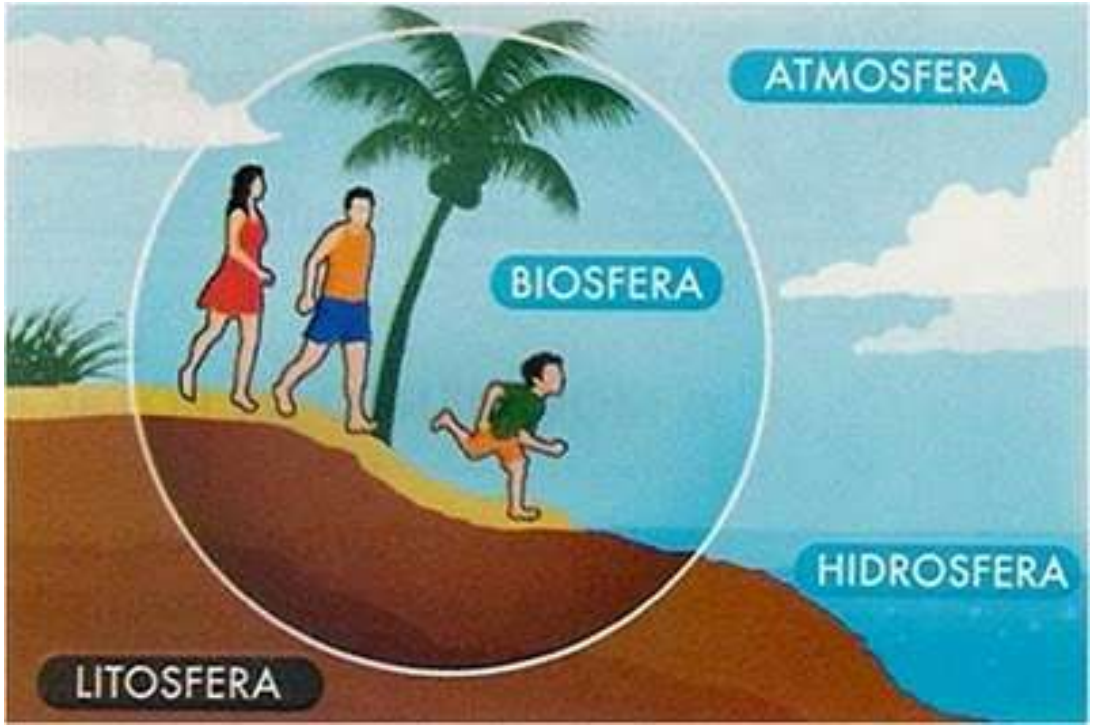
OKOLIŠ I ZDRAVLJE

Dr.sc. Ana Mojsović Ćuić, prof.struč.stud.

Zdravstveno veleučilište, Zagreb

Što je okoliš?

„Okoliš je skup ekoloških čimbenika koji djeluju na organizam ili na zajednicu određujući njezin oblik i preživljavanje.”



Okoliš

„…okoliš je i skup socijalnih i kulturnih uvjeta koji utječu na život pojedinca.”

O OKOLIŠU OVISI I KVALITETA ŽIVOTA

Okoliš

Unutarnji okoliš

- Biološki sustav pojedinca

Vanjski okoliš

- BITNI ČIMBENICI OKOLIŠA
- NEBITNI ČIMBENICI OKOLIŠA

Što je zdravlje?

„Zdravlje je stanje potpunog fizičkog, psihičkog i socijalnog blagostanja, a ne samo odsutnost bolesti ili iznemoglosti.”

Svjetska zdravstvena organizacija

Zdravlje je dinamičan sustav, funkcija međuovisnosti organizma i okoliša.

Zdravstvena ekologija

„Zdravstvena ekologija je onaj dio ekološke i medicinske znanosti koji proučava međuodnose tvari, sila i uvjeta u okolišu s jedne i čovjekova zdravlja s druge strane.”

- Čimbenici okoliša:
- ✓ biološki
- ✓ kemijski
- ✓ fizikalni
- ✓ psihosocijalni

Zadatak zdravstvene ekologije:

…pružiti *brojčane podatke* za zaštitu čovjekova zdravlja od nepovoljnih utjecaja okoliša te, po mogućnosti i za unaprjeđenje zdravlja isticanjem pozitivnih utjecaja okoliša…

Kako dolazimo do tih brojčanih podataka?

- ✓ Epidemiološke studije
- ✓ Testovi na eksperimentalnim organizmima
- ✓ Ekotoksikološke studije

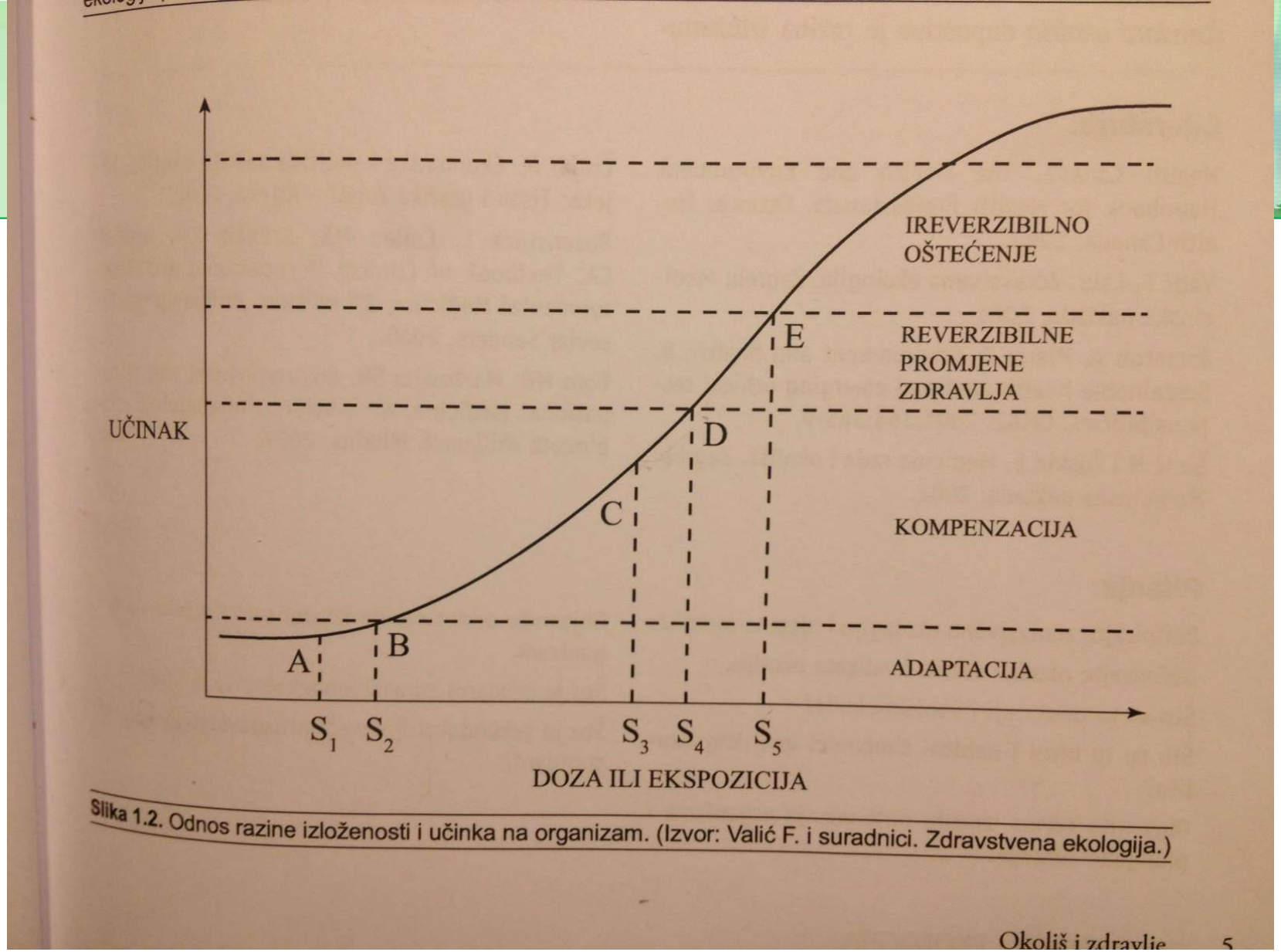
Zadatak zdravstvene ekologije:

- Odrediti razinu izloženosti koja se može prihvatiti uz zanemarujući ili prihvatljiv zdravstveni rizik.

- Rizik?

➢ Očekivana učestalost neželjenih učinaka nastalih uslijed izloženosti određenom čimbeniku okoliša.

➢ Vjerojatnost neželjenog ishoda.



Slika 1.2. Odnos razine izloženosti i učinka na organizam. (Izvor: Valić F. i suradnici. Zdravstvena ekologija.)

Okoliš i zdravlje 5

Odnos razine izloženosti i učinka na organizam

Važnost prilikom određivanja zdravstveno-ekoloških standarda okoliša.

- Zdravstveno-ekološki standardi okoliša: podzakonski propisi kojima se određuju granice izloženosti da bi se zaštitilo zdravlje populacije i potomstva od neželjenih učinaka.
- Iz donjih slajdova zaključite jesu li isti u svim zemljama?

Štavljenje kože u Indiji

Promet-jedan od glavnih uzroka onečišćenja zraka u mnogim svjetskim gradovima

Iz medija…

„REKORDNO ZAGAĐENJE ZRAKA Od smoga ne vide prst pred nosom. Na ulicu ne izlaze bez maski”

Peking, Kina, 2014., 2015…

Odlagalište otpada Jakuševac, Zagreb

FOTOSPECIJAL: ZAGREB POD SMEĆEM Građani su bijesni i ogorčeni: 'Ulice se pretvaraju u Jakuševec'

'U požaru na Jakuševcu oslobođene velike količine kancerogenih dioksina i furana'

- Samo Bog zna što su sve građani udisali - kaže toksikolog Franjo Plavšić.

https://www.vecernji.hr/zagreb/u-pozaru-na-jakusevcu-oslobo-ene-velike-kolicine-kancerogenih-dioksina-i-furana-1348926

Kante za smeće i 'ZG vrećice' u središtu političke bitke u Zagrebu

Klub gradskih zastupnika HDZ-a i HSLS-a ocijenio je u srijedu uoči sutrašnje sjednice Gradske skupštine kako najavljeni model odvoza otpada u ovom obliku nije provediv te upozorio da će dovesti do poskupljenja usluge i novih troškova

VELIKA ZABRINUTOST

Stručnjaci o vrsti plastike koja gori u Osijeku: 'Znate li zašto je dim takav? Nikako nije dobro...'

'Takav dim je, najvjerojatnije, smjesa brojnih, potencijalno opasnih tvari za ljudsko zdravlje i okoliš...'

POBUNA KOD RIJEKE

Prosvjed na Viškovu protiv odlagališta otpada: Zdrav život je ustavno pravo građana!

Spalionica otpada u Beču, Austrija

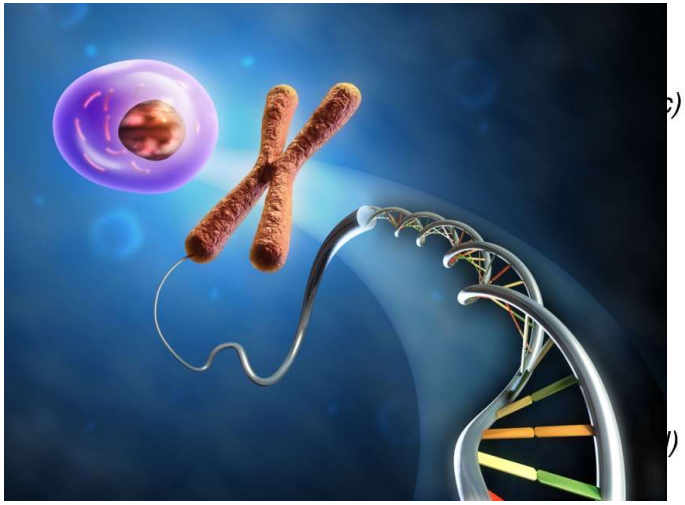
CILJ: **Najveća moguća iskorištenost otpada za proizvodnju energije**

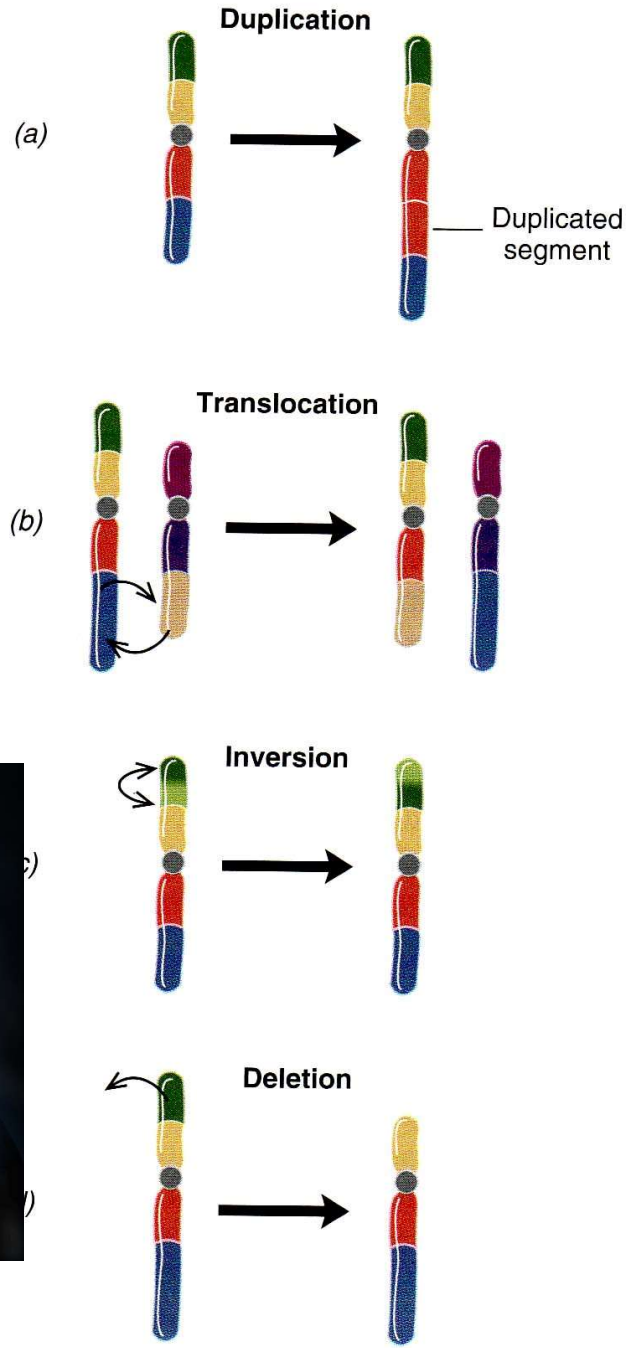
- Kvaliteta okoliša nije samo ekološko pitanje, već i društveno odnosno političko pitanje pojedine zemlje.

EKOTOKSIKOLOGIJA

Toksikanti se pojavljuju u mnogo različitih skupina:

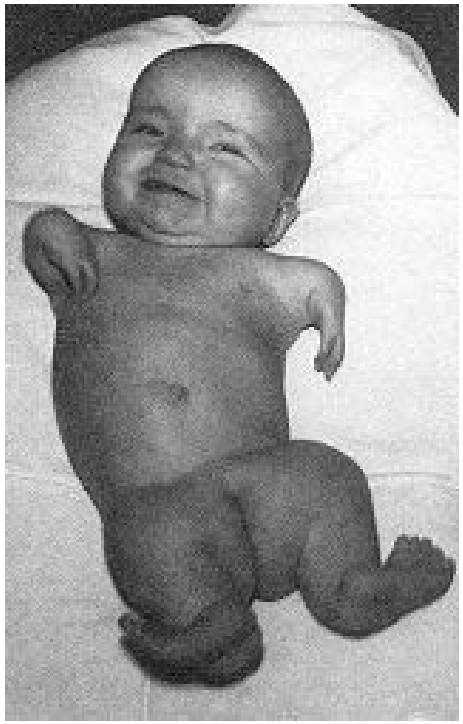
- **Karcinogeni**
- **Mutageni**
- **Teratogeni**

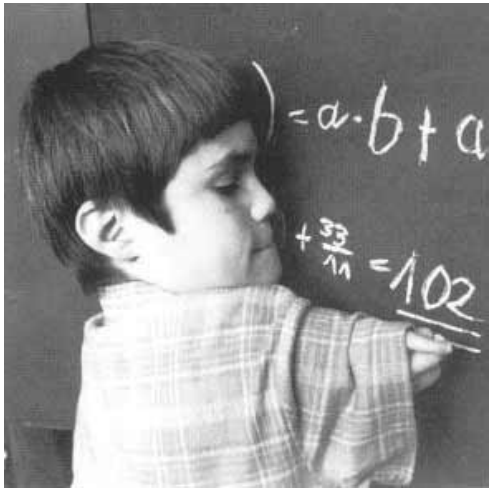




- **Teratogeni**

(talidomid,1950/60 -ih godina!)





Ukoliko toksikant djeluje na diobeno vreteno stanice u proliferaciji, do čega može dovesti?

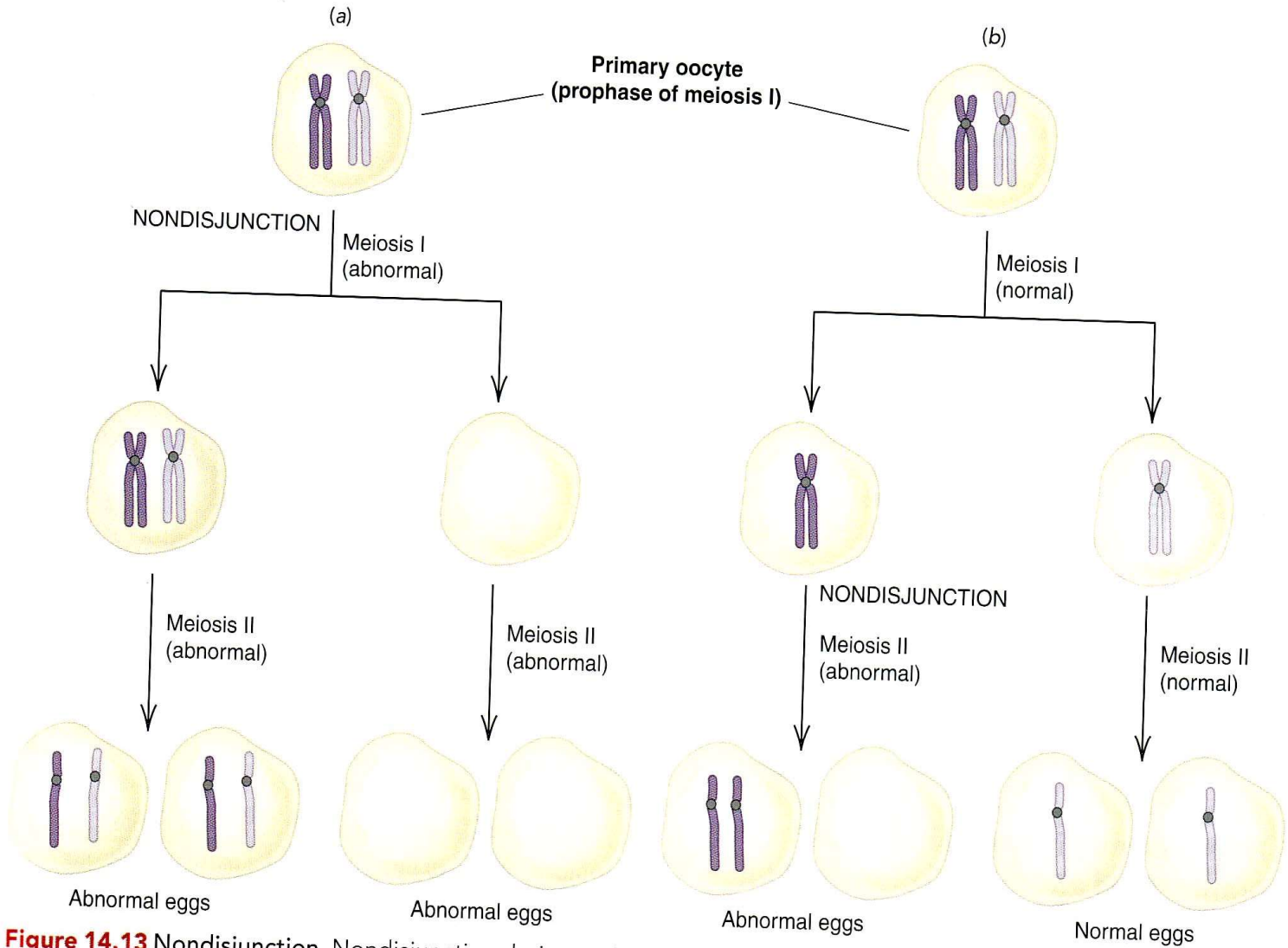


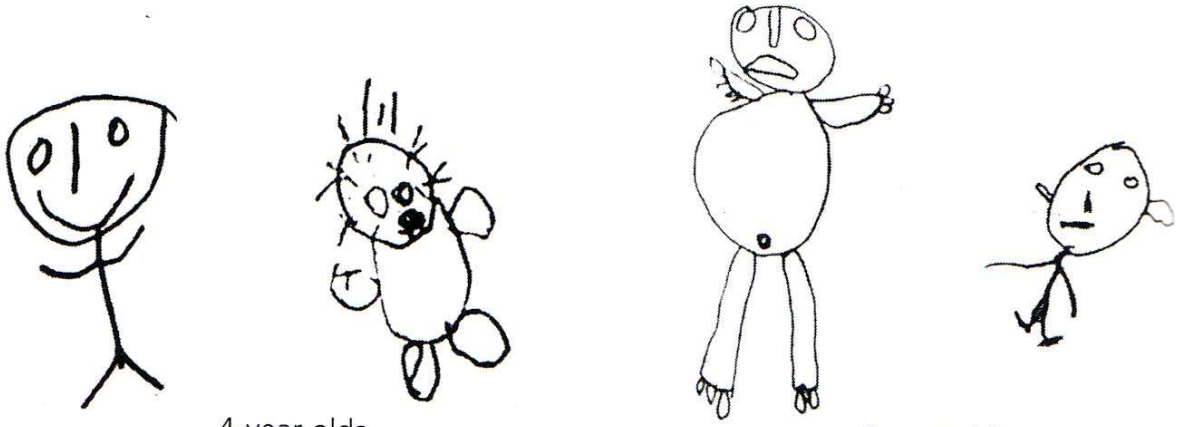
Figure 14.13 Nondisjunction. Nondisjunction during meiosis I (*a*) as compared with nondisjunction during meiosis II (*b*).

