



## 7. KONGRES SLOVENSKEGA TOKSIKOLOŠKEGA DRUŠTVA

### IZZIVI IN PASTI KAJENJA TOBAKA IN POVEZANIH IZDELKOV

### CHALLENGES AND TRAPS OF TOBACCO SMOKING AND RELATED PRODUCTS

Ljubljana, 5. 11. 2024





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## SPREMNA BESEDA

*Spoštovani udeleženci 7. kongresa Slovenskega toksikološkega društva!*

*Kajenje predstavlja resno nevarnost za zdravje, saj povzroča številne bolezni, vključno z rakom, boleznimi srca in ožilja ter dihalnimi težavami. Nove oblike kajenja, kot so elektronske cigarete in segreti tobačni izdelki, prav tako prinašajo tveganja, saj vsebujejo škodljive kemikalije, ki lahko povzročijo zasvojenost in druge zdravstvene težave.*

*Elektronske cigarete, znane tudi kot e-cigarete ali vape, delujejo tako, da segrevajo tekočino, ki običajno vsebuje nikotin, arome in druge kemikalije. Čeprav se pogosto oglašujejo kot manj škodljiva alternativa tradicionalnim cigaretam, raziskave kažejo, da lahko uporaba e-cigaret povzroči poškodbe pljuč, bolezni srca in druge zdravstvene težave. Nekateri tobačni izdelki segrevajo tobak na nižjo temperaturo kot običajne cigarete, kar naj bi zmanjšalo količino škodljivih kemikalij. Kljub temu pa ti izdelki še vedno vsebujejo nikotin in druge škodljive snovi, ki lahko povzročijo zasvojenost in zdravstvene težave. Zmotno je prepričanje da so elektronske cigarete uradno že odobrene s strani zdravstvenih pristojnih organov kot pomoč pri odvijanju od kajenja tradicionalnih cigaret. Zdravju neškodljive oblike kajenja ni.*

*V Sloveniji je uporaba tobačnih izdelkov strogo regulirana. Zakon o omejevanju uporabe tobačnih in povezanih izdelkov (ZOUTPI) iz leta 2017 prepoveduje oglaševanje, sponzoriranje in promocijo tobačnih izdelkov ter prodajo mladoletnim. Prav tako je prepovedano kajenje v zaprtih javnih in delovnih prostorih ter v vozilih, ko so prisotne mladoletne osebe. Poleg tega zakonodaja zahteva, da proizvajalci in uvozniki tobačnih izdelkov redno poročajo o sestavinah in emisijah svojih izdelkov ter zagotavljajo sledljivost tobačnih izdelkov od proizvodnje do prodaje. Slovenija si prizadeva zmanjšati uporabo tobaka in povezanih izdelkov z različnimi ukrepi, vključno z ozaveščanjem javnosti o škodljivosti kajenja in spodbujanjem opuščanja kajenja ter čimhitrejšim vključevanjem novih izdelkov v zakonodajni okvir.*

*Uporaba tobačnih izdelkov predstavlja tudi eno največjih groženj zdravju mladih v Sloveniji. Po podatkih pri nas kadi 24 odstotkov starejših od 15 let, mladi se s tobačnimi izdelki srečajo že v osnovni šoli. Kajenje je za mlade še posebej škodljivo in ima lahko dolgoročne posledice na razvoj možganov in razvoj drugih oblik odvisnosti. Glavna ovira za učinkovito zmanjševanje kajenja je razvoj zasvojenosti, zato sta tako preprečevanje začetka kajenja kot ustrezna strokovna pomoč pri opuščanju kajenja izrednega pomena.*

*V Sloveniji so programi za pomoč pri opuščanju kajenja v obliki skupinskih ali individualnih delavnic v zdravstvenih domovih po celi Sloveniji v Centrih za krepitev zdravja ali v Zdravstvenovzgojnih centrih. Za pomoč je na voljo tudi brezplačni svetovalni telefon 080 2777.*

*Upamo, da boste udeleženci v programu tega kongresa našli teme, ki bodo za vas še posebno zanimive, morda se s podobnim področjem, metodologijo tudi sam raziskovalno ali strokovno ukvarjate. Želimo si, da se znanja s tega področja povezujejo in dograjujejo tudi v slovenskem strokovnem in znanstvenem prostoru.*

Marija Sollner Dolenc  
Smiljana Milošev Tuševljak

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## PROGRAM/PROGRAMME

8.30-9.00	Registracija/Registration
9.00-9.05	Pozdravni nagovor /Welcome address
9.05-10.35	<b>Simpozij 1/Symposium 1</b> <b>Zakonodajni okvir in epidemiološko stanje/Legislative framework and epidemiology</b> Predsedujoči/Chair: Smiljana Milošev Tuševljak
9.05-9.35	Zakonodajni okvir tobaka in povezani izdelki Nataša Blažko, univ dipl soc, Ministrstvo za zdravje
9.35-10.05	Novi tobaki in nikotinski izdelki v Sloveniji Helena Koprivnikar, dr. med. spec., Nacionalni inštitut za javno zdravje
10.05-10.35	»Austrian Guideline for the evaluation of Heated Tobacco Products in a legal Framework» Mag. Dr. Katharina Vejdovsky, MScTox ERT, Austrian Agency for Health and Food Safety (AGES); Predavanje na daljavo/Remote lecture
10.35 -11.05	Odmor: kava, čaj in ogled plakatov/Break: coffee, tea and poster viewing
11.05-12.35	<b>Simpozij 2/Symposium 2</b> <b>Vpliv na človeka – kliničnih primeri, in vpliv na organizme v okolju/Impact on humans - clinical cases, and impact on organisms in the environment</b> Predsedujoči/Chair: Lucija Peterlin Mašič
11.05-11.35	Prizadetost pljuč kot posledica kajenja cigaret in novih tobaka izdelkov – kronične in akutne manifestacije: klinični primeri. prim. Katarina Osolnik, dr. med., specialistka interne medicine in pnevmologije, Univerzitetna Klinika Golnik
11.35-12.05	Zastrupitve v povezavi s tobaknimi izdelki - predstavitev kliničnih primerov asist. Matej Dobravc Verbič, mag. farm., spec., Center za klinično toksikologijo in farmakologijo, Univerzitetni klinični center Ljubljana
12.05-12.35	Vpliv mikroplastike iz cigaretne filtre na vodne in kopenske organizme Prof dr Anita Jemec Kokalj, Univerza v Ljubljani, Biotehniška fakulteta
12.35-13.40	Kosilo in ogled plakatov/Lunch and poster viewing
13.40-14.00	Podelitev častnega priznanja/Ceremony of Honorary Award to /prim. dr. Lucija Perharič, dr. med. s predavanjem/with lecture: Etnotoksikologija- srčika moje poklicne poti/Ethnotoxicology – the pith of my professional path
14.00-16.00	<b>Simpozij 3/Symposium 3</b> <b>Molekularni mehanizmi delovanja na človeka/Molecular mode of action on humans</b> Predsedujoči/Chair: Gorazd Drevenšek
14.00-14.45	Mehanizmi zasvojenosti z nikotinom in pomoč pri opuščanju/ Mechanisms of nicotine addiction and assistance in quitting Dr. Tomaž Čakš, dr. med. spec., Nacionalni inštitut za varovanje javnega zdravja
14.45-15.00	Odmor/Break
15.00-15.30	Kajenje med nosečnostjo: Vplivi na zdravje ploda in vloga nikotinskega nadomestnega zdravljenja ter elektronskih cigaret/ Smoking during pregnancy: Fetal Health Impacts and the Role of Nicotine Replacement Therapy and E-Cigarettes Tina Kek, dr. med., specialistka ginekologije in porodništva, prof. Dr. Ksenija Geršak, dr.med., specialistka ginekologije in porodništva, svet., Univerzitetni klinični center Ljubljana
15.30-16.00	Interakcije med kajenjem in zdravili na recept, Prof. dr. Lucija Peterlin Mašič, mag. farm., Univerza v Ljubljani, Fakulteta za farmacijo
16.00-17.00	<b>Diskusija in zaključne misli/Discussion and final thoughts</b> Predsedujoči/Chair: Jernej Kužner
16.00-16.30	Pogled v preteklost: Zgodovina tobaka in izzivi/ Tobacco history and challenges Dr. Marjan Vračko, Kemijski inštitut, Ljubljana
16.30-17.00	Pogled v naprej: Razprava in zaključek





