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35th Annual Meeting

FESTEM 2019

International Symposium on Trace Elements and Minerals

Program & Book of Abstracts

Potsdam-Griebnitzsee, Germany

2^{nd} to 5^{th} of April 2019

Contents

Welcome

On behalf of AISETOV, SEQCET, GMS, RUSTEM, SFERETE and TraceAge it is our pleasure to invite you to the 7^{th} International Symposium of the Federation of European Societies on Trace Elements and Minerals (FESTEM) together with the 35th Annual GMS Meeting, to be held in Potsdam/Germany from 2^{nd} to 5^{th} of April 2019.
The Scientific Program Committee is preparing an exciting program that will highlight a wide diversity of all aspects in the field of trace elements and minerals. We are proud to be able to present an array of excellent speakers and key note lecturers covering a variety of current aspects in epidemiology, analytical methods, biomarkers, pathophysiology, nutrition and toxicology, interactions of trace elements, human health, aging as well as animal health and diseases. Selected abstracts will be chosen for oral presentations. Poster sessions will be held and we kindly invite all participants to submit their work for any kind of presentation. Participation of young scientists is particularly encouraged, and we offer reduced conference fees.
The venue of the conference, Potsdam University Campus Griebnitzsee, is situated between Potsdam and Berlin within few minutes of major tourist attractions. The conference location offers comfortable space for the exhibition of companies' products. Coffee and lunch breaks will offer a stimulating environment to strengthen old and to foster new collaborations and to emerge new directions of research.

We are looking forward to your participation which will be a key part for an enjoyable and successful conference.

Paola Borella
FESTEM President

Dirk Schaumlöffel
GMS President

Tanja Schwerdtle
Conference Chair

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Scientfic Program

Tuesday, April 2nd 2019

12:00 – 14:00 WORKSHOP I
The latest developments and application of Single Particle ICP-MS and Single-Cell ICP-MS
Michael Petrich *(Perkin Elmer LAS GmbH, Germany)*

12:00 – 13:45 FESTEM BOARD MEETINGS AND ELECTIONS

12:00 – 14:00 *Registration*

14:00 – 14:30 OPENING CEREMONY

14:30 – 15:45 SESSION I: ANIMAL HEALTH AND DISEASE

Chair: Daniel Brugger

14:30 – 15:00
Wilhelm Windisch *(Technical University Munich)*:
Responsibilities in Feeding Trace Minerals to Livestock: Impacts on Environment and Food Quality

15:00 – 15:15
Esther Humann-Ziehank *(LABVETCON)*, A. Menzel, P. Roehrig, B. Schwert, M. Ganter, I. Hennig-Pauka:
Acute and subacute response of iron, zinc, copper and selenium in pigs experimentally infected with Actinobacillus pleuropneumoniae

15:15 – 15:30
Stéphane Durosoy *(CEO Animine, Sillingy)*, D. Cardoso, A. Romeo, N. Meme, Y. Chevalier, A. Narcy:
Physicochemical properties of ZnO correlated to their bioavailability in broilers

15:30 – 15:45
Winfried Arnhold *(H. Wilhelm Schaumann GmbH)*, M. Seifert, B. Ridout:
Titanium Status in Ruminants and Omnivores

15:45 – 16:15 *Coffee break*

16:15 – 18:15 SESSION II: ENVIRONMENT

Chair: Bernhard Michalke, Paola Borella

16:15 – 16:45
Pilar Bermejo-Barrera *(University of Santiago de Compostela)*, M. V. Taboada-Iópez, R. Domínguez-González, A. Moreda-Piñeiro:
Environmental impact of the metal-nanoparticles and their possible effects on health

16:45 – 17:00
Nadine Wiesmann *(University Medical Center Mainz)*, C. Buhr, M. Viel, W. Tremel, J. Brieger:
Nanoparticulated metal oxide - harmful toxicants or promising innovative therapeutics?

17:00 – 17:30
Andrey R. Grabeklis *(RUDN University Moscow)*:
Occupational exposure to metals: issues of choosing biomarkers

17:30 – 17:45
Paola Borella *(University of Modena and Reggio Emilia)*, A. Bargellini, A. Ferrari, S. Paduano, G. Frezza, I. Marchesi:
Neurobehavioral effects of chronic low lead exposure in children: an updated review

17:45 – 18:00
Martin Herzberg *(Martin-Luther-University Halle-Wittenberg)*, L. Bütof, V. Schulz, D. Dobritzsch:
A complex metal homeostasis forces a basic cellular metal composition thought a predefined core metallome in proteobacteria like Cupriavidus metallidurans

18:00 – 18:15
Ulrike Seidel *(University of Kiel)*, E. Baumhof, F. Hägele, A. Bosy-Westphal, M. Birringer, G. Rimbach:
Lithium in German mineral waters and its bioavailability in humans

18:15 – 19:15 WELCOME RECEPTION

19:15 – 20:15 GMS BOARD MEETING

Wednesday, April 3rd 2019

08:00 – 09:00 *Registration*

08:00 – 09:00 WORKSHOP II
Fundamentals of ICP-MS and ICP-MS/MS compared to other atomic spectroscopy techniques and why it is particularly well suited for speciation and other hyphenated application
Jörg Hansmann *(Agilent, Germany)*

09:00 – 10:30 SESSION III: HOT TOPICS IN ANALYTICS AND BIOMARKERS PART I

Chair: Dirk Schaumlöffel, Montserrat Gonzalez Estecha

9:00 – 9:30
Maria Montes-Bayón *(University of Oviedo)*, M. Corte Rodríguez, R. Álvarez-Fernández García, J. Bettmer:
ICP-MS based tools to study the fate of nanostructured elemental species in cells

9:30 – 9:45
Bernhard Michalke *(Helmholtz Zentrum München)*, D. Willkommen, V. Venkataramani:
Iron Redox Speciation Method Using Capillary Electrophoresis coupled to Inductively Coupled Plasma Mass Spectrometry (CEICP- MS)

9:45 – 10:00
Margarita G. Skalnaya *(RUDN University Moscow)*, Yu. N. Lobanova:
Determinants of serum Mn levels: in search for potential biomarkers

10:00 – 10:30
Josiane Arnaud *(University Hospital of Grenoble)*:
External Quality Assessment Schemes: Organizer and user point of view

10:30 – 11:00 *Coffee break*

11:00 – 12:45 SESSION III: HOT TOPICS IN ANALYTICS AND BIOMARKERS PART II

Chair: Marco Vinceti, Kostja Renko

11:00 – 11:30
Dirk Schaumlöffel *(Université de Pau et des Pays de l'Adour/CNRS)*, M. Angels Subirana Manzanares:
Trace element localization in cells and tissue

11:30 – 12:00
Hajo Haase *(TU Berlin)*:
Measurement and physiological relevance of free zinc ions

12:00 – 12:15
Johannes F. Kopp *(University of Potsdam)*, K. Lossow, J. Baudry, S. M. Müller, G. Pohl, M. Schulze, A. Kipp, T. Schwerdtle:
Trace element profiling in very small volumes of human serum for the application in large cohorts

12:15 – 12:30
Doris Kuehnelt *(University of Graz)*, N. Kröpfl, I. Rohn, K. A. Francesconi, T. Schwerdtle:
Analysis of selenoneine and ergothioneine in biological samples – challenges and current applications

12:30 – 12:45
Lutz Schomburg (Charité University Medical School Berlin):
Selenoprotein P as biomarker for selenium status-associated health risks

12:45 – 13:15 WORKSHOP III: INTRODUCTION TO SCHOLARLY PUBLISHING: PUBLICATION AND RESEARCH ETHICS
Silke Guddat *Elsevier JTEMB, Germany*

13:15 – 14:00 *Lunch break*

14:00 – 16:00 SESSION IV: HEALTH BENEFITS AND RISKS

Chair: Margarita G. Skalnaya, Lutz Schomburg

14:00 – 14:30
Wolfgang Maret *(King's College London)*:
A new era of zinc biology

14:30 – 14:45
Maria Maares *(TU Berlin)*, C. Keil, H. Haase:
Addressing molecular factors of intestinal zinc resorption using a Caco-2/HT-29-MTX barrier model

14:45 – 15:15
Marco Vinceti *(University of Modena and Reggio Emilia)*:
Methodologies and uncertainties in risk assessment of trace elements: the selenium example

15:15 – 15:30
Isabelle Rohn *(University of Potsdam)*, N. Kröpfl, J. Bornhorst, D. Kuehnelt, T. Schwerdtle:
Selenoneine, a novel selenium species: lessons learned from cells and worms

15:30 – 15:45
Tommaso Filippini *(University of Modena and Reggio Emilia)*, C. Malagoli, M. Malavolti, M. Vinceti:
Dietary cadmium exposure and risk of melanoma: an Italian population-based case-control study

15:45 – 16:00
Nikolay Solovyev *(Ghent University and St. Petersburg State University)*,
E. Drobyshev, L. Kybarskaya, S. Dagaev:
Intravenous beryllium toxicity – new lessons to take

16:00 – 17:45 POSTER SESSION I and *coffee break*

17:45 – 19:00 GMS GENERAL ASSEMBLY AND ELECTIONS

Thursday, April 4th 2019

08:45 – 10:45 SESSION V: HUMAN HEALTH

Chair: Anna Kipp, Muriel Bost

8:45 – 9:15
Elias S. J. Arnér *(Karolinska Institutet)*:
The selenium dependent thioredoxin system as a target for drug therapy in cancer or oxidative stress

9:15 – 9:30
Laurent Chavatte *(Centre International de Recherche en Infectiologie Lyon)*, C. Vindry, O. Guillin, P. Mangeot, T. Ohlmann:
Regulation of human selenoproteins: development of a new tool mimicking selenium deficiency based on CRISPR-CAS9 virus-like particles

9:30 – 10:00
Alexey A. Tinkov *(RUDN University Moscow)*:
Toxic trace elements in obesity and metabolic syndrome

10:00 – 10:15
Sandra M. Müller *(University of Potsdam)*, J. F. Kopp, G. Pohl, S. Hornemann, J. Spranger, A. F. H. Pfeiffer, K. Mai, T. Schwerdtle:
Biomonitoring in the NutriAct intervention study

10:15 – 10:30
Arturo Corbatón-Anchuelo *(Hospital Clínico San Carlos Madrid)*, M. González-Estecha, N. Martell-Claros, M. Fuentes-Ferrer, M. T. Martínez-Larrad, M. Serrano-Ríos:
Is lead a remarkable component in resistant hypertension? Results from a study in patients of a Spanish hypertension unit

10:30 – 10:45
Anatoly V. Skalny *(RUDN University Moscow)*:
'Macro effects' of 'micro elements': current trends in trace element status of russian population

10:45 – 11:15 *Coffee break*

11:15 – 12:45 SESSION VI: AGING

Chair: Maria T. Llorente Ballesteros, Anatoly V. Skalny

11:15 – 11:45
Vadim Gladyshev *(Harvard Medical School)*:
Systems biology of selenium utilization

11:45 – 12:15
Susan Fairweather-Tait *(University of East Anglia Norwich)*, A. Jennings, NU-AGE consortium:
Effects of consuming a Mediterranean-like dietary pattern on trace element intake, and iron and selenium status, in elderly men and women: results from the NU-AGE study

12:15 – 12:30
Julia Baudry *(German Institute of Human Nutrition)*, J. F. Kopp, T. Schwerdtle, M. Schulze:
Change of trace element profiles during aging: preliminary results from the EPIC-Potsdam study

12:30 – 12:45
Jessica Baesler *(University of Potsdam)*, J. F. Kopp, G. Pohl, M. Aschner, H. Haase, T. Schwerdtle, J. Bornhorst:
Zinc homeostasis in Parkinson's disease - investigations of labile and total zinc levels in aging Caenorhabditis elegans

12:45 – 13:45 *Lunch break*

13:45 – 15:30 SESSION VII: INTERACTIONS OF TRACE ELEMENTS

Chair: Esther Humann-Ziehank, Barbara Witt

13:45 – 14:15
Anna Kipp *(Friedrich Schiller University Jena)*:
Interactions of selenium with other essential trace elements

14:15 – 14:30
Florin Muselin *(BUASMV "King Michael I of Romania" from Timisoara)*, Z. Gârban, R. T. Christina, A. O. Doma, E. Dumitrescu, A. B. Viţălaru, I. Bănăţean-Dunea:
Homeostatic changes of some trace elements in geriatric rats in condition of oxidative stress induced by aluminium and the benefic role of resveratrol

14:30 – 15:00
Julia Bornhorst *(University of Potsdam)*, J. Baesler, M. Nicolai, V. Michaelis, J. F. Kopp, G. Pohl, M. Aschner, A. Bürkle, A. Mangerich, H. Haase, T. Schwerdtle:
Insights in trace element effects and interactions with focus on C. elegans

15:00 – 15:15
Olga P. Ajsuvakova *(RUDN University Moscow)*, D. Willkommen, A. A. Skalnaya, A. A. Tinkov, B. Michalke, V. Danilov, A. V. Skalny:
Assessment of toxic and essential trace element levels in patients with Parkinson's disease

15:15 – 15:30
Nicola Winkelbeiner *(University of Potsdam)*, F. Ebert, M. Martin, K. Lossow, A. Kipp, J. F. Kopp, A. Mangerich, A. Bürkle, J. Bornhorst, T. Schwerdtle:
Trace element profiles and genomic stability in aging: DNA repair and damage response

15:30 – 16:15 WORKSHOP IV
Career Development & Research Funding – Why and how to apply
Georg Munz *(DFG, Germany)*

16:15 – 17:45 POSTER SESSION II and *coffee break*

19:00 – 22:00 DINNER

Friday, April 5th 2019

08:45 – 11:00 SESSION VIII: PATHOPHYSIOLOGY AND TOXICOLOGY

Chair: Tanja Schwerdtle

8:45 – 9:15
Michael Aschner *(Albert Einstein College of Medicine New York):*
Manganese-Induced Neurotoxicity: Lessons from Worms

9:15 – 9:30
Vivek Venkataramani *(UMG Goettingen)*, T. R. Doeppner, D. Willkommen, C. M. Cahill, Y. Xin, G. Ye, Y. Liu, A. Southon, A. Aron, H. Y. Au-Yeung, X. Huang, D. K. Lahiri, F. Wang, A. I. Bush, G. G. Wulf, P. Ströbel, B. Michalke, J. T. Rogers:
Manganese causes neurotoxic iron accumulation via translational repression of Amyloid Precursor Protein (APP) and H-Ferritin

9:30 – 10:00
Rosanna Squitti *(Molecular Markers Laboratory Brescia)*:
Cognitive Dysfunction in neurodegenerative disorders: focus on copper

10:00 – 10:15
Muriel Bost *(Trace Element Analysis Laboratory Lyon)*, G. Piguet-Lacroix, O. Guillaud, E. Broussolle, C. Laurencin, L. F. Lion, A. Lachaux:
Diagnosis of hereditary copper disorders in childhood by Next-Generation Sequencing

10:15 – 10:45
Anne-Laure Bulteau *(IGFL Lyon)*, S. Mounicou, L. Chavatte, J. Malherbe, P. le Costumer, E. Gontier, D. Schaumlöffel, C. Nizard, E. Noblesse, S. Schnebert:
Impact of heavy metal accumulation on skin

10:45 – 11:00
Hannah Finke *(University of Potsdam)*, V. Wandt, F. Ebert, N. Guttenberger, M. Stiboller, G. Raber, K. A. Francesconi, T. Schwerdtle:
Toxicological characterization of an arsenic-containing phosphatidylcholine in human liver cells

11:00 – 11:30 *Coffee break*

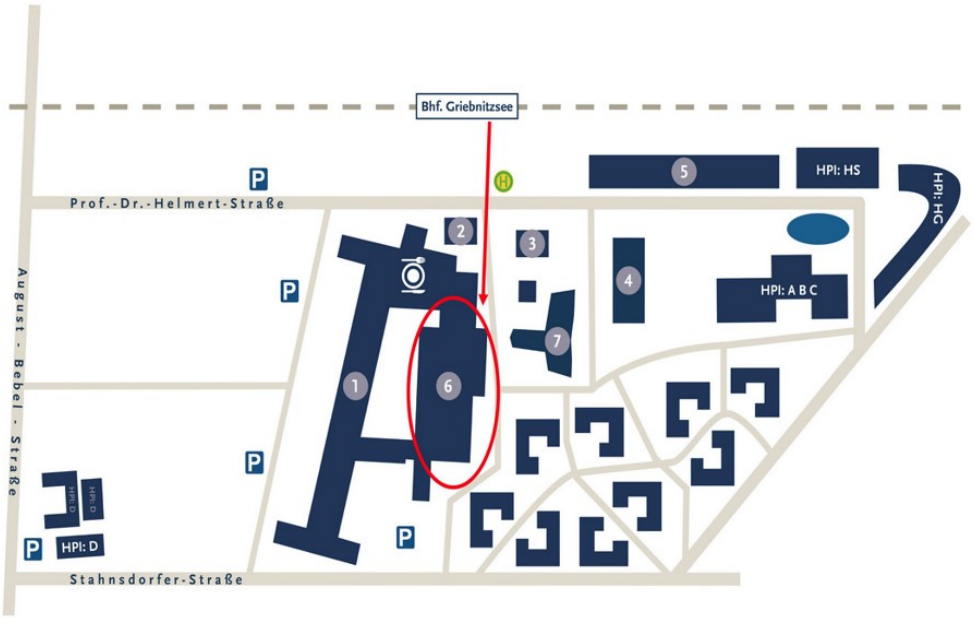
11:30 – 12:30 GABRIEL BERTRAND MEDAL AND PRIZE 2019

12:30 – 13:15 HEINZ-ZUMKLEY-PRIZE 2019

13:15 – 13:30 CLOSING REMARKS AND POSTER AWARDS

General Information

Map of Campus Griebnitzsee



Coffee and lunch breaks

Coffee, tea and refreshments will be served in the exhibition area throughout the conference and are included in the registration fee. The university cafeteria (mensa) is located between building 1 and 6. Upon registration, all conference participants receive lunch vouchers for the university cafeteria. All vouchers include a main dish. You may select between different menus in the university cafeteria.