- **Alergeni**

- **Neurotoksini** (teški metali; živa, kadmij; pesticidi; Minamata katastrofa)

- **Endokrini disruptori** – tvari ili spojevi koji dovode do promjene u funkciji endokrinog sustava.

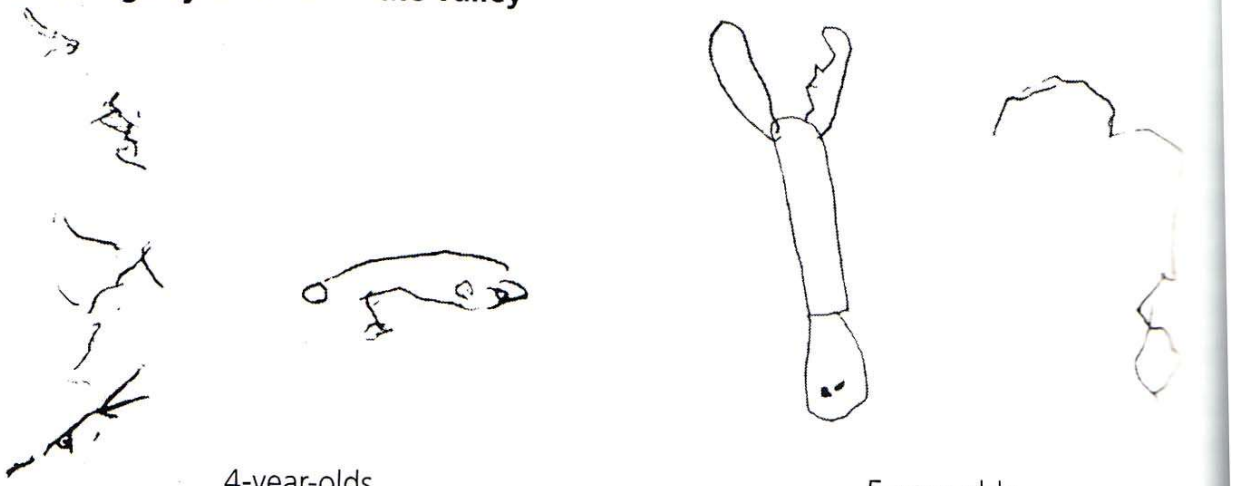
Drawings by children in the foothills



4-year-olds

5-year-olds

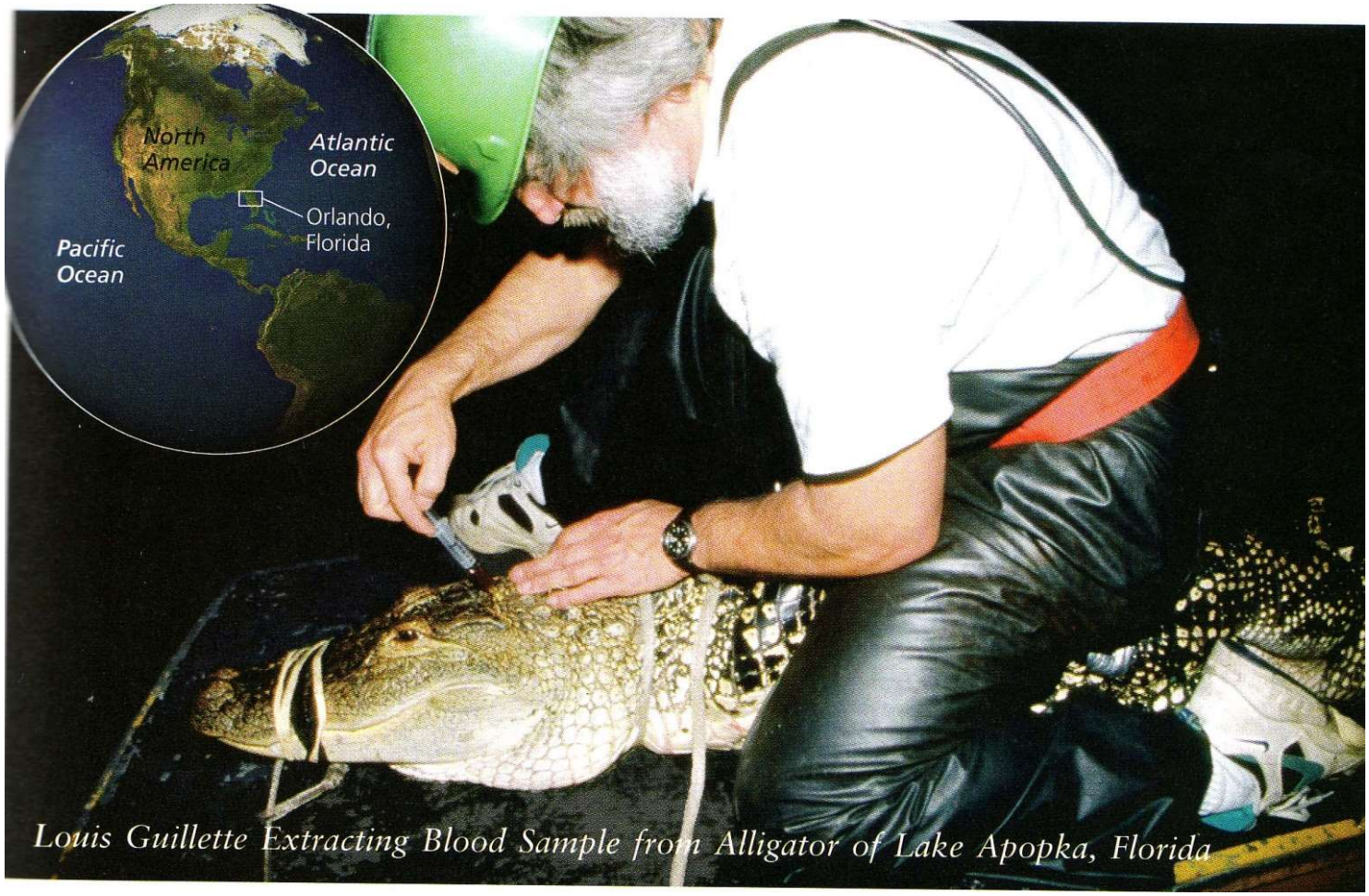
Drawings by children in the valley



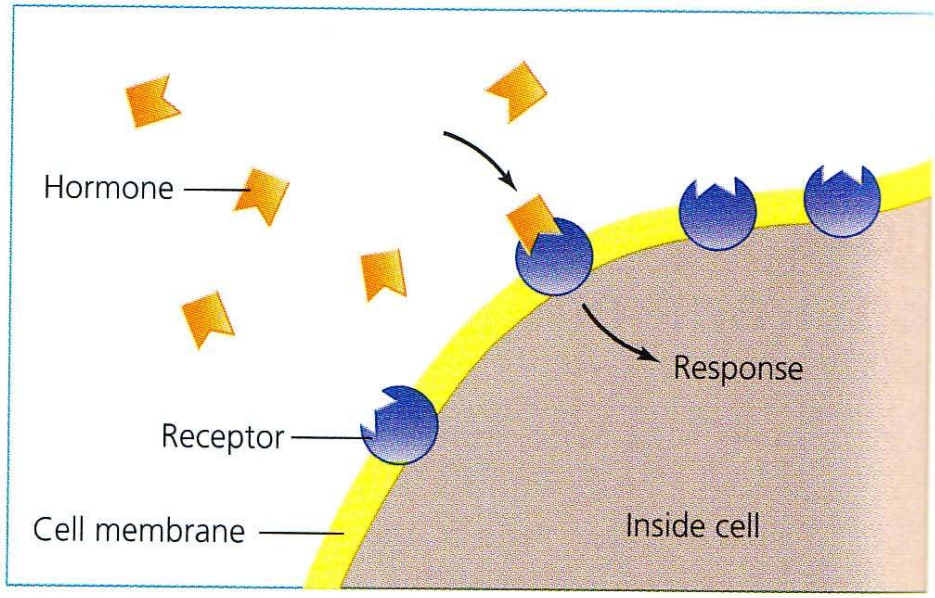
4-year-olds

5-year-olds

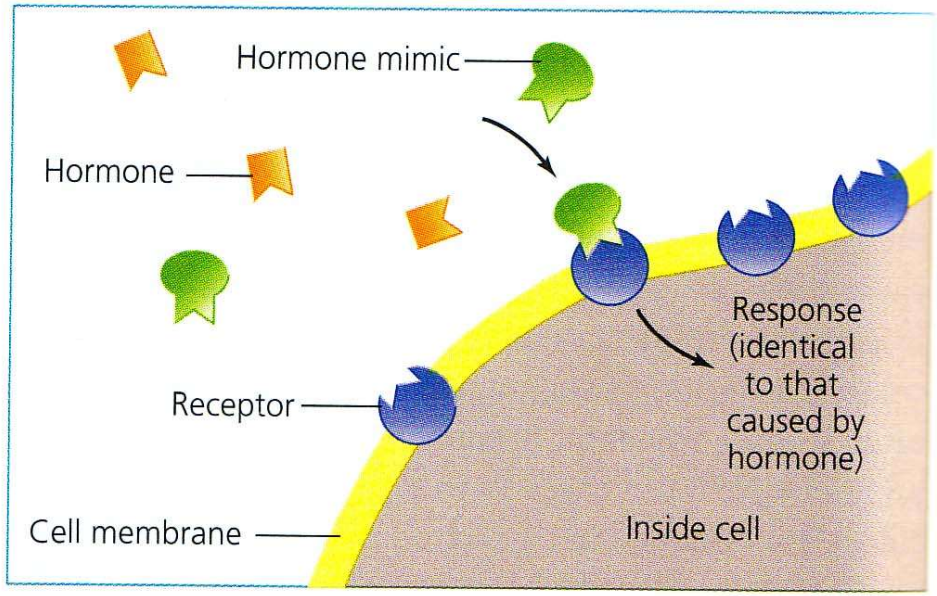
Utjecaj trovanja ostacima pesticida iz hrane koji su djelovali kao neurotoksini; potvrđeno i pomoću crteža.



Prvi slučajevi posljedica djelovanja endokrinih disruptora dokazani su na životinjama (gmazovima i vodozemcima).



(a) Normal hormone binding



(b) Hormone mimicry

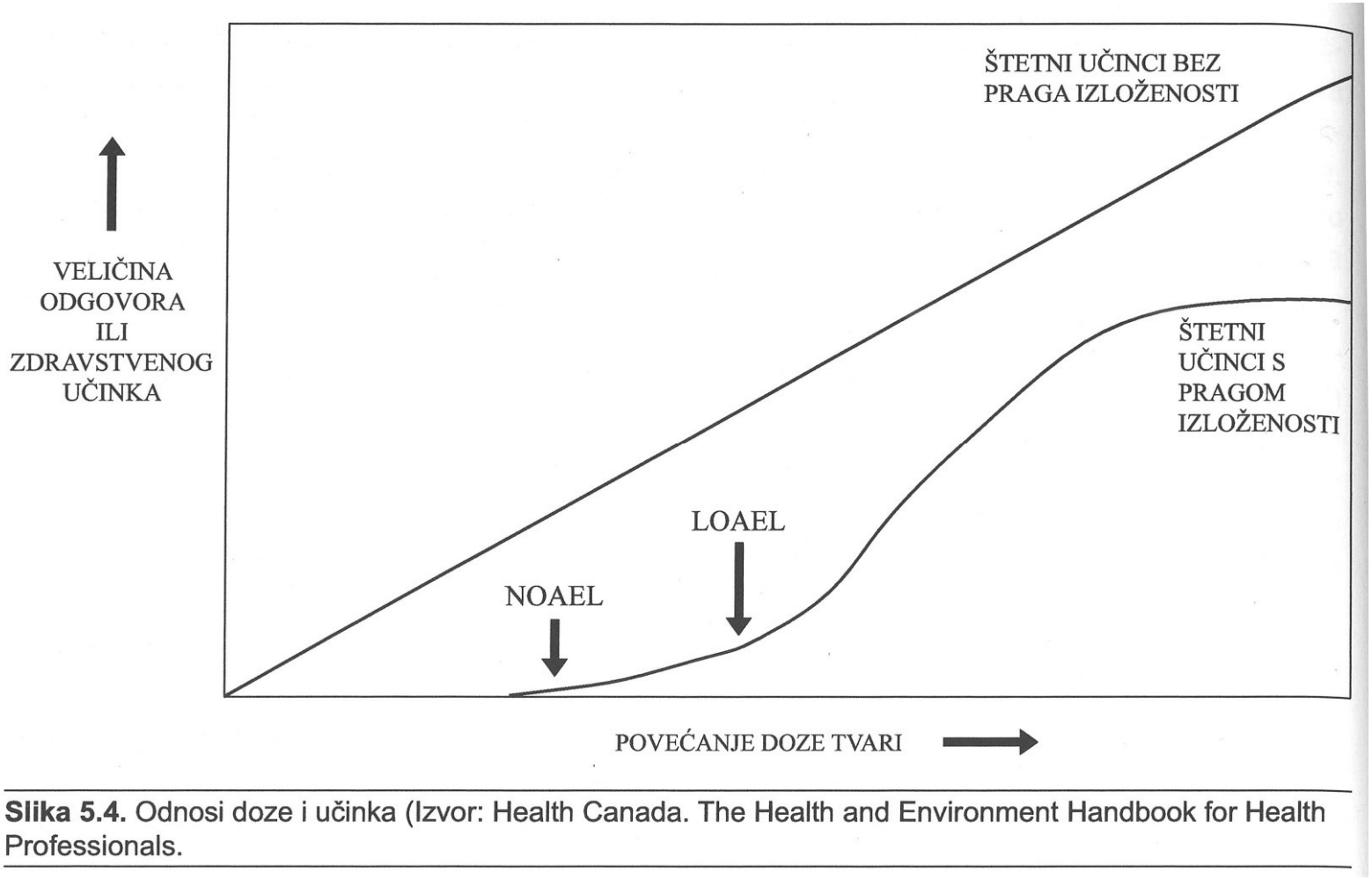
Način djelovanja endokrinih disruptora

Vežu se za jezgrine i membranske receptore i maju direktan utjecaj na stanične signalne puteve regulirane hormonima. Veliki broj endokrinih disruptora veže se za **estrogenske i testosteronske receptore.**

RAZLIKUJEMO DVIJE VRSTE NEGATIVNIH ZDRAVSTVENIH UČINAKA:

- ***UČINCI S PRAGOM IZLOŽENOSTI*** **(UČINCI S GRANIČNOM VRIJEDNOŠĆU)**

- ***UČINCI BEZ PRAGA IZLOŽENOSTI*** **(UČINCI BEZ GRANIČNE VRIJEDNOSTI)**



Slika 5.4. Odnosi doze i učinka (Izvor: Health Canada. The Health and Environment Handbook for Health Professionals.

NOAEL (*No Observed Adverse Effect Level*) - razina izloženosti ili doza bez opaženog štetnog zdravstvenog učinka/ispod koje nema štetnih učinaka

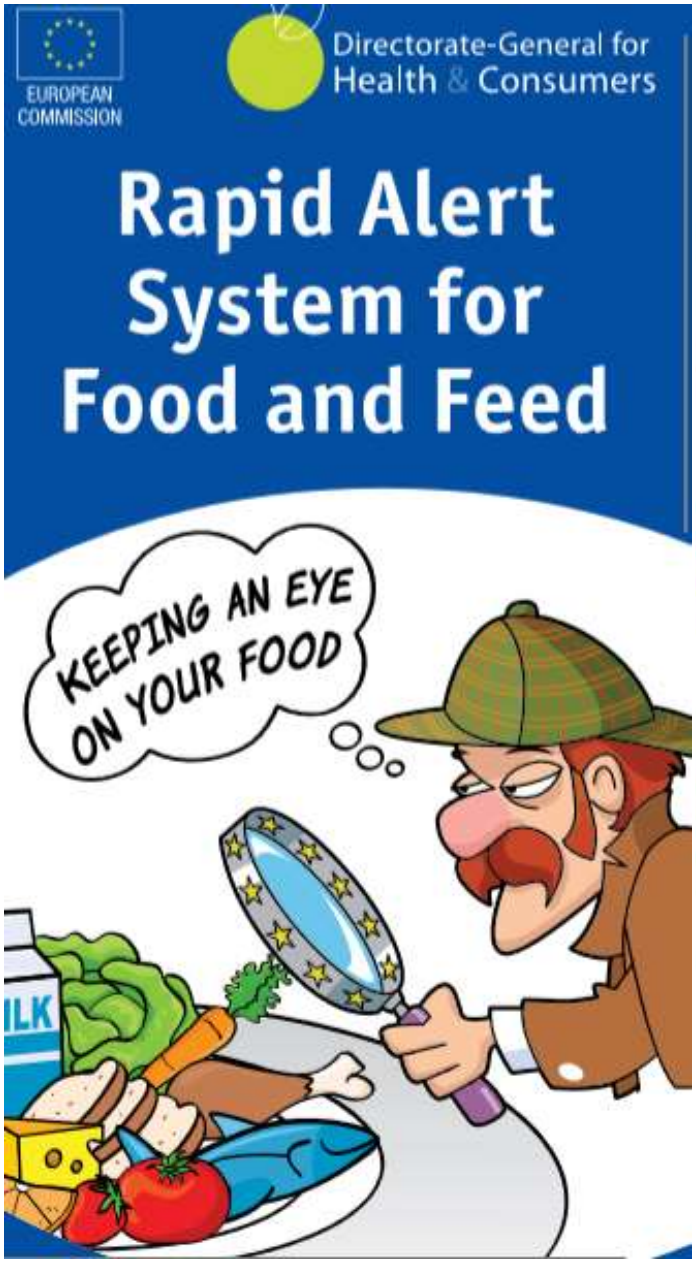
LOAEL (*Lowest Observed Adverse Effect Level*) – najniža razina izloženosti s opaženim štetnim učinkom

Granične vrijednosti (MDK) = NOAEL (ili LOAEL)/umnožak faktora nesigurnosti

Tablica 5.1. Najčešće upotrebljavani faktori nesigurnosti

Razlog primjene	**Vrijednost faktora**
Ekstrapolacija sa životinje na čovjeka (*interspecies variability*)	10
Varijabilnost rezultata unutar pojedinih životinjskih vrsta (*intraspecies variability*)	10
Nedovoljna dužina izloženosti (utvrđivanje supkroničnih, a ne kroničnih učinaka)	do 10
Slabost u planiranju ili izvedbi pokusa (npr. primjena LOAEL-a umjesto NOAEL-a)	do 10

RASFF - SUSTAV BRZOG UZBUNJIVANJA ZA HRANU I HRANU ZA ŽIVOTINJE



- European Union
- EFTA
- Austria
- Belgium
- Bulgaria
- Croatia
- Cyprus
- Czech Republic
- Denmark
- Estonia
- Finland
- France
- Germany
- Greece
- Hungary
- Iceland
- Ireland
- Italy
- Latvia
- Liechtenstein
- Lithuania
- Luxembourg
- Malta
- Netherlands
- Norway
- Poland
- Portugal
- Romania
- Slovakia
- Slovenia
- Spain
- Sweden
- Switzerland
- United Kingdom

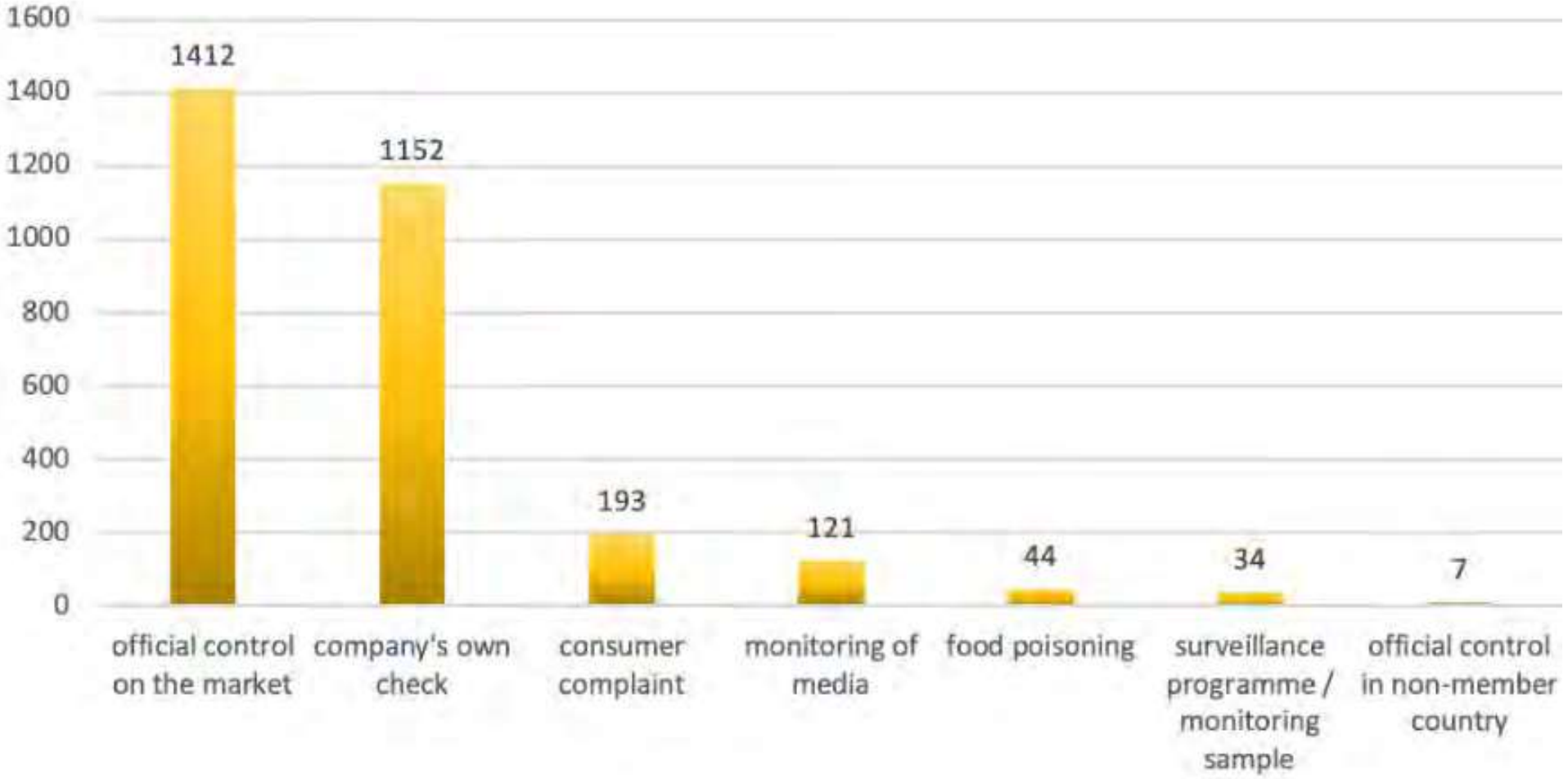
https://ec.europa.eu/food/safety/rasff-food-and-feed-safety-alerts_en