POVZETKI VABLJENIH PREDAVANJ

## **Zakonodajni okvir in epidemiološko stanje/Legislative framework and epidemiology**

## Zakonodajni okvir tobačnih in povezanih izdelkov

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**Ključne besede:** tobačni izdelki, povezani izdelki, nikotinski izdelki, zakonodaja

V Sloveniji že vrsto let zakonsko omejujemo uporabo tobačnih izdelkov, v zadnjem obdobju pa tudi povezanih izdelkov. Pri tem sledimo tako Okvirni konvenciji Svetovne zdravstvene organizacije za nadzor nad tobakom, katere pogodbenica smo že od leta 2005, kot evropskim direktivam na tem področju. Leta 2017 smo, poleg tobačnih izdelkov, na podlagi Direktive 2014/40/EU Evropskega parlamenta in Sveta z dne 3. aprila 2014 o približevanju zakonov in drugih predpisov držav članic o proizvodnji, predstavitvi in prodaji tobačnih in povezanih izdelkov in razveljavitvi Direktive 2001/37/ES, začeli zakonsko regulirati tudi povezane izdelke, kot so npr. elektronske cigarete, tobačni izdelki za segrevanje in zeliščni izdelki za kajenje, ki ne vsebujejo tobaka, ampak rastline, zelišča ali sadje. V letu 2024 smo na nacionalni ravni med povezane izdelke dodali še nove nikotinske izdelke, ki vsebujejo nikotin, a ne vsebujejo tobaka in niso registrirani izdelki za nikotinsko nadomestno zdravljenje.

Z različnimi zakonskimi ukrepi kot so: prepoved kajenja in uporabe povezanih izdelkov v vseh zaprtih javnih in delovnih prostorih in v vseh vozilih v navzočnosti mladoletnih, prepoved privlačnih arom v cigaretah, tobaku za zvijanje, ogrevanih tobačnih izdelkih in elektronskih cigaretah; popolna prepoved oglaševanja, promocije, doniranja ali sponzoriranja in razstavljanja tobačnih in povezanih izdelkov na mestih prodaje; prepoved prodaje tobačnih in povezanih izdelkov mladoletnim, obvezna dovoljenja za prodajo v poslovnih prostorih ter prepoved prodaje tobačnih in povezanih izdelkov po spletu oz. na daljavo ter ukrepi za preprečevanje črnega trga, prispevamo k omejevanju uporabe teh izdelkov in se pridružujemo najnaprednejšim državam na tem področju.

Velik izziv pa predstavlja pojavljanje novih izdelkov na tržišču, ki še niso zakonsko regulirani in so narejeni tako, da so posebej privlačni za otroke in mladostnike, zato je v prihodnje izredno pomembno hitro ukrepanje in dobro mednarodno sodelovanje.



## Legislative framework for tobacco and related products

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**Key words:** tobacco products, related products, nicotine products, legislation

In Slovenia, we have been legally restricting the use of tobacco products for many years, and more recently also related products. In doing so, we follow both the World Health Organization Framework Convention on Tobacco Control, to which we have been a party since 2005, and European directives in this area. In 2017, in addition to tobacco products, on the basis of Directive 2014/40/EU of the European Parliament and of the Council of 3 April 2014 on the approximation of the laws, regulations and administrative provisions of the Member States concerning the manufacture, presentation and sale of tobacco and related products and repealing Directive 2001/37/EC, we also began to legally regulate related products, such as electronic cigarettes, heated tobacco products and herbal products for smoking that do not contain tobacco but contain plants, herbs or fruits. Since 2024, at the national level, we also regulate novel nicotine products, which contain nicotine but do not contain tobacco and are not registered products for nicotine replacement therapy as related products.

With various legal measures such as: ban on smoking and ban on using related products in all enclosed public places and workplaces and in all vehicles in the presence of minors, ban on attractive flavors in cigarettes, roll-your-own tobacco, heated tobacco products and electronic cigarettes; a total ban on advertising, promotion, donation or sponsorship, including a point-of-sale display ban of tobacco and related products; ban on the sale of tobacco and related products to minors, mandatory tobacco and related products retail licensing system and ban on domestic and cross-border distance sales of tobacco and related products and measures to prevent illicit trade, we contribute to limiting the use of these products and join the most advanced countries in this field.

A major challenge represent novel and emerging tobacco and nicotine products appearing on the market that are not yet regulated by law and are especially designed to be particularly attractive to children and young people, therefore quick action and good international collaboration in this field are extremely important in the future.

## Novi tobačni in nikotinski izdelki v Sloveniji

Helena Koprivnikar, Tina Zupanič, Maruša Rehberger, Darja Lavtar

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**Ključne besede:** tobačni izdelki, nikotin, elektronski sistemi za dovajanje nikotina

V Sloveniji so dostopni različni novi tobačni in nikotinski izdelki (elektronske cigarete, ogrevani tobačni izdelki, nikotinske vrečke). Odstotki uporabnikov teh izdelkov naraščajo ter so praviloma višji med mladostniki in mladimi odraslimi kot v starejših starostnih skupinah prebivalcev. Ob uporabi teh izdelkov je uporabnik izpostavljen znatnim ravnem zdravju škodljivih snovi ter podobnim ali celo višjim ravnem nikotina kot pri kajenju cigaret. Dolgoročni učinki njihove uporabe niso v celoti raziskani, saj so v uporabi prekratek čas. Dostopni podatki kažejo, da imajo škodljive učinke na zdravje, nekateri že kratkoročno, in da bodo pri dolgoročni uporabi zvišali tveganje za različne bolezni, npr. srca in ožilja. O manjši škodljivosti teh izdelkov v primerjavi s kajenjem cigaret ni možno govoriti, saj je na voljo še premalo kakovostnih raziskav. Pogosto se promovirajo kot učinkoviti pripomočki za opuščanje kajenja, a o tem ni na voljo zadosti kakovostnih raziskav in se jih v te namene ne priporoča.

V Sloveniji so vedno bolj popularni tudi brezdimni tobačni izdelki. Vsi omenjeni izdelki vsebujejo nikotin (razen elektronskih cigaret brez nikotina, ki se redkeje uporabljajo) in uporabnika lahko zasvojijo ali vzdržujejo zasvojenost. Uporaba izdelkov z nikotinom predstavlja dodatna pomembna tveganja za otroke, mladostnike in mlade odrasle, poleg zasvojenosti, ki se lahko razvije hitreje in močnejše kot pri odraslih, so to še škodljivi učinki na razvoj možganov, pri elektronskih cigaretah in brezdimnih tobačnih izdelkih pa tudi zvišano tveganje za kajenje cigaret.

Zakonodaja v Sloveniji regulira omenjene izdelke, med ključnimi ukrepi so prepoved oglaševanja, razstavljanja in promocije, prepoved prodaje mladoletnim, dovoljenja za prodajo, prepoved prodaje po internetu v Sloveniji ter prepoved uporabe ogrevanih tobačnih izdelkov in elektronskih cigaret povsod, kjer je prepovedano kajenje tobaka. V ogrevanih tobačnih izdelkih so, tako kot v cigaretah, prepovedane značilne arome, v elektronskih cigaretah pa bo aprila 2025 začela veljati prepoved vseh arom, z izjemo določenih tobačnih.

## **New tobacco and nicotine products in Slovenia**

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**Key words:** tobacco products, nicotine, electronic nicotine delivery systems

New tobacco and nicotine products (e-cigarettes, heated tobacco products, nicotine pouches) are available in Slovenia, the percentage of their users increases and is generally higher among adolescents and young adults than among older inhabitants. The user is exposed to significant levels of harmful substances and to similar or higher levels of nicotine compared to cigarette smokers. The long-term effects of use have not been fully investigated. The available data suggest that they have adverse health effects, some already short-term, and that their long-term use will increase the risk of various diseases, e.g. cardiovascular. It is not possible yet to speak of lower harmfulness of these products compared to cigarette smoking, as there is still a lack of good quality research. They are often promoted as effective smoking cessation aids, but there is insufficient good quality research and they are not recommended for this purpose.

Smokeless tobacco products are also increasingly popular in Slovenia. All of these products contain nicotine (except nicotine-free e-cigarettes, which are less commonly used) and can addict the user or maintain addiction. The use of nicotine products poses additional significant risks to children, adolescents and young adults, including, in addition to addiction, which can develop more quickly and more strongly than in adults, adverse effects on brain development, and, in the case of e-cigarettes and smokeless tobacco, an increased risk of cigarette smoking.