Internet access for participants

Wireless LAN is provided in all university buildings. If you cannot get access via the “eduroam” network, you can receive a username and password for the “UP-Conference” network at the conference office. This account expires at the end of the conference. You will also receive a detailed instruction in which the configuration procedure is described. If you need help with the configuration procedure, our staff members will gladly assist you.

Name badges

Participants and exhibitors are kindly requested to wear their name badges throughout the conference.

Smoking

Smoking is prohibited in all buildings of the University. However, ashtrays are provided outside the buildings next to the entrances.

Conference dinner

As part of the FESTEM symposium, the social evening will take place at „Brauhaus Lemke am Alex“ starting from 7 to 10 pm on Thursday, April 4^{th} 2019. All registered conference participants will be informed and will receive a query concerning their participation. The regular registration fee already includes the participation in the social evening, however the participants need to sign in for the social evening.

Venue:
BRAUHAUS LEMKE AM ALEX
KARL-LIEBKNECHT-STR. 13
10178 BERLIN

Travel information:

Nearest stop for public transport: Alexanderplatz (Berlin)

From Griebnitzsee: take the S7 (direction Ahrensfelde), get off at Alexanderplatz Bhf (Berlin); travel time: approx. 40 min, every 10 min

From Potsdam Hauptbahnhof (central station Potsdam): take the S7 (direction Ahrensfelde) or regional trains [direction Frankfurt (Oder)], get off at Alexanderplatz Bhf (Berlin); travel time 30-45 min, every 10 min

Information for authors

Oral presentations
Oral presentations are given in the lecture hall.
Morning speakers are kindly requested to provide their presentation on USB **by the end of the day before their lecture will be given.**
Afternoon speakers are kindly requested to provide their presentation on USB **by the beginning of the lunch break**.
Our technical staff will assist you in installing your presentation. The conference computers are equipped with Windows and PowerPoint.

Poster sessions
Approved abstract authors may hang up their posters on Tuesday, April 2^{nd}, but latest on Wednesday, April 3^{rd} 9 am. Please remove the posters until Friday, April 5^{th} 2 pm. Otherwise, they will be disposed by the conference staff.

Poster award
A poster jury will evaluate all posters and select the best proceedings. The poster award session is scheduled at the end of the conference.

Abstracts: Oral Proceedings

Responsibilities in Feeding Trace Minerals to Livestock: Impacts on Environment and Food Quality

Wilhelm Windisch*

Chair of Animal Nutrition, Technical University Munich, Liesel-Beckmann-Str. 2, 85354 Freising, Germany

**Wilhelm.windisch@wzw.tum.de*

Many trace elements (TE) are essential minerals to food producing livestock. Native dietary TE contents are often too low to meet the metabolic requirements of highly producing animals and hence must be actively added to the feed rations, particularly in case of Zn, Cu, Mn, Se, I, and partially also Fe. On the other hand, high dietary doses of these TE may affect gut health. While in the first instance such effects may be considered undesirable (e.g. in Fe), they have been utilized in case of Zn and Cu since decades to control diarrhea in young pigs. Such pharmacological practices, however, cause collateral damages not only to treated animals through metabolic stress when counteracting excessive intakes, but also to the environment. The latter arises from the fact that Zn und Cu are heavy metals with high ecologically hazardous potential. They may accumulate in soils, when emitted from livestock production in excessive amounts via dung. Furthermore, such practices may indirectly provoke antibiotic resistances already in the hind gut of animals and later on during storage of manure.
Quality of animal derived food (milk, meet, eggs), however, remains comparably constant over a wide range of dietary supplies with Zn, Cu, Mn, and Fe. On the contrary, food quality is a major issue in case of Se and I since these TE may effectively accumulate in milk, eggs, and meat (only Se). Indeed, a certain level of accumulation has been generally considered as a tool to rise the overall Se and I intake of humans in areas at risk of insufficient natural supplies. On the other hand, accumulations need to be controlled as they may easily reach undesirably high levels.
In total, feeding trace minerals to livestock is subject to several tradeoffs, the necessity to guarantee the animals' health and productivity in terms of supplying essential trace minerals, the protection of the environment, and the proper control of TE contents in animal derived food.

Acute and subacute response of iron, zinc, copper and selenium in pigs experimentally infected with Actinobacillus pleuropneumoniae

[1]**Esther Humann-Ziehank*,** [1]**Anne Menzel,** [1]**Petra Roehrig,** [1]**Barbara Schwert,** [1]**Martin Ganter and** [b]**Isabel Hennig-Pauka**

[1]*Klinik für kleine Klauentiere und Forensische Medizin und Ambulatorische Klinik, Stiftung Tierärztliche Hochschule Hannover, Bischofsholer Damm 15, D-30173 Hannover, Germany*
[b]*Universitätsklinik für Schweine, Veterinärmedizinische Universität Wien, Veterinärplatz 1, 1210 Wien, Austria; current affiliation: Aussenstelle Bakum, Stiftung Tierärztliche Hochschule Hannover, Büscheler Str. 9, 49456 Bakum*

**LABVETCON, Burgdorf, Germany (www.labvetcon.de), mail@labvetcon*

The acute phase response (APR) affects several systemic reactions including trace elements. It is mandatory to consider these facts also in routine clinical diagnostics to avoid misinterpretation. However, especially studies focusing on farm animals are rare. Therefore, this topic was integrated into an experimental study of a widespread, bacterial-induced porcine lung disease. Twenty piglets were challenged by aerosolic infection with *Actinobacillus pleuropneumoniae (A.pp.)* serotype 2, ten piglets serving as controls. Blood sampling was done initially and at day 4 and 21 after infection, collection of liver tissue was done at day 21 (autopsy). *A.pp.*-infection caused fever and respiratory symptoms. APR at day 4 after infection was marked by an increase in total white blood cells, granulocytes and monocytes in whole blood samples and an increase in globulin/albumin ratio (G/A), a2-globulins, C-reactive protein, haptoglobin, ceruloplasmin, Cu and Se in serum. Concurrently, there was a decrease in haemoglobin (Hb) and packed cell volume (PCV) in whole blood as well as a decrease in albumin, transferrin, total iron binding capacity and Fe in serum and Zn in plasma. The subacute stage at day 21 was characterised by progressively increased concentrations of G/A, β-globulins and γ-globulins reflecting the specific immune reaction. Hb and PCV showed further decreases, all other parameters returned to the initial concentrations. Glutathione peroxidase activity in plasma and liver tissue remained unaffected by *A.pp.*-infection. The liver concentration (day 21) of Zn was found to be higher, that of Se was lower in the *A.pp.*.-group, whereas hepatic concentrations of Cu and Fe were not affected by *A.pp.*-infection. For more details, see [1].
In summary, the model of experimental *A.pp.*-infection proved advantageous for evaluating trace element response showing a distinct impact of a bacteria-induced APR on Fe, Zn, Cu and Se metabolism in pigs. The APR was verified by typical reactions of acute phase proteins. For diagnostic use, these samples collected during APR are unsuitable for assessing the current trace element status of the herd and may cause misleading interpretations. Prospectively, the development and application correcting factors as being already discussed for men, might be desirable for farm animal laboratory diagnostic as well.

[1] Humann-Ziehank, E., et al., *Acute and subacute response of iron, zinc, copper and selenium in pigs experimentally infected with Actinobacillus pleuropneumoniae.* Metallomics, 2014. 6(10): p. 1869-79.

Physicochemical properties of ZnO correlated to their bioavailability in broilers

[1,2,3]**D.Cardoso***, [3]**A. Romeo,** [3]**S. Durosoy,** [2]**N. Meme,** [1]**Y. Chevalier,** [2]**A. Narcy**

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[2]*INRA, UR83 Recherches Avicoles, F-37380, Nouzilly*
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The bioavailability of a trace mineral source is related to its in vivo solubility, which in turn is determined by its physicochemical properties. It is still not clear which characteristics are more relevant in affecting solubility and bioavailability of feed compounds. Zinc oxide (ZnO) is a common feed additive used to supplement zinc in the diet of monogastric animals. However, different sources have shown different responses on animal bioavailability. This study hypothesized that different sources of feed grade ZnO have various physicochemical features that lead to distinct bioavailability values. Over 40 samples of ZnO have been collected from the feed industry worldwide. Samples were analyzed for density, tapped density, particle size, shape, specific surface area and dissolution kinetics. A principal component analysis (PCA) was performed to define the most relevant physicochemical characteristics and categorize the samples into groups. Representative products from each family were selected for in vivo trial to measure the effect of their characteristics on the zinc bioavailability in broilers. 135 animals were fed a standard starter diet from day 1 after hatching up to day 7. At day 8, animals were allocated in individual cages and fed one of each treatment. Treatments consisted of a basal diet with 23 ppm of Zinc from the feed and 7 diets with supplement zinc oxide or sulfate at 6 or 12 ppm. Animals were slaughtered at day 22 and 23. Bone zinc was used to assess zinc bioavailability. The bioavailability of the different sources varied from 37 to 132% in relation to zinc sulfate. In conclusion, ZnO sources have variable physicochemical properties, which affects its dissolution kinetics and bioavailability.

Titanium Status in Ruminants and Omnivores

[1]Winfried Arnhold*, [2]Mathias Seifert, [3]Bruce Ridout

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[2]*Hochschule Fresenius – University of Applied Sciences, Idstein, Germany*
[3]*San Diego Zoo Global, Institute for Conservation Research, San Diego, California, USA*

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Titanium is regarded as essential for animals and man. A titanium poor diet caused reduced feed intake and life weight gain in growing goats, enhanced the mortality in kids and reduced the titanium content of milk by 50%. However, the requirement is met worldwide and titanium deficiency is not a practical problem. There is evidence that the titanium concentration of animal tissues is not homeostatically controlled. Mice, voles and screws from areas with a different titanium content of feed accumulated species specific different high amounts of titanium. Whereas a few results exist on the tissues concentration in organ tissues of monogastric animals no data are available in ruminants. Due to the species specificity of the titanium status, organ tissues of different wild ruminant species and omnivores were analysed and compared.
The investigated wild ruminants which were kept in captivity came from the Zoological Society of San Diego, and from the Zoo Leipzig. Furthermore, organ tissues were taken from road killed mule deer from the Sacramento area. Wild living opossums and foxes were trapped at the San Diego Zoo and euthanized due to diseases screening. For the presentation of the results the various species of ruminants were morphophysiologically classified as the ruminant feeding types concentrate selectors, intermediate mixed feeders, and grass and roughage eaters.
After dry ashing of the samples the Ti-concentration was analyzed by optical emission spectroscopy with inductively coupled plasma.
Although the Ti concentration in the organ tissues was highly variable the Ti amounts per kg tissue dry matter were quite low compared to the Ti amount to that the ruminants are exposed.
The Ti concentration in organ tissues and hair showed a very high variability. Nevertheless, the habitat seems to be of high influence to the Ti status. Adult concentrate selectors of Northern California accumulated 3 times more Ti in the liver than in Southern California. Furthermore, the feeding types took effect on the Ti content of organ tissues. As a rule grass and roughage eaters stored less Ti in different tissues than the other feeding types. This was true for adults ruminants as well as for neonatal but to a lower extend. Except ribs and lungs neonatal ruminants accumulated significantly more Ti in the organ tissues than adults.
There is a tendency, that with exception of heart, female adult ruminants accumulate up 3 times more Ti in organ tissues than male ruminants, and the differences become significant in the case of kidney, rib, skeletal muscle, spleen and hair. The same tendency was observed in wild living mule deer.
Whereas the liver of adult opossums contained similar high Ti-concentration like concentrate selectors and intermediate feeders, foxes accumulated mush higher amounts. It seems that the reproduction status takes also effect on Ti-status. Lactating opossums contained less Ti in the organ tissues than non lactating.

Environmental impact of the metal-nanoparticles and their possible effects on health

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Nowadays the potential of Engineered nanomaterials (ENM) to improve the quality of life and to contribute to economic growth is widely recognized. But, due to the fast expansion in the production of ENM and products incorporating these materials, there is a concern about their potential risks. Between these ENM metal-nanoparticles such as titanium dioxide nanoparticles (TiO_2 NPs) and silver nanoparticles (AgNPs) are of a special concern because both NPs have many different applications, and they are present in manufactured products widely used for animals and human. For this, today it is necessary to develop new analytical methods to perform studies about the presence of metal-nanoparticles in foods, in consume products, and in biological samples. The ICP-MS in the mode single particle, sp-ICP-MS is an adequate analytical technique because with this technique it is possible to obtain information about the NPs concentration and about the size distribution of the nanoparticles. The use of this technique has been applied to study the TiO_2 NPs and Ag NPs in (NPs concentration and size distribution) in different type of mollusks (mussels, edible cockles, oysters, razor clams, variegated scallops, and clams) after applying ultrasound assisted enzymatic hydrolysis and ultrasound assisted alkaline extraction of the NPs. Moreover, the same edible mollusks have been subjected to an in vitro bioaccesibility procedure for TiO_2 NPs and Ag NPs human risk evaluation. In addition, permeability and transport experiments by using caco-2 cells were also tested. On the other hand, a mesocosm model was developing to evaluate the possible accumulation of TiO_2 NPs in mollusks. Clams have been exposed to TiO_2 NPs (50 nm TiO_2 NPs, 100 nm TiO_2 NPs, and at the food additive E171) under laboratory conditions (2 tanks for each condition, each tank containing 30 organisms). After exposition to TiO_2 NPs (5 μg L^{-1} each two days for 15 days) samples were analyzed for total Ti and for TiO_2 NPs. Results have shown that the TiO_2 NPs of small size (50 nm) are absorbed by the organisms.

Nanoparticulated metal oxides – harmful toxicants or promising innovative therapeutics?

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Nanoparticles as innovative therapeutic agents have enormous potential to supplement current state of the art therapeutics in the treatment of numerous diseases. Nevertheless, the risks that could be associated with their application in a medical setting have to be carefully considered as the interaction between nanomedical agents and the human body is still poorly understood. Our goal was to contribute to the bridging of that gap and gain a better understanding of the toxicity of metal oxide nanoparticles *in vitro* and *in vivo* using zinc oxide nanoparticles as a model substance.
Toxicity of zinc oxide nanoparticles was assessed *in vitro* with the help of immortalized tumor cell lines and primary cell lines stemming from healthy tissue. Additionally, the Chorioallantoic Membrane Assay (CAM Assay) was employed to gain insights into the *in vivo* toxicity of the nanoparticles. The CAM Assay provides the opportunity to study the effect of nanoparticles on a complete organism with fertilized hen's eggs.
We were able to show that zinc oxide nanoparticles evoke toxic effects on human cells *in vitro* as well as on chicken embryos in the CAM assay. These toxic effects could be traced back to the dissolution of zinc ions from the nanoparticles as well as to the direct interaction of the nanoparticles with the cells. By the use of silica coated zinc oxide nanoparticles, we could show the attachment and cellular internalization of the particles. We could demonstrate that an increase in reactive oxygen species (ROS) within the cells follows treatment with zinc oxide nanoparticles and is central to the mechanism of toxicity.
ZnO as an example of metal oxide nanoparticles showed Janus-faced characteristics. However, depending on the applied concentrations selective tumor cell killing seams feasible. Careful determination of the therapeutic window will be mandatory.