Food Safety (europa.eu)

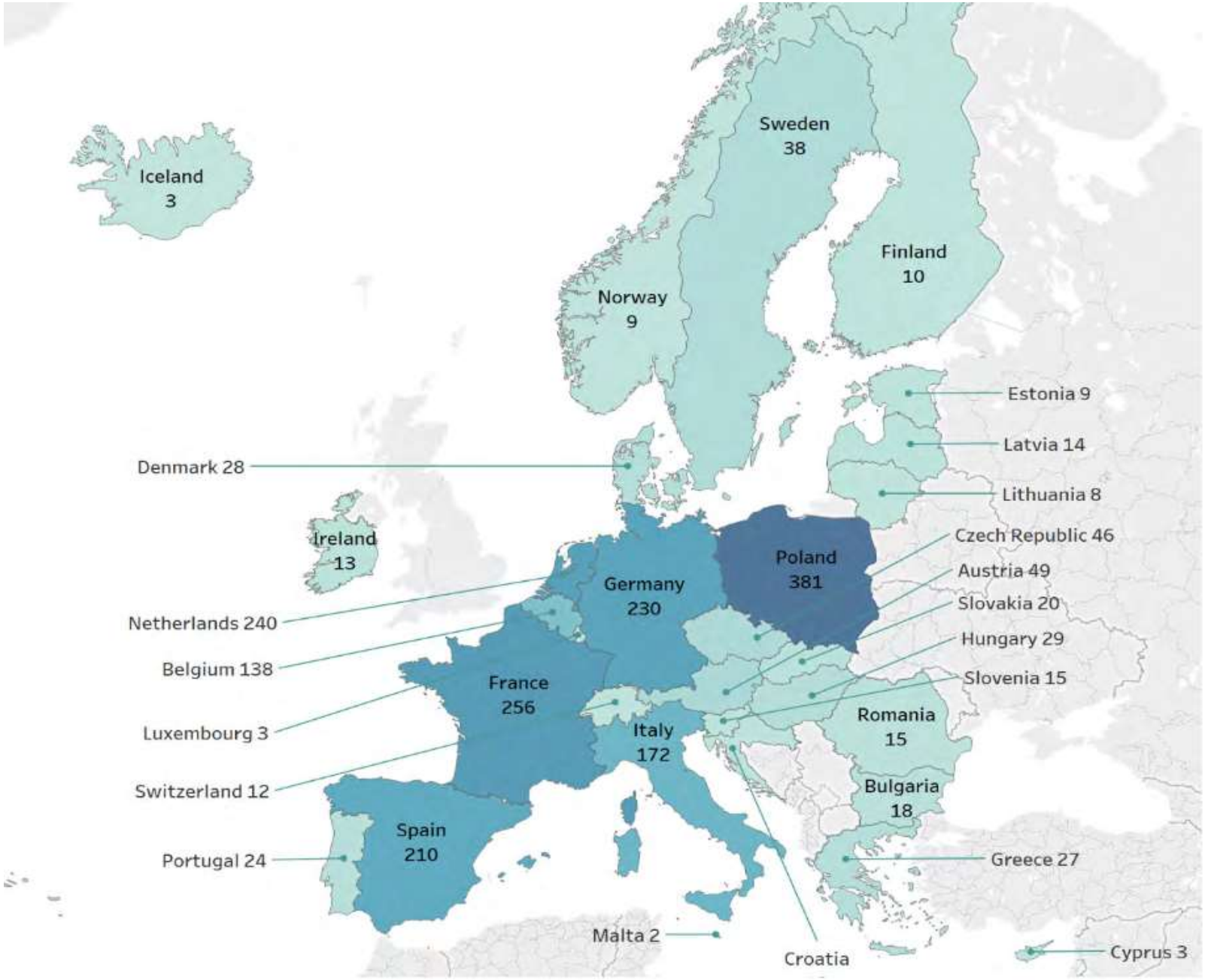
U 2021. godini od 4607 RASFF objava:

- ✓ 4102 se odnose na hranu
- ✓ 236 na hranu za životinje
- ✓ 269 na materijale koji dolaze u kontakt s hranom

*407 slučajeva: sumnja na prevaru



Izvor: 2021 Annual Report, Alert and Cooperation Network, European Union, 2022.



RASFF objave prema zemlji podrijetla proizvoda u Europi

Izvor: 2021 Annual Report, Alert and Cooperation Network, European Union, 2022.

Zemlje koje nisu članice RASFF, a čiji su proizvodi uključeni u objave:

- Turska – zemlja s najviše objava (613), od kojih se najveći broj odnosi na pesticide
- Slijedi Indija (383), s 272 objava pesticida
- Kina (331), od kojih se gotovo pola odnosi na materijale i predmete koji dolaze u neposredan dodir s hranom (160)

Hazard	Product category	Origin
Pesticide residues	Fruits and vegetables	Turkey
Salmonella	Poultry meat and poultry meat products	Poland
Salmonella	Herbs and spices	Brazil
Pesticide residues	Nuts, nut products and seeds	India
Pesticide residues	Fruits and vegetables	Egypt
Aflatoxins	Fruits and vegetables	Turkey
Aflatoxins	Nuts, nut products and seeds	Iran
Aflatoxins	Nuts, nut products and seeds	Egypt
Aflatoxins	Nuts, nut products and seeds	United States
Aflatoxins	Nuts, nut products and seeds	Turkey

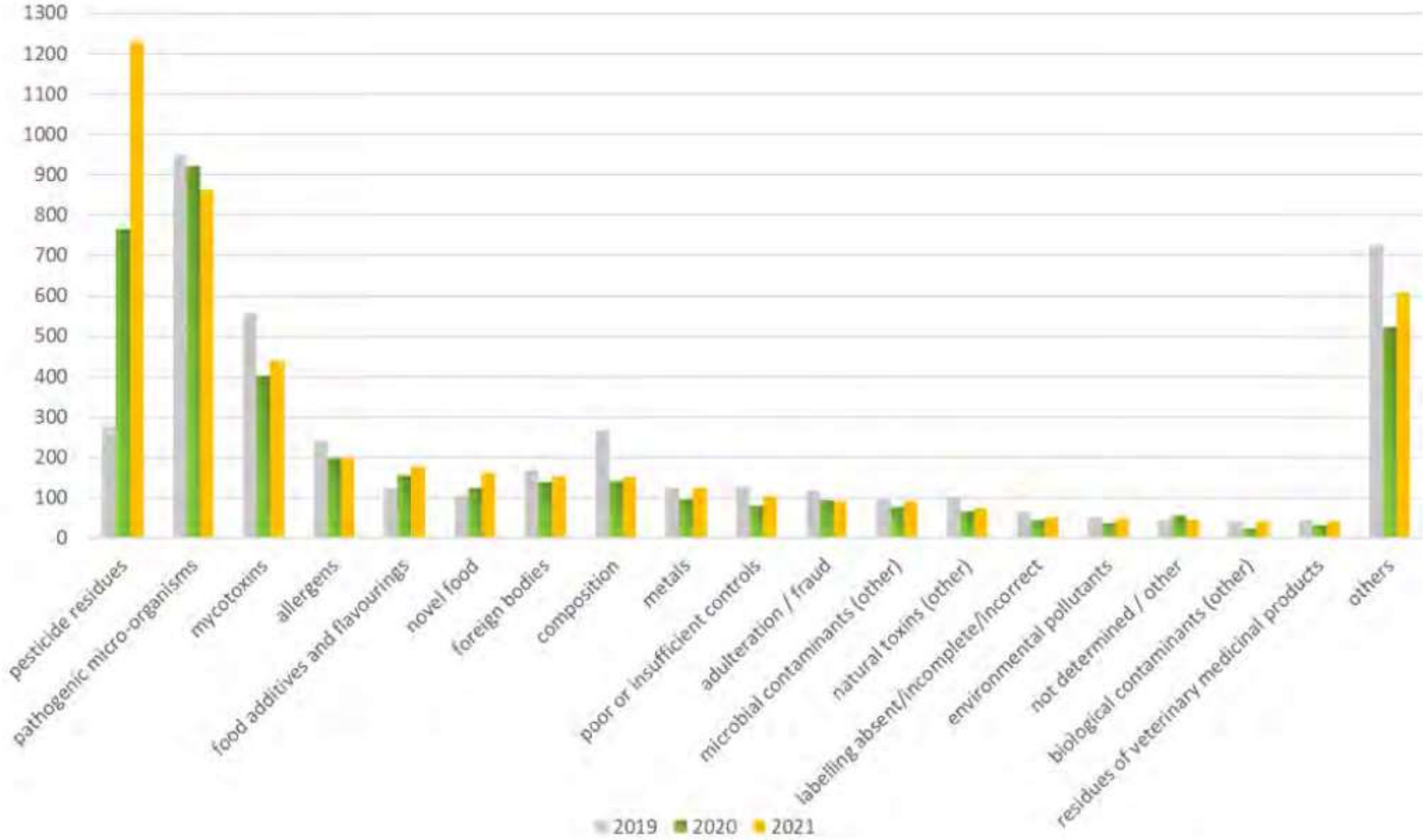


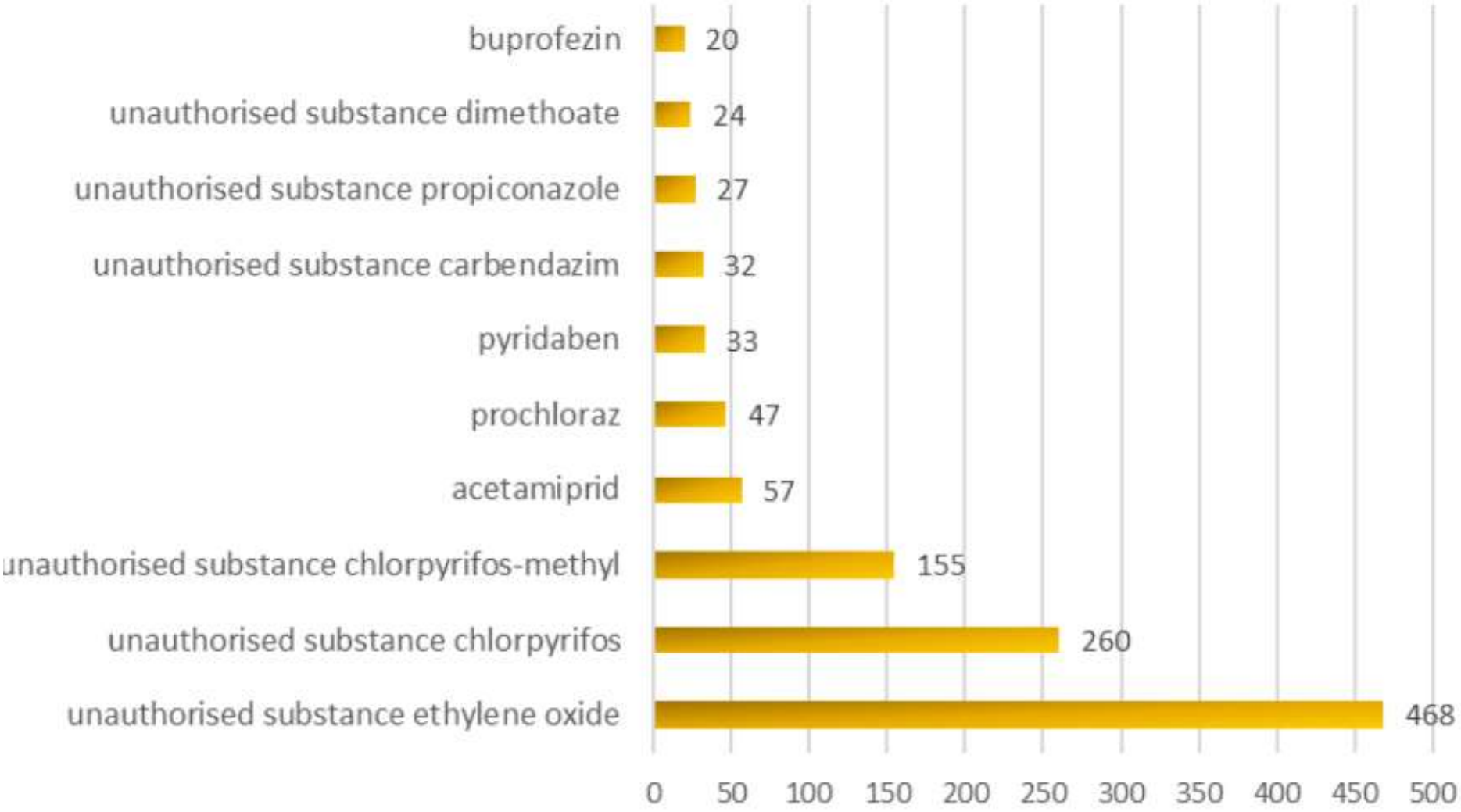
Fig.7: RASFF notifications by food hazard category 2019-2021

Objave prema kategorijama opasnosti od hrane

Ostaci pesticida su ostaci koji su prisutni u ili na proizvodima biljnog podrijetla, posebice uključujući ostatke koji se pojavljuju kao **rezultat uporabe u zaštiti bilja, u veterinarskoj medicini i kao biocidi.** Ostaci pesticida uključuju i aktivne tvari, njihove metabolite i/ili produkte razgradnje ili produkte reakcije aktivnih tvari.

Ostaci pesticida..

- U 2021. ostaci pesticida po objavama na prvom mjestu u RASFF
- 1231 objava što predstavlja povećanje od 61% u usporedbi s 2020.
- Najviše objava – Turska; limun, naranče, mandarine, grejp



Broj RASFF objava ostataka pesticida u hrani (top 10) u 2021. godini

MDK – najveća dopuštena količina ostataka pesticida (u miligramima po kilogramu hrane) koja se može pojaviti u ili na hrani nakon upotrebe pesticida prema principima dobre poljoprivredne prakse.

PREDSTAVLJA PROVJERU ISPRAVNE UPOTREBE PESTICIDA – ODREĐUJU ZDRAVSTVENU ISPRAVNOST HRANE

TO NISU TOKSIKOLOŠKE/SIGURNOSNE GRANICE!!

ODREĐENA JE KAO ZAKONSKI ODREĐENA GRANICA

„Činitelj sigurnosti, a ne rizika”
„Smanjuje se sigurnost i barem blago povećavaju rizici”

Popis registriranih sredstava za zaštitu bilja u Republici Hrvatskoj – tražilica

Proučite koje sve informacije možete dobiti o određenom proizvodu ili aktivnoj tvari!

- https://fis.mps.hr/trazilicaszb/

- U HR testiranja namirnica provode ovlašteni laboratoriji zavoda za javno zdravstvo
- Dva laboratorija za analizu ostataka pesticida sudjeluju u provedbi Nacionalnog programa praćenja rezidua pesticida u i na hrani: Nastavni zavod za javno zdravstvo Andrija Štampar (za proizvode biljnog podrijetla) i Hrvatski veterinarski institut (za proizvode životinjskog podrijetla).

- Nacionalni program praćenja ostataka pesticida u i na proizvodima biljnog podrijetla (započeo 2007. godine)
- Na temelju dostavljenih informacija nadležna uprava izrađuje godišnje izvješće o ostacima pesticida

Rezultati Nacionalnog programa praćenja ostataka pesticida u i na hrani u 2021. godini

Godina	Broj uzoraka	Bez ostataka	S ostacima ispod MDK	Višestruki ostaci	Prekoračenje MDK
2014	374	323 (86%)	70 (19%)	28	0
2015	483	348 (72%)	134 (28%)	74	1
2016	547	331 (60,51%)	216 (39,49%)	108	10 (1,83%)
2017	608	423 (69,57%)	170 (27,96%).	95	15
2018	595	356 (59,83%)	226 (37,98%)	155	13 (2,18%)
2019	290	166 (57,24 %)	116 (40%)	94	8 (2,7 %)
2020	311	202 (60 %)	107 (35 %)	69	3 (1 %)
2021	549	255 (46,45 %)	259 (47,18 %)	193	35 (6,38 %)

Višestruki ostaci pronađeni u: bananama, grejpu, grožđu, patlidžanima, kultiviranim gljivama, dinjama, slatkoj paprici, jabukama, kiviju, limunu, mandarinama, breskvama, malinama, jagodama, mrkvi, špinatu.

Izvor: Ministarstvo poljoprivrede, Izviješće o rezultatima Nacionalnog programa monitoringa ostataka pesticida u hrani za 2021. godinu

- Većina toksikoloških istraživanja do prije 10-ak godina, promatrala je svaki pesticid zasebno

- **Čovjek svakodnevnom prehranom konzumira proizvode tretirane s različitim pesticidima (“kombinacije”; “mješavine”; “kokteli”**) – time smo izloženi **kumulativnom izlaganju** više ostataka pesticida odjednom!

- Procjena rizika izlaganju kombinacijama u svakodnevnom životu je time mnogo teža u odnosu na izlaganje jednoj tvari!

RASFF izvještaj za 2012. godinu **prvi puta** navodi sljedeće:

„Takve **mješavine ili kokteli pesticida** trebali bi se **izbjegavati gdje god je to moguće**, s obzirom da se smatra da znanstvenici još uvijek **nisu u potpunosti sposobni procijeniti rizik** koji oni predstavljaju **za zdravlje potrošača**.”

- Poznato je, iako nedovoljno istraženo, da izlaganje kombinacijama pesticida može rezultirati u:

✓ aditivnom (2+2=4)

✓ sinergističkom (2+2=20) ,

✓ antagonističkom (4+6=8) ili

✓ potencijacijskom (2+0=10) utjecaju na stanice.

- U niže prikazanom istraživanju, odabrana su tri pesticida koji su prema višegodišnjim izvještajima RASFF (*Rapid Alert System for Food and Feed*), dokazani u hrani u koncentracijama višim od MDK: **imazalil, karbendazim i cipermetrin.**
- Imazalil - fungicid široko rasprostranjen u proizvodnji agruma

- Cipermetrin – jedan od najviše korištenih insekticida u poljoprivredi
- Karbendazim - fungicid širokog spektra djelovanja u kontroli raznih gljivičnih patogena u agrikulturi.
- Združeno i interaktivno djelovanje mješavina ovih triju pesticida u hrani bilo je neistraženo te se o njihovom međudjelovanju znalo vrlo malo.

- Može li kombinirano izlaganje niskim dozama tvari koje pojedinačno ne dovode do neželjenih učinaka na zdravlje, inducirati toksične učinke kada se pojavljuju u mješavinama odnosno „koktelima“??

