laboratory practice. The reliability is assessed using the Klimicsh score. A draft monograph subsequently commented upon by all MS is prepared. The EFSA and ECHA expert committees discuss the comments and questions. The ECHA decides on classification of the substance, i.e. on hazard, which is binding for all MS until the next assessment. Besides hazard, the EFSA also assesses the risk for consumers, workers, bystanders, and residents living in the vicinity of the agricultural sites where PPP are used. Once assessed, a.s. may be used for ten years provided it does not pose risk to human health, wildlife or groundwater. Thereafter, it is reassessed. The applicant is bound to submit all the data accumulated since the previous assessment. In the European Union, glyphosate remains classified as irritant (causes serious eye damage) and toxic to aquatic life with long lasting effects, but not classified as mutagen, carcinogen or toxic to reproduction.

Regardless of how extensive the pre-registration studies may be it is impossible to precisely predict all possible adverse effects. Therefore, after the registration toxicovigilance is essential. Talking of glyphosate, that is toxicovigilance of the eventual effects due to repeated exposure.

Bi morali uporabo glifosata prepovedati? Vprašanje, ki razdvaja Evropo!

Metka Filipič

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Glifosat je zelo učinkovit širokospektralni herbicid. Pripravki z glifosatom so prišli na tržišče leta 1974. Sprva je bila njegova uporaba zaradi neselektivne toksičnosti za rastline zelo omejena. Uvedba gensko spremenjenih na glifosat odpornih kultur, ter uporaba glifosata za desikacijo pred žetvijo, pa je njegovo uporabo močno povečala. Zaradi tega se njegovi ostanki pojavljajo v različnih živilih kar povečuje izpostavljenost potrošnikov s tem pa tudi tveganja za škodljive posledice. Zgodnje toksikološke raziskave so kazale, da ima nizko toksičnost za ljudi in netarčne okoljske organizme ter, da je biorazgradljiv zato so ga smatrali, da je »idealen herbicid«. Vendar pa novejše raziskave kar nekaj teh predpostavk zavračajo in postavljajo pod vprašanje varnost njegove uporabe. Tako je IARC je nedavno opredelila glifosat kot verjetno karcinogen za ljudi, vendar so mnenja glede njegove genotoksičnosti in karcinogenosti so deljena. EFSA in nedavno ECHA sta zaključili, da ga na osnovi razpoložljivih podatkov ni mogoče opredeliti kot genotoksičnega in karcinogenega za ljudi. Ne smemo zanemariti tudi potencialnih dolgoročnih negativnih vplivov glifozata na okolje. Vse več je podatkov o njegovih škodljivih vplivih na ne tarčne organizme, zaradi prisotnosti ostankov v okolju pa prihaja do selekcijske prednosti na glifosat odpornih rastlin in plevelov, kar vse vpliva na biološko raznolikost. Dejstvo je, da so herbicidni pripravki na osnovi glifosata v svetovnem merilu najbolj uporabljani herbicidi, njihova uporaba pa še narašča. Različni pogledi glede varnosti uporabe glifosata, ki pa niso vedno nepristranski, kažejo, da je problem zapleten in zahteva nadaljnje raziskave. V skladu s principom previdnosti je potrebno njegovo uporabo omejiti in strogo nadzorovati. Uvesti bo potrebno sistematski monitoring prisotnosti ostankov glifosata v živilih, kot tudi biomonitoring izpostavljenosti ljudi.

Should the use of glyphosate be banned? A question that divides Europe!

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Glyphosate is extremely effective wide spectrum herbicide. The glyphosate based preparations have entered the market in 1974. The use of glyphosate was at the beginning rather limited due to its non-selective phytotoxicity. The introduction of genetically modified agricultural plants resistant to glyphosate and the pre-harvest application for desiccation highly increased its use. As a result, its residues occur in different foods, which increases the exposure of consumers and hence the risks of harmful health effects. Early toxicological studies indicated that for humans and non-target organisms the toxicity of glyphosate is low and that it is readily biodegradable and was thus considered as "ideal herbicide". However, recent studies contradicted some of these assumptions and raise question of the safety of its use. Thus, IARC classified glyphosate as probable carcinogen for humans, while EFSA and ECHA recently concluded that it cannot be classified as genotoxic and carcinogenic for humans on the basis of the available data. In addition, we should not neglect potential long-term adverse effects of glyphosate to the environment. Data on its adverse effects on non-target organisms is accumulating and due to the presence of glyphosate residues glyphosate resistant plants and weeds geined selective advantage, which all affect biodiversity. The fact is that glyphosate is the most used herbicide worldwide and its use is still increasing. Various views on the safety of glyphosate use, which are not always unbiased, indicate that the problem is complex and requires further research. In accordance with the precautionary principle, its application should be limited and thoroughly controlled. Moreover, a systematic monitoring of the presences of glyphosate residues in food as well as biomonitoring of human exposure should be introduced.