Occupational exposure to metals: issues of choosing biomarkers

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Modern industry inevitably generates risk for peoples' health due to occupational and environmental contact with toxic substances, particularly heavy metals and other toxic chemical elements. Heightened load with toxic elements can lead to various health problems, as because of direct effects like intoxications, as because of indirect effects like secondary deficiencies of essential elements. Therefore it is important to have an effective monitoring system, which could detect the exposure including effects of so-called "low-intensity pollution" using appropriate biomarkers in order to estimate risk of diseases connected to it. The proposed methodology of such monitoring includes: (1) screening estimation of exposure to toxic and potentially toxic chemical elements using multielement hair analysis with the subsequent determination of risk groups; (2) indepth assessment of the elemental status within risk groups using analyses of whole blood, serum, urine or other biological samples. The methodology is based on the following findings. Hair analysis is a suitable method for screening of human elemental status due to non-invasiveness of sampling, simplicity of storage, irreversible binding of trace elements into the hair matrix, and high degree of mineralization. Assessment of exposure by hair samples is most informative for those chemical elements whose background content in hair is comparable to the amounts taken from the pollution. Generally, determination of toxic elements is more effective for exposure assessment than determination of essential elements. For assessment of the risk of disease development due to occupational exposure, relative content of toxic elements to antagonistic essential elements (e.g. pairs like Hg/Se, As/Se, Pb/Ca, Cd/Zn, Be/Mg, etc.) may be more informative than the levels of individual elements. In-depth assessment of occupational harmful influence on mineral metabolism requires simultaneous investigation of at least two biosubstances: hair and whole blood, or hair and blood plasma, with whole blood being more preferable due to its better indicative ability towards alterations in exchange of most chemical elements.

Neurobehavioral effects of chronic low lead exposure in children: an updated review

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Despite the implementation of many public policies and laws aimed to reduce the use of lead, numerous studies suggest that in children also a low exposure to this toxic element may be associated with the development of neurobehavioral effects. In the 80's, lead was widely used in industries and added in gasoline, thus human exposure was particularly high through both air and diet. For instance, at that time, in the ceramics district of Sassuolo (Italy), air contamination reached 10.3 μg/m^3 and assumption with diet was approximately 600 μg/die. A large survey on Pb-exposed women highlighted higher rates of spontaneous miscarriage and dysmenorrhea compared to controls. By examining school pupils with an average of 17.5 μg/dl, the most exposed children showed a significant increase in restlessness, distractibility and decreased scholar performance. The effects of Pb on central nervous system was evaluated in a consistent number of children attending the first year of primary school. Lead in deciduous teeth, representing the global exposure since the intrauterine life, was found significantly associated with impairment of cognitive functions. At present, Pb exposure is decreased and the neurological effects of lead in children well established, but recent studies open other perspectives in this field, including relevant effects on behavioral. These studies emphasize that Pb exposure is associated with antisocial and risky behavior among children and adolescents at any measurable concentration of lead in blood. A current major challenge is identification of children who may be uniquely susceptible to lead toxicity because of genetic predisposition. In conclusion, no threshold level can guarantee the absence of detrimental effect on the child nervous system, an observation useful for future strategies aimed at protecting children and adolescents from potential health risks associated with low-level lead exposure.

A complex metal homeostasis forces a basic cellular metal composition thought a predefined core metallome in proteobacteria like *Cupriavidus metallidurans*

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The biochemistry of cellular life depends on metals as catalytically cofactors in a multitude of enzymes, structurally cofactors in proteins or involved in signaling and regulation. The entirety of synthesized metalloproteins in a proteome, comprise the metallome. An adapted and controlled interplay of efflux, uptake and storage systems is required to maintain cellular metal homeostasis. Bioinformatically approaches suggest an average of 10 - 20% in bacterial proteins depend on metals. It is predicted that nearly all of these biometals are tightly bond in cells. As a model organism for investigation of metal fluxes, the β-Proteobacterium *Cupriavidus metallidurans* possesses a large number of metal efflux systems and a repertoire of secondary and primary uptake systems of divalent transition metal cations such as zinc. In nearly all-living organisms, zinc is an essential metal and in most prokaryotes the second most abundant transition metal after iron. A quantitative proteomic approach revealed that the overall number of predicted zinc-binding proteins is higher than the zinc content of a cell. Thus, one zinc pool in the cytoplasm of *C. metallidurans* is probably composed of zinc bound to these binding sites, which in their totality form the "zinc repository". The transition metal ratio in this bacterium during the exponential phase of growth does not depend on the growth medium used, from complex to minimal media. This indicates a direct connection between the predicted metallome and the content of biometals under strictly controlled metal homeostasis in the face of the changing metal concentrations in the surrounding environment.

Lithium in German mineral waters and its bioavailability in humans

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Lithium is an important trace element in human nutrition mediating potential health benefits. Mineral water is widely consumed by the German population and may represent a significant source of dietary lithium intake. Therefore, we determined the lithium concentration in more than 300 German mineral waters collected throughout the country. We observed considerable differences in the lithium concentrations of the analyzed mineral waters. Especially mineral waters from the South-West of Germany exhibited high lithium concentration, whereas in Eastern and Northern parts of the country rather low lithium concentration in the analyzed mineral waters were evident. The lithium concentration in mineral water was significantly correlated with its sodium, potassium and magnesium but not with its calcium concentration. The declared lithium concentration were mostly in accordance with its analyzed values. There were no differences in terms of the analyzed lithium concentration between the same mineral waters filled either into glass or plastic bottles. Based on our systematic screening we choose three different mineral waters exhibiting a low (1.7 µg/l), medium (171 µg/l) and high lithium (1723 µg/l) concentrations for an acute bioavailability study in male healthy volunteers. Volunteers (n = 3 x 10 each) drank 1.5 liter of the respective mineral waters and lithium concentrations in plasma and urine were monitored over 24 hours. Consumption of the three different mineral waters resulted in a in a dosedependent response in plasma lithium concentrations and total urinary lithium excretion. Thus lithium-rich mineral water may be an important and highly bioavailable lithium source for human consumption.

ICP-MS based tools to study the fate of nanostructured elemental species in cells

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The term nutraceutical is used to describe any product derived from food sources with extra health benefits in addition to the basic nutritional value found in foods. They can be considered non-specific biological therapies used to promote general well-being, control symptoms and prevent malignant processes. In this regard, two different nutraceuticals have been the focus of our research work: selenized yeast and nanoparticulated Fe-nutritional supplements. The understanding of the Se-involving biochemical processes during Se-rich yeast production, the optimization of the Se-incorporation during the yeast growth and the characterization of the final products in terms of the selenium chemical forms present requested by regulatory agencies, require the development of suitable methods of analysis. Thus, novel strategies have been developed to address the presence of Se in single cells by using single cell analysis with elemental detection (SC-ICP-MS).
The second nutraceutical that will be revised within this presentation is nanoparticulated iron (NPFe). The use of iron NPs as Fe supplements is of increasing interest, in particular to overcome anaemia which is one of the World Health Organization's top 10 target diseases for cure and prevention. According to recent publications, Fe (III)-based nanoparticles deserve careful attention as potential therapeutic agents for three reasons. First, iron oxo-hydroxides represent one of the luminally-formed digestion products of dietary non-haem iron. Secondly, dietary ferritin is a commonly ingested protein-encapsulated Fe (III) nanoparticle and thirdly, nano Fe (III)-based supplements could provide bioavailable iron with minimal gastrointestinal and systemic adverse-effects. Thus, the development of analytical strategies that permit to monitor the fate of this class of particles in biological systems including cell cultures and animal tissues are highly demanded and some of them will be illustrated on this presentation.

Iron Redox Speciation Method Using Capillary Electrophoresis coupled to Inductively Coupled Plasma Mass Spectrometry (CE-ICP-MS)

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Oxidative stress (OS) and ferroptosis (FPT) are important causes of neurodegeneration, being closely related to Fe(II)/Fe(III) status. Therefore, Fe (II)/(III) redox-speciation is important in neurodegeneration research.
Fe(II)/(III) speciation methods with LC-ICP-MS need 8-10 min per sample and excessive purge times between runs. New LC columns for iron speciation turned out to vary from batch to batch and need timeconsuming re-optimizations of elution conditions. These problems hamper high-throughput and increased reoptimization time is needed again for acceptable reliability and an evaluated method.
We describe a CE-ICP-MS based method for Fe(II)/Fe(III) redox-speciation. Advantages of CE count the lack of stationary phase, thus being independent on capillary-batch. When aged or blocked capillaries are replaced quickly with unchanged performance. Purge steps between samples are effective, short and sample analysis time is below 3 minutes.
The developed method employed a simple acidic background electrolyte (20 mM HCl, pH 1.7) with +25 kV. No complexation of Fe-species with PDCA was applied, since PDCA hampered CE-separation of iron redox species. A combined conductivity-pH-stacking was introduced, improving peak focusing and allowed even higher injection volumes for extremely low concentrated samples while maintaining good separation of iron species. With 13 nL injection volume detection limits of 3 µg/L were calculated for both iron species. ICPMS was operated in dynamic reaction cell mode, with ammonia as reaction gas at 0.6 ml/min. Calibration curves from LOD – 150 µg/L showed high linearity. At higher concentrations Fe (II) curve flattened significantly.
Measurement precision in 1:2 diluted human neuroblastoma cell lysates (line SH-SY5Y) were 3.5% (Fe (II) at 62 µg/L) or 2.2% (Fe (III) at 112 µg/L). Migration time precision in SH-SY5Y lysates was 2% for Fe (III) and 3% for Fe (II).
Concentration accuracy was checked by parallel measurements of SH-SY5Y lysates with validated LC-ICPMS method and by recovery experiments after standard addition. Taking LC-ICP-MS values as 100%, accuracy (n=6) was 97.6 $\pm$ 3.7% for Fe (III) and 105 $\pm$ 6.6% for Fe (II).
Recovery of standard addition was 101 $\pm$ 4.5% (n=3). For proof of principle, the method finally was applied to cerebrospinal fluid samples.

Determinants of serum Mn levels: in search for potential biomarkers

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Mn deficiency and intoxication are both associated with adverse metabolic effects. Assessment of Mn status of the organism is essential, although data on the potential biomarkers of Mn status and the relationship between metabolic parameters and Mn status are contradictory. The objective of the present study was to assess the determinants of serum Mn levels. A total of 1318 adult women and 915 adult men were enrolled in the present study. Serum and urinary Mn levels were assessed using inductively-coupled plasma massspectrometry at NexION 300D+NWR213 (Perkin-Elmer, USA). Laboratory quality control was performed using the certified reference materials of plasma and urine (ClinChek, Recipe, Germany). Ageing (> 65 y.o.) was significantly associated with a 15% increase in serum Mn levels in women as compared to 18-year olds, but not in men. In women, pregnancy and infertility were associated with a 15% increase and 10% decrease in serum Mn as compared to healthy non-pregnant women of reproductive age. Higher serum Mn (> 0.0024 μg/ml) was associated with a more than 2.5-fold decrease in C-reactive protein levels ($r = -0.48$; $p < 0.001$), whereas no relationship with fasting glucose, triglycerides, or HbA1c was observed. Serum Mn values inversely correlated with transferrin saturation ($r = -0.16$; $p < 0.05$). Protein metabolism biomarkers were also interrelated with serum Mn levels. Particularly, higher Mn levels were associated with a 19% and 12% increase in uric acid and citrulline levels, whereas serum glutamine levels were characterized by a 8% decrease as compared to the lower Mn concentrations (< 0.0017 μg/ml). Generally, the obtained data demonstrate that serum Mn concentration is associated with ageing and reproductive health in women, whereas CRP and the levels of citrulline, arginine, and glutamine may be used as additional biomarkers of Mn status.

External Quality Assessment Schemes: Organizer and user point of view

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A fundamental feature of clinical biology is the measurement of markers in specimens such as whole blood, plasma, serum or urine. The results of inorganic elements are used to protect and to monitor workers exposed to these elements, to identify undue exposures from the natural environment, to detect essential trace element deficiencies and to diagnose and monitor patients suffering from inherited disorders.
It is vital, therefore, that the laboratory work should be carried out with an emphasis on ensuring accurate and consistent results. External Quality Assessment Schemes (EQAS) are one of the resources that help laboratories to achieve these goals. ISO/IEC 17043:2010 specifies general requirements for the competence of providers of EQAS and for the development and operation of EQAS. The objectives of organisers of EQA schemes in clinical biology are to stimulate improvements in analytical data and to verify accuracy of participant results. For this purpose, they must prepare and validate homogeneous and stable samples with concentrations within the range of that observed in the clinical laboratories (from deficient to toxic levels) and with matrix as similar as that received in participant laboratories. They must inform the participants how assigned values and acceptable range are calculated and reply to participant claims. In addition, they must inform and as far as possible give advice to poor performers.
The participation to EQAS is essential for clinical biological laboratory but the interpretation of EQAS results must be critical and cautious. The EQAS choose takes into account the concentration range and nature of samples, the number of participants, the statistics used, the acceptable ranges, the number of samples per year and the frequency of controls. With the results obtained, the laboratory can evaluate the performance of its methods, particularly accuracy. In addition, EQAS contribute to the determination of the uncertainty of the laboratory measurements.
These different points will be developed and discussed with emphasize on the differences between schemes.

Trace element localization in cells and tissue

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Trace element imaging in biological cells and tissues provide an important contribution to the investigation of biochemical functions, biosorption and bioaccumulation processes at cellular and subcellular level. Scientific progress in this field is directly related to new instrumental and methodological analytical developments. Nano secondary ion mass spectrometry (NanoSIMS) is an analytical technique which relies on the sputtering of ions from a solid surface by focused positive or negative primary ion beams and the subsequent analysis of the produced secondary ions by a mass spectrometer under high vacuum. NanoSIMS allows chemical imaging of a sample surface with lateral resolution below 50 nm combined with high sensitivity and thus it is perfectly suited to localize the distribution of chemical elements with high spatial resolution in biological samples. This lecture highlights advantages and challenges of NanoSIMS bioimaging of trace elements at nanometer level. Recent examples from our laboratory will be presented showing divers fields where NanoSIMS element bioimging can be applied.

Measurement and physiological relevance of free zinc ions

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Zinc is a crucial trace element for various physiological functions and zinc deficiency or a disturbance of zinc homeostasis is associated with many different diseases. In this context, it becomes increasingly clear that free or loosely bound zinc is of particular importance, as it represents the rapidly exchangeable and thereby bioavailable form of zinc. The quantification of free zinc is hindered by the fact that is represents only a small fraction of total zinc, while the vast majority is tightly bound to proteins. E.g., in cytosol and serum, sub-nanomolar concentrations of free zinc have to be investigated in the presence of micromolar concentrations of total zinc. Consequently, the use of classical methods for metal quantification, such as atomic absorption or mass spectrometry, is not a viable option. Our lab utilizes various forms of fluorescent probes for the quantification of free zinc. To measure intracellular free zinc levels, this includes low molecular weight fluorescent probes, but also stable transfection of cells with genetically encoded proteinbased zinc sensors. This allows investigating various aspects of free zinc homeostasis, ranging from its role as a second messenger in immune cells to the transport of zinc within intestinal epithelial cells in *in vitro* models of the intestinal barrier. Comparable techniques are also applicable for extracellular free zinc, where it gives important information about the availability of zinc to cells, the buffering capacity for zinc during sepsis, and where it may represent a potential biomarker for zinc status, which is currently under evaluation. These examples highlight the potential of measuring free zinc to enable a better understanding of many physiological functions of this important trace element.