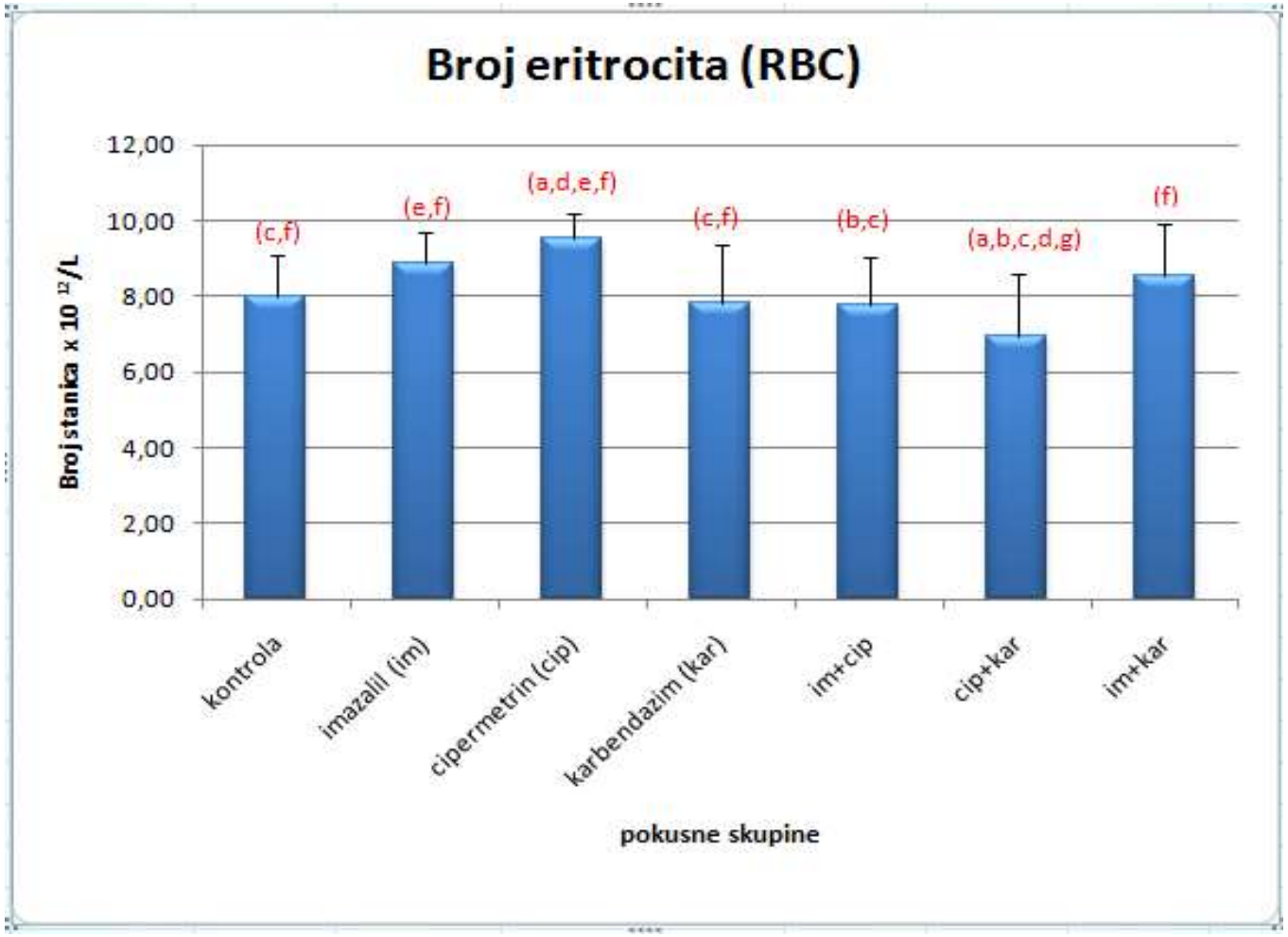
NOAEL (*No Adverse Effect Level*) - razina izloženosti ili doza bez opaženog štetnog zdravstvenog učinka/ispod koje nema štetnih učinaka – istraživanja koja se rade s NOAEL dozama od posebne su važnosti jer simuliraju svakodnevnu koncentraciju unosa različitih tvari u organizam čovjeka

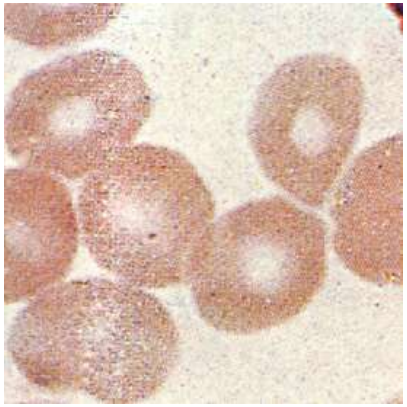
Što možemo saznati iz ekotoksikoloških istraživanja na životinjskom modelu (ovdje na primjeru kombinacija pesticida)**?**

- ODREĐIVANJE TJELESNE TEŽINE POKUSNIH ŽIVOTINJA
- ANALIZA PERIFERNE KRVI
  - BROJ ERITROCITA, KONCENTRACIJA HEMOGLOBINA, BROJ LEUKOCITA - DIFERENCIJALNA KRVNA SLIKA (DKS),
- KOMET TEST LEUKOCITA, MIKRONUKLEUS –TEST *IN VIVO*
- ODREĐIVANJE BIOKEMIJSKIH PARAMETARA U SERUMU I HOMOGENATU JETRE
- ANALIZA LIMFOHEMATOPOETSKIH ORGANA (TIMUS, SLEZENA, LIMFNI ČVOR); TEŽINA, STANIČNOST, KOMET TEST, APOPTOZA, NEKROZA, VITALNOST (TIMOCITA, SPLENOCITA, STANICA LIMFNOG ČVORA)
- ODREĐIVANJE OKSIDATIVNOG STRESA TIMUSA, SLEZENE I LIMFNOG ČVORA
  - ANTIOKSIDATIVNI ENZIMI: KATALAZA (CAT), SUPEROKSID DISMUTAZA (SOD), REDUCIRANI GLUTATION (GSH)
  - LIPIDNA PEROKSIDACIJA; MALONDIALDEHID (MDA), 8-ISO-PROSTAGLANDIN F2α
- HISTOLOŠKA ANALIZA ORGANA

Analiza periferne krvi

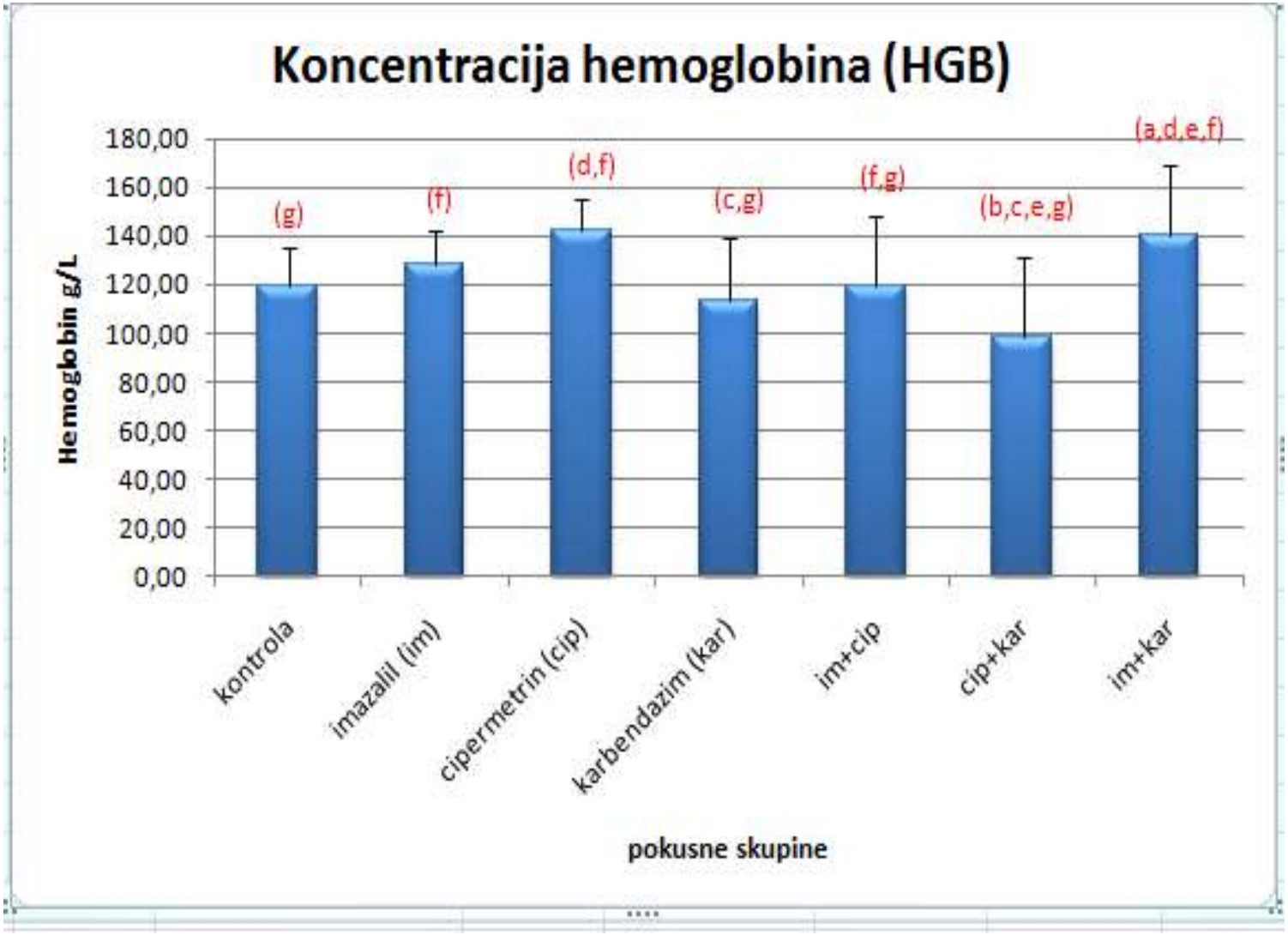
Ukupan broj eritrocita u perifernoj krvi životinja izloženih imazalilu, cipermetrinu, karbendazimu i njihovim kombinacijama





Statistički značajna promjena ukupnog broja eritrocita – u skupini tretiranoj cipermetrinom i kombinaciji cipermetrin + karbendazim

Koncentracija hemoglobina u krvi životinja izloženih imazalilu, cipermetrinu, karbendazimu i njihovim kombinacijama



Kombinacija imazalila i karbendazima izazvala je statistički značajan porast koncentracije hemoglobina. U ostalim pokusnim skupinama nije dokazana promjena

VAŽNO!

- U novije vrijeme u ekotoksikološkim/biološkim istraživanjima sve se više uvode istraživanja genotoksičnosti, iz razloga što nam analiza na nivou organa može dati sasvim drugačije rezultate..

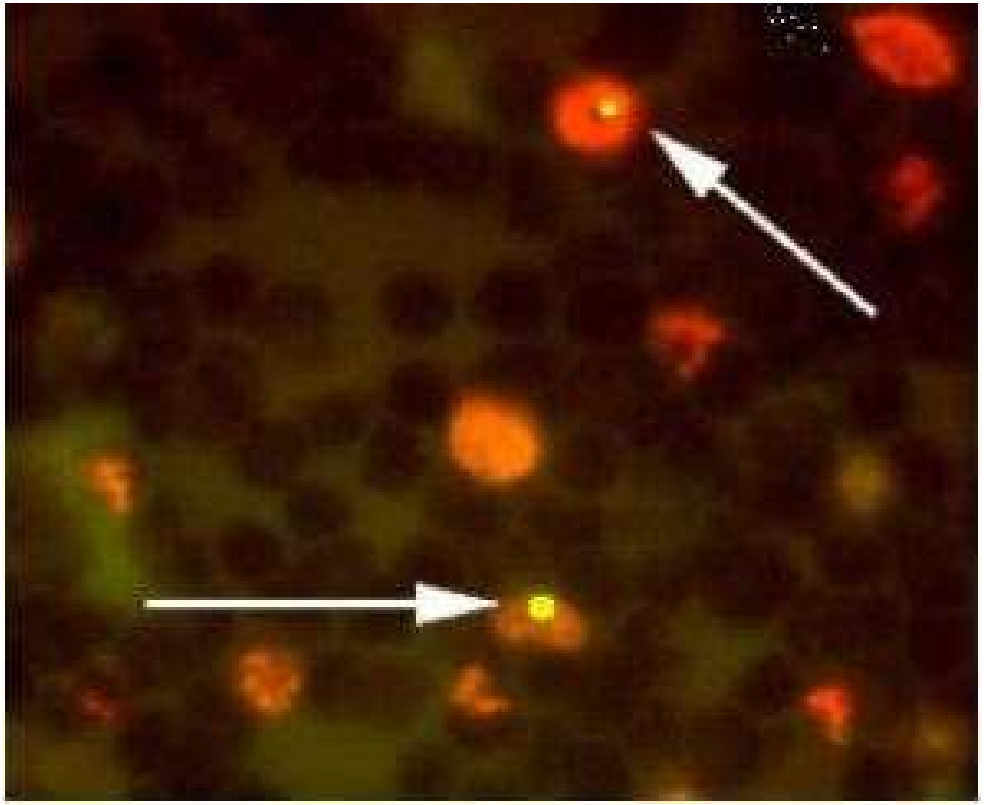
- Neki od testova genotoksičnosti:

✓ MIKRONUKLEUS TEST

✓ KOMET TEST

MIKRONUKLEUS TEST

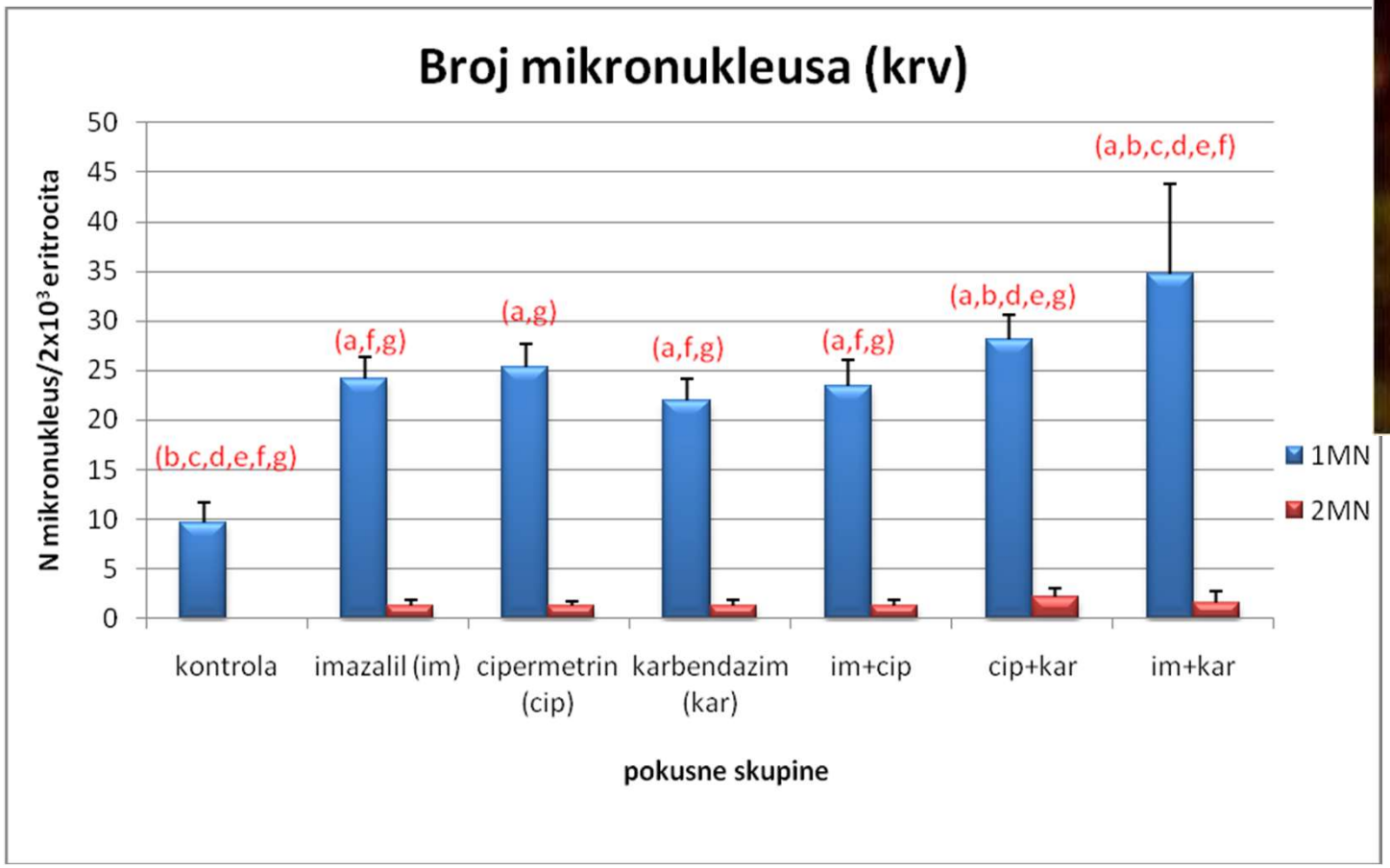
MIKRONUKLEUSI - male kromatinske strukture koje nalikuju jezgri, smještene su unutar interfazne citoplazme.

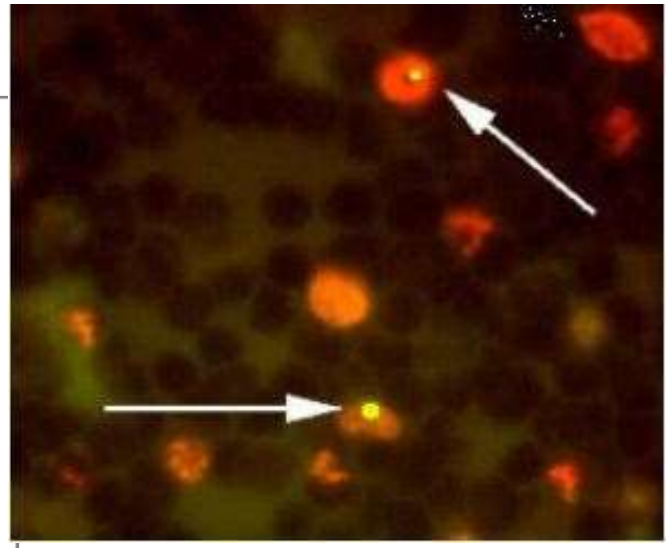


- ✓ Učestalost mikronukleusa - kvantitativna mjera strukturnih i numeričkih aberacija kromosoma u stanicama, u uvjetima *in vitro* i *in vivo* pod utjecajem različitih genotoksičnih tvari.

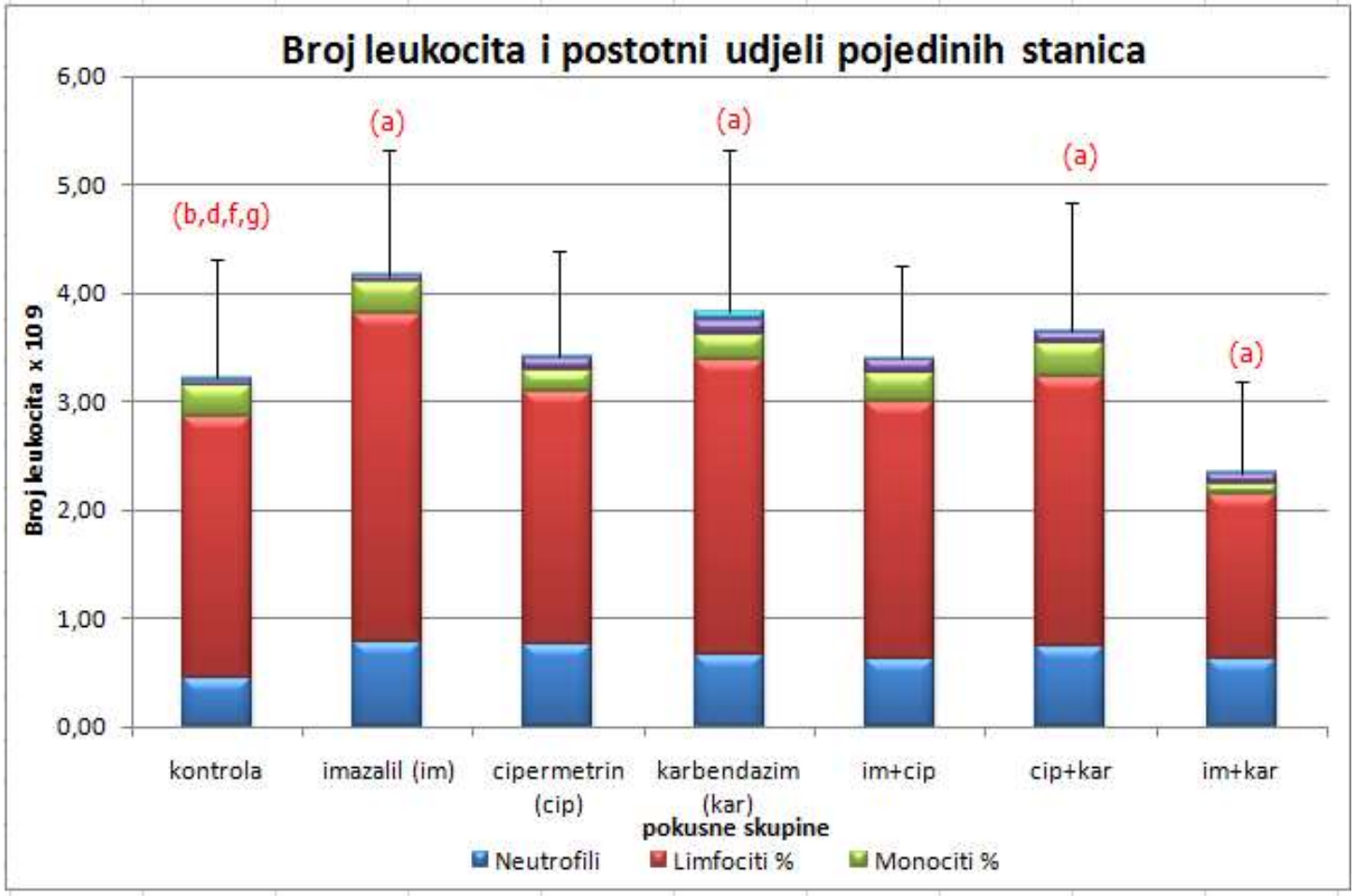
- **Aneugeni učinak**
- **Klastogeni učinak**

Broj mikronukleusa u krvi životinja izloženih imazalilu, cipermetrinu, karbendazimu i njihovim kombinacijama