POVZETKI POSTERJEV

Učinki dioksinu podobnega PCB-169 in dioksinu nepodobnega PCB-155 preko materinega mleka na razvoj stegnenice mladičev podgan

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Proizvodnja polikloriranih bifenilov (PCB), obstojnih lipofilnih onesnaževalcev okolja, je prepovedana že desetletja, a jih še dandanes najdemo v okolju in organizmih. V času gravidnosti prehajajo v plod in po skotitvi v mleko. V predhodni raziskavi predpubertetnih smo ugotovili zaviralne vplive PCB-169 na rast in mineralizacijo stegnenic (1). Namen nadaljnje raziskave je bil proučiti vpliv dioksinu podobnega PCB-169 in dioksinu nepodobnega PCB-155, individualno in v kombinaciji, na razvoj stegnenic pubertetnih podgan, izpostavljenih PCB preko materinega mleka.
Podganam seva Wistar smo po kotitvi intraperitonealno aplicirali PCB raztopljene v olivnem olju po predhodno opisanem protokolu (1, 2). Celokupno je prva skupina prejela 3 mg PCB-169/kg telesne mase (tm), druga skupina 12 mg PCB-155/kg tm ter tretja skupina 3 mg PCB-169 in 12 mg PCB-155/kg tm. Kontrolna skupina je prejela olivno olje. Samičkam žrtvovanim 42. dan po skotitvi, smo odvzeli kri za biokemične analize ter stegnenice za geometrične (μCT) in biomehanične (tri-točkovni upogibni test) meritve.
Pri 42-dni starih podganah so bile krvne koncentracije PCB v posameznih poskusnih skupinah 6- do 13-krat nižje v primerjavi z 22-dnevnimi mladiči (2). Nadalje tudi ugotavljamo, da so bile najbolj prizadete stegnenice podgan izpostavljenih PCB-169, ki so bile krajše in tanjše (zunanji in notranji premer ter debelina scelne kostnine v diafizi) v primerjavi z ostalimi poskusnimi skupinami, nakazuje pa se tudi negativni trend rasti v skupini PCB-155, ki ni bil zaznan v predpubertetnem obdobju (1). Od serumskih kazalnikov sta bila povišana kalcij v skupinah PCB-169 in PCB-155 v primerjavi s kontrolo in aktivnost alkalne fosfataze v skupini PCB-169 (nakazuje hepatotoksičnost).
Rezultati kažejo, da je zaviralni vpliv laktacijske izpostavitve dioksinu podobnemu PCB-169 na rast stegnenic prisoten tudi med navalom pubertetne rasti, nasprotno pa se pri dioksinu nepodobnemu PCB-155 pojavi šele v pubertetnem obdobju.

Ključne besede: poliklorirani bifenili; stegnenica; biokemijski parametri; geometrija; biomehanika; podgane

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Effects of dioxin-like PCB-169 and non-dioxin-like PCB-155, through dam's milk, on rat offspring femur development

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Production of polychlorinated biphenyls (PCBs), persistent lipophilic environmental pollutants, has been banned for decades, but still they can be found in the environment and organisms. PCBs can be transmitted to the fetus and after delivery excreted into milk. Our preceding study on prepubertal rat offspring showed inhibitory effects of PCB-169 on femur growth and mineralization (1). The aim of this study was to evaluate the effects of dioxin-like PCB-169 and non-dioxin-like PCB-155 individually and in combination, on femur development of pubertal female rat offspring, lactationally exposed to PCBs.

After delivery, female Wistar rats were intraperitoneally administered PCBs dissolved in olive oil according to the previously described protocol (1, 2). In total, group 1 received 3 mg of PCB-169 per kg body mass (bm), group 2 12 mg of PCB-155 per kg bm, group 3 3 mg of PCB-169 and 12 mg of PCB-155 per kg bm, and the controls received olive oil. Offspring were sacrificed on postnatal day 42, blood samples collected for biochemical analyses and femurs for geometrical (μCT) and biomechanical (three-point bending test) measurements.

On postnatal day 42, PCB blood levels were 6- to 13-fold lower compared to 22-day-old offspring (2). Further, markedly reduced femur length and width (outer/inner diameter at diaphysis and cortical bone thickness) was observed in the PCB-169 group compared to the other experimental groups, however a negative growth trend was also seen in the PCB-155 group that was not detected in the prepubertal period (1). Among serum parameters, calcium was increased in the PCB-169 and PCB-155 groups and the alkaline phosphatase activity in the PCB-169 group (suggesting hepatotoxicity).