Trace element profiling in very small volumes of human serum for the application in large cohorts

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Trace elements (TE) play an important role for a myriad of bodily functions and thus need to be adequately supplied via nutritional intake. There are multiple studies on changing TE-homeostasis during aging, due to both, pathologic and natural changes. However, in most studies, the focus usually lies on one or two elements. Interactions of multiple elements at the same time are rarely investigated, although most endpoints are dependent on a combination of TE.
This issue is addressed by the DFG research unit #2558 TraceAge. We aim to investigate the interactions and status of six TE, namely manganese (Mn), iron (Fe), copper (Cu), zinc (Zn), selenium (Se) and iodine (I). Therefore, we analyze TE-profiles in large cohorts, such as the EPIC-Potsdam cohort, investigate the effects of TE-adequate and subadequate conditions in *in vivo* models like *M. musculus* and *C. elegans*. Furthermore, we attempt to establish novel biomarkers for the determination of i.e. oxidative stress and DNA-damage caused by the disruption of TE-homeostasis. Hereby, the focus lies on age-related changes.
In most large cohorts, the total amount of sample available is limited, due to the large number of different analyses that are conducted on the same sample. For this study, we adopted a published ICP-MS/MS method capable of determining 33 elements in 500 µL of serum (Konz et al., J. Proteom Res., 2017). Instead of acidic digestion, the sample is diluted using a special diluent containing EDTA, butanol, ammonia and surfactant. These conditions also allow for the determination of iodine levels.
We miniaturized and optimized this method for the measurement of the six aforementioned TE in as little as 50 µL of serum, including an isotope dilution approach for the determination of Se. The modified method was validated against serum reference materials and compared to a conventional acid-digestion method and found to be in excellent agreement. The achieved detection and quantification limits are sufficient for concentrations expected in human serum from central European study participants. After validation, we applied the method to samples from the EPIC Potsdam cohort as well as to murine serum samples from the TraceAge animal study.

Analysis of selenoneine and ergothioneine in biological samples – challenges and current applications

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Selenoneine, a selenium species discovered about 10 years ago as the major water soluble form of selenium in several marine fish species, is the selenium analogue of the purported sulfur-containing antioxidant ergothioneine and has been discussed to exhibit similar health effects.[1] Furthermore, selenoneine has been suspected to be involved in mercury detoxification.[2,3] Its potential health benefits have sparked interest in the investigation of this selenium species, which is hampered by the lack of accessibility of the pure species as well as of analytical methods for its accurate determination. In this work, analytical challenges regarding the determination of selenoneine as well as its methylated metabolite Semethylselenoneine by HPLC coupled to mass spectrometry are addressed. These comprise the availability of standards, sample preparation, as well as the simultaneous measurement of selenoneine and ergothioneine. Furthermore, application to selected sample matrices will be discussed.

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Selenoprotein P as biomarker for selenium status-associated health risks

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Selenium (Se) is one of a few essential trace elements, i.e., life without a sufficiently high Se supply is not possible. Transgenic and knockout mouse models have indicated that certain selenoproteins are of utmost importance for mammalian development, e.g., the thioredoxin reductases or glutathione peroxidase-4. Selenoprotein P (SELENOP) constitutes a very special member of the family of selenocysteine (Sec)-containing selenoproteins, as it carries multiple Sec residues in its primary sequence and contributes a major share to blood Se concentrations. Knockout of the murine Selenop gene has indicated that SELENOP is not essential for development and survival, but that it facilitates Se transport and organ supply, becoming of health-relevance when the mice are raised under Se restricted conditions. From these data, it can be concluded that SELENOP contributes decisively to Se metabolism and homeostasis, and that a severe deficiency in SELENOP causes health risks, especially in geographical regions where the Se supply is marginal, like in the EU or in large parts of Africa and Asia.
SELENOP biosynthesis and circulating SELENOP concentrations increase in response to Se supplementation, further supporting the notion that SELENOP may constitute the most relevant and accessible Se status biomarkers in mammals. This notion is supported by a number of our recent clinical studies relating SELENOP concentrations in patients to disease course and severity, as well as mortality rate, and in healthy subjects to disease risks. Collectively, these studies conducted in marginal supplied populations support the notion that Se status is of high relevance for maintaining health and increasing survival odds in disease, and that SELENOP analysis offers a meaningful way for Se status assessment and identification of a health-relevant Se deficiency.

Conflict of Interest Statement: LS holds shares in selenOmed GmbH.

A new era of zinc biology

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Zinc ions have intercellular, intracellular and subcellular functions in regulatory networks at different hierarchical levels. Regulatory functions of zinc in transient interactions with proteins are in addition to its already impressive functions as a catalytic and structural cofactor in about three thousand human zinc proteins. A molecular basis for zinc regulation emerged only in the last decade with recognition of the unique properties of Zn^{2+} as a signalling ion. Cellular zinc itself is regulated by a complex system of at least two dozen transporters and one dozen metallothioneins. These proteins are fully integrated into cellular biology. They control the buffering and muffling of high micromolar concentrations of cellular zinc in such a way that the concentrations of available "free" zinc ions are only in the picomolar range but can increase transiently by about one order of magnitude. The Zn^{2+} transients affect gene expression, phosphorylation, redox, and calcium signalling, protein-protein and other biomolecular interactions, and the assembly of protein complexes. The proteins involved in the homeostatic control of cellular zinc have a relatively large number of mutations. The resulting variants change the metabolism of zinc and other metal ions and are risk factors for many diseases. Owing to the complex genetics of the proteins controlling zinc, one-for-all recommendations of daily requirements for zinc need to be re-examined and additional biomarkers of zinc status are needed. It is expected that the new knowledge can be translated soon into significant advances in medicine and in the nutritional and pharmaceutical sciences.

Addressing molecular factors of intestinal zinc resorption using a Caco-2/HT-29-MTX barrier model

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The essential trace element zinc is predominantly resorbed in the small intestine [1]. Despite years of research, the distinct molecular mechanisms controlling intestinal zinc transport are not yet fully understood. Mucins are essential for the absorption of nutrients and suggested to play a beneficial role in luminal zinc availability [2]. For this purpose, suitable *in vitro* intestinal models, closer to the *in vivo* situation regarding their cellular composition and medium constituents, are much-needed. Thus, this study aims to investigate the role of intestinal mucins as well as of basolateral serum albumin on the luminal zinc uptake into enterocytes and its zinc transfer into the blood [3-4].
Zinc binding properties of mucins were determined with colorimetric assays and flame atomic absorption spectrometry (FAAS). Intestinal mucins showed a high zinc binding capacity, indicating several zinc binding sites with physiologically relevant affinity. Short-term zinc uptake into enterocytes, analyzed with the fluorescent zinc probe Zinpyr-1, was significantly impaired by zinc-depleted mucins. However, this does not represent their status in the intestine *in vivo* under zinc adequate conditions. Impact of zinc-loaded mucins on enterocytes' zinc uptake indicated that intestinal mucins more likely act as a zinc buffer and delivery system for the intestinal epithelium. This was supported by zinc transport studies using a co-culture of Caco-2 and mucin-producing HT-29-MTX goblet cells and inductively coupled plasma mass spectrometry (ICP-MS). Basolateral application of serum demonstrated that a zinc acceptor in the plasma considerably affects intestinal zinc resorption.
This study demonstrates that the luminal and basolateral matrix composition is crucial when investigating intestinal zinc transport using *in vitro* cell culture. Moreover, it provides a first comprehensive assessment of the molecular role of mucins for zinc uptake and their relevance for intestinal zinc resorption.

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Methodologies and uncertainties in risk assessment of trace elements: the selenium example

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Risk assessment for human selenium exposure is difficult, complex, and fascinating, like for other essential minerals with both nutritional and toxicological relevance, such as manganese, iodine, copper and iron. Selenium is ubiquitous in the environment, and primary sources of exposure include diet (generally the most important one), drinking water, air pollution from motorized traffic, coal combustion and industrial facilities, hair care products, and selected occupations. Selenium exists in inorganic and organic chemical forms, each having distinctive nutritional and toxicological properties.
Any risk assessment of this trace element, including effects of deficiency and overexposure, should take into consideration the rapidly evolving evidence in both epidemiology and laboratory sciences. Interestingly, the agencies addressing selenium risk assessment have yielded markedly different recommendations for the safe range of intake, depending on their interpretation of the literature. In addition, such agencies did not generally use epidemiologic human data for assessment, mainly relying on biochemical evidence based on proteomic endpoints (selenoprotein upregulation). Results of the most recent experimental and nonexperimental epidemiologic studies have yielded concerning results about selenium safety, contrary to results based on biochemical endpoints, challenging the reliability of current assessments of the safe range of selenium exposure. These results have not yet been incorporated into risk assessment of the health effects of selenium exposure by regulatory and scientific agencies.
This new evidence generated by the epidemiology must therefore be carefully assessed, weighting the evidence yielded by both experimental and nonexperimental studies, and bridging such evidence with that yielded by recent nutritional and toxicologic investigations. Such a reassessment should be mainly driven by the evidence from the low-bias controlled randomized trials, and by the dose-response relations from meta-analyses of epidemiologic studies.

Selenoneine, a novel selenium species: lessons learned from cells and worms

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The recently identified selenium (Se) species selenoneine (2-selenyl-N_α,N_α,N_α-trimethyl-L-histidine) constitutes a major form of organic Se in marine fish [1]. Being the Se analogue of ergothioneine, a sulfurcontaining antioxidant, selenoneine has been speculated to have similar properties. Moreover, the detection of selenoneine and its methylated derivative Se-methylselenoneine in human blood indicate its bioavailability and potential metabolism in humans [2]. However, data on its relevance to human health are lacking in general. Our project aims to elucidate the bioavailability, metabolism and potential protective properties of selenoneine in mammalian cell cultures and the in vivo model Caenorhabditis elegans (*C. elegans*).
Applying the Caco-2 *in vitro* intestinal barrier model, the bioavailability of selenoneine was studied in comparison to the reference compounds selenite and methylselenocysteine. Selenoneine was transferred across the *in vitro* intestinal barrier in higher amounts, but with similar kinetics compared to selenite, while methylselenocysteine showed the highest permeability. The transfer of selenoneine occurred in a sidedirected manner, and cellular Se contents demonstrate an efficient uptake by Caco-2 cells. Additionally, Se speciation studies revealed a partial metabolism to Se-methylselenoneine, the previously detected human metabolite [2]. In the roundworm *C. elegans*, a comparative *in vivo* characterization of selenoneine and ergothioneine was performed regarding their bioavailability, protective properties towards the induction of reactive oxygen and nitrogen species, and impact on the expression of antioxidant genes. Further planned Se speciation studies will reveal a possible metabolism of selenoneine in *C. elegans*.
Taken together, our results indicate that selenoneine might be a non-toxic, highly bioavailable Se species with possible antioxidant effects, and further studies will help to elucidate its role in human Se metabolism.

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Dietary cadmium exposure and risk of melanoma: an Italian population-based casecontrol study

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Background and aim. The heavy metal cadmium could be highly toxic to humans, and its environmental exposure has been linked to many adverse health effects, such as atherosclerosis, diabetes, and cancer, including melanoma. Although the underlying mechanisms need yet to be clearly identified, recent findings suggested that cadmium can specifically promote the malignant transformation of melanoma cells through the aberrant DNA methylation inducing dysregulation of specific gene expression. Since in the nonoccupationally exposed population, in addition to smoking, food intake is the major source of cadmium exposure, we aimed at assessing the risk of cutaneous melanoma in relation to dietary cadmium intake.
Methods. Using a population-based study design, we recruited 380 incident cases of newly-diagnosed melanoma and 719 sex- and age-matched controls in the Emilia-Romagna Region, Northern Italy. We evaluated their dietary habits through a semi-quantitative food frequency questionnaire and we computed the odds ratio (OR) and its 95% confidence interval (CI) for melanoma according to quintile distribution of cadmium intake, using a conditional logistic regression model, matching by sex, age and province of residence, and adjusting also for phototype, non-alcoholic energy intake, body mass index, and Italian Mediterranean Diet Index.
Results: Median intake of cadmium was 5.81 µg/day (interquartile range 4.46-7.59) in cases, and 5.63 µg/day (4.46-7.34) in controls. OR of melanoma associated with 1-unit increase in cadmium intake was 1.11 (95% CI 1.00-1.24). Melanoma risk increased with increasing quintile of cadmium exposure, with ORs of 1.55 (95% CI 0.99-2.42), 1.54 (95% CI 0.99-2-40), 1.75 (95% CI 1.12-2.75), and 1.65 (95% CI 1.05-2.61) in the second to the highest quintile compared to the lowest quintile. Sex-stratified analysis showed substantially comparable results and a generally higher risk in female population, with continuous ORs of 1.10 (95% CI 0.93-1-29), and 1.15 (95% CI 0.99-1.33) in men and women, respectively.
Conclusions: Our results suggest a positive association between cadmium exposure through diet and risk of cutaneous melanoma in a Northern Italy population. Such association started to occur at a level of exposure lower than the tolerable intake established by the World Health Organization, and considered to be safe for humans.

Intravenous beryllium toxicity – new lessons to take

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The rare element beryllium (Be) is highly toxic for humans and, due to steadily increasing technological use of this metal, the potential intoxications in both occupationally exposed and general populations become more probable. The well-explored aspect of Be toxicity is related to the exposure of metal-containing aerosols, causing chronic inflammation in respiratory tract, lung sarcoidosis, and neoplasia. However, it seems that such events might be mainly caused by Be-containing nanoparticles rather than the effect of Be^{2+} ions per se. Exposure routes other than inhalation are somewhat hard to investigate, in case of Be. The reasons for that are: very low per oral bioavailability of Be salts and their poor solubility under pH values, close to physiological conditions (around 7.4). The parenteral Be introduction usually causes extreme acidic and osmotic damage to the tissue, seriously biasing the observations. Thus, the exact toxicity mechanisms of Be^{2+} were not properly studied.
We designed a new model of Be toxicity including intraperitoneal administration of neutralized Be^{2+} – amino acid glycine composition. High penetration of Be into the bloodstream and accumulation in the tissue was observed in adult male Wistar rats. Under acute poisoning, we found scarce accumulation of Be in lungs, whereas primary target organs were found to be testes. The most prominent histological changes were also found in these tissues. Our findings showed the prominent toxic effect of Be to the male fertility. Liver and kidney were found to be responsible for Be excretion. Low Be penetration through the bloodbrain barrier was also detected. LD_{50} of the Be^{2+} – glycine composition was found to be 50.61 ± 0.06 mmol kg^{-1}. The data obtained shows the necessity to explore Be^{2+} toxicity in more details.

The selenium dependent thioredoxin system as a target for drug therapy in cancer or oxidative stress

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Selenium is an essential trace element for mammals due to essential roles of several selenoproteins that contain a selenocysteine residue. This is often acting as the catalytic site in oxidoreductases that employ the high chemical reactivity and unique characteristics of selenium to catalyze redox reactions. Thioredoxin reductases belong to the family of essential selenoprotein oxidoreductases that carry out a wide range of reductive reactions in cells, either directly through their main substrates thioredoxins or thioredoxin related protein of 14 kDa, or indirectly via peroxiredoxins, methionine sulfoxide reductases, ribonucleotide reductase, or other reductive pathways dependent upon a functional thioredoxin system. A number of observations have revealed that drug targeting of thioredoxin reductases, often through irreversible derivatisation of the Sec residue, not only inhibits normal function of these oxidoreductases, but can also induce an oxidative NADPH oxidase activity in the otherwise inhibited enzyme. This, in turn, can have a number of major consequences. In normal cells, the main effect is typically a strong activation of the Nrf2 transcription factor, which upregulates protective glutathione-dependent enzyme systems that, paradoxically, can *protect* such cells from further oxidative stress. Cancer cells, in contrast, typically do not survive without thioredoxin reductase and its drug targeting therefore displays wide direct anticancer efficacy. It is thus possible that drugs targeting the Sec residue in thioredoxin reductases can find clinical applicability in cancer treatment as well as in therapies requiring increased protection of normal cells against oxidative stress. The latest developments in this field will be discussed in this presentation.

Regulation of human selenoproteins: development of a new tool mimicking selenium deficiency based on CRISPR-CAS9 virus-like particles

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Selenium is an essential trace element in mammals, which shares many similarities with its above neighbor element in the periodic table, the atom sulfur. Therefore, many aspects of selenium chemistry, reactivity, and metabolism are similar to those of sulfur. However, one key difference between these two elements is the insertion of selenium in the group of selenoproteins in the form of a rare amino acid, the selenocysteine. The translation of selenoprotein mRNAs involves a non-canonical ribosomal event in which an in-frame UGA is recoded as a selenocysteine (Sec) codon, instead of being a stop codon. Dedicated cellular machinery achieves this recoding process based on two central RNA components: the selenocysteine insertion sequence (SECIS) located in 3'UTR of the mRNA and the selenocysteine-tRNA (SectRNA$^{[Ser]Sec}$). This translational UGA recoding event by the ribosome is a limiting stage of selenoprotein expression. Its efficiency is controlled by the SECIS, the Sec-tRNA$^{[Ser]Sec}$ and their interacting proteins partners. In the present work, we used a recently developed strategy based on murine leukemia virus-like particles (VLPs) loaded with Cas9/sgRNA ribonucleoproteins to inactivate the Sec-tRNA$^{[Ser]Sec}$ gene in human cell lines. We showed that these CRISPR-Cas9-VLPs were able to induce efficient genome-editing in several cell lines and a robust reduction of selenoprotein expression. The alteration of selenoprotein expression was the direct consequence of lower levels of Sec-tRNA$^{[Ser]Sec}$, and therefore a decrease in translational recoding efficiency of the ribosome. Interestingly, the decrease in Sec-tRNA$^{[Ser]Sec}$ levels mimics several aspect of selenium deficiency. This novel strategy opens many possibilities to study the consequence of selenoprotein deficiency in hard-to-transfect cells since these CRISPR-Cas9-VLPs are able to target many cell lines.