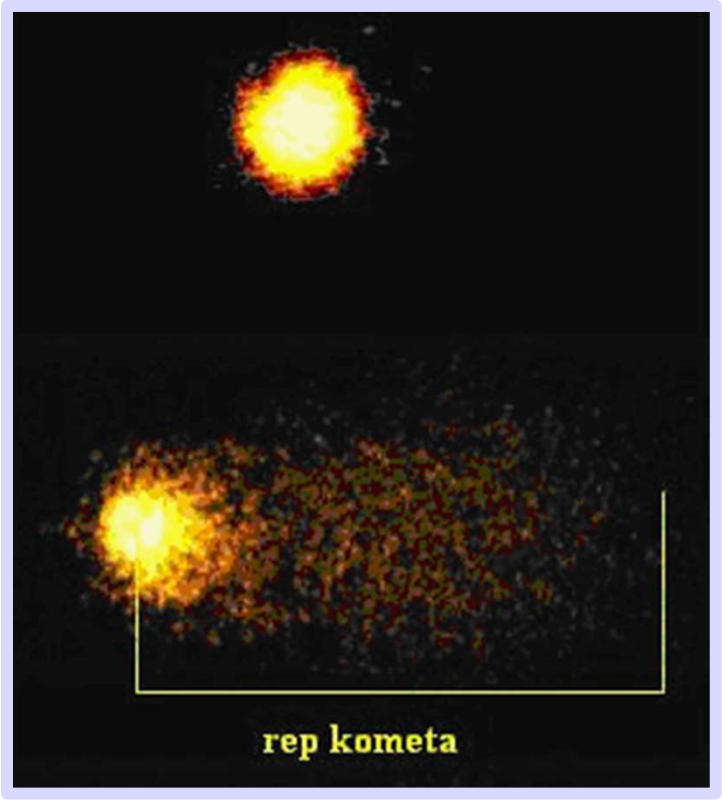


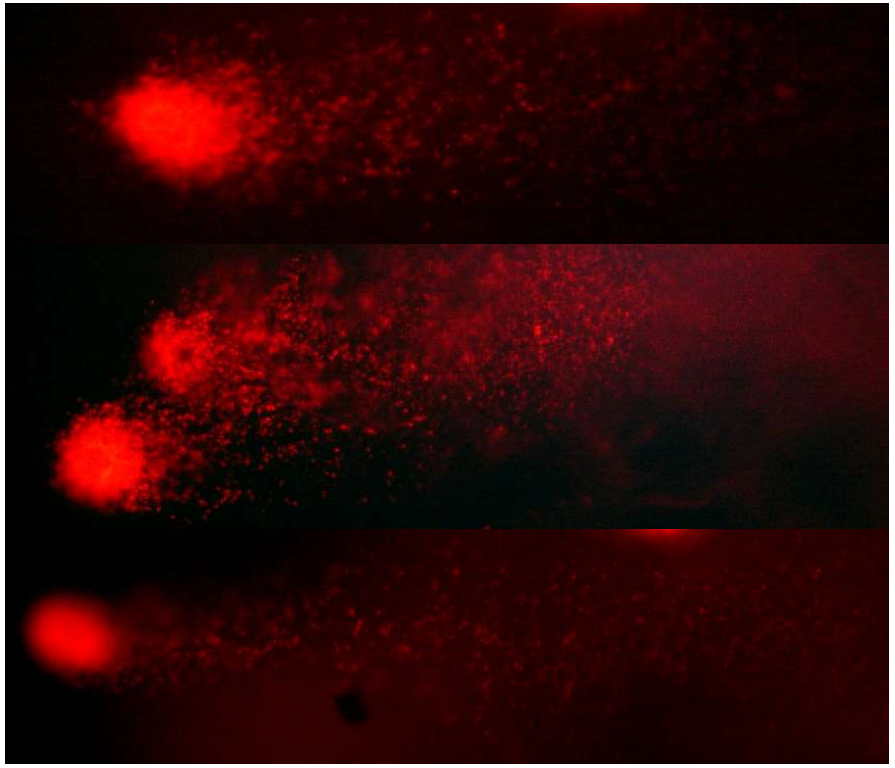
Ukupan broj leukocita u perifernoj krvi životinja izloženih imazalilu, cipermetrinu, karbendazimu i njihovim kombinacijama



Imazalil i karbendazim doveli su do statistički značajnog povećanja broja leukocita u krvi, a jedna kombinacija (imazalil i karbendazim) ih je smanjila

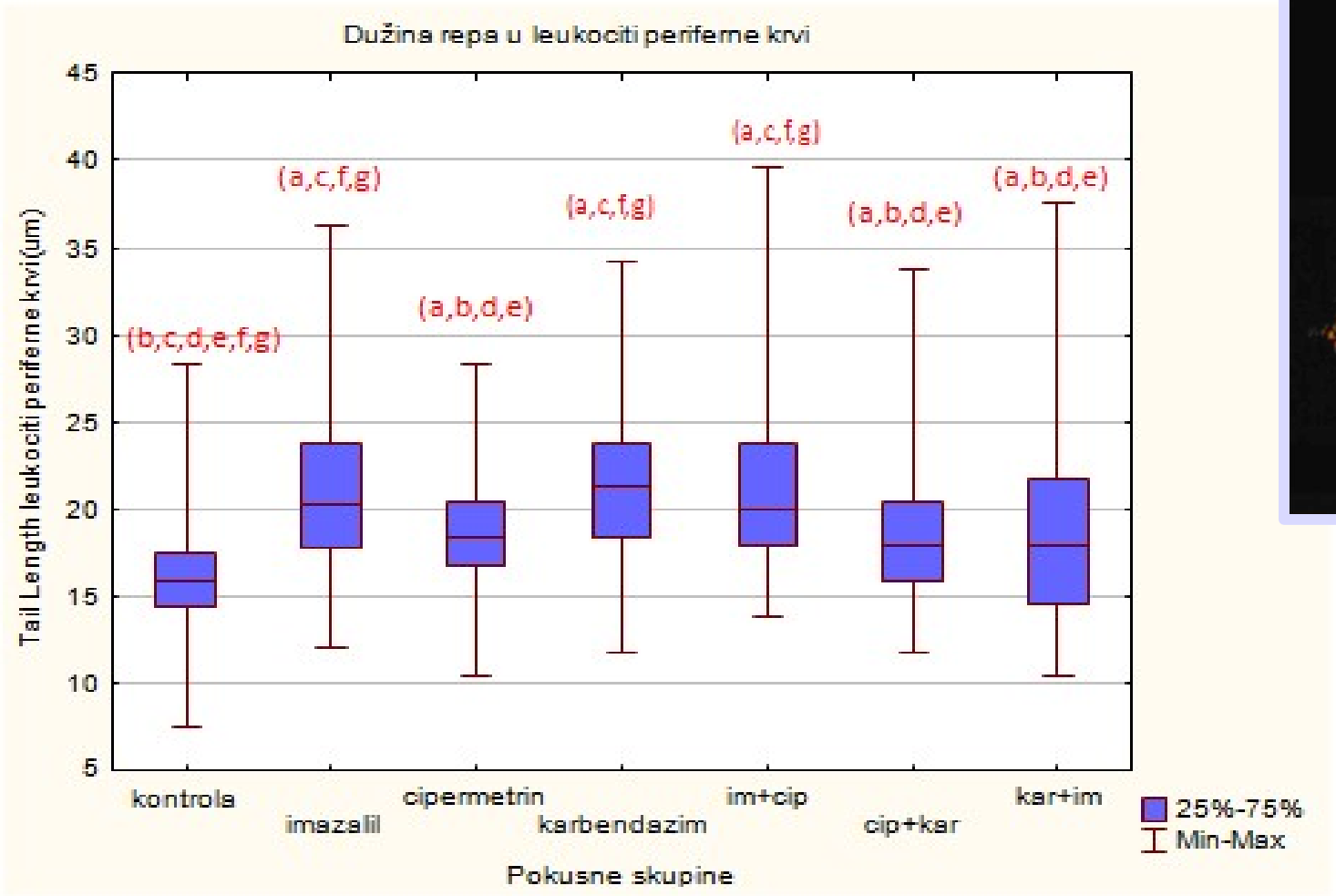
KOMET TEST

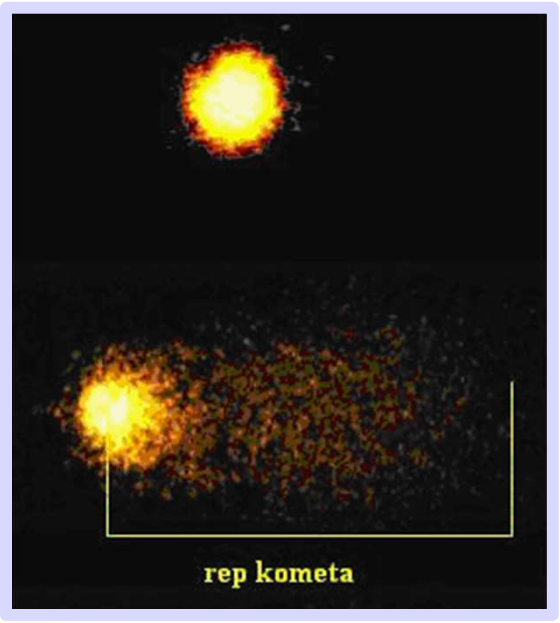




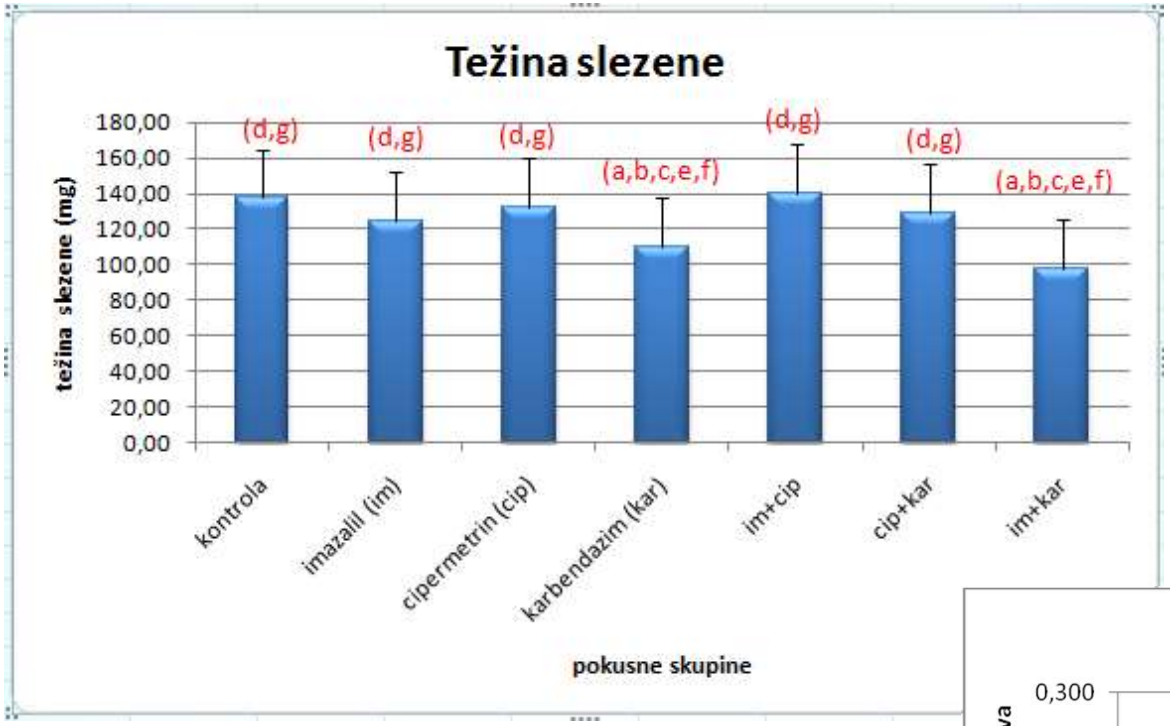
- Mikrogel elektroforeza - procjenjuje se oštećenje DNA u pojedinim stanicama različitih tkiva

Učinak imazalila, cipermetrina, karbendazima i njihovih kombinacija na stabilnost DNA u leukocitima periferne krvi izmjerena alkalnim komet testom

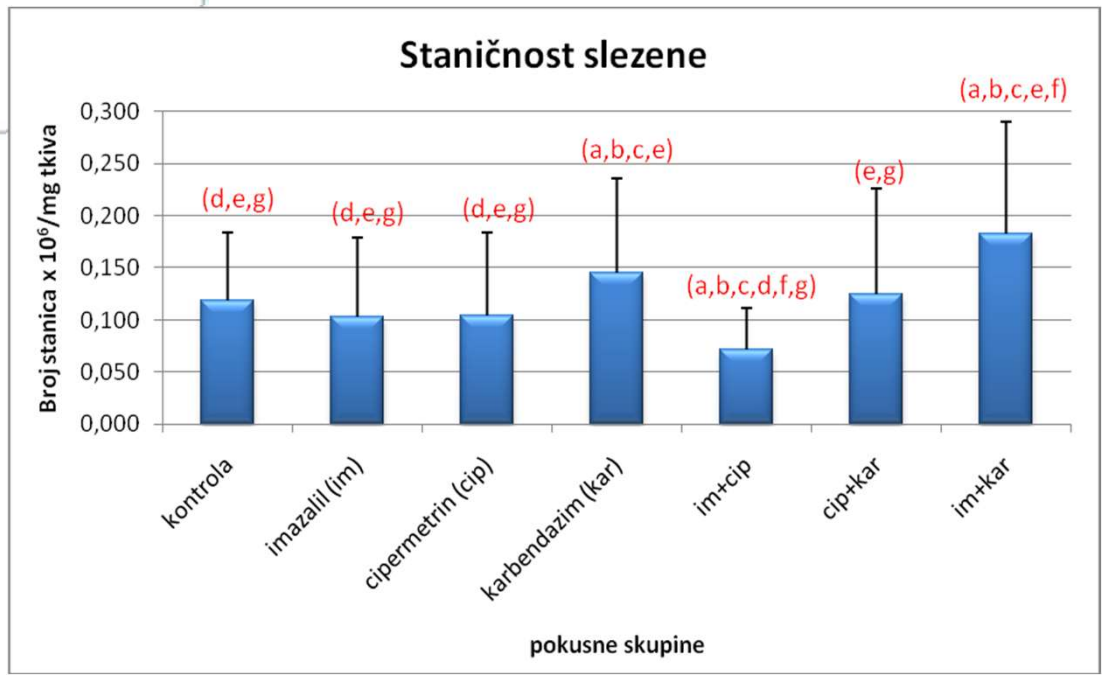




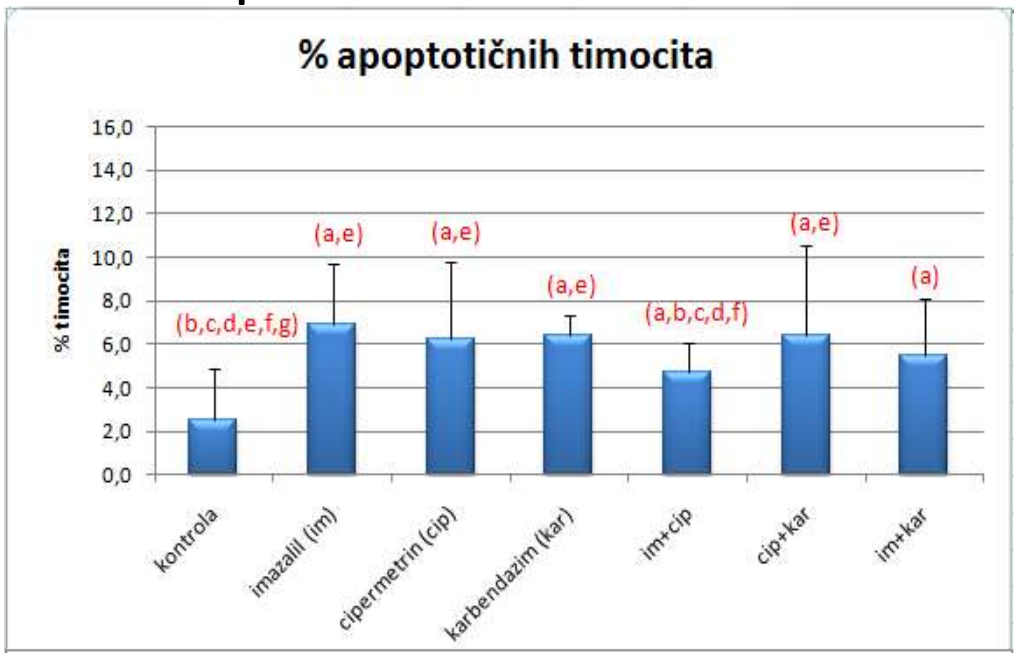
Analizira se još… učinak na organe imunosnog sustava

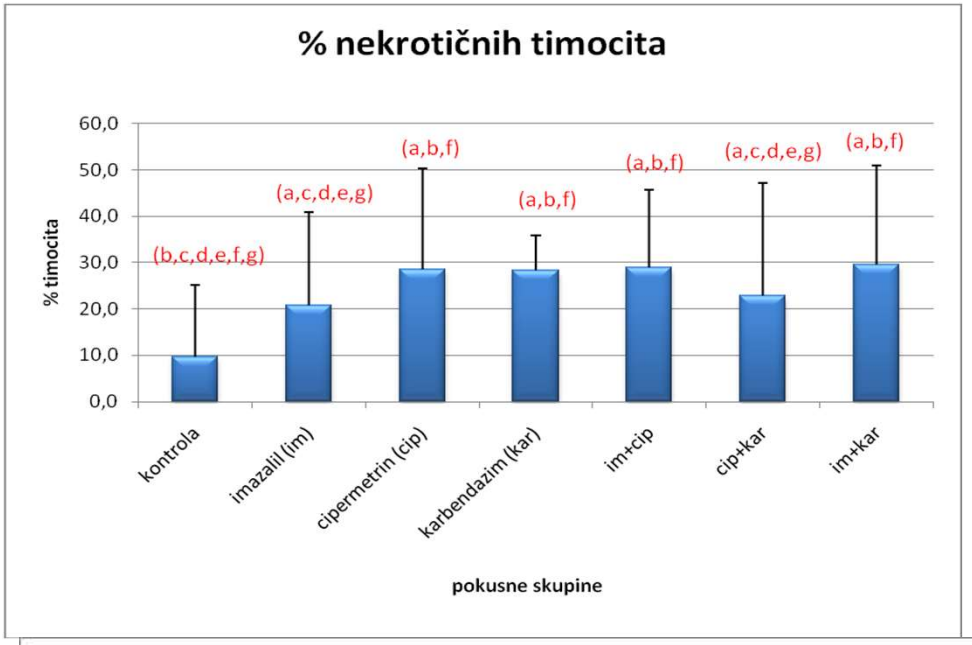


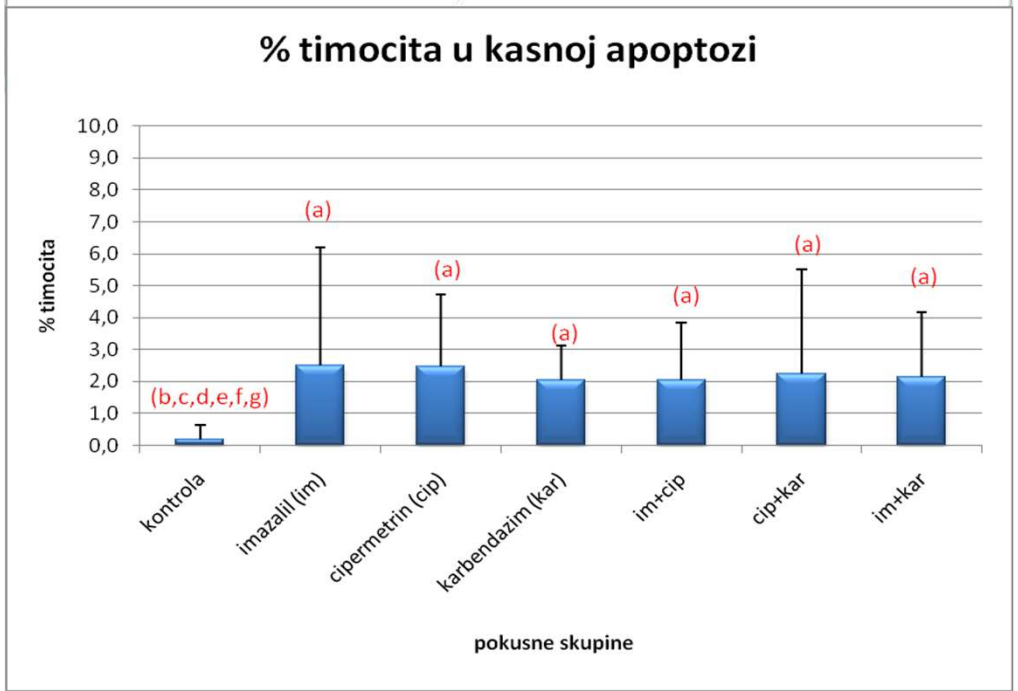
Na razini organa, statistički značajna promjena dokazana samo u slučaju karbendazima i kombinaciji imazalil+karbendazim (težina slezene), a u staničnosti još i kombinacija imazalil+cipermetrin

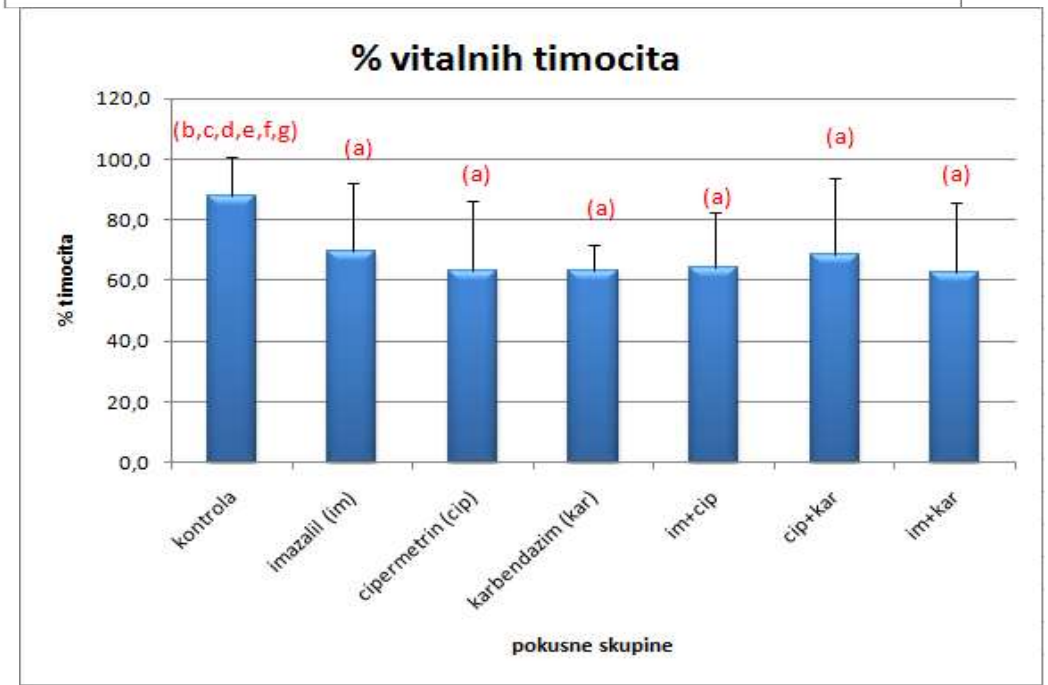


Učinak na **apoptozu, nekrozu i vitalnost** stanica timusa, slezene i poplitealnog limfnog čvora izmjerena metodom protočne citometrije