Slovene legislation regulates these products, key measures include a ban on advertising, display, promotion and sales to minors, sales permits, a ban on internet sales in Slovenia and a ban on the use of heated tobacco products and e-cigarettes wherever tobacco smoking is prohibited. Characteristic flavours are banned in heated tobacco products, in e-cigarettes a ban on all flavours (except certain tobacco) will come into force in April 2025.

## **Austrian Guideline for the evaluation of Heated Tobacco Products in a legal Framework**

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**Keywords:** heated tobacco products, novel tobacco products, authorisation, emissions, *in vitro* toxicological tests

Heated Tobacco Products are tobacco products that produce aerosols containing nicotine and other chemicals, which are inhaled by users, through the mouth. According to European legislation they are novel tobacco products and are so far, the only ones known on the European market. In Austria all novel tobacco products need to be authorised by the Federal Ministry of Health before they can be marketed. As a prerequisite for approval, it must be demonstrated for the respective novel tobacco product that its consumption does not raise greater health concerns than the consumption of already available, comparable tobacco products like e.g. cigarettes. In order to provide clarity for applicants and, above all, to ensure that different applications are treated equally, a guideline has been drawn up setting out the exact requirements for the data regarding toxicological information to be included in an authorisation application. In specific, detailed data on the emissions of the novel tobacco products as well as *in vitro* toxicological tests of the aerosol fractions must be provided and compared with those of conventional tobacco products (e.g. cigarettes).

Finally, it must be pointed out that the data, stated in the guideline as requirements for novel tobacco products in the frame of the Austrian authorisation process, do not enable an exhaustive and conclusive evaluation of health risks of heated tobacco products but serve to comply with the authority's request within the legal framework.



## POVZETKI VABLJENIH PREDAVANJ

**Vpliv na človeka – kliničnih primeri, in vpliv na organizme v okolju/  
Impact on humans - clinical cases, and impact on organisms in the  
environment**

## **Prizadetost pljuč kot posledica kajenja cigaret in novih tobačnih izdelkov – kronične in akutne manifestacije: klinični primeri**

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**Ključne besede:** kajenje, pljučna prizadetost, klinični znaki in simptomi

Znane s kajenjem povezane pljučne bolezni so: kronična obstruktivna pljučna bolezen, pljučni rak, histiocitoza pljučnih Langerhansovih celic, zgodnji razvoj emfizema pri osebah z genetskimi motnjami: npr. genetska motnja pomanjkanja alfa-1-antitripsina, respiratorni bronhiolitis in respiratorni bronhiolitis-intersticijska pljučna bolezen ter deskvamativna intersticijska pljučnica.

Kajenje je tudi rizični ali sprožilni dejavnik za več različnih intersticijskih pljučnih bolezni: idiopatska pljučna fibroza, intersticijska pljučna bolezen pri revmatoidnem artritisu, kombinacija fibroze in emfizema, akutna eozinofilna pljučnica, sindrom alveolarne hemoragije.

Problem je, da se kajenje elektronskih cigaret pogosto med laiki smatra za manj škodljivo kot kajenje cigaret in ga nekateri celo uporabljajo za odvajanje od cigaret, kar lahko vodi v sočasno uporabo obeh vrst izdelkov.

Zaradi relativno kratkega obdobja uporabe elektronskih cigaret za zdaj še ni na voljo dolgoročnih raziskav, ki bi pokazale njihov vpliv na zdravje, so pa v klinični praksi že zaznane akutne posledice, ki nakazujejo, kakšne daljnosežne učinke lahko pričakujemo.

V prispevku bo predstavljenih nekaj kliničnih primerov, ki so povezani s kajenjem klasičnih cigaret in novih tobačnih izdelkov.



## **Lung damage as a result of smoking cigarettes and new tobacco products - chronic and acute manifestations: clinical cases**

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**Key words:** smoking, pulmonary involvement, clinical signs and symptoms

Known smoking-related lung diseases are: chronic obstructive pulmonary disease, lung cancer, pulmonary Langerhans cell histiocytosis, early development of emphysema in people with genetic disorders: e.g. genetic disorder of alpha-1-antitrypsin deficiency, respiratory bronchiolitis and respiratory bronchiolitis-interstitial lung disease as well as desquamative interstitial pneumonia.

Smoking is also a risk or trigger factor for several different interstitial lung diseases: idiopathic pulmonary fibrosis, interstitial lung disease in rheumatoid arthritis, combined fibrosis and emphysema, acute eosinophilic pneumonia, alveolar hemorrhage syndrome.

The problem is that electronic cigarette smoking is often perceived by layman as less harmful than cigarette smoking and is even used by some individuals for smoking cessation, which can lead to dual use of both types of products.

Due to the relatively short period of use of electronic cigarettes, there are currently no long-term studies available that would show their impact on health, but acute consequences have already been detected in clinical practice, which indicate what far-reaching effects can be expected.

We want to present some clinical cases related to smoking classic cigarettes and new tobacco products.

## Zastrupitve v povezavi s tobačnimi izdelki - predstavitev kliničnih primerov

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**Ključne besede:** tobačni izdelki, zastrupitev, izpostavljenost, klinični primeri

Zastrupitve s tobačnimi izdelki, podobno kot zastrupitve z drugimi povzročitelji, v Republiki Sloveniji beležimo v nacionalnem Registru zastrupitev, ki ga vodi Center za klinično toksikologijo in farmakologijo Univerzitetnega kliničnega centra Ljubljana. Podatki o zastrupitvah so celostno vodeni v elektronskem Registru od leta 2019. V zadnjih 10 letih je bilo v elektronskem Registru zavedenih 57 primerov nenamerne izpostavljenosti, zlorabe ali zastrupitev s tobačnimi izdelki. Pri veliki večini primerov so bili udeleženci mladoletne osebe. V 51 primerih je šlo za zaužitje tobačnega izdelka, od tega v 27 primerih za zaužitje cigaret, v preostalih primerih za fuge ali elemente elektronskih cigaret – tekočino, kartušo ali filter. V sedmih primerih so bili udeleženci izpostavljeni tudi drugim snovem, največkrat alkoholu. Šest je bilo primerov zlorabe tobačnih izdelkov pri otrocih in mladostnikih, starih od 8 do 16 let. Med slednjimi je šlo v petih primerih za uporabo fuge. V 42 primerih je šlo za nenamerno izpostavljenost pri otrocih, mlajših od treh let.

23/57 primerov izpostavljenosti je bilo asimptomatskih. V preostalih primerih so bili najpogosteje opisani simptomi zmedenost, nemir, somnolenca, vrtoglavica, bledica, znojenje, tahikardija, slabost, bruhanje in oslavljen mišični tonus. Pri nobenem izmed primerov ni šlo za hudo zastrupitev. Štirje so bili primeri zmerne zastrupitve. Pri teh so bili opisani pomembna motnja zavesti (z oceno po Glasgowski lestvici pod 8 – ob sočasnem uživanju alkohola), sinkopa, cianoza, oslavljen mišični tonus, tahikardija, bledica in bruhanje.

Akutne zastrupitve s tobačnimi izdelki so redke, klinična slika zastrupitev pa je običajno blaga. V zadnjih 10 letih so v Sloveniji prevladovali primeri nenamerne zaužitja tobačnih izdelkov pri dojenčkih in majhnih otrocih. Najučinkovitejši ukrep za preprečevanje zastrupitev je tako shranjevanje tobačnih izdelkov na mestu, ki je nedosegljivo otrokom.

## Tobacco-related poisonings: Case series presentation

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**Keywords:** tobacco products, poisoning, exposure, case reports

Poisoning cases with tobacco products in Slovenia are recorded in the National Register of intoxications, which is managed by the Centre for Clinical Toxicology and Pharmacology at the University Medical Centre Ljubljana. All poisoning data are entered in the electronic Register since 2019. In the last 10 years, there were 57 electronically registered cases of unintentional exposure, abuse or poisoning related to tobacco products. The vast majority of cases involved patients under 18 years of age. A tobacco product or part of it was ingested in 51 cases. 27 cases involved the ingestion of cigarettes, and the remaining cases involved nicotine pouches or elements of electronic cigarettes – liquid, cartridge or filter. In seven cases, the patients were concomitantly exposed to other substances, mainly alcohol. There were six cases of abuse of tobacco products among children and adolescents aged 8 to 16 years. In five of these cases, nicotine pouches were used. 42 cases were unintentional exposures in children under 3 years of age.