Toxic trace elements in obesity and metabolic syndrome

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Obesity is a worldwide epidemic affecting approximately 13% of the total population, whereas up to 39% (1.9 billion) adults are overweight. Recent studies have demosntrated a significant role of environmental factors and especially persistent organic pollutants (POPs) in obesity pathogenesis. At the same time, data on adipogenic activity of inorganic toxins is insufficient. The existing data demonstrate that toxic metals and trace elements may have a significant impact on adipogenesis being endocrine disruptors. However, the existing human data are rather contradictory. Therefore, the objective of a series of studies was to investigate the association between toxic metal exposure and obesity and metabolic syndrome. Hair metal content of more than 10,000 adults was assessed using inductively-coupled plasma mass-spectrometry (ICP-MS). Hair mercury (Hg), cadmium (Cd), lead (Pb), and tin (Sn) levels were significantly associated with overweight and obesity as assessed by body mass index (BMI) values, although the association was age- and genderspecific. Hair toxic metals were also associated with adverse trends in lipid spectrum (total cholesterol, triglycerides, LDL and HDL cholesterol), carbohydrate metabolism, and blood pressure. Although being relatively unrelated to BMI, hair aluminium (Al) content is independently associated with atherogenic lipid spectrum. At the same time, serum metal levels were differentially associated with metabolic syndrome components. In addition, hair metal (loid) and especially Hg content in the inhabitants of different regions of Russia were associated with the incidence of diabetes and obesity. Comparative analysis of the association between metal levels and BMI has been performed using multiple stepwise regression. The mechanisms involved in metal-induced adipose tissue dysfunction may include endocrine disruption (e.g. adiponectin and leptin production and signaling), adipogenesis dysregulation (PPARs), insulin resistance, local and systemic inflammation (NF-κB, and AP-1 activation) and oxidative stress, endoplasmic reticulum stress, antagonism with essential elements (Zn, Se), alteration of gut microbiota, etc. Certain metals including tin may have central effects on neural circuits of appetite regulation leading to the prevalence of proorexigenic signals and resulting in hyperphagia. Hypothetically, these mechanisms may be considered as the potential targets for management of obesity and metabolic syndrome in a population exposed to toxic metals.

Biomonitoring in the *NutriAct* intervention study

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Based on the current demographic status of Germany, the cluster project *NutriAct* creates a scientific framework for healthy aging. It is one of the leading questions of the *NutriAct* intervention study to assess how nutrition benefits health in the elderly. In this case, averting age-associated diseases is one of the common assumptions that joins nutrition and health together. The competence cluster 'nutritional intervention: food patterns, behavior, and products' (*NutriAct*) monitors 50 – 70 year-olds for three consecutive years. While the intervention group consumes foods according to the specially designed *NutriAct* food pattern, the control group keeps a balanced diet based on recommendations of the German Society for Nutrition (DGE). Samples (plasma, serum, blood, stool) are collected at regular intervals to check for short- and long-time changes of exposure or effect biomarkers. In the blood serum of the study participants, we determined the element profile comprising the following bulk and trace elements: Mg, Ca, Mn, Fe, Cu, Zn, Se, Cd, As, Mo, and I. The ICP-MS/MS-based multi-element method provides insights into the element supply of the elderly, by which also any deficiency state can be recognized. The limited sample volume and the demand for a high throughput system are without doubt very challenging for analytics. The elements Mg, Ca, Mn, Fe, Cu, Zn, Mo, Cd and I are measured with helium as collision gas to eliminate disturbing interferences, while As and Se are analyzed with oxygen as reaction gas. Here, Se is quantified by isotope dilution analysis. To avoid memory effects regarding I, serum samples are prepared under alkaline conditions. The analysis of blood serum samples of three visiting points (until now: 0, 6 months, and 12 months) show no significant differences between the control group and intervention group with regards to their total elements concentration. However, both groups profit from a shift to adequate supply regarding Se. As the element serum level is not a convincing biomarker for every element analyzed, one must also differentiate between the species (e.g. total Se vs. Selenoprotein P).

Is lead a remarkable component in resistant hypertension? Results from a study in patients of a Spanish hypertension unit

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BACKGROUND. Lead is a toxic element that represents a public health problem and that could be not only a risk factor for hypertension (HT) but contribute to the development of resistant hypertension (rHT). The aims of this study are to describe and compare the blood lead levels in a group of subjects - from a hospital hypertension unit - with resistant hypertension and non-resistant hypertension and to define a blood lead threshold related with resistant hypertension.
MATERIAL AND METHODS. Cross-sectional study. Resistant hypertension was defined according to the American Heart Association (AHA) criteria. Blood pressure was taken in two successive consultations according to the European Society of Hypertension (ESH) guidelines with at least one week interval and then the measures were averaged. Obesity was defined as body mass index (BMI) $\geq$ 30 kg/m^2. Creatinine clearance was obtained from a 24 hour urine (ml/min). The blood lead concentration was measured by electrothermal atomization atomic absorption spectrometry with Zeeman background correction in a Perkin-Elmer spectrometer Aanalyst 800.
RESULTS. We included 73 unrelated subjects (46.6% men) recruited during the years 2003-2004, 44 (61.7%) were rHT cases. Age: HT 57.7 vs. rHT 61.7 y (p = 0.06). Blood pressure: HT 132.5 $\pm$ 15.4/75.8 $\pm$ 8.5 vs rHT 155.6 $\pm$ 23.5/82.45 $\pm$ 11.9 mm Hg (p < 0.05). BMI: HT 30.2 $\pm$ 3.9 vs. rHT 31.7 $\pm$ 6.6 kg/m^2 (p = 0.236). Glucose: HT 107.1 $\pm$ 21.1 vs. rHT 121.5 $\pm$ 46.3 mg/dl (p = 0.077). Creatinine clearance: HT 90.7 $\pm$ 25.85 vs. rHT 74.6 $\pm$ 26.2 ml/min, p = 0.013). Blood lead median: HT 3.7 (IQR 2.7-5.1) vs. rHT 3.7 (IQR 2.8-6.1) µg/dl (p = 0.885). Multiple step logistic regression analysis introducing a blood lead threshold of 5.5 µg/dl (75th percentile) and adjusting for age, obesity and creatinine clearance showed: OR 4.29 (1.07 $\pm$ 17.16, p = 0.04).
CONCLUSION: Subjects with a blood lead threshold of $\geq$ 5.5 µg/dl have higher risk for resistant hypertension independently of age, obesity and creatinine clearance.

'Macro effects' of 'micro elements': current trends in trace element status of Russian population

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Multiple studies have demonstrated significant effects of both essential and toxic trace elements in a particular organism. However, data on demographic effects of trace elements are insufficient. Moreover, data on the current trends in both essential and toxic trace element status of the Russian population for the last decades are absent. Therefore, the objective of a series of studies performed during the last decades (2000s – 2010s) was to assess the current trends in element status of Russian population, as well as its interaction with demography and population health. Data on hair trace elements content assessed by inductively-coupled plasma mass-spectrometry from more than 60,000 adults and 15,000 children living in 80 regions of Russia were obtained. Based on these data on the time-dependency of the rate of trace element deficiency and excess in Russia was evaluated. Generally, it has been revealed that high content of hair toxic trace elements is associated with reduced birth rate, increased mortality and morbidity. At the same time, the legislative and technical efforts undertaken since the early 2000s have resulted in a significant decrease in hair toxic trace element content, being indicative of the trend to improvement of metal pollution. However, the data on essential trace elements are contradictory thus being indicative of the high risk of essential trace element deficiency in the Russian population. The cost-effectiveness of iron supplementation for prevention of irondeficiency anemia based on the results on national-wide hair iron analysis was estimated (Russian experience confirmed the worldwide estimate of 1:10 cost-benefit ratio). The impact of Hg on populational health and demography was demonstrated. In turn, essential trace element deficiency (Se) is associated with decreased life expectancy and higher morbidity and mortality. At the same time, the relationship with demographic parameters was more significant after consideration for interaction (ratios) between particular trace elements (e.g. Hg/Se). The potential benefits and approaches of trace element status regulation are discussed.

Systems biology of selenium utilization

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Selenium is a trace element that exhibits both beneficial and toxic effects in human health. Importance of this micronutrient in the diet is primarily due to the fact that selenium is used in selenoproteins in the form of selenocysteine. In this presentation, discussion will be focused on the occurrence and evolution of selenium and selenoproteins. We recently found selenoproteins in fungi, the last kingdom where these proteins were thought to be absent. Comparative and functional genomics methods allow assessing its use at the levels of proteins, cells, organs and entire organisms. Selenoproteins with known functions are oxidoreductases, and the tight link between selenium and redox biology offers an opportunity to better understand protein function and use this information to examine questions central to the thiol-based redox control of cellular processes. We use high-throughput approaches, including genome sequencing, transcriptomics, and metabolomics, to better understand the systemic role of selenium in diverse animals and other organisms. We also apply these methods to conditions of varying selenium levels in the diet, revealing elements of antagonistic pleiotropy of this element.

Effects of consuming a Mediterranean-like dietary pattern on trace element intake, and iron and selenium status, in elderly men and women: results from the NU-AGE study

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A one year Randomised Controlled Trial (NU-AGE) was carried out in older Europeans to investigate the effects of consuming a Mediterranean-style diet on indices of inflammation and changes in nutritional status. Reducing meat intake, an important supply of bioavailable iron, plus higher consumption of phytatecontaining wholegrain cereals, could result in a lower iron status. Conversely, increased intakes of fish, a good source of selenium, could increase selenium status. We tested these hypotheses using dietary and biochemical data from a total of 1,294 people aged 65-79 y from 5 European countries (France, Italy, Netherlands, Poland and the UK) who were randomly allocated to an intervention (MD) or control group for a period of one year. As it has been reported that lower serum selenium is associated with anaemia in older women, we also examined associations between iron and selenium status.
Selenium intakes increased significantly in the intervention group ($p < 0.01$), but changes in selenium intake were not accompanied by with changes in selenium status. Iron intakes were increased by the intervention ($p < 0.001$) but there was no change in iron status. When stratified by study centre there was a positive effect of the intervention on iron status for serum ferritin in Italy ($p=0.04$), the Netherlands ($p=0.05$) and France ($p=0.04$) and sTfR in Poland ($p < 0.01$), relative to controls. Meat intake decreased and fish intake increased to a greater degree in the intervention group relative to the controls ($p < 0.01$ for both), but the overall effect of the intervention on meat and fish intake were mainly driven by data from Poland and France. Change in selenium status in the intervention group was associated with a greater change in serum ferritin ($p=0.01$) and body iron ($p=0.01$) but not sTfR ($p=0.73$), but there were no study centre x selenium status interactions for the iron biomarkers. We conclude that consuming a Mediterranean-style diet for one year had no overall effect on iron or selenium status, although there was a positive effect on biomarkers of iron status in some study sites.

Change of trace element profiles during aging: preliminary results from the EPIC-Potsdam study

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Studies on age-related differences in trace element (TE) status have been mostly conducted cross-sectionally and few have considered several TE simultaneously. Within the framework of the DFG-research group #2558 TraceAge, we aim to evaluate age-dependent changes of TE profiles (selenium (Se), zinc (Zn), iron (Fe), copper (Cu), iodine (I) and manganese (Mn)) over a 20 year period.
TE levels were determined in serum samples taken at baseline and after 20y of follow-up from 219 healthy participants of the EPIC-Potsdam study, using an ICP-MS/MS approach. For each TE, absolute differences were calculated between the two time points, as well as the proportion of individuals within normal reference ranges. Interdependence between age-related TE differences was investigated using principal component analysis (PCA). Relationships between selected factors (TE concentration at baseline, lifestyle, sociodemographic, anthropometric factors and hypertension) and corresponding TE longitudinal variability were examined using multivariable linear regression models.
Median age of our study sample was 58.32y (4.42) at baseline and 40% were female. Median Mn, Zn, Se concentrations and Se/Cu ratio significantly decreased during aging while median Fe, Cu, I concentrations and Cu/Zn ratio significantly increased. A substantial percentage of the participants, at both time points, had Zn concentrations outside the reference ranges (almost half at baseline and 60% after 20y). The first PCAextracted factor was positively correlated with decreased Mn, Fe and Zn over time and the second factor positively with decreased Cu and I. Overall, none of the investigated factors were strong determinants of TE longitudinal variability, except the respective TE baseline concentration.
In this preliminary study, aging was characterized by specific changes in TE profile. Further research is required to investigate food determinants and markers of TE status as well as the relationships between TE profiles and the risk of age-related diseases. This is planned as part of the TraceAge project.

Zinc homeostasis in Parkinson's disease – investigations of labile and total zinc levels in aging *Caenorhabditis elegans*

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Parkinson's disease (PD) is one of the most prevalent neurodegenerative disorders in the elderly. While genetic factors can be identified in up to 10% of PD patients, environmental factors, such as metal exposure are assumed to play a role in PD's etiology. Nevertheless, the role of the essential trace element zinc (Zn) in this regard remains controversially discussed.
In this study we analyzed labile ($[Zn]^{2+}$) and total Zn concentrations in PD models of the nematode *Caenorhabditis elegans* (*C. elegans*). Strains studied were N2 (wild type, WT), as well as the PD mutants *pdr-1* (orthologue of the mammalian parkin (PARK2)) and *catp-6* (orthologue of the mammalian ATP13A2 (PARK9)). Young (day 2 of adulthood) and middle-aged worms (day 5 of adulthood) were analyzed after 24 h exposure to Zn-elevated (200 µM or 500 µM) or Zn-chelated (200 µM TPEN) food.
Inductively coupled plasma tandem mass-spectrometry (ICP-MS/MS) analysis revealed an age-dependent Zn accumulation from day 2 to day 5. Both, day 2 and day 5 adults of the tested strains showed comparable trends of TPEN-decreased and Zn-increased total Zn concentrations. A marked increase in total Zn was found in day 2 adult PD mutants compared to WT controls upon feeding with 500 µM Zn for 24 h.
Loosely bound, and referred to as labile Zn, is of particular interest as a potential biomarker of zinc bioavailability. It is known that intracellular $[Zn]^{2+}$ changes affect signal transduction pathways in mammals. Therefore, we performed a multi-well in vivo analysis of $[Zn]^{2+}$ in *C. elegans* using the fluorescent probe ZinPyr-1. In young worms a dose-independent rise of $[Zn]^{2+}$ after Zn feeding in WT, and decreased $[Zn]^{2+}$ after TPEN supplementation in all strains were noted. An increase in $[Zn]^{2+}$ of the PD models was only detected in pdr-1 mutants following 500 µM Zn exposure. Those Zn- or TPEN-induced changes in $[Zn]^{2+}$ were not observed in older day 5 adults.
Taken together, these results establish the need for further investigation of Zn's role in PD and indicate that $[Zn]^{2+}$ and total Zn should be mentioned as two independent biomarkers in the field of aging studies.

Interactions of selenium with other essential trace elements

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Selenium is an essential trace element with various functions in the human body which are mainly mediated by selenoproteins. The selenoproteins of the deiodinase family are, besides others, important mediators of thyroid hormone activity. Those hormones are produced by iodination of thyroglobulin which needs to be degraded and deiodinated to become active T3. This example nicely documents a strong interrelationship between the two trace elements selenium and iodine.
Most other selenoproteins are antioxidant enzymes and thus regulate the cellular redox status. Also this way, selenium acts in concert with other essential trace elements such as zinc and copper. We could show that copper interferes with selenoprotein synthesis resulting in lower amounts of selenoproteins upon copper treatment. If provided in excess all of these trace elements, however, have pro-oxidant effects as well. Thus, we aimed to address the question whether combinations of selenium, copper, and zinc are able to modulate redox-sensitive transcription factors such as Nrf2 differently than under conditions of separate stimulation.
Vice versa, Nrf2 mediates a plethora of genes involved in maintaining trace element homeostasis e.g. transporters and binding proteins. To identify systemic effects, Nrf2 knockout mice were used to understand how this transcription factor modulates profiles of several redox-active trace elements in different tissues. Overall, we aim to clarify the mode of further interactions of essential trace elements to better understand their function and regulation under physiological as well as under pathophysiological conditions.