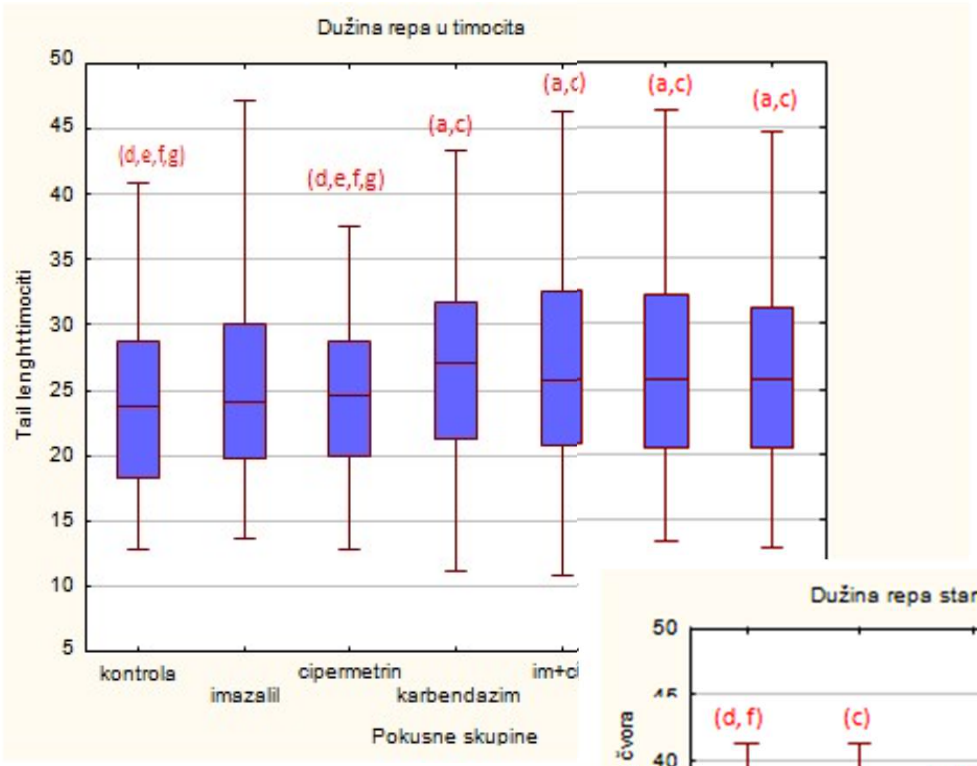


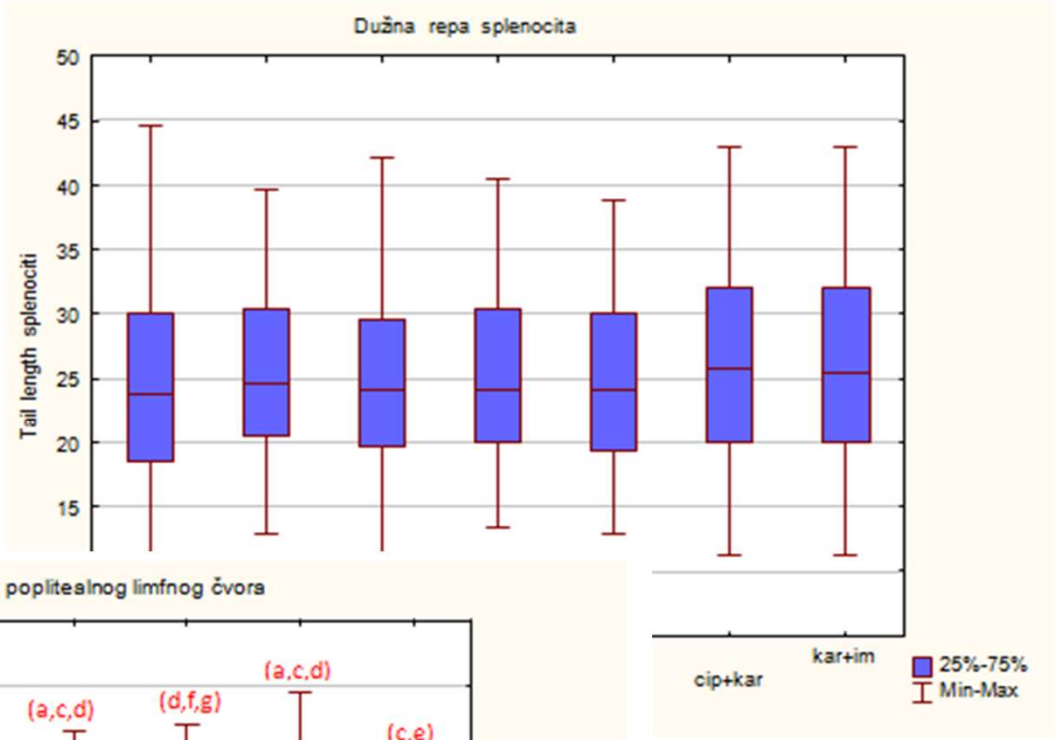


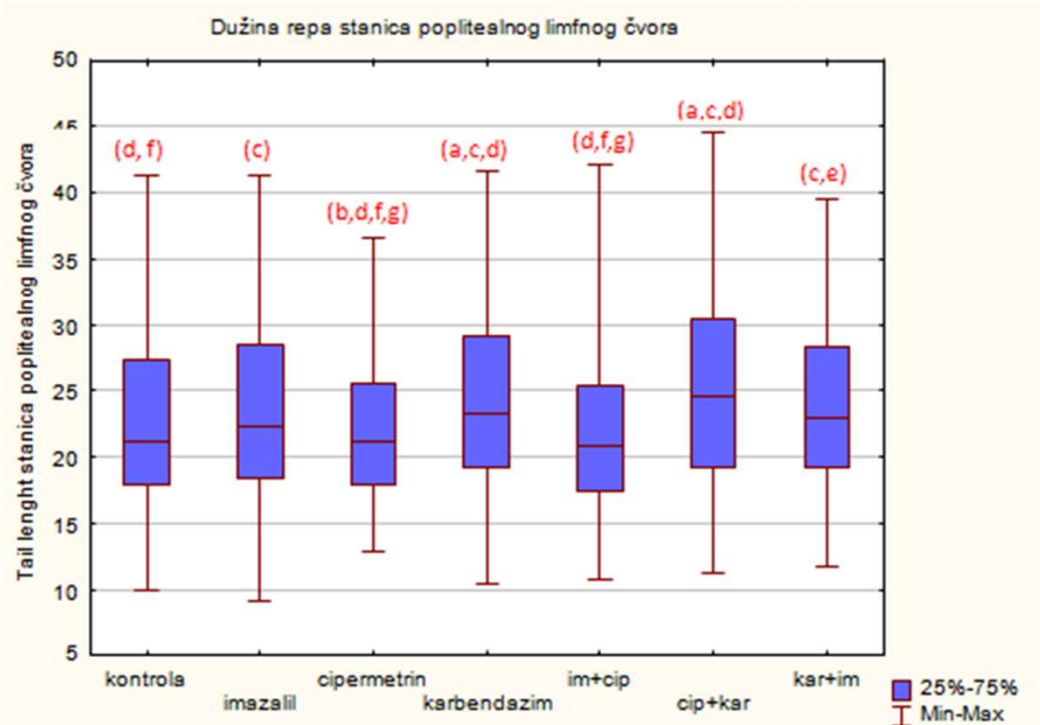


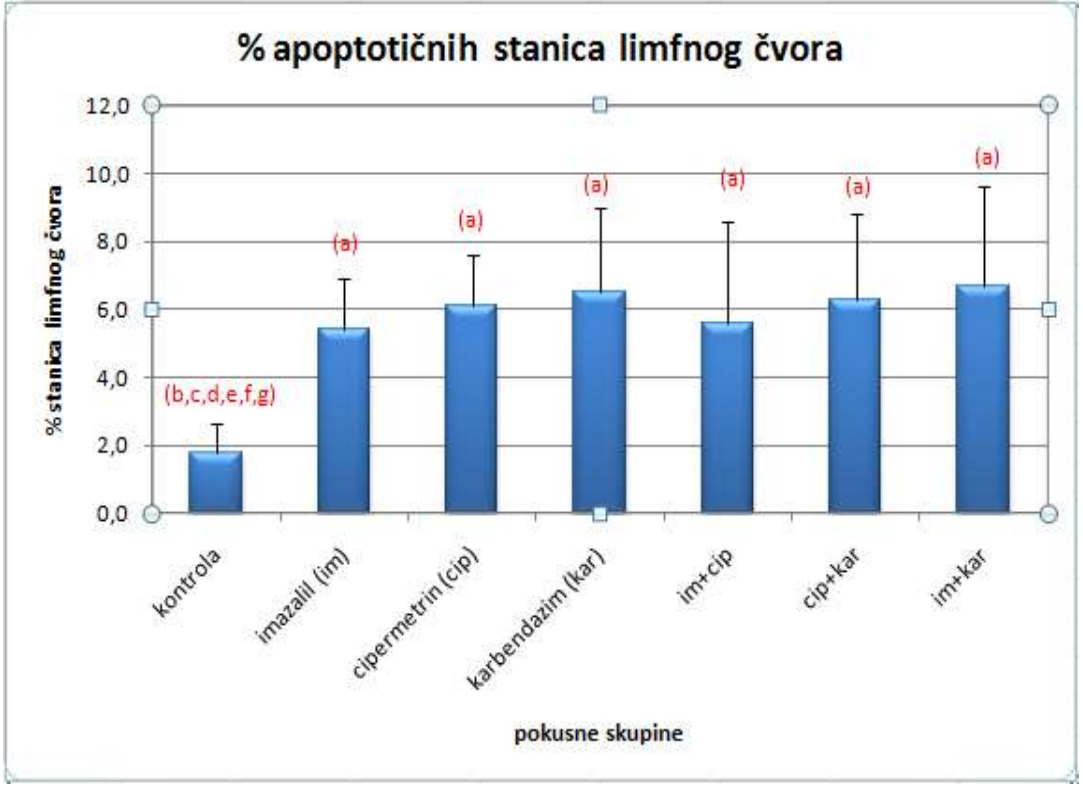


- **Učinak imazalila, cipermetrina, karbendazima i njihovih kombinacija na stabilnost DNA timusa, slezene i poplitealnog limfnog čvora izmjerena alkalnim komet testom**

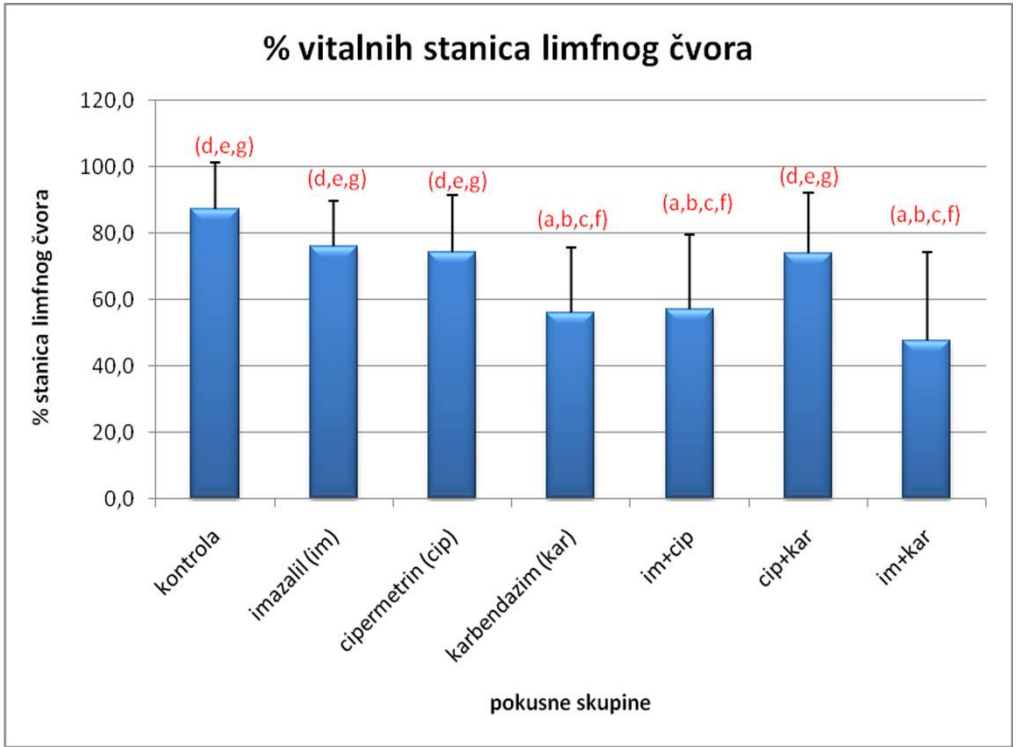




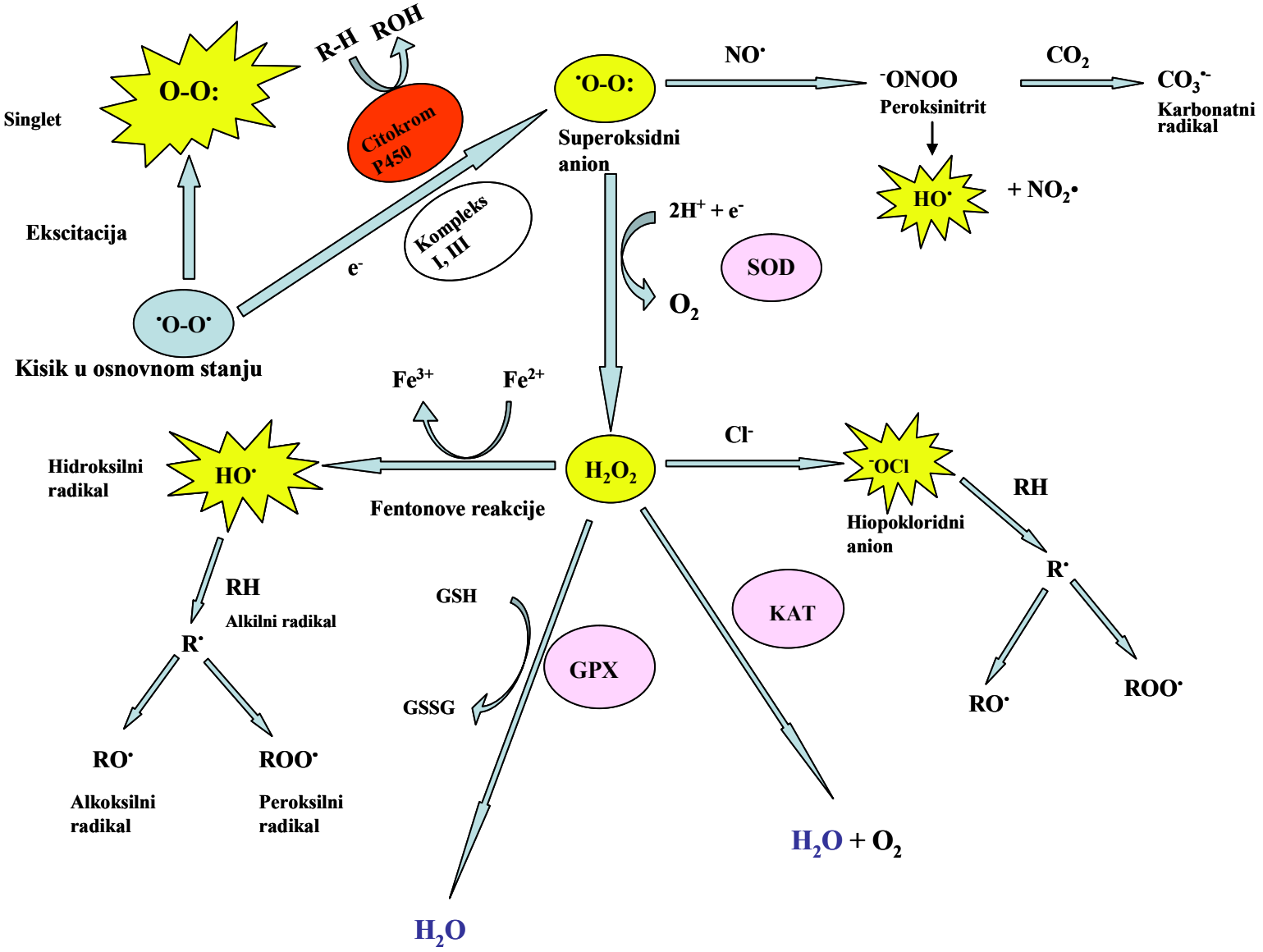




Limfni čvor-najosjetljiviji od analiziranih organa; svi pesticidi i njihove kombinacije dovele su do statistički značajnog porasta stanica u apoptozi

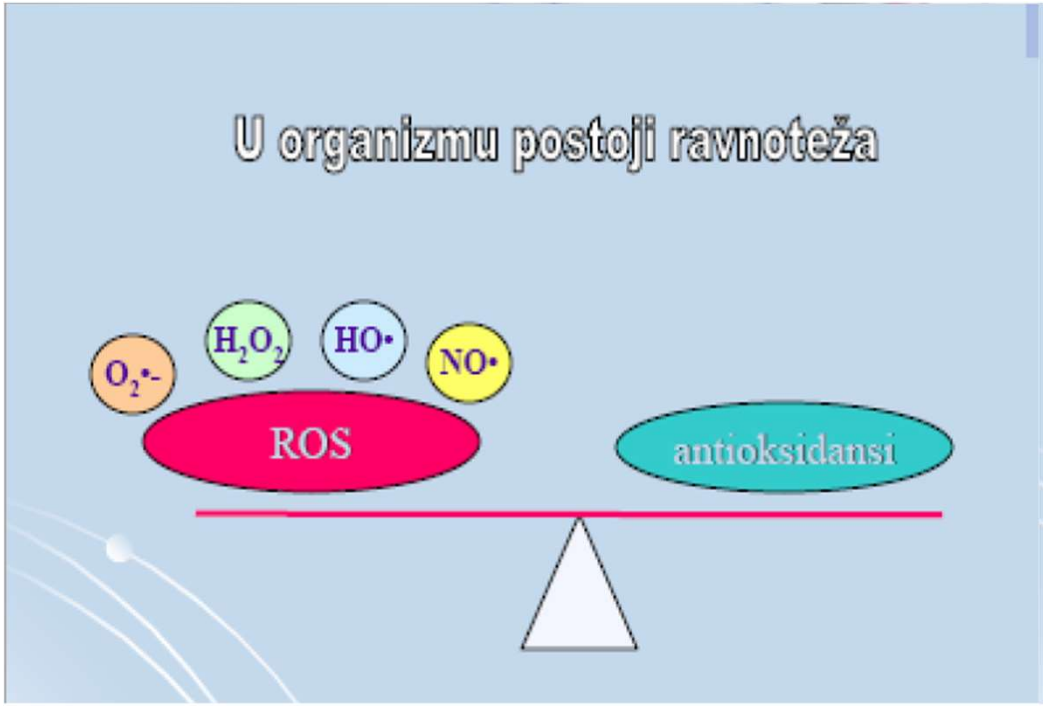


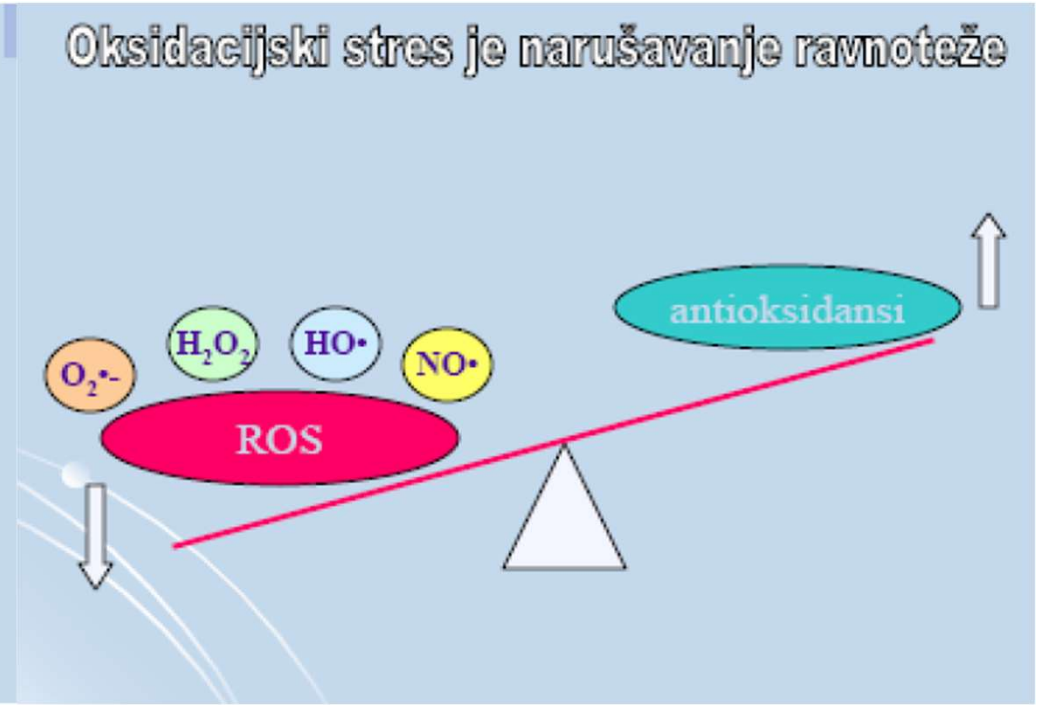
Sjećate li se što je oksidativni stres i do kojih promjena dovodi na staničnoj razini?