23/57 registered cases were asymptomatic. In the remaining cases, confusion, restlessness, somnolence, dizziness, pallor, sweating, tachycardia, nausea, vomiting and decreased muscle tone were the most frequently described symptoms. Severe poisoning was not reported in any of the cases, whereas four cases demonstrated moderate poisoning, with one or several of the following signs and symptoms described: significant disturbance of consciousness (Glasgow Coma Scale of less than 8 - with concomitant alcohol consumption), syncope, cyanosis, weakened muscle tone, tachycardia, pallor and/or vomiting.

Acute poisonings with tobacco products are rare, and the clinical picture is usually mild. In Slovenia, accidental ingestions of tobacco products by infants and young children were most commonly implicated in the poisonings in the past 10 years. The most effective measure to prevent future poisonings is to store tobacco products in places inaccessible to children.

## Vpliv mikroplastike iz cigaretnih filtrov na vodne in kopenske organizme

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**Ključne besede:** mikroplastika, cigaretni filtri, ogorki, kovine, odziv organizmov, raki, žuželke, kotačniki

Cigaretni ogorki oz. pokajeni cigaretni filtri (SCF) predstavljajo enega najpomembnejših virov mikroplastike, saj se pojavljajo po vsem svetu in so v okolju v znatnih količinah. Njihovi učinki na organizme, zlasti na talne nevretenčarje, niso dobro raziskani. Raziskali smo učinke mikroplastike iz pokajenih cigaretnih filtrov (SCF-MPs) in nepokajenih cigaretnih filtrov (UCF-MPs) na vodne nevretenčarje (*Daphnia magna* in *Brachionus calyciflorus*) in kopenske nevretenčarje (*Porcellio scaber* in *Tenebrio molitor*). Cigaretni filtri so bili podvrženi krio-mletju, iz njih pa je nastala mikroplastika za testiranje: SCF-MP ( $9,06 \pm 4,1 \mu\text{m}$ ) in UCF-MP ( $12,71 \pm 6,82 \mu\text{m}$ ). Obe vrsti mikroplastike sta bili kemijsko opredeljeni. Oba vzorca sta vsebovala triacetin in različne potencialno strupene kovine, SCF-MPs pa tudi nikotin in večje število spojin v sledovih. Obe vrsti MP sta bili akutno strupeni za vodne nevretenčarje in sta do 100 mg/L znatno zmanjšali stopnjo preživetja organizmov *B. calyciflorus* in *D. magna*. Medtem ko izpostavljenost SCF-MP ali UCF-MP (do 1,5 % m/m MP v tleh) ni vplivala na preživetje kopenskih nevretenčarjev *T. molitor* in *P. scaber* v 14 oziroma 28 dneh, smo opazili več fizioloških odzivov. Ti so vključevali spremembe imunskih parametrov, spremembe detoksifikacijskih encimov, aktivnosti acetilholinesteraze in z energijo povezanih biomarkerjev. MP iz pokajenih filtrov je na splošno povzročila izrazitejše učinke kot iz nepokajenih filtrov. Ta študija poudarja kompleksno naravo tovrstne mikroplastike, ki vsebuje mešanico kovin in organskih kemikalij, ki vplivajo na zdravje organizmov. Izpostaviti želimo potrebo po učinkovitem okoljskem upravljanju cigaretnih filtrov, ki obravnava tako MP iz pokajenih in nepokajenih cigaretnih filtrov.

**Zahvala:** Študijo podpira ARIS (J1-50014, P1-0184, P2-0273, P1-0153, P1-0175).

# The effects of cigarette filter microplastics on aquatic and terrestrial organisms

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**Keywords:** microplastics, cigarette butts, filters, metals, organism's response, crustaceans, insects, rotifera

Cigarette butts or smoked cigarette filters (SCF) represent one of the most important microplastic sources due to their global occurrence and the significant amounts found in the environment. Their effects on organisms, especially soil invertebrates, are not well studied. We have investigated the effects of microplastics from smoked cigarette filters (SCF-MPs) and unsmoked cigarette filters (UCF-MPs) on aquatic invertebrates (*Daphnia magna* and *Brachionus calyciflorus*) and terrestrial invertebrates (*Porcellio scaber* and *Tenebrio molitor*). Cigarette filters were subjected to cryo-milling and microplastics were produced for testing: SCF-MPs and UCF-MPs had a size of  $9.06 \pm 4.1 \mu\text{m}$  and  $12.71 \pm 6.82 \mu\text{m}$ , respectively. Both types of microplastics were chemically characterised. Both samples contained triacetin and a variety of potentially toxic metals, while SCF-MPs also contained nicotine and a larger number of trace compounds. Both types of MP were acutely toxic to aquatic invertebrates and significantly reduced the survival rates of *B. calyciflorus* and *D. magna* up to 100 mg/L. While exposure to SCF-MPs or UCF-MPs (up to 1.5% MPs, w/w in soil) did not affect the survival of the terrestrial invertebrates *T. molitor* and *P. scaber* over 14 and 28 days, respectively, several physiological responses were observed. These included changes in immune parameters, changes in detoxification enzymes, the activity of acetylcholinesterase and energy-related biomarkers. SCF-MPs generally caused more pronounced effects than UCF-MPs. This study emphasises the complex nature of these microplastics, which contain a mixture of metals and organic chemicals that affect the health of organisms, and highlights the need for effective environmental management that addresses both smoked and unsmoked cigarette filter MPs.

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POVZETKI VABLJENIH PREDAVANJ

**Predavanja ob prejemu častnega priznanja/Lecture upon honorary  
award receipt**

**prim. dr.Lucija Perharič, dr. med**



## Etnotoksikologija- srčika moje poklicne poti

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**Ključne besede:** etnofarmakologija, etnotoksikologija, rastlinski toksini, rastlinska zdravila, tropanski alkaloidi

Etnotoksikologija proučuje škodljive posledice etnofarmakoloških učinkovin in pripravkov, ki navadno izvirajo iz rastlin, živali, gliv, mikroorganizmov in mineralov. Uporabljajo se v ljudskem ali tradicionalnem zdravilstvu za preprečevanje in zdravljenje bolezni. Etnofarmakologija in etnotoksikologija sta me očarali med potovanjem po Južni Ameriki leta 1982, čeprav sta me v gojenje, nabiranje in uporabo zdravilnih rastlin vpeljali že mama in babica. Med magistrskim študijem toksikologije na Univerzi Surrey sem raziskovala teratogenost polifenolov pravega čaja. Leta 1990 sem na tedanjem Centru za zastupitve londonske bolnišnice *Guy's* vzpostavila projekt *Toksikološki problemi zaradi izpostavljenosti tradicionalnim zdravilom in prehranskim dopolnilom*, za katerega sem 1993 prejela nagrado fundacije *Rank Prize*. V letih 1998/99 sem sodelovala pri mednarodni klinični študiji, v kateri smo ugotavljali prednosti in tveganja različnih kombinacij antihipertenzivnih zdravil. V študijo smo poleg britanskih »staroselcev« vključili tudi bolnike različnih etničnih skupin, kar je predstavljalo poseben izziv z vidika njihove etnično pogojene percepcije škodljivih učinkov in interakcij med zdravili. Leta 2003 je v Sloveniji prišlo do množične zastupitve zaradi onesnaženosti ajdovega zrnja s semeni kristavca (*Datura* sp.) iz družine razhudnikovk. Kristavec vsebuje atropin in skopolamin, ki sta starodavni zdravili in starodavna strupa. *Datura*, ki so jo v preteklosti pogosto obredno uporabljali v številnih predelih sveta, še vedno uživajo šamani v Amazoniji. Iz množične zastupitve sem spletla globalno odmevno raziskavo *Opredelitev odnosa med odmerkom in učinkom pri oralnem vnosu nizkih odmerkov mešanice atropina in skopolamina v živilih*. Raziskava je postala temelj znanstvenega stališča o tropanskih alkaloidih v hrani Evropske agencije za varnost hrane (<https://www.efsa.europa.eu/en/efsajournal/pub/3386>) ter smernic Organizacije za hrano in kmetijstvo in Svetovne zdravstvene organizacije (<https://openknowledge.fao.org/server/api/core/bitstreams/cdcafea7-63dd-4c7c-a767-34b168a3904c/content>). Etno-učinkovine sem presojala tudi med desetletnim sodelovanjem z nacionalno skupino za opozarjanje o novih psihoaktivnih snoveh. V zadnjem času sem za učbenik *Kemijski dejavniki tveganja v prehrani* napisala poglavje *Rastlinski toksini v hrani* in pregledala primere uporabe zeliščnih preparatov kot dopinga.