Homeostatic changes of some trace elements in geriatric rats in condition of oxidative stress induced by aluminium and the benefic role of resveratrol

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The aim of the study was to assess the protective role of resveratrol upon the homeostatic changes of some trace elements in geriatric rats in condition of oxidative stress induced by aluminium exposure. Forty Wistar rats, 18-20 months old, were divided randomly in four groups (n=10): control (C) – receiving 1 ml of physiologically saline (P.S) via intraperitoneal (i.p) administration, E1 – 1ml of P.S and 1000 ppb aluminium sulphate (AS) in drinking water *ad libitum*, E2 – 20 mg/kg^{-1} resveratrol, i.p. and 1000 ppb AS in drinking water, E3 – 20 mg/kg^{-1} resveratrol i.p. The groups C and E3 received distillated water as drinking water *ad libitum*. The i.p administration was once a week for four weeks. Levels of oxidative stress markers (glutathione, glutathione peroxidase, superoxide dismutase and catalase) in serum and the levels of main trace elements (copper, zinc, iron, selenium, manganese and magnesium) in blood, liver, kidney and spleen were analyzed. The results revealed significant ($p < 0.05$) decrease of catalase (CAT), glutathione (GSH), glutathione peroxidase (GPx) and significant ($p < 0.05$) increase of superoxide dismutase (SOD) in E1 groups comparative with control, E2 and E3 groups. There were also observed significant ($p < 0.05$) decreases of Cu, Zn, Fe and Mg, not significant ($p > 0.05$) increase of Se and Mn in blood, significant ($p < 0.01$) increase of Cu, Zn, Mg, Se, Mn in kidney and liver and Fe in spleen of rats from E1group comparative to control. In groups that received resveratrol (E2 and E3) there were recorded not significant ($p > 0.05$) differences comparative to control group, and significant differences ($p < 0.05$) especially in blood comparative to E1, suggesting that the resveratrol can prevent the homeostatic imbalance of trace elements in geriatric rats in condition of oxidative stress induced by aluminium exposure.

Insights in trace element effects and interactions with focus on C. elegans

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Excessive and prolonged exposure to the plentiful and naturally abundant trace element manganese (Mn) may lead to devastating neurological impairment, which is termed as "manganism". The underlying mechanisms have yet to be conclusively clarified. The nematode *Caenorhabditis elegans* (*C. elegans*) constitutes a distinguished model organism for the essential further investigation of *in vivo* mechanisms. Quantifying various oxidative stress/damage markers following Mn exposure underlined that Mn-induced oxidative stress contributes to the disease etiology. Accordingly, DNA damage caused by oxidative stress may further trigger an induction or dysregulation of DNA repair pathways, as well as DNA damage response. The expression of genes acting in the initiation of the base excision repair (BER), a pathway correcting small base lesions, was induced after exposing the worms towards Mn. Studies with transgenic animals further showed that loss of a DNA glycosylase, which normally removes oxidized purines and pyrimidines resulted in hypersensitivity towards Mn-induced lethality compared to wildtype worms. Currently we are striving to unravel the underlying molecular mechanisms. Our quantification of cellular poly(ADP-ribosyl)ation (PARylation), a DNA damage response involved in DNA repair, suggest a role of PAR in Mn-induced mitochondrial-derived oxidative stress. Since the trace element zinc (Zn) is involved in these pathways and Mn might impact other trace elements' homeostases, metal co-exposure will be of central importance for identifying novel targets in the etiology of manganism. Co-exposing worms to Mn and Zn chronically through middle age should point out a possible interaction of the homeostases of the two metals. Since manganism shares a similar neuropathology with Parkinson's disease (PD) and PD genes have been implicated in Zn and Mn homeostases, the role of PD-associated genes in these metal homeostases is currently studied. In particular, the bioavailability of Zn and Mn after co-exposure is quantified in N2 (wildtype, WT), as well as the PD mutants pdr-1 (orthologue of the mammalian parkin [PARK2]) and catp-6 (orthologue of the mammalian ATP13A2 [PARK9]).

Assessment of toxic and essential trace element levels in patients with Parkinson's disease

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The existing data demonstrate a significant role of trace element dyshomeostasis in pathogenesis of Parkinson's disease (PD). The large body of data demonstrate the role of Mn exposure in PD development through disruption of dopamine system, neuroinflammation, and synaptic transmission (Aschner et al., 2009). Other metals may be also involved in PD pathogenesis. At the same time, the existing data on trace element status in PD are rather contradictory. Therefore, the objective of the present study was to assess essential and toxic trace element levels in hair, serum, and urine, as well as manganese, iron, copper, and zinc speciation in serum of patients with PD disease. The preliminary study involved a total of 40 patients including 20 patients with diagnosed PD (Parkinsonism excluded) and age- and gender-adjusted controls. All procedures performed within the study were in agreement with the ethical principles. The obtained hair samples were subjected to microwave digestion. Total levels of essential and toxic trace elements in hair, serum and urine were assessed using ICP-DRC-MS and NexION 300D (PerkinElmer, USA). The obtained data demonstrate significantly decreased hair (0.169(0.087-0.255) μg/g vs 0.412(0.232-0.499) μg/g), serum (0.0002 ± 0.0002 μg/ml vs 0.0005 ± 0.0003 μg/ml), and urine (0.0002 ± 0.0001 μg/ml vs 0.0006 ± 0.0004 μg/ml) Hg levels in the patients as compared to the controls, being in agreement with the study by Gellein et al., 2008. Hair (0.386 (0.382-0.437) μg/g vs 0.4590.401-0.513) μg/g) and urine (0.019 ± 0.015 μg/ml vs 0.036 ± 0.021) Se levels were also characterized by a significant decrease when compared to the respective control values. Hair Cu and urinary Cd were also found to be decreased in the patient group by 22% and 20%, respectively. In addition, Mn, Fe, Zn, and Cu speciation analysis was performed using HPLC-ICPDRC-MS. It has been demonstrated that the relative abundance metal species vary significantly between the PD and control groups. Finally, trace element status in Parkinson's disease was found to be associated with disease severity as assessed by Hoehn-Yahr scale. The obtained data demonstrate that trace element metabolism may be interrelated with PD pathogenesis, although the intimate mechanisms are still to be studied both in vivo and in vitro. The project was supported by RFBR (19-015-00212).

Trace element profiles and genomic stability in aging: DNA repair and damage response

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The DFG research unit TraceAge (#2558) aims at elucidating the impact of trace element (TE) profiles on endpoints of genomic stability regarding the essential TEs copper, iron, zinc, selenium, manganese and iodine as well as interactions among these elements in the context of aging. Until today, knowledge is scarce about the relationship between the dietary TE intake and the TE homeostasis, neither is known how the health status, especially within aging, is affected. However, it is well known that the TE status plays a crucial role in a multitude of diseases.
We hypothesize that an impaired TE homeostasis disturbs DNA repair processes thereby contributing to the aging process and eventually causing genomic instability. Hence, we investigate the correlation between the age-dependent TE profiles and the genomic stability related endpoints base excision repair (BER) incision and poly(ADP-ribosyl)ation (PARylation) in murine liver.
BER is the major cellular repair mechanism for oxidative DNA lesions. By removing a broad and frequently occurring spectrum of DNA lesions, base excision repair contributes greatly to the maintenance of genomic stability. Based on a previous publication (Hamann, Schwerdtle, Hartwig; Mutat Res 2009, 669, 122-30) we established a non-radioactive cleavage assay to assess BER incision activity via determination of the repair capacity towards an AP site and the common oxidative DNA lesions 8-oxoguanine and 5-hydroxyuracil in murine liver.
PARylation is a post-translational modification, catalysed by poly(ADP-ribose)polymerases (PARPs), occurring as an immediate response to DNA lesions and, among others, taking part the DNA damage response, chromatin reorganisation and transcription, thereby contributing to genome maintenance. PARP-1, a DNA damage sensor, forms poly(ADP-ribose) chains at the damage site that are used for localizing further DNA repair factors at the damage site. PARylation status in murine liver was determined via stable isotope dilution liquid chromatography tandem mass spectrometry (LC-MS/MS) using an approach that was modified from a previous publication (Martello, Mangerich, Sass, Dedon, Bürkle; ACS Chem Biol 2013, 8(7), 1567-1575). The TE status in serum and murine liver was determined via inductively coupled plasma tandem mass spectrometry (ICP-MS/MS) to find correlations between the TE status and investigated genomic stability related endpoints.

Manganese-Induced Neurotoxicity: Lessons from Worms

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Manganese (Mn), is a trace metal required for normal physiological processes in humans. Mn levels are tightly regulated, as high levels of Mn result in accumulation in the brain and cause a neurological disease known as manganism. Manganism shares many similarities with Parkinson's disease (PD), both at the physiological level and the cellular level. Exposure to high Mn-containing environments increases the risk of developing manganism. Homozygous mutations in SLC30A10 cause familial parkinsonism associated with manganese (Mn) retention. We recently identified SLC30A10 as a cell surface-localized Mn efflux transporter and demonstrated that parkinsonism-causing mutations block its intracellular trafficking and efflux function. In *C. elegans*, SLC30A10 over-expression protected against Mn-induced lethality and dopaminergic neurotoxicity, consistent with results in mammalian systems. SLC30A10 expression did not protect worms against ZnSO4 toxicity, suggesting that SLC30A10 does not mediate Zn export in *C. elegans*.

The mechanisms underlying the selective dopaminergic (DAergic) neurodegeneration are poorly understood; however, genetic factors, as well as environmental and endogenous toxins have been implicated. Additional studies show that DA and additional genetic factors are intrinsically required to render DAergic neurons susceptible to environmental risk factors of PD. We have recently successfully converted in *C. elegans* serotonergic (5HT) to DAergic neurons by genetic modification, and these neurons show analogous sensitivity to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) as VTA DAergic classical neurons. Intrinsic expression of DA in these pseudoneurons renders them susceptible to classical model neurotoxins of PD, leading to neurodegeneration.

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Manganese causes neurotoxic iron accumulation via translational repression of Amyloid Precursor Protein (APP) and H-Ferritin

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For more than 150 years it is known that occupational overexposure of manganese (Mn) causes movement disorders resembling Parkinson's disease (PD) and PD-like syndromes. However, the mechanisms of Mn toxicity are still poorly understood. Here, we demonstrate that Mn dose- and timedependently blocks the protein translation of amyloid precursor protein (APP) and heavy-chain Ferritin (H- Ferritin), both iron homeostatic proteins with neuroprotective features. APP and H-Ferritin are post-transcriptionally regulated by iron responsive proteins (IRPs), which bind to homologous iron responsive elements (IREs) located in the 5'-untranslated regions (5'-UTRs) within their mRNA transcripts. Using reporter assays, we demonstrate that Mn exposure repressed the 5'-UTR-activity of APP and H-Ferritin, presumably via increased IRP-IRE binding, ultimately blocking their protein translation. Using two specific Fe^{2+}-specific probes (RhoNox-1 and IP-1) and ion chromatography inductively coupled plasma mass spectrometry (IC-ICP-MS), we show that loss of the protective axis of APP and H-Ferritin resulted in unchecked accumulation of redox-active ferrous iron (Fe2+) fueling neurotoxic oxidative stress. Enforced APP expression partially attenuated Mn-induced generation of cellular and lipid reactive oxygen species (ROS) and neurotoxicity. Lastly, we could validate the Mn-

mediated suppression of APP and H-Ferritin in two rodent *in vivo models* (C57BL6/N mice and RjHan:SD rats) mimicking acute and chronic Mn exposure. Together, these results suggest that Mn-induced neurotoxicity is partly attributable to the translational inhibition of APP and H-Ferritin resulting in impaired iron metabolism and exacerbated neurotoxic oxidative stress.

Cognitive Dysfunction in neurodegenerative disorders: focus on copper

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Trace metal dyshomeostasis has been linked to cognitive deterioration and in particular a disturbance in the regulation of copper (Cu). Excess Cu not bound to ceruloplasmin (non-Cp Cu, also referred to as 'free' Cu), is thought to play a role in the development of Alzheimer's disease (AD). Non-Cp Cu is redox active and its toxicity results from its ability to accelerate oxidative stress via Fenton-like and Haber Weiss chemistry reactions. The plasma component of non-Cp Cu is composed of Cu loosely bound to albumin, transcuprein, peptides and amino acids and it is exchanged among them. It makes up 5-10% of plasma Cu in in normal condition. If the non-Cp Cu pool becomes expanded, this Cu becomes toxic, as exemplified by Wilson's disease and reported in AD and other neurodenerative diseases. Non-Cp Cu may serve as a biomarker for cognitive impairment in AD. Elevated levels of non-Cp Cu in serum increase the probability of having AD by approximately three-fold. Subjects with mild cognitive impairment (MCI, a prodromal stage of AD) have elevated non-Cp Cu levels and a hazard rate of conversion to AD three times higher than those with normal non-Cp Cu values. These results suggest that abnormalities of Cu act at early stages of the disease. This concept is further supported by the finding that an increased frequency of variants in the ATP7B gene, which is a major regulator of non-Cp Cu levels, associates with the risk (odds ratio from 1.63 to 5.16) of having AD. Non-Cp Cu appears to be increased also in Parkinson's disease and in corticobasal degeneration but not in frontotemporal lobar dementia. Recent studies support the existence of a Cu subtype of AD, typified by increased levels of non-Cp Cu, exhibiting peculiar ATP7B gene, neurophysiological and neuroimaging patterns.

Diagnosis of hereditary copper disorders in childhood by Next-Generation Sequencing

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Background: Next-Generation sequencing (NGS) has opened up novel diagnostic opportunities for children with unidentified, but suspected copper inherited diseases as Wilson Disease, Aceruloplasminemia or other copper rare diseases. We describe the experience of our Reference Center for Wilson Disease and other Rare Copper Disease.
Objectives: The aim of this study was to use a panel of copper genes or modifier genes to diagnose copper rare diseases.
Materials and Methods: This study included 49 children with abnormal copper analysis. After automated DNA extraction, the DNAs were screened by NGS using a panel of genes [*ATP7B* (NM_000053), *Cp* (NM_000096), *ATOX1* (NM_004045), *COMMD1* (NM_152516), *APOE* (NM_001302688), *MTHFR* (NM_005957), *PRNP* (NM_000311), *XIAP* (NM_001167), *IL1RN* (NM_173841), *BDNF* (NM_001143810), *SLC33A1* (NM_004733)]. Library preparations for NGS were established using a hybrid capture system for sequencing on the Miseq sequencer. The bioinformatics is performed with pipeline plateform of Hospices Civils de Lyon. All pathogenic variant were confirmed by Sanger sequencing.
Results: In 39 of these children we detected pathogenic or likely pathogenic variants in three different copper genes (*ATP7B, CP, SLC 33A1*) Conclusions: Our multi-gene panel is a fast and comprehensive tool to diagnose inherited pediatric copper disorders. We also illustrate the challenge of dealing with genetic variants and highlight arising clinical questions, especially in patients with atypical phenotypes.

Impact of heavy metal accumulation on skin

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Pollution is a global problem and skin is the first line of defense against environmental stressors in the body. In addition to exposure to diesel particulates and other air pollutants, the skin may be exposed to many topically applied substances that are absorbed through the skin into the blood and tissues of the body. Once in the skin, they can accumulate and induced the formation of free radicals, DNA and protein lesions that can cause severe premature aging. We used skin explants, exposed to 32 pollutants (27 heavy metals and 5 hydrocarbons) placed under a patch. This model is a good ex-vivo model for studying pollution damage on skin. We have studied the penetration of these metals in the different layers of the epidermis and dermis by LA-ICP and correlated this penetration with the expression of oxidation markers (lipid peroxidation, protein oxidation) and cell response to metal stresses. We also used a cultured keratinocyte model to understand the mechanisms of metals toxicity. For this, we treated keratinocytes in culture with different concentrations of cobalt, nickel, copper and aluminum for 24 hours. We measured cell respiration and cell oxidation markers. Our results indicate that nickel is the most toxic metal for keratinocytes and by NanoSIMS imaging we have shown that it accumulates preferentially in the nucleus of the cells.