Reaktivne kisikove vrste (ROS)

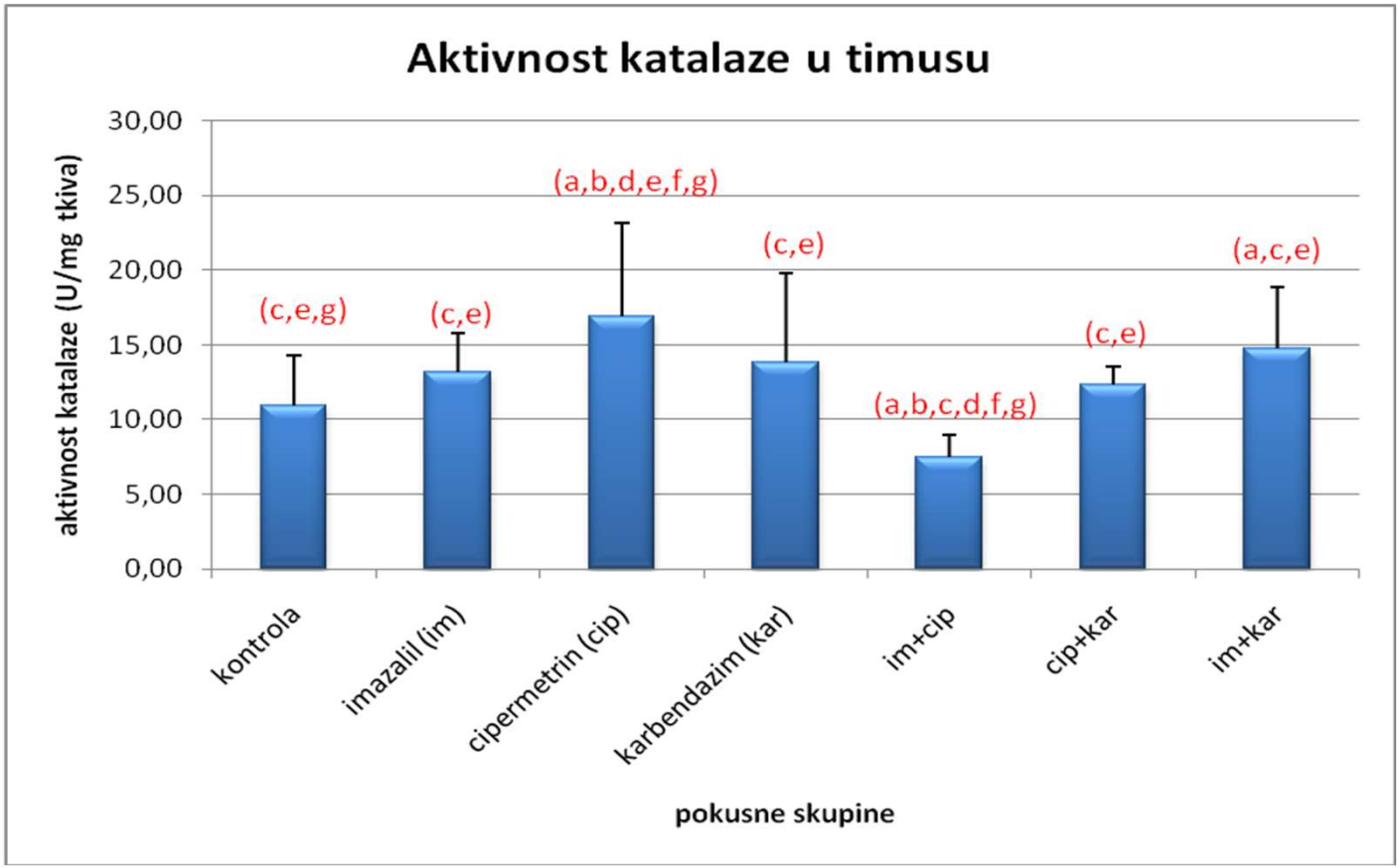
- **OKSIDATIVNI STRES** definiran je kao **pomak ravnoteže u staničnim oksidativno–redukcijskim reakcijama prema oksidaciji**, odnosno to je ***stanje prekomjernog stvaranja slobodnih radikala kisika***, pri čemu dolazi do gubitka ravnoteže stvaranja slobodnih radikala i mogućnosti neke stanice da ih razgradi, a ***rezultira promjenama vezanim za oštećenje stanica***. Ovu promjenu ravnoteže mogu uzrokovati različiti procesi koji potiču stvaranje reaktivnih kisikovih i dušikovih spojeva (engl. *Reactive Oxygen Species*, ROS; *Reactive Nitrogen Species*, RNS). (Žarković, 2001).
- Kao pokazatelj oksidativnog stresa koristi se i lipidna peroksidacija


U organizmu postoji ravnoteža
$O_2^{\bullet-}$
H_2O_2
HO•
NO•
ROS
antioksidansi

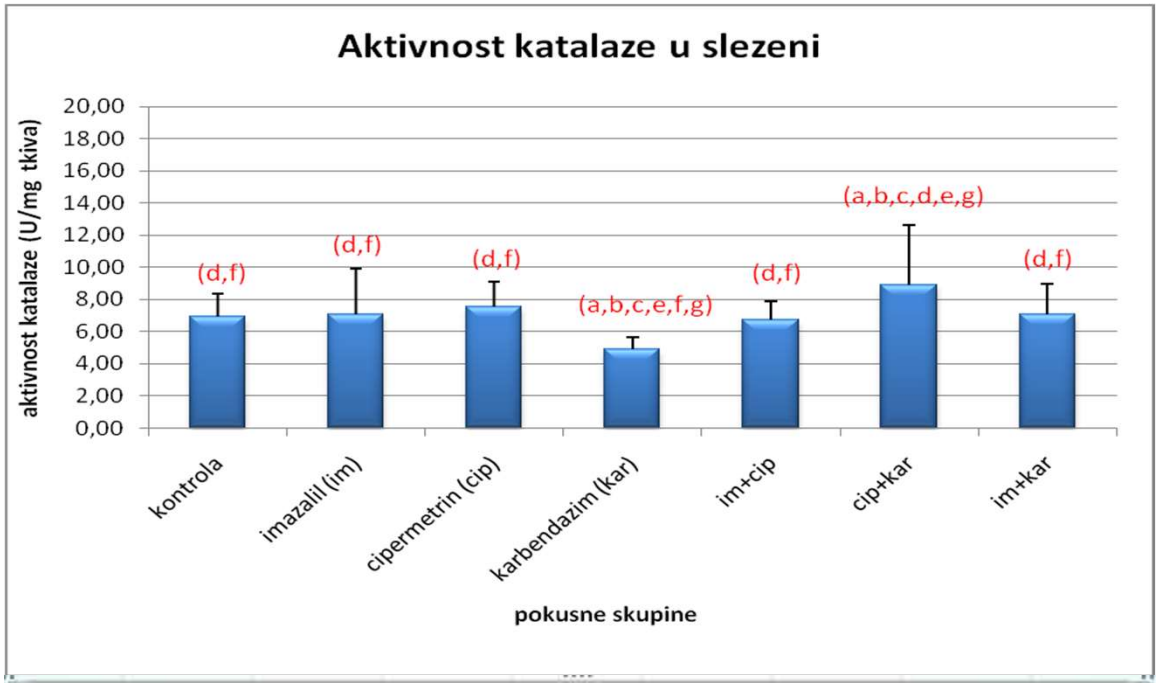

Oksidacijski stres je narušavanje ravnoteže
$O_2^{\bullet-}$
H_2O_2
HO•
NO•
ROS
antioksidansi

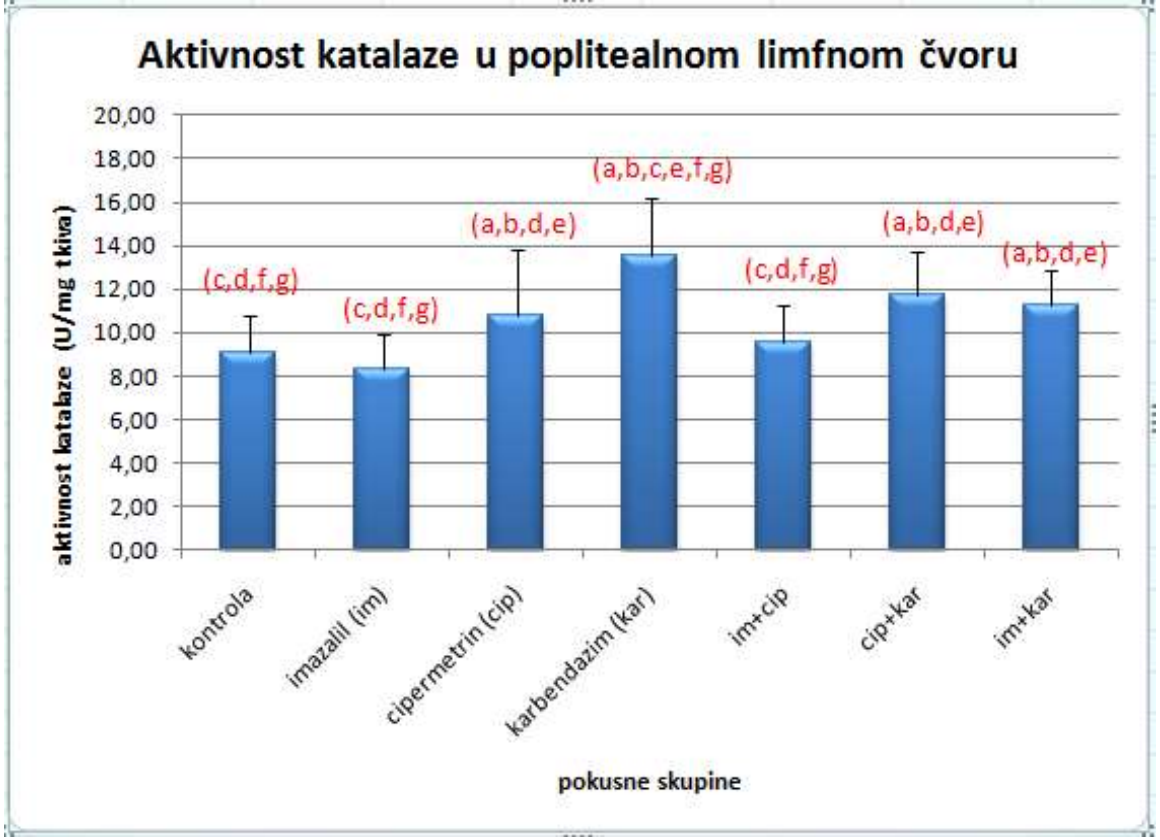
Učinak imazalila, cipermetrina, karbendazima i njihovih kombinacija na aktivnost antioksidativnih enzima u timusu, slezeni i poplitealnom limfnom čvoru

Učinak na aktivnost katalaze (CAT)



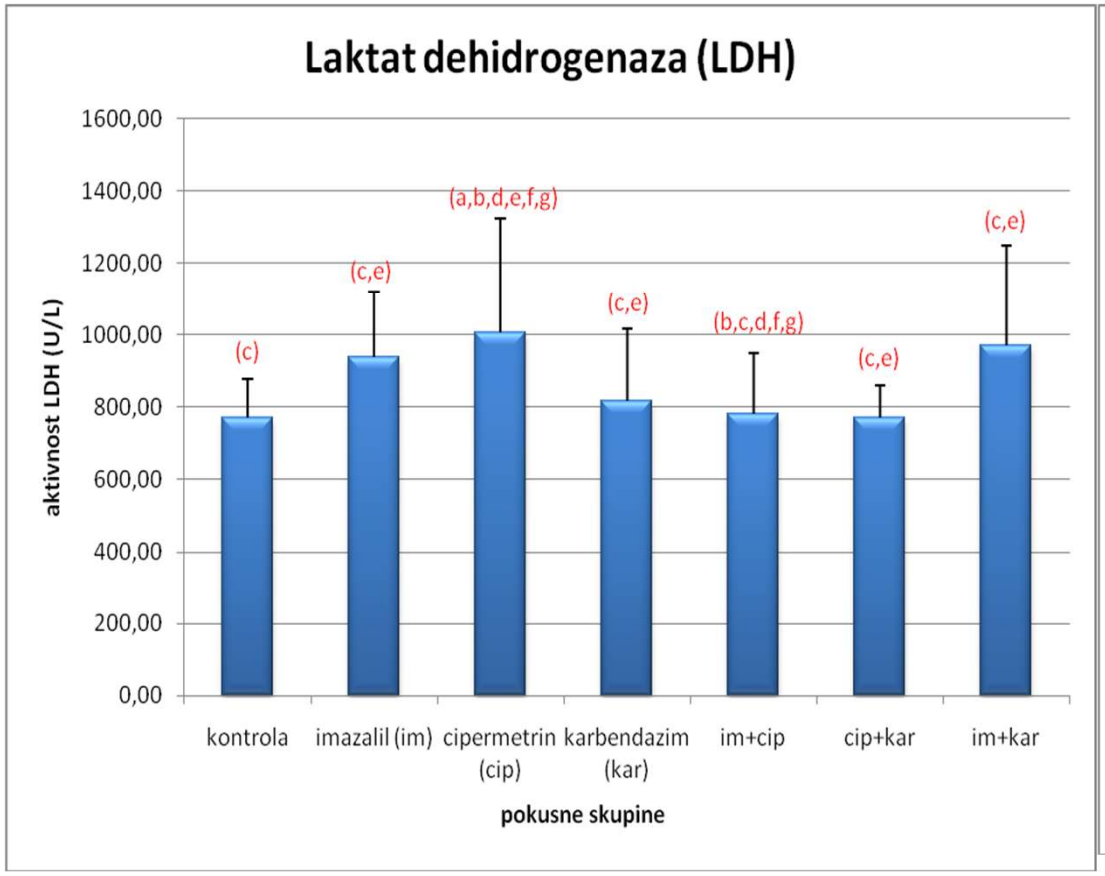
Slovo a u zagradi označava statistički značajnu razliku u odnosu na kontrolnu skupinu

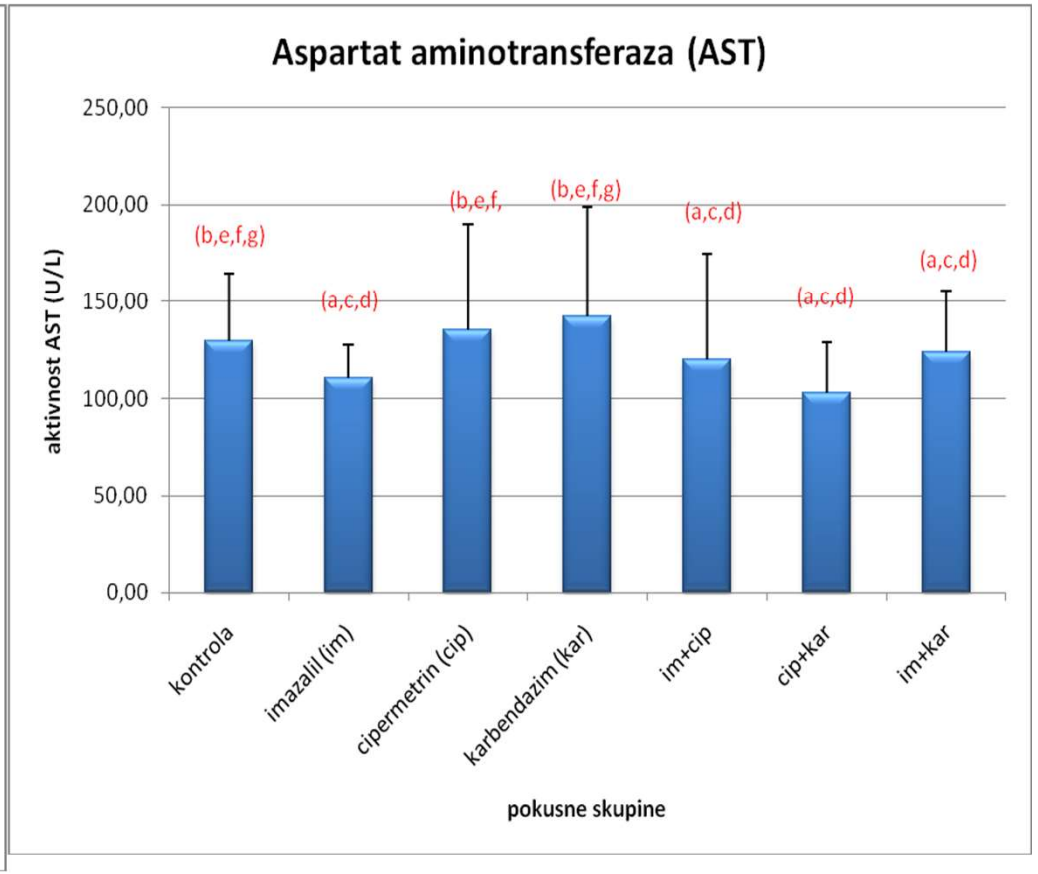




Koji organ od tri analizirana je imao najviše statistički značajnih promjena razine katalaze odnosno dokaza oksidativnog stresa uzrokovanih pesticidima i njihovim kombinacijama?

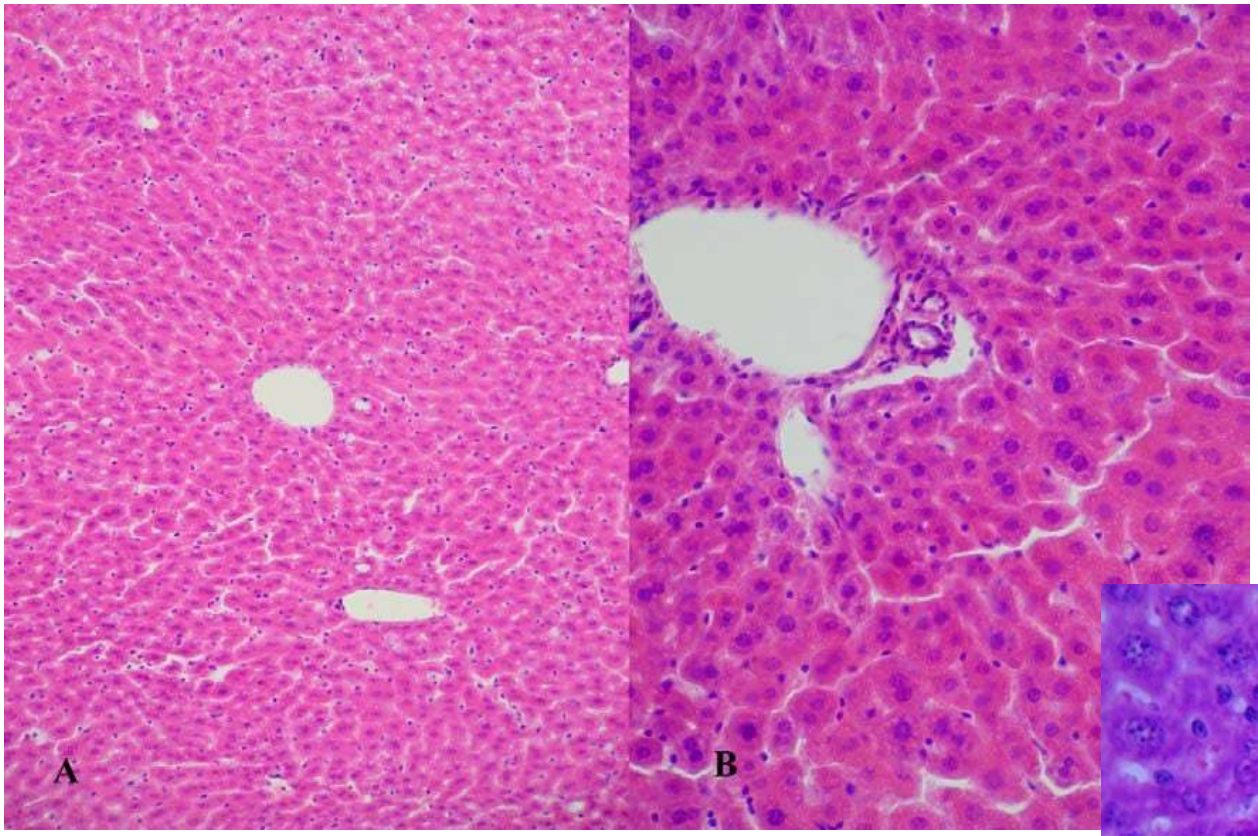
Rezultati mjerenja aktivnosti serumskih enzima u životinja izloženih imazalilu, cipermetrinu, karbendazimu i njihovim kombinacijama