## **Ethnotoxicology – the pith of my professional path**

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**Keywords:** ethnotoxicology, ethnopharmacology, herbal medicines, plant toxins, tropane alkaloids

Ethnotoxicology studies adverse effects of ethnopharmacological substances and products, usually originating from plants, animals, fungi, microorganisms and minerals, used in folk or traditional medicine for disease prevention and treatment. I became spellbound by ethnopharmacology and ethnotoxicology in 1982, during a voyage in South America, although I was previously introduced to cultivating, gathering and using of herbal medicines by my mother and grandmother. During my Master's in toxicology at the University of Surrey, I researched the teratogenicity of tea-extract polyphenols. In 1990, I initiated a project, *Toxicological problems resulting from exposure to traditional medicines and food supplements*, at the then Guy's Hospital's Poisons Unit in London. The project was awarded a Rank Prize in 1993. During 1998/99 I was employed on an international clinical trial, studying the advantages and risks of various combinations of antihypertensive medications. Besides the indigenous British patients, we also included those from ethnic minorities, facing particular challenges in view of their ethnicity driven perception of adverse effects and drug interactions. In 2003, a mass poisoning incident occurred in Slovenia due to contamination of buckwheat grain with seeds of thorn-apple (*Datura* sp.) of the nightshade's family. *Datura* contains atropine and scopolamine, both ancient medicines and poisons. Historically, *Datura* was often used ceremonially in many parts of the world, and is still ingested by shamans of the Amazon region. A study, *Oral dose-response relationship of low-dose atropine and scopolamine mixture as food contaminant*, knit from the poisoning incident was met with a wide response and provided the basis for the European Food Safety Authority's Scientific opinion on tropane alkaloids in food (<https://www.efsa.europa.eu/en/efsajournal/pub/3386>), as well as Food and Agriculture Organization's and World Health Organization's Guidance document (<https://openknowledge.fao.org/server/api/core/bitstreams/cdcafea7-63dd-4c7c-a767-34b168a3904c/content>). During my 10-year collaboration with the National group for warning on the new psychoactive substances I assessed a number of ethno-compounds. Lately, I contributed a chapter, *Plant toxins in food*, for the textbook *Chemical risk factors in nutrition* and surveyed the selected case studies of herbal substances used as doping.



## **Molekularni mehanizmi delovanja na človeka/Molecular mode of action on humans**

## Mehanizmi zasvojenosti z nikotinom in pomoč pri opuščanju

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**Ključne besede:** nikotin, zasvojenost, pomoč pri opuščanju

Nikotin je psihoaktivna snov, ki ima veliko vplivov na organizem. Potrjen je obstoj bioloških in vedenjskih mehanizmov, ki so odgovorni za nastanek zasvojenosti. Ponavljajoče se izpostavljanje učinkom nikotina vodi do nevroadaptacije, ta pa podpira vedenje, usmerjeno v potrebo po drogi ter vzpostavljanje tolerance. Nikotin se zelo hitro absorbira skozi pljuča, kožo in sluznico. Deluje preko acetilholinskih receptorjev, ki pospešujejo sproščanje različnih nevroloških prenašalcev. Nikotin se veže prednostno na nikotinske acetilholinergične receptorje (nACh) v mezolimbičnem dopaminskem sistemu, pri čemer je najpomembnejši nikotinski receptor  $\alpha 4\beta 2$  v ventralnem tegmentalnem področju (VTA). Po vezavi nikotina na nikotinski receptor  $\alpha 4\beta 2$  pride do sproščanja dopamina v nucleusu accumbensu, ki je povezan s sistemom nagrajevanja v možganih. Pri zasvojenosti z nikotinom so poleg dopamina pomembni tudi drugi nevrološki prenašalci, ki se najpogosteje sproščajo po vezavi nikotina na receptorje: noradrenalin, acetilholin, serotonin, vazopresin,  $\beta$ -endorfin, glutamat. Dopamin vpliva na občutje ugodja in nagrajevanja, podobno kot pri zlorabi kokaina, heroina in alkohola; noradrenalin povzroča budnost in je prisoten pri obrambni reakciji telesa v stresnih situacijah; serotonin oblikuje razpoloženje in apetit. Živčni sistem se pri mnogih drogah, ki se danes uporabljajo, adaptira na njihovo stalno prisotnost. Ob prekinitvi dovajanja droge se pojavijo telesni odtegnitveni simptomi kot odziv na zmanjšanje sproščanja nevroloških prenašalcev. Poleg tega, da zasvaja, ima sam nikotin vrsto negativnih vplivov na zdravje organizma, saj vpliva na razvoj bolezni srca in žilja, pospešuje razvoj malignih obolenj ter vpliva na vrsto drugih sistemov v organizmu. Vpliva tudi na razvoj mladih možganov in jih lahko dolgoročno okvari. V Sloveniji so programi za pomoč pri opuščanju kajenja v obliki skupinskih ali individualnih delavnic v zdravstvenih domovih po celi Sloveniji v CKZ/ZVC (Centri za krepitev zdravja /Zdravstvenovzgojni centri). Za pomoč je tudi brezplačni svetovalni telefon "QuitLine" 080 2777. Od farmakoterapije obstajajo različne oblike nikotinskega nadomestnega zdravljenja ter zdravila vareniklin, citisin in bupropion.

## Mechanisms of nicotine addiction and assistance in quitting

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**Keywords:** nicotine, addiction, cessation support

Nicotine is a psychoactive substance that has many effects on the body. The existence of biological and behavioural mechanisms responsible for addiction has been confirmed. Repeated exposure to nicotine leads to neuroadaptation, which in turn supports craving behaviour and tolerance building. Nicotine is rapidly absorbed through the lungs, skin and mucous membranes. It acts through acetylcholine receptors, which stimulate the release of various neurotransmitters. Nicotine binds preferentially to nicotinic acetylcholinergic receptors (nACh) in the mesolimbic dopamine system, the most important being the  $\alpha 4\beta 2$  nicotinic receptor in the ventral tegmental area (VTA). After nicotine binds to the receptor, dopamine release occurs in the nucleus accumbens, which is linked to the reward system in the brain. In addition to dopamine, other neurotransmitters are also important in nicotine addiction and are most frequently released after nicotine binding to receptors: noradrenaline, acetylcholine, serotonin, vasopressin,  $\beta$ -endorphin, glutamate. Dopamine influences feelings of pleasure and reward, similar to cocaine, heroin and alcohol abuse; noradrenaline induces wakefulness and is involved in the body's defensive reaction in stressful situations; serotonin shapes mood and appetite. The nervous system adapts to their constant presence in many of the drugs used today. In addition to being addictive, nicotine itself has a number of negative effects on the health of the organism, affecting the development of cardiovascular disease, accelerating the development of malignancies and affecting a number of other systems in the organism. It also affects the development of the young brain and can damage it in the long term. In Slovenia, smoking cessation support programmes are available in the form of group or individual workshops in health centres throughout Slovenia at the Primary health care units (in CKZ/ZVC). There is also a free "QuitLine" 080 2777. Pharmacotherapy includes various forms of nicotine replacement therapy and the drugs varenicline, cytisine and bupropion.