Our data demonstrate that the inhibitory effect of lactational exposure to dioxin- like PCB-169 on femoral growth is also present during the onset of pubertal growth spurt, while in the case of non-dioxin-like PCB-155 it was first observed in the puberty.

Key words: polychlorinated biphenyls; femur; biochemical parameters; geometry; biomechanics; rats.

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Usposobljenost izvajalcev zdravstvene nege za posredovanje informacij o okoljskih dejavnikih tveganja

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Izvajalci zdravstvene nege so kot delavci v zdravstvu zaradi svoje prisotnosti ob pacientu pogosto tiste osebe, ki dajejo pacientom informacije. Od njih se pričakujejo le strokovne in relevantne informacije. Namen raziskave je bil proučiti znanje izvajalcev zdravstvene nege o najpogostejših okoljskih dejavnikih tveganja za zdravje in ugotoviti, ali lahko korektno posredujejo te informacije pacientom.

Na podlagi pregleda literature in drugih virov je bil izdelan anketni vprašalnik sestavljen iz sociodemografskega sklopa, sklopa kjer smo ugotavljali poznavanje delovanja zdravstveno-ekološke dejavnosti v Sloveniji, sklopa v katerem smo spraševali u uporabi znanja na njihovem delovnem mestu in željah po izobraževanju ter testa znanja o okoljskih dejavnikih tveganja. Anketiranje je potekalo preko spletnega orodja 1.ka. Anketirali smo izvajalce zdravstvene nege, do katerih smo dostopali preko spleta (elektronska pošta, Facebook) po principu snežne kepe. V analizo je bilo vključenih 113 vprašalnikov, ki jih je rešilo 14 % moških in 86 % žensk s srednješolsko ali višjo izobrazbo in različno dolgo delovno dobo. Podatki so bili analizirani s programom IBM SPSS 19.0 z vidika opisne statistike in bivariatne analize podatkov.

Izvajalci zdravstvene nege v večini poznajo organiziranost zdravstveno ekološke dejavnosti v Sloveniji, vendar slabše same naloge službe. Njihovo znanje o okoljskih dejavnikih tveganja je dobro (v povprečju doseženih 71,4 % ± 8,6 % na testu znanja). Statistično značilnih razlik ($p > 0,05$) glede na starost, stopnjo izobrazbe in delovno dobo nismo opazili. Redko svetujejo pacientom o okoljskih dejavnikih tveganja, vendar si vseeno želijo dodatnih izobraževanj s tega področja. Izvajalci zdravstvene nege so usposobljeni za podajanje osnovnih informacij o okoljskih dejavnikih tveganja. Glede na pokazan primanjkljaj na nekaterih področjih, bi bilo dobro raziskavo ponoviti na večjem vzorcu in z bolj kompleksnim vprašalnikom. Tako bi natančneje ugotovili, katere so tiste teme, ki jim je potrebno posvetiti več pozornosti med rednim in vseživljenjskim izobraževanjem izvajalcev zdravstvene nege.

Ključne besede: medicinske sestre, komunikacija tveganja

Competency of nurses for forwarding information on environmental risk factors

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Nurses are as health workers, because of their presence by the patient, those who provide information to the patient. Only professional and relevant information is expected from them. The purpose of the study was to explore the knowledge of nurses about the most common environmental risk factor for health and to determine whether they can correctly communicate this information to patients.

A questionnaire was compiled on the basis of a literature review. It consisted of a sociodemographic set, a set where we determined the knowledge about functioning environmental health service in Slovenia, a set in which we asked about the use of knowledge at their workplace and the wishes for education and the test of knowledge about environmental risk factors. The survey was conducted via online tool 1.ka. We surveyed nurses that we accessed online (e-mail, Facebook) according to the principle of snowball. 113 questionnaires, which were resolved by 14 % of men and 86 % of women with secondary or higher education and a different length of service, were included in an analysis. Data were analysed with IBM SPSS 19.0 program in terms of descriptive statistics and bivariate data analysis.

Nurses mostly know the organisation of environmental health service in Slovenia, but poorer their tasks. Their knowledge of environmental risk factors is good (on average 71.4% ± 8.6% in the knowledge test). There were no statistically significant differences ($p > 0.05$) with respect to age, level of education and length of service. They advise patients about environmental risk factors rarely, but they still want additional training in this field.

Nurses are qualified to provide the basic information on environmental risk factors. According to the deficit in some fields, it would be good to repeat research on a larger sample and with more complex questionnaire. We would find out that way more carefully, which are those topics that need to be given more attention during regular and lifelong education of nurses.

Keywords: nurses, risk communication