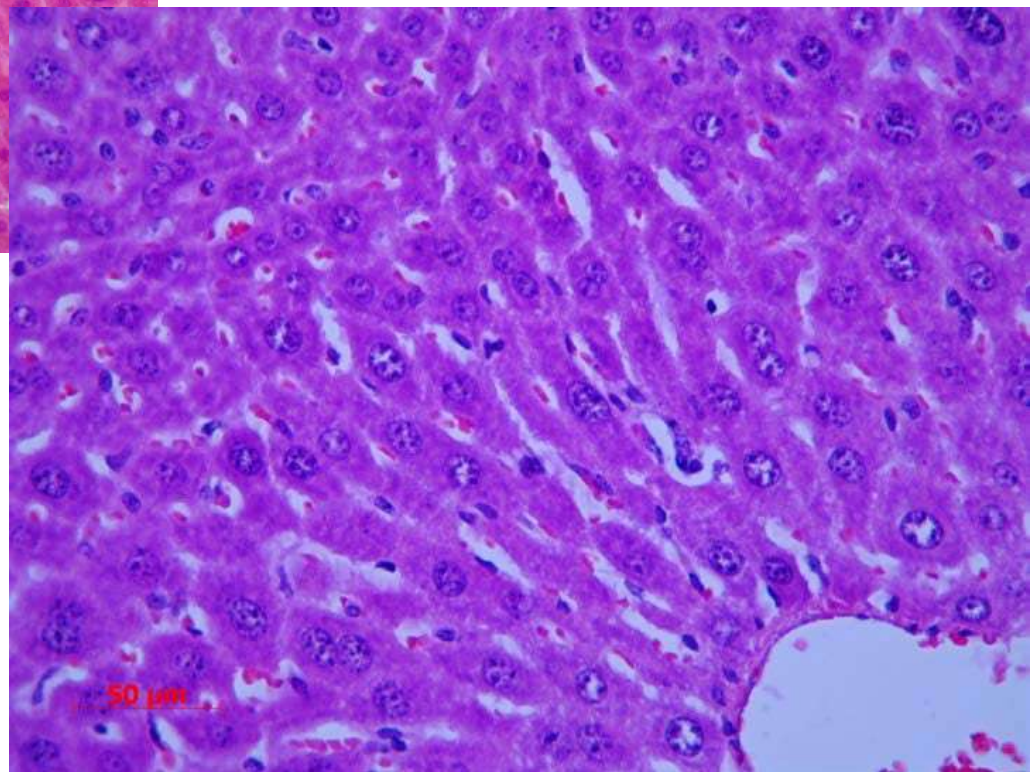


Različiti toksični potencijal: cip>im>kar

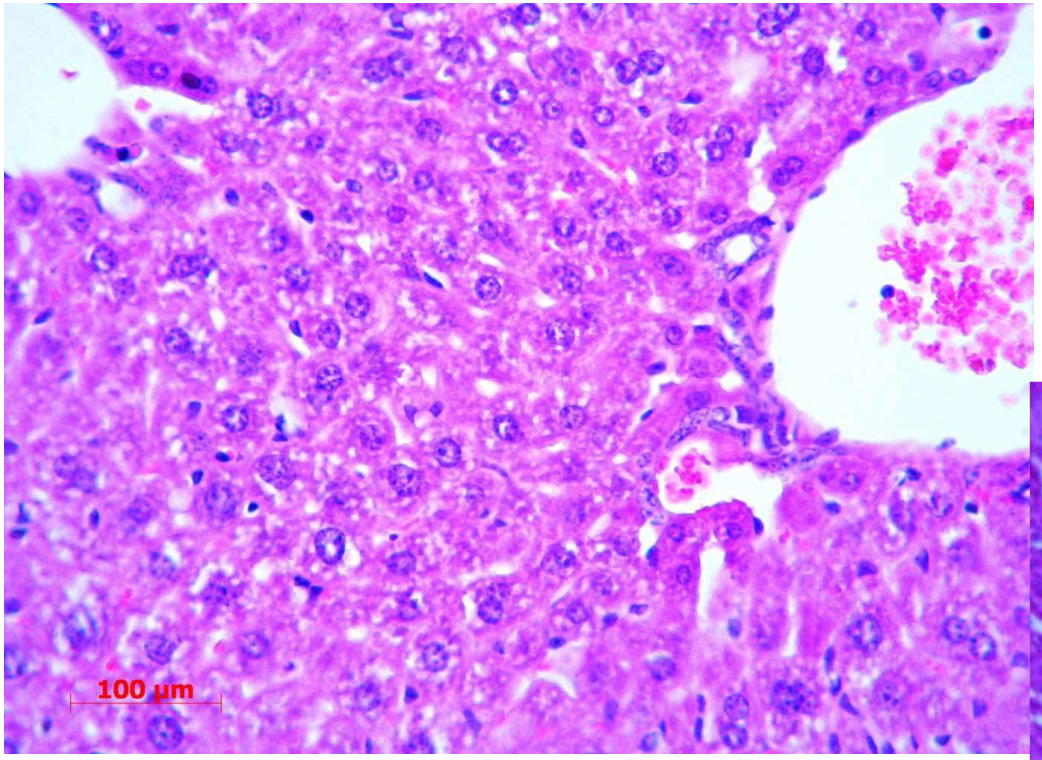
Histologija jetre



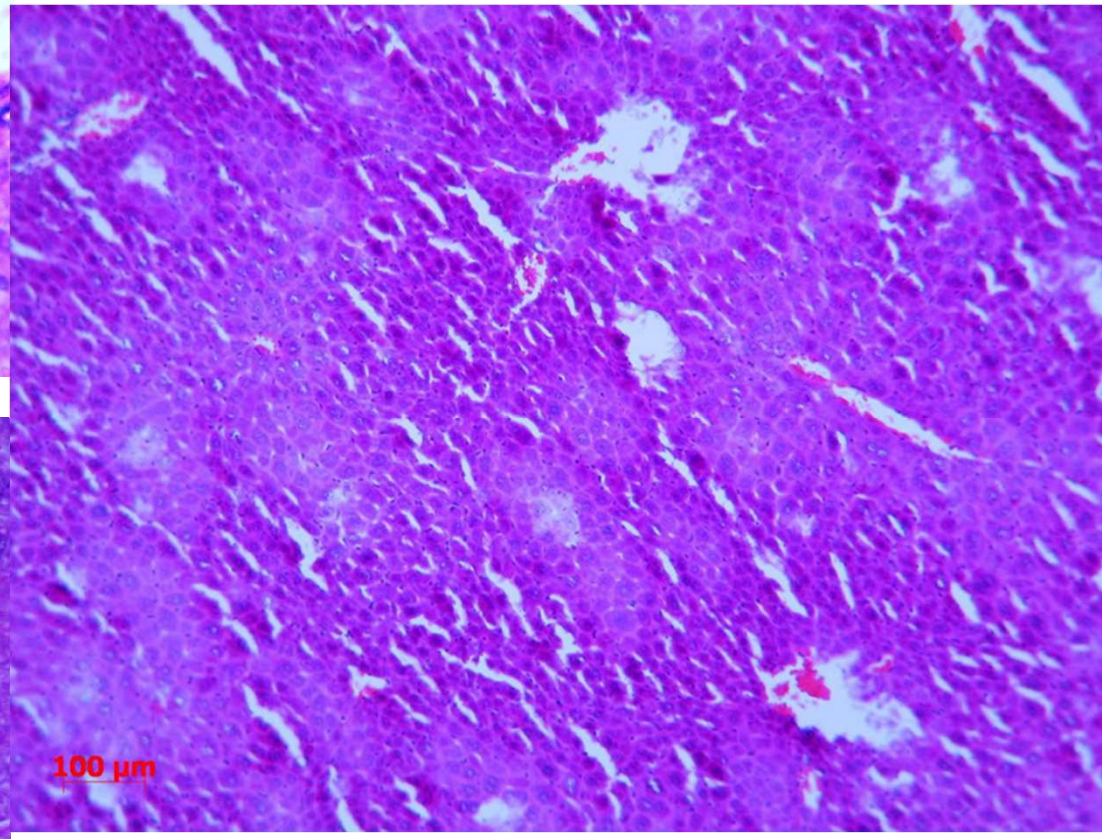
kontrolna skupina

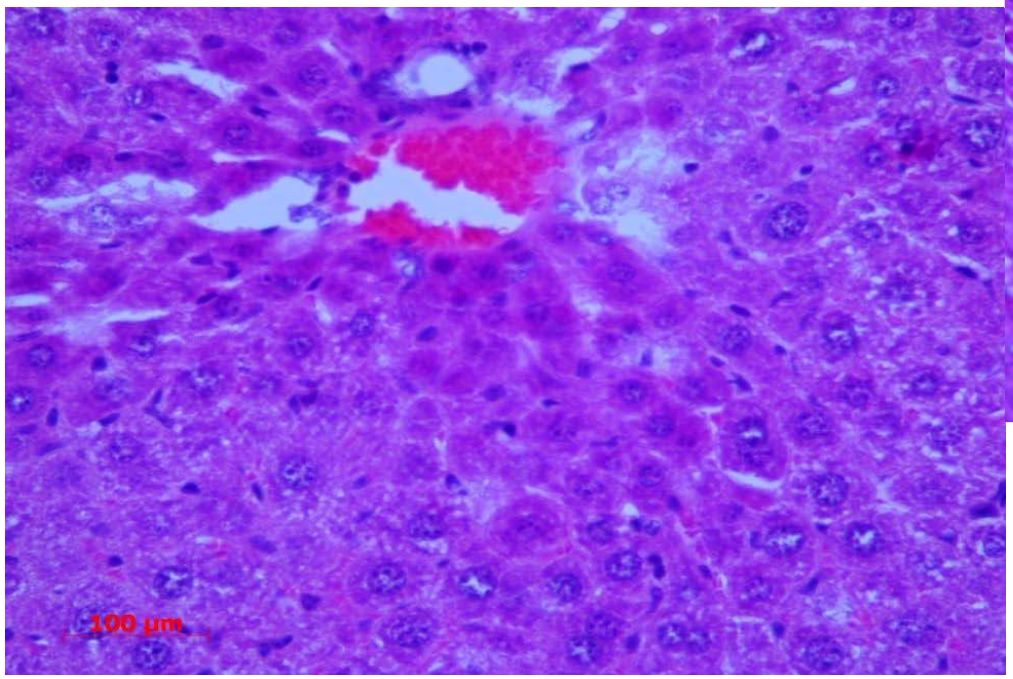


skupina tretirana imazalilom
(najslabiji stupanj oštećenja)



Imazalil+cipermetrin





Imazalil+karbendazim (najjači stupanj oštećenja jetre)

Cipermetrin+karbendazim

BCPT Basic & Clinical Pharmacology & Toxicology

Basic & Clinical Pharmacology & Toxicology Doi: 10.1111/j.1742-7843.2011.00831.x

Carbendazim Impends Hepatic Necrosis when Combined with Imazalil or Cypermethrin

Domagoj Đikić[1], Irena Landeka[2], Fabijan Knežević[3], Ana Mojsović-Ćuić[4], Vesna Benković[1], Anica Horvat-Knežević[1], Goran Lončar[1], Renata Teparić[2] and Dunja Rogić[5]

[1]Department of Animal Physiology, Faculty of Science, University of Zagreb, Zagreb, Croatia, [2]Faculty of Food Technology and Biotechnology, University of Zagreb, Zagreb, Croatia, [3]Department of Pathology, Sestre Milosrdnice University Hospital for Tumours, Zagreb, Croatia, [4]School of Applied Health Sciences, Zagreb, Croatia, and [5]Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia

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Abstract: Imazalil, cypermethrin and carbendazim are detected in plants for human nutrition. To explore whether their combinations, applied orally in low doses, would induce changes in metabolic patterns and hepatotoxicity, a subchronic *in vivo* experiment was conducted. Doses of 10 mg/kg of imazalil (im) and cypermethrin (cy) and 20 mg/kg of carbendazim (car) and their combinations (im, 10 mg/kg + cy, 10 mg/kg; im, 10 mg/kg + car, 20 mg/kg; car, 20 mg/kg + im, 10 mg/kg) were given to Swiss mice daily over 28 days. After 24 hr from the last dose, the relationships of cytotoxicity biomarkers were analysed: serum lactate dehydrogenase, aspartate transaminase, alanine transferase, amylase, alkaline phosphatase, creatine kinase, creatinine and total proteins. Individual pesticides showed different toxic potential (cy > im > car) generally characterized by increase in enzyme activities. Histological analysis showed that cypermethrin, but not imazalil or carbendazim, alone can cause mild necrosis. Combinations generally caused decrease in the activity of enzymes, indicating liver damage. Low doses of carbendazim in combination with low doses of imazalil or cypermethrin caused very pronounced hepatic necrosis, more than any of the three individually applied pesticides or combination of imazalil and cypermethrin. In fruits and vegetables for human consumption, residues of these three pesticides and prolonged combined intake of low doses, which by themselves acutely would not cause any effect, may have similar hepatotoxic effects.

The toxic potential of different pesticides is usually established for each compound individually to extrapolate risk estimation to human beings by exposure to food-borne traces or residues. In reality, exposure to a single pesticide via food or water residues is rare [1]. Usually, it is the combinations of all remaining traces of pesticides and other pollutants that cause toxic effects [2] acting as synergists, agonists or antagonists. There is a growing evidence of various mutual actions of common pesticide residues from designed toxicological experiments [3]. In the countries of the European Union, in recent years, significant traces of imazalil, cypermethrin and carbendazim have frequently been documented in food plants (https://webgate.ec.europa.eu/rasffwindow/portal/index.cfm?event=searchResultList/2011). Imazalil or (+)-1-(2-(2,4-dichlorophenyl)-2-(2-propenyloxy)ethyl)-1*H*–imidazole (CAS No., 73790-28-0, 35554-44-0) is a widely used imidazole-antifungal pesticide. Traces of this pesticide are mainly found in citrus fruits and sporadically detected in other fruits and vegetables in significant concentrations This compound is used as a drug (enilconazole) [6]. It has a potential of disturbing hepatocyte homeostasis [7]. Cypermethrin or (RS)-α-cyano-3 phenoxybenzyl-(1RS)-cis, trans-3-(2,2-dichlorovinyl)-2,2 dimethylcyclopropane carboxylate (CAS No., 52315-07-8) is the most worldwide used type II pyrethroid insecticide in agriculture, home pest control, protection of foodstuff and disease vector control [6]. It is highly accumulative, and one of the best examples of this is that traces of it were found alongside dichlorodiphenyltrichloroethane (DDT) in breast milk in endemic areas of South Africa [8]. The toxicity of cypermethrin is well studied in fruit fly, fish, rats and mice and is reported to cause neurotoxicity and endocrine disruption [9–12]. Carbendazim or methyl benzimidazol-2-ylcarbamate (CAS No., 10605-21-7) is a systemic broad-spectrum fungicide controlling a wide range of pathogens [6]. It is also used as a preservative in paint, papermaking and in the leather industry and further used as a preservative of fruits. It is known that carbendazim may cause endocrine disruption and oxidative stress [13,14]. It has

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Carbendazim combined with imazalil or cypermethrin potentiate DNA damage in hepatocytes of mice

D Đikić[1], A Mojsović-Ćuić[2], I Čupor[1], V Benković[1], A Horvat-Knežević[1], D Lisičić[1] and N Oršolić[1]

Abstract

Traces of pesticides imazalil, cypermethrin and carbendazim are detected in plants used for human consumption. To explore whether their application in oral combinations will induce DNA breaks in hepatocytes, a subchronic in vivo experiment was performed in Swiss mice. Doses of 10 mg kg^{-1} of imazalil (im) and cypermethrin (cy), and 20 mg kg^{-1} of carbendazim (car) and their combinations (im, 10 mg kg^{-1} + cy, 10 mg kg^{-1}; im, 10 mg kg^{-1} + car, 20 mg kg^{-1}; car, 20 mg kg^{-1} + cy, 10 mg kg^{-1}) were applied daily for 28 days. Afterward, DNA damage in hepatocytes was evaluated by comet assay. Individually, imazalil and cypermethrin damaged DNA at alkali-labile sites, while the tail moment (TM) of carbendazim alone was similar to control but with higher tail length. In combination with carbendazim clastogen, properties of imazalils and cypermethrins were potentiated compared to all other treatments and control. There were pronounced sex differences in pattern of fragmentation between treated groups. Higher long tail nuclei (LTN) in females indicate that certain cells in females were especially prone to total nucleus disintegration. Due to synergistic effects, low environmentally present concentrations of imazalil and cypermethrin in food, and especially their mixtures with carbendazim have genotoxic potential that could be particularly dangerous over prolonged exposure in mammalian organism.

Keywords

pesticide mixtures; genotoxicity; liver; fungicides; insecticides

Introduction

Significant traces of imazalil, cypermethrin and carbendazim are frequently documented in plants used for human consumption.[1] Imazalil or (+)-1-(2-(2,4-dichlorophenyl)-2-(2-propenyloxy)ethyl)-1H-imidazole (CAS No. 73790-28-0, 35554-44-0) is a widely used imidazole-antifungal pesticide and a food contaminant. Traces of this pesticide are mainly found in citrus fruits,[2] but they are sporadically detected in other fruits and vegetables in significant concentrations.[3] The best

pyrethroid insecticide used worldwide in agriculture, home pest control, protection of foodstuff and disease vector control.[6] It is highly accumulative and traces of cypermethrin may be found alongside dichlorodiphenyltrichloroethane (DDT) far from original application sites, for example in breast milk in endemic areas of South Africa.[7] The toxicity of cypermethrin is well studied in various animal models and is reported to cause neurotoxicity, endocrine disruption and hepatotoxicity.[8–11] Carbendazim or methyl benzimidazol-2-ylcarbamate (CAS No. 10605-21-7) is a systemic

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Research Article

The Risk Assessment of Pesticide Ingestion with Fruit and Vegetables for Consumer's Health

Gordana Jurak,[1] **Jasna Bošnir**,[1] **Domagoj Đikić**,[2] **Ana Mojsović Ćuić**,[3] **Iva Pavlinić Prokurica**,[4] **Aleksandar Racz**,[3] **Tomislav Jukić**,[5] **David Stubljar**,[6,7] **and Andrej Starc**[8]

[1]*Dr. Andrija Štampar Teaching Institute of Public Health, Zagreb, Croatia*
[2]*Department of Biology, Faculty of Science, University of Zagreb, Zagreb, Croatia*
[3]*University of Applied Health Sciences, Zagreb, Croatia*
[4]*Croatian Agency for Agriculture and Food, Zagreb, Croatia*
[5]*Department of Internal Medicine, History of Medicine and Medical Ethics, Faculty of Medicine Josip Juraj Strossmayer, Osijek, Croatia*
[6]*Department of Research & Development, In-Medico, Metlika, Slovenia*
[7]*Institute of Microbiology and Immunology Faculty of Medicine Ljubljana, Ljubljana, Slovenia*
[8]*Faculty of Health Sciences, University of Ljubljana, Ljubljana, Slovenia*

Correspondence should be addressed to Aleksandar Racz; aleksandar.racz@zvu.hr

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Pesticides are chemicals used in agriculture to protect crops from pests. In addition to protection during cultivation, they are also used after harvesting to extend the shelf life of products. Postharvest control stands out, especially when it comes to products imported from distant countries, resulting in increased concentration of pesticides and risk to human health consuming such products. In this study, analyses of pesticide residues were performed on 200 samples of fruits and vegetables. Pesticide residues were identified and quantified in 30 out of 200 samples. Study results revealed imazalil to be the most frequently detected pesticide. Risk assessment was performed on the obtained results, and it was carried out separately for adults and for children under 6 years of age. Imazalil showed the highest ARfD percentage for adults (max% ARfD 251%), and these values were especially high on risk assessment for children, where they amounted up to max% ARfD 1087%. The study of imazalil impact was performed on 16 Swiss albino mice divided into two groups and 4 subgroups. Experimental group animals were treated with the corresponding NOAEL dose of imazalil (10 mg/kg) for 28 days. Body weight was measured before each pesticide application on a digital electronic Sartorius scale. Peripheral blood analysis was performed after 28-day animal exposure to pesticides. Animals were anesthetized, blood samples were obtained by cardiac puncture, and red blood cell (RBC) count, hemoglobin (Hb) concentration, and white blood cell (WBC) count were determined by standard hematological methods. The organs for determination of imazalil concentration were extracted immediately upon animal sacrifice and stored in a freezer at -80°C until analysis. Results show difference in gain weight, and an increase in WBC count was recorded in the experimental group as compared with a control group of animals. The highest imazalil levels were recorded in adipose tissue (45.2‰) which proves tendency to accumulate.

Table 2: Pesticide residue concentration and assessment of acute exposure of adults and children to detected pesticides.

Pesticide	Matrix	Concentration (mg/kg)	% of acute ARfD adults	IESTI (μg/kg bw/day)	% of acute ARfD children	IESTI (μg/kg bw/day)
Imazalil	Orange	4.10	251	126	1087	544
Imazalil	Orange	3.50	215	107	928	464
Imazalil	Orange	3.30	202	101	875	438
Imazalil	Orange	3.33	204	102	883	442
Imazalil	Orange	2.90	178	89	769	385
Imazalil	Orange	2.55	156	78	676	338
Imazalil	Orange	2.55	156	78	676	338
Imazalil	Orange	0.90	55	28	239	119
Imazalil	Orange	0.72	44	22	191	95
Imazalil	Orange	0.096	6	2,9	25	13
Imazalil	Clementine orange	2.40	86	43	285	142
Imazalil	Pomelo fruit	0.02	0,7	0,36	2	1,2
Imazalil	Mandarin orange	2.40	86	43	285	142
Imazalil	Mandora fruit	3.80	136	68	451	225
Imazalil	Lemon	1.53	27	14	105	52
Imazalil	Lemon	0.33	6	3	23	11
Imazalil	Grapefruit	0.37	13	6,6	58	29
Imazalil	Grapefruit	0.14	5	2,6	23	11
Ethion	Orange	0.27	41	0,83	179	3,6
Chlorpyriphos	Orange	0.22	135	6,7	584	28
Chlorpyriphos	Grapefruit	0.21	75	3,8	330	16
Chlorpyriphos	Grapefruit	0.09	32	1,6	141	7,1
Chlorpyriphos	Lemon	0.08	14	0,72	55	2,7
Chlorpyriphos	Grapefruit	0.11	39	2,0	173	8,6
Chlorpyriphos	Pear	0.03	18	0,92	83	4,2
Chlorpyriphos	Peach	0.27	101	5,1	513	26
Chlorpyriphos	Grapes	0.07	47	2,4	102	5,1
Phorate	Potato	0.014	14	0,42	72	2,2
Phorate	Tomato	0.019	10	0,30	37	1,1
Phorate	Tomato	0.011	6	0,17	21	0,64

IESTI = international estimated short-term intake; bw = body weight; ARfD = acute reference dose.

Zaključak

- Kontrola cjelokupnog puta hrane „od polja do stola”
- Izuzetna važnost kontrole (sustavnih monitoringa) prehrambenih proizvoda
- Suradnja mnogih stručnjaka i organizacija
- Znanstvena istraživanja moraju biti sveobuhvatna (obuhvaćati mnogo parametara!) za donošenje konkretnih zaključaka
- Prikupljanjem rezultata ovakvih i sličnih istraživanja odlučuje se koji se pesticid više ne smije koristiti i eliminira se s liste komercijalno dostupnih (karbendazim – zabranjen za upotrebu!)

HVALA NA PAŽNJI!