# Kajenje med nosečnostjo: Vplivi na zdravje ploda in vloga nikotinskega nadomestnega zdravljenja ter elektronskih cigaret

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**Ključne besede:** Kajenje med nosečnostjo, Nikotin, Teratogenost, Nikotinsko nadomestno zdravljenje, Elektronske cigarete

Izpostavljenost cigaretnemu dimu med nosečnostjo predstavlja pomemben in preprečljiv vzrok obolenosti nosečnic in plodov. Med številnimi škodljivimi snovmi v cigaretnem dimu je nikotin najbolj raziskan. Ob izpostavljenosti se nikotin hitro absorbira in prehaja skozi posteljico, pri čemer je plod izpostavljen višjim koncentracijam kot nosečnica. Za razliko od mnogih drugih teratogenov se škodljivi učinki nikotina najmočneje izrazijo v pozni nosečnosti, predvsem v tretjem trimesečju. Negativni učinki kajenja med nosečnostjo so lahko vidni že med nosečnostjo ali zgodaj po rojstvu, v obliki odstopa posteljice, prezgodnjega poroda, nizke porodne teže, zastoja rasti ploda, mrtvorojenosti ali sindroma nenadne smrti dojenčka. Dolgoročno se pri otrocih poveča tveganje za motnjo pozornosti in hiperaktivnosti, debelost ter zlorabo substanc v mladostništvu. Raziskave, vključno s kohorto slovenskih otrok z orofacialnimi shizami in prirojenimi srčnimi napakami, kažejo na večjo pogostost prirojenih nepravilnosti pri otrocih, ki so bili pred rojstvom izpostavljeni cigaretnemu dimu. Čeprav natančni patofiziološki mehanizmi še niso popolnoma pojasnjeni, se domneva, da k temu prispevajo oksidativni stres, hipoksija, vazokonstrikcija ter nepravilnosti v delovanju mitohondrijev in posteljice. V zadnjih desetletjih se je stopnja kajenja med nosečnostjo zmanjšala. Podatki slovenske kohorte nosečnic kažejo, da se je delež nosečnic, ki kadijo, zmanjšal z 18 % v osemdesetih letih na 10 % v obdobju med 2011 in 2015. Ocenjuje se, da več kot polovica kadilk (~ 55 %) nadaljuje s kajenjem tekom nosečnosti. Vedenjske intervencije in svetovanje, zlasti v kombinaciji s farmakoterapijo, dokazano povečujejo uspešnost opustitve kajenja med nosečnostjo. Kljub temu pa skrb glede možnih škodljivih vplivov na plod omejuje uporabo nikotinskih nadomestkov (NRT) in elektronskih cigaret pri nosečnicah. Cochrane pregled raziskav uporabe NRT v nosečnosti je, sicer z nizko stopnjo zanesljivosti dokazov, pokazal, da NRT lahko poveča opustitev kajenja brez vpliva na izide nosečnosti. Potrebne so dodatne raziskave za opredelitev varnosti in učinkovitosti uporabe NRT in elektronskih cigaret med nosečnostjo.



# Smoking During Pregnancy: Fetal Health Impacts and the Role of Nicotine Replacement Therapy and E-Cigarettes

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**Keywords:** smoking during pregnancy, nicotine, teratogenicity, nicotine replacement therapy (NRT), electronic cigarettes

Exposure to cigarette smoke during pregnancy is a significant and preventable cause of adverse pregnancy outcomes. Among the harmful substances in cigarette smoke, nicotine is the most extensively studied. Nicotine is rapidly absorbed and crosses the placenta, exposing the fetus to higher concentrations than the mother. Unlike many teratogens, nicotine's harmful effects are most pronounced in the later stages of pregnancy, particularly in the third trimester. Maternal smoking is associated with both immediate and long-term adverse outcomes. Short-term effects include placental abruption, preterm birth, low birth weight, fetal growth restriction, stillbirth, and sudden infant death syndrome. Long-term effects include an increased risk for attention-deficit/hyperactivity disorder, obesity, and substance abuse during adolescence. Studies, including a cohort of Slovenian children with orofacial clefts and congenital heart defects, show a higher prevalence of congenital abnormalities in infants prenatally exposed to cigarette smoke. Although the underlying pathophysiological mechanisms remain unclear, oxidative stress, hypoxia, vasoconstriction, mitochondrial dysfunction, and placental abnormalities are hypothesized contributors. Globally, smoking rates during pregnancy have declined in recent decades. Data from a Slovenian cohort of pregnant women shows smoking rates among pregnant women dropped from 18% in the 1980s to 10% between 2011 and 2015. It is estimated that more than a half of pregnant woman continues to smoke during the pregnancy. Behavioural interventions and counseling, particularly when combined with pharmacotherapy, have been shown to improve smoking cessation rates during pregnancy. However, concerns about potential fetal harm limit the use of nicotine replacement therapy (NRT) and electronic cigarettes in pregnant women. The Cochrane review found low-certainty evidence that NRT may increase smoking cessation during pregnancy, with no significant effects observed on birth outcomes. Further research is required to determine the safety and efficacy of these interventions for both pregnant women and their infants.

# Interakcije med kajenjem in zdravili na recept

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**Ključne besede:** policiklični aromatski ogljikovodiki, indukcija encimov, kadilski status, farmakokinetične interakcije

Interakcije med kajenjem in zdravilnimi učinkovinami so večinoma farmakokinetične narave, kar pomeni, da kajenje vpliva na metabolizem učinkovin preko indukcije določenih encimov v jetrih, pljučih in placenti. Glavna komponenta tobačnega dima, policiklični aromatski ogljikovodiki, delujejo kot močni induktorji izoencimov citokroma P450, predvsem CYP1A2 in CYP2E1, kar vpliva na hitrejši metabolizem določenih učinkovin. S kajenjem povzročena indukcija CYP1A2 encima pomembno vpliva na učinkovitost določenih učinkovin. Pri kadilcih pride do večjega metabolizma in posledično očistka zdravilnih učinkovin, zato je potreben višji odmerek za doseg ustreznega učinka. Razlog za farmakodinamične interakcije so predvsem farmakološki učinki nikotina in sicer so posledica stimulativnega delovanja nikotina na osrednji živčni sistem.

V primeru, da pacient prične s kajenjem, je treba spremljati plazemske koncentracije učinkovin, saj je že pet pokajenih cigaret dnevno lahko dovolj, da pride do indukcije encimov. To je pomembno predvsem za zdravila z ozkim terapevtskim oknom, kot so teofilin, klozapin, olanzapin in erlotinib, ki zahtevajo spremljanje plazemskih koncentracij in prilagajanje odmerkov ob spremembi kadilskega statusa. Če pacient nenadno preneha kaditi, je priporočljivo v naslednjih 2-3 dneh zmanjšati odmerek učinkovin, ki so substrati za CYP1A2, za približno 10 % na dan v štirih dneh, saj se učinki prenehanja kajenja začnejo izraziteje pojavljati šele po nekaj dneh ali po enem tednu. Pri obravnavi pacientov se svetuje rutinsko preverjanje (ne)kadilskega statusa pacienta in vrste izdelka, ki ga pacient uporablja. Pri kadilcih je potrebno spremljati koncentracije zdravil z ozkim terapevtskim oknom in prilagoditi odmerke glede na status kajenja. Nenadna prekinitev kajenja lahko povzroči neželene učinke zaradi povečanih plazemskih koncentracij učinkovin.

## Interactions between smoking and prescription medicines

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**Keywords:** polycyclic aromatic hydrocarbons, enzyme induction, smoking status, pharmacokinetic interactions

Interactions between smoking and prescription medicines are mostly pharmacokinetic in nature, meaning that smoking affects the metabolism of drugs through the induction of certain enzymes in the liver, lungs, and placenta. The main component of tobacco smoke, polycyclic aromatic hydrocarbons, act as strong inducers of cytochrome P450 isoenzymes, particularly CYP1A2 and CYP2E1, which leads to faster metabolism of certain drugs. The smoking-induced induction of the CYP1A2 enzyme significantly affects the effectiveness of certain drugs. In smokers, the metabolism and subsequent clearance of drugs are increased, requiring higher doses to achieve the desired effect. Pharmacodynamic interactions are mainly due to the pharmacological effects of nicotine, primarily its stimulatory action on the central nervous system.

If a patient starts smoking, it is important to monitor plasma concentrations of active substances, as even five cigarettes per day can be sufficient to induce enzymes. This is especially important for drugs with a narrow therapeutic window, such as theophylline, clozapine, olanzapine, and erlotinib, which require monitoring of plasma concentrations and dose adjustments when the smoking status changes. If a patient suddenly stops smoking, it is recommended to reduce the dose of substances that are substrates of CYP1A2 by approximately 10 % per day over four days, as the effects of smoking cessation become more pronounced only after a few days or a week. During patient treatment, it is advised to routinely check the patient's (non)smoking status and the type of product they use. For smokers, it is necessary to monitor the concentrations of drugs with a narrow therapeutic window and adjust doses based on smoking status. Sudden cessation of smoking can lead to adverse effects due to increased plasma concentrations of active substances.