Toxicological characterization of an arsenic-containing phosphatidylcholine in human liver cells

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Arsenolipids, a subgroup of organic arsenicals, can account for a large part of the total arsenic (As) content in fish and seafood. They include among others As-containing hydrocarbons (AsHC), As-containing fatty acids (AsFA) and As-containing glycophospholipids. In previous studies AsHCs were found to be highly toxic, comparable to inorganic As, in *in vitro* studies [1, 2]. AsFAs showed less toxicity [3]. In 2016 Viczek et al. identified five As-containing phosphatidylcholines (AsPC) in herring caviar [4]. We undertook a first toxicological characterization of AsPC 840, a synthesized AsPC-standard [5]. We assessed general cytotoxicity (lysosomal integrity, dehydrogenase activity, cell number), genotoxicity (micronucleus test, comet assay), bioavailability and metabolism in human liver carcinoma cells (HepG2). Speciation studies showed that the AsPC 840 undergoes hydrolysis. Consequently, the observed toxicity effects must be attributed to AsPC 840 and its hydrolysis products AsFA 362 and lyso-AsPC 602. Speciation studies of incubated cell media taken after different time points showed that at 0 h mainly AsPC 840 (28%) and lyso-AsPC 602 (62%) were found in the media. After 48 h 47% were present as AsFA 362 and 54% as lyso-AsPC 602 while only 0.1% remained as AsPC 840. Bioavailability, measured as total As, revealed a high uptake (AsPC 840: 100 µM incubation > 3000 µM As cellular). The cytotoxicity assays indicated a slightly higher toxicity of the AsPC 840 incubation compared to the AsFA 362 incubation. There was no indication of genotoxicity. Gene expression analysis regarding stress response, cell death and autophagy will be performed via RT-qPCR.
The lability of the compound raises the question whether AsPCs reach any organs after oral consumption. Further studies will therefore have to determine stability and toxicity of AsPCs when taken up in combination with other lipids. Furthermore the mechanism and site of formation of the AsPCs should be investigated, which could lead to a local toxicity.

[1] Meyer et al., 2014, Metallomics, 6
[2] Witt et al., 2017 Archives of Toxicology, 91
[3] Meyer et al., 2015, Toxicology Research, 4
[4] Viczek et al., 2016, Angewandte Chemie, 128
[5] Guttenberger et al., 2017, Tetrahedron Letters, 58

Abstracts: Poster Proceedings

Distribution of trace elements in mice depending on age and sex

P 1

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Essential trace elements are key players in a plethora of enzymatic reactions and are therefore involved in various signaling pathways. Especially Se, Cu, Zn, and Fe act as cofactors or prosthetic groups of antioxidative or protective enzymes, such as glutathione peroxidases, Cu/Zn superoxide dismutases, catalases or metallothionein. Accordingly, deficiencies but also overloads of trace elements contribute to various diseases. Vice versa, health and age affect the homeostasis of trace elements. The DFG research unit TraceAge (#2558) studies the impact of the six trace elements Mn, Se, Cu, Zn, Fe and I, thereby considering all of them in parallel and not just individually. To establish age-specific trace element profiles, we compared the total concentration in serum and various tissues of adult and old, male and female mice, fed with adequate amounts of trace elements. Quantification of trace elements was performed by means of ICPMS/MS. The data revealed that age had a significant impact on half of the analyzed trace elements in serum. Thereby, older animals showed significantly higher serum levels for Cu and I, whereas Zn concentration was decreased. Gender difference only affected Zn levels in serum, with higher concentrations in male mice. Comparable age-dependent effects were observed for humans based on data of the EPIC-Potsdam cohort as well as for old C. elegans worms. Our results indicate that age in particular has a consistent impact on trace element profiles across different species.

Dietary Calcium Affects the Progression of Non-Alcoholic Fatty Liver Disease in Rats

P 2

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Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in North America with a prevalence of 20-30% in adults. NAFLD occurs when fat accumulates in the liver. It typically develops with the intake of Western-style high-fat, high-cholesterol, and high-sugar diets and insufficient physical exercise. In 10-30% of cases the disease progresses to non-alcoholic steatohepatitis (NASH), a more severe condition characterized by inflammation and liver cell damage. Toxicity from free fatty acids (FFA) or cholesterol may play a role in the progression to NASH. Calcium is a mineral nutrient that modulates fat absorption and metabolism, thus it may affect NAFLD development. This study examined the effects of calcium intakes below and above nutritional requirements on NAFLD development in rats. Male Sprague Dawley rats (42 days old) were fed a diet high in fat (~42% of energy), cholesterol and sugar containing low (LCa, 2 g/kg), normal (NCa, 5 g/kg) or high (HCa, 20 g/kg) calcium. After 16 weeks, rats were killed and blood was collected for hematological and biochemical analyses. Livers were removed for histopathological examination using hematoxylin and eosin staining. Total lipids were extracted from the liver and separated into different fat molecules using thin layer chromatography. Fatty acid profiles in isolated triglycerides (a fatty acid storage molecule) and FFA fractions were determined by gas chromatography. NAFLD was induced in all rats with 16-41% of the liver weights being fat. Rats fed the HCa diet had lower ($p < 0.05$) liver weights relative to body weight, less accumulation of small fat vacuoles (microsteatosis) in livers, and less liver cell hypertrophy compared to rats fed the NCa diet suggesting less advanced NAFLD. White blood cell counts and alkaline phosphatase activity (marker of liver damage) were also lower in rats fed the HCa diet. In general, proportions of different fatty acids in liver triglycerides or FFA fractions did not differ among groups. The results suggest that higher dietary calcium may have a protective effect on the progression of NAFLD in rats.

The selenium status of HepG2 cells has no major impact on cardamonin-induced Nrf2 activation

P 3

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Many secondary plant metabolites are thought to be beneficial for human health, with respect to prevention of oxidative stress-related disorders including cancer and diabetes mellitus. For investigating the molecular mechanism of their actions, it is advantageous to test combinations of different substances. Recently, the chalcone cardamonin was identified as inducer of Nrf2-regulated genes. Two proteins, whose expression in enterocytes is stimulated by cardamonin, glutathione peroxidase-2 (GPx2) and thioredoxin reductase-1 (TrxR1), are selenoenzymes.
The potential interaction between the cellular selenium (Se) status and the Nrf2 signalling pathway was investigated in HepG2 human hepatoma cells. Cardamonin was detected in HepG2 cell lysates by HPLC. By real-time qRT-PCR and immunoblotting, mRNA and protein levels of selected Nrf2 target genes were measured after short- and long-term cardamonin treatment of HepG2 cells cultured in Se-deficient, Seadequate and high-Se medium. The localization of Nrf2 was detected by immunoblotting after cytoplasmic/nuclear fractionation. Sulforaphane (SFN), a well-known Nrf2-activator, served as positive control.
Treatment with both SFN and cardamonin resulted in rapid induction of Nrf2 and its enrichment in the nucleus, independent of the cellular Se status. Both compounds up-regulated Nrf2 target genes, although with differences regarding the extent and the time course of their induction. The most pronounced induction of all investigated Nrf2 target genes, with up to 20-fold increase in expression, was observed for HMOX1/HO-1. Cardamonin increased GSTA1 gene expression better than SFN, whereas SFN appeared to be a better stimulator of gene expression of TXN, SOD2 and SQSTM1/p62 after 16 h of treatment. The increase of protein levels was time-dependent: induction of SQSTM1/p62 was detected after 8 h, whereas the TrxR1 levels were increased only after 24 h. The Se status did not significantly affect gene or protein expression of the Nrf2 target genes. As expected, gene expression of the selenoenzyme GPX1 was higher under Se-adequate and high-Se conditions, as compared to Se-deficient cells. In conclusion, the Se status of the HepG2 cells appears to exert only a minor and statistically non-significant effect on the observed alterations in mRNA and protein levels of Nrf2 target genes induced by SFN and cardamonin.

GTP-Cyclohydrolase I: The second member of the Zur-regulated *cobW*$_1$-gene cluster

P 4

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Zinc is an important trace element and about 5% of all proteins in prokaryotes are zinc-binding proteins, even though zinc is toxic in high concentration. The ß-proteobacterium *Cupriavidus metallidurans* can cope with both - zinc excess and starvation conditions. To understand the mechanism of zinc homeostasis, we characterized an operon-like clusters that are regulated by Zn^{2+}- dependent repressor Zur. The Zur-dependent *cobW*$_1$ gene cluster contains six genes, which are strongly expressed under zinc starvation conditions and contains two Zur-boxes in the operator region. The first gene of this cluster is *cobW*$_1$, which encodes a zinc chaperone of the G3E family of P-loop GTPases. The genes downstream of cobW1 encode paralogs of zincdependent proteins. Rmet_1099 or *folE*$_{1B2}$ is the gene directly downstream of *cobW*$_1$. This gene product is a putative class I GTP-cyclohydrolase (GCHY I) converting GTP into 7,8-dihydroptyrine, which subsequently enters different metabolic pathways, e.g. leading to tetrahydrofolate or modified tRNA nucleosides such as queuosine and archaeosine. *C. metallidurans* contains three GTP-cyclohydrolases I, the type IB proteins FolE$_{1B2}$ (Rmet_1099) and FolE$_{1B1}$ (Rmet_2614), and the type IA protein FolE$_{1A}$ (Rmet_3990). The IA-type enzymes contain usually Zn and are constitutively expressed, this was verified in the case of FolE1A by ICPMS and reporter-gene studies. The proteins of the type IB may use different transition metals as a cambialistic enzymes. A main aim is to investigate the interplay of GCHY-I-paralogues in the various deletion strains and under different conditions, the role of CobW1 for the cofactor delivery and the activity of those three proteins with regard to the different metal cations cofactors.

Iodine status changes with epidemiological variables in an Australian population. P 5

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The World Health Organisation (WHO) recognizes Iodine deficiency as an important health issue. Iodine status is regulated by diet and is essential for the production of the thyroid hormones Thyroxine and Triiodothyronine. Low production of these hormones due to chronic Iodine deficiency is clinically manifested by thyroid goiter and a wide spectrum of mental and physical disorders. The WHO recommends the measurement of urinary Iodine concentration as the best indicator of a populations iodine status with a median urinary Iodine of <100 µg/L being indicative of insufficient Iodine.
Iodine concentration was measured using Inductively Coupled Plasma Mass Spectrometry to assess the Iodine status of a randomly selected population from Northern Adelaide (North West Adelaide Health Study) resulted in a median urinary Iodine of 111.4 µg/L (n=1067). Male participant median = 117.5 µg/L (n=517) and female median = 108.5 µg/L (n=550). These urine Iodine levels satisfy the WHO criteria for Iodine sufficiency. However epidemiological variables indicate that some groups of this population had insufficient Iodine intake according to the WHO guidelines. The sub population of males and females with ages greater than 70 years (median 96.1 µg/L), income of <$20,000 (median 94.7 µg/L) and the severely obese (median 94.6 µg/L).
The data shows that some epidemiological sub populations of the North West Adelaide Health Study have inadequate Iodine intake with median urinary Iodine concentrations below the WHO recommended levels of 100 µg/L and would likely benefit from dietary Iodine fortification.

The interaction between trace element status, neuroinflammation, and amino acid metabolism in children with autism spectrum disorder (ASD)

P 6

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Altered metal-ligand homeostasis was shown to play a significant role in ASD pathogenesis. Particularly, Zn deficiency is highly prevalent in ASD, being associated with increase oxidative stress, reduced detoxification, as well as impaired synaptic plasticity. Other essential metals were also found to play a role in ASD. At the same time, the existing data on trace element status and its association with clinical severity are insufficient. Therefore, the objective of the present study was to assess the levels of trace elements in hair and serum of children with ASD in association with markers of neuroinflammation, being the one of key mechanisms of ASD pathogenesis, and altered amino acid metabolism. The content of the elements in hair and serum of 76 children with ASD and 76 control subjects was assessed by inductively-coupled plasma mass spectrometry using NexION 300D (PerkinElmer Inc., USA). Markers of neuroinflammation were assessed using ELISA. In turn, serum amino acid levels were evaluated using high-pressure liquid chromatography (HPLC) at Perkin Elmer S200 (USA). A significant increase by 33% in the level of Co in the hair, as well as a decrease in the level of calcium and selenium by 23% and 15%, respectively, were detected in children with ASD. A significant increase in the concentration of Co, Mg, and V by 17, 4%, and 29% was observed in the serum of children with ASD, while the concentration of manganese and selenium decreased by 16% and 8%, respectively. Trace element levels were associated both with markers of neuroinflammation and clinical severity of ASD. At the same time, adjustment for neuroinflammation markers did not improve or reduce the significance of the interaction between trace elements and ASD clinical characteristics, being indicative of the significant role of altered metal-ligand homeostasis in ASD. Moreover, essential metal levels were associated with serum amino acid profile in children suffering from ASD. Generally, it is proposed that metal-ligand whether modulation of trace element status may be an effective tool for ASD management. The project was supported by RFBR (18-315-00103).

Influence of 3-months iron and folic acid supplementation on iron status in reproductive-age women

P 7

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The aim of this study was to determine the iron status and folic acid concentration in serum and complete blood count (CBC) parameters after 3-months iron and folic acid supplementation in women with deficiency of this micronutrients. The research was carried out with the approval of the Local Ethical Committee (approval no. 917/16). The study population consisted of 163 European white women of average age 25.9±3.7 (range 20 to 35).
During the recruitment for this research all women participated in medical consultation and interview examination with the gynecologist. Blood samples were taken from antecubital vein after an overnight fast. Morphological parameters were assayed in whole blood and total iron binding capacity (TIBC), UIBC (unsaturated iron binding capacity) and C-reactive protein (CRP), iron and folic acid concentration were assayed in serum using a biochemical analyzer. Body composition was measured using BOD POD. Statistical analysis was performed with Statistica 12.0 using nonparametric tests ($p<0.05$). Women were divided into two groups, the first group (SS) with iron and folic acid deficiency and the second group (NS) without deficiency (SS (n=100) vs NS (n=63): UIBC 315.41 vs 230.99 μg/dl; folic acid 6.20 vs 8.26 ng/ml). Group SS received Fe and folic acid supplement (Fe 14 mg (iron gluconate) and folic acid 200 μg) for 3 months. Before and after the study blood was collected from both groups.
It was found that morphological parameters and also UIBC, TIBC, CRP did not change significantly after 3 months supplementation. Iron concentration increased but not significant during the study. Folic acid concentration increased markedly in one-month intervals (6.20 before supplementation, 9.40 after 1^{st} month, 10.56 after 2^{nd} month, 11.31 ng/ml after 3^{rd} month). In NS group TIBC and UIBC increased significantly after 3 months (TIBC 343.60 vs 387.92 μg/dl; UIBC 230.99 vs 281.41 μg/dl). Body mass index (BMI) and body composition did not differ between groups.
Conclusion: 3-months iron and folic acid supplementation did not change iron status in young women deficient in these micronutrients. It is suggested that folic acid may negatively affect iron accessibility in women.
This research was supported by a grant from the National Science Center, Poland (2015/17/B/NZ7/02952).

Folic acid affects iron status in female rats

P 8

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The aim of this study was to determine the influence of folic acid alone and combined with iron in long-term supplementation on iron concentration in tissues of rats.
The research was carried out with the approval of the Local Ethical Committee (approval no. 59/2016). The experiment was performed on 50 eight-week old female Wistar rats. In the first stage of the experiment (28 days) animals were randomly assigned to a control group CC (10 rats) fed the standard diet (AIN-93M) and a study group (40 rats) fed an iron and folate deficit diet. Then the study group was randomly divided to four groups: DEF group was fed a deficit diet, DFE group was fed a deficit diet with iron gluconate, the DFOL group was fed a deficit diet with folate acid, the DFEFOL group was fed a deficit diet with iron gluconate and folate acid. After 21 days of supplementation animals were killed. Iron concentration was assayed in liver, spleen, pancreas, heart, kidney, brain and hypothalamus by AAS. Statistical analysis was performed with *Statistica* 12.0 using ANOVA test ($p<0.05$).
It was observed that folic acid supplementation significantly decreased iron concentration in pancrease than in DEF group and markedly increased in hypothalamus compared to CC, DEF and DFE group. Moreover, combination of iron with folic acid (DFEFOL) markedly decreased iron level in liver and spleen and increased iron concentration in hypothalamus in comparison to iron alone supplement (DFE). Interestingly, neither deficiency in iron and folic acid nor supplementation of these micronutrients change iron level in brain and heart in rats.
In conclusion, long-term folic acid supplementation may affect iron status in female rats deficient in these micronutrients.
This research was supported by a grant from the National Science Center, Poland (2015/17/B/NZ7/02952).