POVZETKI VABLJENIH PREDAVANJ

**Razprava in zaključne misli/discussion and final thoughts**

## **Zgodovina tobaka in izzivi**

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V prispevku bomo prikazali kratko zgodovino gojenja in predelave tobaka v predkolumbijski Ameriki, ZDA in Evropi. Podali bomo nekaj statističnih podatkov o proizvodnji, predelavi tobaka in tobačnih politikah ob koncu 19. stoletja v ZDA in Evropi. Opisali bomo prve študije, ki so bile objavljene v 50 letih 20. stoletja in ki nakazujejo na povezavo med določenimi boleznimi (pljučni rak, kardio-vaskularne bolezni, itd.) in kajenjem. Vnela se je bitka med tobačnim lobijem in zagovorniki omejitve oglaševanja in prodaje tobaka, ki se je v ZDA končala leta 1970 s prepovedjo oglaševanja cigaret na televiziji. Predstavili bomo tudi Johna Blatnika, ameriškega senatorja slovenskih korenin, ki je odigral ključno vlogo pri naporih za omejevanje oglaševanja cigaret.

## **Tobacco history and challenges**

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In this paper, we present a brief history of tobacco cultivation and processing in pre-Columbian America, the USA and Europe. We will provide some statistics on tobacco production, processing, and tobacco policies at the end of the 19th century in the United States and Europe. We will describe the first studies that were published in the 1950s and that indicate a link between certain diseases (lung cancer, cardiovascular diseases, etc.) and smoking. A battle broke out between the tobacco lobby and advocates of restricting tobacco advertising and sales, which ended in the USA in 1970 with the banning of cigarette advertising on television. John Blatnik will be also introduced, a US senator with Slovenian roots who played a key role in efforts to limit cigarette advertising.





## POVZETKI POSTERJEV/POSTER ABSTRACTS

# Izkustveno učenje dijakov o škodljivih posledicah uporabe tobačnih izdelkov za okolje: učinki mikroplastike iz nepokajenih in pokajenih cigaretnih filtrov na vodne organizme

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**Ključne besede:** cigaretni filtri, mikroplastika, vodni organizmi, vodne bolhe *Daphnia magna*

Srednješolske raziskovalne naloge dijakom omogočajo izkustveno učenje. To pomeni, da s sistematičnim raziskovanjem in nadzorovanimi poskusi pridobijo odgovore na zastavljena znanstvena vprašanja, ki naslavlja aktualne globalne probleme. Onesnaženost okolja z mikroplastiko (MP), ki nastaja z razgradnjo filtrov cigaretnih ogorkov na manjše delce, predstavlja okoljski problem, vreden širše pozornosti. Da bi ugotovili potencialne posledice uporabe tobačnih izdelkov za okolje, smo z dijaki 3. letnika GSSRM Kamnik izvedli poskuse, s katerimi smo preučili vpliv MP iz pokajenih (SCF-MP) in nepokajenih filtrov (UCF-MP) navadnih cigaret na vodne organizme - vodne bolhe (*Daphnia magna*) in kotačnike (*Brachionus calyciflorus*). En dan stare organizme smo izpostavili različnim koncentracijam MP (1, 10 in 100 mg L<sup>-1</sup>) in po 24 h (kotačniki) in 48 h ter 96 h (vodne bolhe) preverili njihovo preživetje. Preživetje poskusnih organizmov je upadlo z naraščajočo koncentracijo MP. V primeru SCF-MP je bilo preživetje vodnih bolh in kotačnikov izrazito manjše, kot pa v primeru UCF-MP. Domnevamo, da so k zmanjšanemu preživetju testnih organizmov prispevale tudi anorganske in organske snovi, ki se izlužujejo iz obeh tipov MP. Slednje smo potrdili s poskusom, v katerem smo vodne bolhe izpostavili izključno izlužkom, ki smo jih pripravili iz obeh tipov MP. Rezultati so pokazali zmanjšano preživetje vodnih bolh v primeru izlužkov SCF-MP, medtem ko izlužki UCF-MP niso imeli vpliva na preživetje. Nadalje smo z analizo aktivnosti encima acetilholinesteraze (AChE) v vodnih bolhah, ki smo jih za 7 dni izpostavili mediju s SCF-MP (100 mg L<sup>-1</sup>), posredno dokazali nevrotoksični učinek nikotina. V preteklih raziskavah je bilo dokazano, da se nikotin v velikih količinah kopiči v pokajenih CF (Lucia et al., 2023). Na podlagi rezultatov predpostavljamo, da je povečana aktivnost encima AChE v vodnih bolhah posledica povečane koncentracije nikotina v SCF-MP. Rezultati raziskave dokazujejo, da lahko ne le SCF-MP, ampak tudi UCF-MP, vpliva na okolje. Posebno pozornost je potrebno posveti preprečevanju onesnaževanja okolja s cigaretnimi ogorki.

## Literatura:

Lucia, G., Giuliani, M.E., d'Errico, G., Booms, E., Benedetti, M., Di Carlo, M., Fattorini, D., Gorbi, S., Regoli, F., 2023. Toxicological effects of cigarette butts for marine organisms. *Environment International*, 171, 107733. <https://doi.org/10.1016/j.envint.2023.107733>.

# Experiential learning of students on the environmental consequences of tobacco products use: impacts of microplastics from smoked and unsmoked cigarette filters on aquatic organisms

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**Key words:** cigarette filters, microplastics, aquatic organisms, water fleas *Daphnia magna*

Research assignments in high school allow students to learn experientially. This means that through systematic research and controlled experiments, they obtain answers to scientific questions concerning current global problems. Environmental pollution caused by microplastics (MP), which result from the decomposition of cigarette butt filters into smaller particles, is one such environmental problem that deserves greater attention. In order to find out the possible consequences of the use of tobacco products for the environment, we conducted experiments with the 3rd year students of GSSRM Kamnik to investigate the influence of MP from smoked (SCF-MP) and unsmoked filters (UCF-MP) of ordinary cigarettes on aquatic organisms - water fleas (*Daphnia magna*) and water fleas (*Brachionus calyciflorus*). One-day-old organisms were exposed to different concentrations of MP (1, 10 and 100 mg L<sup>-1</sup>) and their survival was tested after 24 h (rotifers), 48 h and 96 h (water fleas). The survival of the test organisms decreased with increasing concentration of MP. In the case of SCF-MP, the survival rate of water fleas and rotifers was significantly lower than in the case of UCF-MP. We assume that inorganic and organic substances leached from both types of MP also contributed to the lower survival of the test organisms. The latter was confirmed by an experiment in which water fleas were exposed exclusively to extracts prepared from both types of MP. The results showed a reduced survival rate of water fleas in the case of SCF-MP extracts, while UCF-MP extracts had no effect on survival. By analysing the activity of the enzyme acetylcholinesterase (AChE) in water fleas exposed for 7 days to a medium containing SCF-MP (100 mg L<sup>-1</sup>), we have also indirectly demonstrated the neurotoxic effect of nicotine. Previous studies have shown, for example, that nicotine accumulates in large quantities in smoked CF (Lucia et al., 2023). Based on the results, we assume that the increased activity of the AChE enzyme in water fleas is due to the increased concentration of nicotine in SCF-MP. The research results show that not only SCF-MP but also UCF-MP can affect the environment. Special attention must be paid to the prevention of environmental pollution from cigarette butts.

## Reference:

Lucia, G., Giuliani, M.E., d'Errico, G., Booms, E., Benedetti, M., Di Carlo, M., Fattorini, D., Gorbi, S., Regoli, F., 2023. Toxicological effects of cigarette butts for marine organisms. *Environment International*, 171, 107733. <https://doi.org/10.1016/j.envint.2023.107733>.

# Nanodelci kot nosilci nikotinskih antagonistov za izboljšanje zdravljenja pljučnega raka

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**Ključne besede:** Kemorezistenca, nanodelci iz želatine, nAChR, antagonisti nAChR, nikotin

Za izboljšanje učinkovitosti kemoterapije raka pljuč je potreben razvoj novih strategij zdravljenja. Pri napredovanju raka pljuč imajo pomembno vlogo nikotinski acetilholinski receptorji (nAChR), ki jih lahko uporabimo kot farmakološke tarče pri zdravljenju. Celice pljučnega raka pogosto prekomerno izražajo podtipe nAChR, ki ob vezavi nikotina aktivirajo signalne poti, ki stimulirajo proliferaciji in migracijo celic ter zavirajo apoptozo rakavih celic. Poznavanje vloge nAChR pri kemoresistenci pljučnega raka je privedlo do ideje o uporabi antagonistov nAChR za povečanje učinkovitosti kemoterapije. Da se izognemo neželenim učinkom antagonistov, ki bi nastali zaradi njihovega delovanja na nAChR izražene na normalnih celicah, je pomembno zagotoviti ciljno delovanje antagonista. To je možno doseči z uporabo nanodostavnih sistemov, ki prednostno dostavijo učinkovino v rakave celice.

Z uporabo človeških celic pljučnega adenokarcinoma (A549) smo pokazali, da 3-alkilpiridinijska sol APS7 (sintetični analog toksina iz morske spužve *Haliclona sarai*) deluje kot antagonist nAChR, ki inhibira pro-proliferativne in anti-apoptotske učinke nikotina. Kot nanodostavni sistem za APS7 smo pripravili želatinaste nanodelce (APS7-GNP). Učinke APS7-GNP smo primerjali z učinki prostega APS7. Pri poskusih na celicah A549, ki smo jih stimulirali z nikotinom, je uporaba tako APS7 kot tudi APS7-GNP povečala učinkovitost kemoterapevtika cisplatina. Vendar pa je uporaba nanodostavnega sistema APS7-GNP pokazala pomembne prednosti v primerjavi s prostim APS7: intenzivnejše zmanjšanje proliferacije A549 celic pljučnega raka ter večjo selektivnost delovanja na rakave celice v primerjavi z netumorigenimi pljučnimi celicami BEAS-2B.

## Referenca:

Kononenko, Veno, et al. (2024). *Biomedicine & Pharmacotherapy*, 177, 117007.

# Nanoparticle-mediated delivery of nicotinic antagonist to improve lung cancer therapy

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**Keywords:** Chemoresistance, gelatine nanoparticles, nAChR, nAChR antagonist, nicotine

Chemotherapy for lung cancer often yields suboptimal outcomes, necessitating the development of innovative therapeutic strategies. Nicotinic acetylcholine receptors (nAChRs) play important role in lung cancer progression, making them attractive therapeutic targets. Binding of nicotine on nAChRs, which are often overexpressed in lung cancer cells, activates signaling pathways leading to stimulation of cell proliferation, cell migration and angiogenesis and in preventing cancer cell apoptosis. Knowledge of the role of nAChRs in lung cancer chemoresistance has led to the idea of using nAChR antagonists to make tumors more susceptible to chemotherapy. To avoid adverse effects of antagonist acting on nAChRs expressed on normal cells, it is important to deliver antagonist predominantly to cancer cells. This can be achieved by nanodelivery systems that are preferentially taken up by cancer cells.

We showed that the 3-alkylpyridinium salt, APS7, a synthetic analog of a toxin from the marine sponge *Haliclona* (*Rhizoneira*) *sarai*, acts as an nAChR antagonist that inhibits the pro-proliferative and anti-apoptotic effects of nicotine on A549 human lung a denocarcinoma cells. Gelatin-based nanoparticles filled with APS7 (APS7-GNPs) were prepared and their effects on A549 cells were compared with that of free APS7. Both APS7 and APS7-GNPs enhanced the efficacy of cisplatin chemotherapy in nicotine-stimulated A549 cells. However, APS7-GNPs exhibited significant advantages. They demonstrated a more pronounced reduction in A549 lung cancer cell proliferation and exhibited far greater selectivity in cytotoxicity towards cancer cells compared to non-tumorigenic BEAS-2B lung epithelial cells.

## Reference:

Kononenko, Veno, et al. (2024). *Biomedicine & Pharmacotherapy*, 177, 117007.

## Jetrni 3D celični modeli – občutljiv pristop za zaznavanje škodljive (geno)toksične aktivnosti benzo(a)pirena

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**Ključne besede:** *in vitro* 3D celični model, policiklični aromatski ogljikovodiki, genotoksičnost

Kajenje predstavlja pomemben javno-zdravstveni problem, ki je povezan z mnogimi zdravstvenimi težavami, med katere sodijo astma in rak v mnogih organih, kot so npr. jetra in pljuča. Med škodljive snovi v tobačnem dimu sodijo tudi policiklični aromatski ogljikovodiki (PAH), zlasti benzo[a]piren (B[a]P), ki je znan karcinogen (Skupina 1) (IARC). Ljudje so izpostavljeni B[a]P s kajenjem, pa tudi preko onesnažene hrane in zraka, saj B[a]P nastaja kot stranski produkt pri nepopolnem gorenju. Trenutne raziskave škodljivih učinkov B[a]P pogosto uporabljajo dvodimenzionalne (2D) celične kulture jetrnih modelov, ki imajo nekaj pomanjkljivosti, zlasti odsotnost encimov faze II., ki so ključni pri presnovi ksenobiotikov. Tridimenzionalni (3D) celični modeli (sferoidi) imajo po drugi strani izboljšano interakcijo med celicami in matriksom ter izkazujejo višjo raven jetrno specifičnih encimov. V tej študiji so bili uporabljeni sferoidi, pridobljeni iz človeške celične linije hepatocelularnega karcinoma (HepG2), pripravljene s prisilno plavajočo metodo in gojeni v statičnih pogojih, na njih pa smo preizkusili genotoksično aktivnost B[a]P. Sferoide smo po 72h inkubacije izpostavili ne-citotoksičnim koncentracijam B[a]P za 24h (0.1, 1, 10, and 20  $\mu$ M) in 72h (0.001, 0.01, 0.1, 1, and 10  $\mu$ M). Po izpostavitvi smo sferoidom izmerili živost (test MTS), proliferacijo (Anti-Ki67), določili rast sferoidov (mikroskopija) ter delež živih in mrtvih celic (konfokalna mikroskopija). S pretočno citometrijo smo analizirali porazdelitev celic v celičnem ciklu (barvilo Hoechst 33258) in preučili nastanek dvojnih prelomov DNA (Anti- H2AX pS139). Ugotovili smo, da je B[a]P vplival na zmanjšano rast HepG2 sferoidov z zaustavljanjem v S fazi celičnega cikla. Prav tako smo zaznali nastanek DNA poškodb po 24 in 72h izpostavitve B[a]P. Raziskava, izvedena na modelu onesnaževalca zraka B[a]P, je pokazala, da 3D jetrni celični model omogoča občutljiv pristop, ki se lahko uporablja za ocenjevanje genotoksične aktivnosti onesnaževalcev zraka.

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## Hepatic 3D cell model - a sensitive approach for determining the adverse (geno)toxic activity of benzo(a)pyrene

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**Key words:** *in vitro* 3D cell model, polycyclic aromatic hydrocarbons, genotoxicity

Tobacco smoking is a significant public health concern, associated with a wide range of health issues that can lead to many diseases including asthma and cancer in a number of target organs such as liver, lungs and other. Among the many harmful substances in tobacco smoke are polycyclic aromatic hydrocarbons (PAHs), particularly benzo[a]pyrene (B[a]P), a known human carcinogen (Group 1) (IARC). People are exposed to B[a]P not only through smoking but also from contaminated food and polluted air. While current research often uses two-dimensional (2D) liver cell cultures to study B[a]P effects, these models fall short in mimicking the relevant hepatic properties, in particular the phase II enzymes, required for the metabolism of xenobiotics. Three-dimensional (3D) cell models (spheroids), on the other hand, have improved cell-cell and cell-matrix interactions and exhibit higher levels of liver-specific functions. In the present study, the spheroids formed from human hepatocellular carcinoma (HepG2) cell line developed by forced floating method and cultured under static conditions were used to test the genotoxic activity of benzo(a)pyrene (B[a]P), a by-product of incomplete combustion, including cigarette smoke. After 3 days of cultivation, spheroids were exposed to non-cytotoxic B[a]P concentrations for 24 (0.1, 1, 10, and 20  $\mu$ M) and 72 (0.001, 0.01, 0.1, 1, and 10  $\mu$ M) hours. After exposure, viability (MTS assay) and division of cells (Ki67 marker) in the spheroids, growth of spheroids (microscopy) were examined, and live/dead staining (confocal microscopy) and cell cycle analysis (Hoechst 33258) were performed. In addition, flow cytometry was used to detect the induction of DNA double strand breaks (Anti-H2AX pS139). The results showed that B[a]P affected the growth of HepG2 cells by arresting them in the S phase of the cell cycle, reduced growth of spheroids, and induced DNA damage after 24 h and 72 h of exposure. In summary, the research conducted using the model air pollutant B[a]P demonstrated that the hepatic 3D cell model is a sensitive approach that can be used for the assessment of genotoxic activity of air pollutants.

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