Al migration from aluminum foil to food: bioaccessibility and risk assessment P 9

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Introduction: Aluminum (Al) foil is widely used to wrap or store food, or during cooking, in treatments such as baking, covering surfaces to prevent losing moisture of foods. However, in spite of Al foil is widely use, the possible migration of this metal from foil to foods has been scarcely studied.
Material and methods: Cooked ham slices 10x10x1 mm were wrapped in Al foil in two ways (bright surface in contact with food or matte surface in contact with food). They were stored for 2 weeks, at different temperatures (4, 18 and 35 °C) simulating refrigeration, standard and high temperature conditions. In addition, bioaccessibility assays simulating the human digestive system, were performed in order to evaluate how bioaccessible this Al is in the intestinal lumen. Electrothermal atomic absorption spectroscopy was used for Al determinations (total, soluble and dialyzable). Finally, a probabilistic model was developed (@Risk model) to study the potential toxicological of this activity on the health of the general population.
Results and discussion: The amount of Al initially present in cooked ham slices (0.33 μg/g) increased for all tests. After 2 weeks, Al content enhanced to 45.9 and 49.4 μg/g (matte surface) and 44.6 and 42.1 μg/g (bright surface) for refrigeration and standard conditions respectively. On the other hand, the most significant increase took place under high temperature conditions for which Al content in cooked ham slices, after 2 weeks, reached 106 and 122 μg/g for matte and bright surface (around 350 higher). Bioaccesibility assays revealed values close to 30% as much as for solubility assay as for dializability assay. These results show that Al migrating from foil to food would be moderately absorbable by enterocites of intestinal lumen and would have a medium toxicological role. A risk assessment model considering a serving size of 1 -2 slices stored for 1 week have an intake of up to 3.1 mg; and slices storing for 2 weeks have an intake of up to 6.0 mg.
Conclusions: The domestic practice of temporarily wrapping many foods with aluminum foil both at home and in ultramarines should be questioned.

Selenium and cadmium in bioaccessible fraction of organic weaning food: risk assessment and influence of dietary components

P 10

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Introduction: Organic food production is one of the fastest growing markets, with an increase of around 250% in the last 10 years. The principal reason is that organic agriculture can enhance human and environmental health because there is no use of synthetic fertilizers or pesticides. However, research data on the nutritional benefits of organic food compared to their non-organic homologues is scarce and contradictory.
Material and methods: Se and Cd contents were analyzed in 10 commercialized weaning foods characterized with the "organic" attribute. The bioaccessible fraction of weaning food was obtained through an in vitro process of gastrointestinal digestion. Se and Cd determinations were performed by electrothermal atomic absorption spectroscopy. Risk assessment (@Risk) was used in order to evaluate the nutritional/toxicological value of these products.
Results and discussion: Se content ranged between 2.44 – 15.4 μg/Kg. Samples with meat ingredients showed the highest Se contents, while weaning foods consisting of fruits or vegetables presented the lowest concentrations. Se bioccessible concentration ranged between 1.90 – 4.35 μg/Kg with a greater uniformity among analyzed samples. Bioaccessibility of Se of samples does not seem to be dependent on macronutrients content (protein, fat and fiber). Regarding Cd, concentrations of this heavy metal ranged between 1.23 - 3.64 μg/Kg. These concentrations are considerably lower than those reported in weaning formulas which were not categorized as organic. Furthermore, Cd bioaccessibility of organic weaning foods ranged between 0.17 - 1.38 μg/Kg. The solubility of all samples studied was around 20% from the initial Cd concentration. A negative statistical correlation between fat content - Cd bioaccesible ($p < 0.05$; $r = - 0.756$) and Cd content – Se bioaccesible ($p < 0.05$; $r = - 0.777$) were also found. Results derived from the simulation of the probabilistic model indicated that the intake level of Se through the consumption of one jar of these organic weaning foods would be below 2.91 μg for the 95% of infant population (15% of DRI for Se). For Cd, in the worst – case scenario, infants consuming one jar only reach 0.79 μg (3.1 – 2.0% of PTWI).

Mineral content from enteral formulas, its sufficiency, and nutritional status of patients after short and mid-term enteral nutrition.

P 11

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The present work assessed trace elements sufficiency of enteral nutrition formulas according to European Dietary Reference Intakes (DRI). Then, it determined whether there were differences between initial and follow-up measurements of several analytical parameters (hemoglobin, hematocrit, alkaline phosphatase, etc.) of patients fed with enteral nutrition during short (<6 months) and mid (<12 months) periods of time. Finally, correlations between enteral formulas composition (total and dialyzed trace elements) from our lab analysis and the biochemical parameters of patients consuming them were evaluated.
Retrospective data of 56 patients receiving enteral nutrition (short n = 47 and mid n = 9 term) was collected from a hospital in southern Spain. Mineral and trace element bioaccessibility was determined with an in vitro gastrointestinal digestion that replicate human digestive conditions. Total and bioaccessible Ca, Mg, Fe, Zn, Cu and Mn were measured with flame atomic absorption spectrometry. The probabilistic assessment showed that the analyzed enteral nutritional formulas meet the DRI of all micronutrients assessed, except for Mg, while Fe, Mn and Cu supply >120% of DRI.
On the other hand, biochemical data from patients with short-term enteral nutrition showed statistically significant differences between initial and follow-up values of multiple parameters. This difference was not observed in patients with enteral nutrition for more than 6 months. Besides the smaller sample, the absence of significant differences in the second group could be due to other factors such as initial clinical state.
However, when the percentage of change between the first and second measure is considered, statistically significant correlations ($p < 0.05$) were found between hemoglobin and dialyzed Fe ($r = 0.756$), and hemoglobin and dialyzed Cu ($r = 0.756$) in the mid-term group. It has been already shown a synergistic effect on the bioavailability of Fe and Cu. Moreover, Cu plays a role in Fe metabolism influencing, together with other factors, hemoglobin biosynthesis.
According to our model, most trace elements are supplied by EF in the amounts suggested by the EFSA. Changes on biochemical outcomes have been observed in some, but not in all biochemical parameters, which makes us reconsider efficiency of this nutrition intervention.

Copper-mediated changes in selenoprotein synthesis

P 12

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Selenium (Se) and Copper (Cu) are essential trace elements for humans. Trace elements play major roles in redox homeostasis by modulating enzyme activities and signaling pathways. So far, interactions of both elements have not been studied in detail. Se is important for the expression of selenoproteins. Glutathione peroxidases (GPX) and thioredoxin reductases (TXNRD) are both responsible for protection against oxidative stress. Cu itself is a redox active element, but it is also important for superoxide dismutase 1 (SOD1) activity.
We aimed to address the question how Cu and Se interfere with each other to modulate the cellular redox status. To this end, we analyzed Cu-dependent proteins and various selenoproteins. HepG2 cells were treated with 50 nM sodium selenite and with or without 100 µM copper sulfate for 72 h. After 48 h of incubation, Bathocuproine disulfonate (BCS) or Tetrathiolmolybdate (TTM) were added to chelate Cu. Accordingly, enzyme activities (GPX, TXNRD) were measured photometrically in freshly prepared cell lysates. Cu content was measured via TXRF and Cu-dependent protein by PCR and Western Blot.
Cu decreased the activity of selenoproteins (e. g. SELENOP, GPX, TXNRD). The Cu chelators, BCS and TTM, were able to diminish Cu under basal conditions and BCS reduced Cu content up to 70% in the Cu treated groups. Interestingly, upon Cu chelation the activity of TXNRD was increased by decreasing Cu content. GPX activity was not affected by the chelators.
Cu is a redox active element which plays a major role for the intracellular redox tone. We could show that Cu decreases the activity of selenoproteins. Vice versa a Cu chelation increases the activity of TXNRD, indicating an important interconnection between both trace elements.

Polymer/silica hybrid membranes loaded with gold/silver nanoparticles: Physicochemical characterization and study of their extraction efficiency toward trace toxic elements

P 13

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Sol-gel approach was applied for the preparation of thin membranes based on hybrid organic/ inorganic polymer matrix loaded down with pre-synthesized gold (Au) or silver (Ag) nanoparticles (NPs). The employed organic polymers poly(vinyl alcohol) (PVA) and poly(ethylene oxide) 400 (PEO) are not toxic and biocompatible in nature; tetraethoxysilane (TEOS) or γ-aminopropyl-triethoxysilane (APTEOS) were used as precursors for the inorganic polymer silica in the hybrid sol-gel matrix. AuNPs and AgNPs have been preliminary prepared by green chemical reduction utilizing D-glucose and starch as reducing and capping agents, respectively. Solutions of PVA, PEO and pre-hydrolyzed TEOS/APTEOS were mixed with AuNPs/AgNPs dispersions and cast hybrid membranes were obtained under drying at suitable temperature. The structure, morphology and optical properties of pre-synthesized nanoparticles and membranes prepared were studied by X-ray diffraction, TEM, SEM and AFM observations, FTIR and UV-vis spectroscopy, DSC analysis. Swelling behavior and mechanical properties of membranes were also characterized. Both noble metal NPs were uniformly distributed in the hybrid PVA-PEG-TEOS/APTEOS membranes without any aggregation. Even more the presence of metal NPs in the membranes remarkably increases their mechanical stability, the most probably because of the crosslinking role of noble metal nanoparticles. Sorption behavior of the most frequently determined trace toxic elements was studied with each of the materials prepared: PVA-PEG-TEOS/APTEOS-Starch, PVA-PEG-TEOS/APTEOS-AgNPs-Starch, and PVA- PEGTEOS/APTEOS-AuNPs-Starch. Sorption experiments were performed at pH 3-8, achieved with hydrochloric acid and ammonia (these pH values ensured protonation/ deprotonation of the functional groups). After sorption time of 12 h, the effluate solution was easy decanted; the membrane was washed twice with doubly distilled water and dissolved in c.HNO3/aqua regia followed by dilution to desired volume and ETAAS measurements. Results obtained undoubtedly confirmed high extraction efficiency of PVAPEG-APTEOS-AgNPs-Starch membrane ensuring quantitative sorption of all studied trace elements at optimal conditions. Promising results were obtained regarding speciation analysis of chromium and manganese. Preconcentration procedure developed based on sorption properties of this smart material was applied for the determination of priority pollutants in surface waters. Determination limits achieved satisfy permissible limits which makes developed analytical procedure suitable for routine laboratory practice. Acknowledgements:

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In vivo exposure of bivalve molluscs to titanium dioxide nanoparticles and food additive E171 in a mesocosms system

P 14

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Titanium dioxide nanoparticles (TiO_2 NPs) are hugely used in industry as an inorganic UV filter in sunscreens and personal care products, and also as a food additive (E171). The remarkably increase in the preparation and use of engineered nanomaterials implies the presence of nanomaterials, mainly metallic nanoparticles, in the environment [1]. In addition, the potential risks of NPs on human health, animals and the environment are still unknown [2]. So, precautions for their responsible use and relevant toxicity studies should be considered. Bivalve molluscs, as filter feeding organisms, are excellent sentinells of marine environmental health. In this communication, a mesocosm system is proposed to evaluate de accumulation of TiO_2 NPs of different size distributions and food additive E171 (nano- and micro-titanium) in clams. Eight independent pools were settled (2 controls, 2 TiO_2 NPs 50 nm, 2 TiO_2 NPs 100 nm, and 2 E171) with 30 specimens per each one. Filtered marine water was used as medium and an aeration system was installed in every tank. Clams were fed every two days with in vitro cultured phytoplankton which was composed by two flagellate species (Tetraselmis suecica and *Isochrysis galbana*) and one diatom (*Chaetoceros igafa*). Concentrations of TiO_2 NPs were increased progressively from 0 to 35 mg L^{-1} (5 mg L^{-1} increments) for 18 days. Three specimens per tank were sampled before every addition. Extracted samples were washed, blend and homogenized independently. An ultrasound assisted enzymatic hydrolysis procedure was applied before sp-ICP-MS measurements [3]. An enzymatic solution (2 mg L^{-1}/2 mg L^{-1} pancreatine:lipase) was added to samples and continuously ultrasonicated during 10 minutes (amplitude 60%). Different TiO_2 NPs uptake rates have been observed depending on the type of TiO_2 NPs (size distribution). In addition, total titanium content was also assessed by ICP-MS after a microwave assisted acid digestion procedure.

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Presence of other rare earth metals in Gadolinium-based contrast media P 15

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Gadolinium-based contrast media (CM) are widely used to enhance tissue contrast during magnetic resonance imaging (MRI) procedures. However, free gadolinium is undesirable as a drug substance due to its high toxicity. Moreover, Gd hydrolyzes under physiological conditions and precipitates in the presence of phosphate and carbonate. Consequently, a coordinating ligand is required to keep it in solution and to increase tolerance. In order to achieve an adequate performance, CM must be administered in relatively large amounts. Chelate amounts are around 13 – 20 grams and, for Gd alone, this amount may reach 3.3 grams. Taking into account the via of administration, impurities in Gd CM may significant. Gadolinium occurs in nature along with 16 other elements known collectively as rare earth metals (RE), which are found throughout the earth's crust in minerals such as monazite. Gadolinium oxide corresponds to 0.7-4.0% of the RE present in minerals, and the sum concentration of RE in minerals is around 4%. Rare earth metals are difficult to separate as the chemical and physical properties between one RE and another are significantly similar. In this study, the presence of other RE in Gd-based CM formulations was investigated. Different lots of Magnevistan®, Viewgam®, OptiMARK®, Omniscan®, Dotarem®, and Gadovist were analyzed. Atomic absorption spectrometry and inductively coupled plasma mass spectrometry were used for RE determination. Procedure optimization included sample decomposition and method validation for element determination.
The results showed that Sc, Y, La, Ce, Pr, Nd, Eu, Tb, Tm, Dy, Ho, and Er were present in the 21 samples analyzed. Terbium, Thulium, and Europium were, in average, found in the highest amounts, which were 0.4 mg/L, 0.2 mg/L, and, 0.15 mg/L, respectively. These results are expected since Europium, Gd, and Terbium are in sequence in the periodic table and therefore present very close ionic radii, which restrict their separation. Considering the sum of all RE, Viewgam® was the most contaminated formulation (mean of 2.1 mg/L) and Magnevistan® the least (mean of 0.6 mg/L). Although the RE are chemically similar, the other RE do not perform as Gd as a CM, therefore, their presence in formulations may be a matter of concern.
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Selenium as an important trace element for coordinating inflammatory processes P 16

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Selenium is an essential trace element, which is required for the synthesis of selenoproteins. Studies indicate that suboptimal supply with selenium might be associated with inflammation as well as carcinogenesis which might be of special interest for the European population, since plasma markers for selenium indicate a low selenium status in this region. We recently i) identified the selenium-sensitive SELENOH to be highly expressed in undifferentiated, proliferating epithelial cells of the intestine as well as in murine immune cells *in vivo*, ii) confirmed an important role of SELENOH for proliferation and differentiation processes in human epithelial adenocarcinoma cells and iii) showed that SELENOH itself is downregulated by differentiation [1]. We therefore hypothesized that selenium and selenoproteins such as SELENOH also affect macrophage differentiation and concomitant changes in immunomodulatory lipid mediator profiles. Here, we report on the role of selenium during macrophage differentiation and function of human monocytic THP-1 cells. As observed for intestinal epithelial cells, SELENOH protein expression also depends on the selenium supply as well as the differentiation state of immune cells. Moreover, selenium impacts on monocyte/macrophage proliferation and differentiation and causes shifts in the lipid mediator network, which suggests that selenium might have key functions in coordinating inflammation and resolution.

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Monitoring study and thermodynamic modeling of trace metal species in Pomorie Lake, Bulgaria

P 17

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Pomorie Lake is a hypersaline lagoon of natural origin, located on the southern coast of the Bulgarian Black Sea. It is a protected area since 2001. That is why the precise assessment of its ecologic state and of the influence of trace metals on the ecosystem is very important. This study covers the period from December 2014 to November 2018 and combines monitoring studies with thermodynamic modeling. Surface and deep water samples were examined and their physicochemical stability was established at each sampling session. The largest variations in the salinity of the lake were recorded during the period December 2014 (14,4 ‰) - October 2015 (38.4 ‰). The total content of the trace metals Al, Fe, Mn, Cu, Zn, Co, Ni, Cd, Pb during the years 2014 and 2016-2018 was low, in many cases below their permissible limits. In June 2015 their total contents were significantly higher in all stations, for Al, Fe, Zn, Pb and Cd reaching and exceeding the recorded limit values for coastal marine waters. The inorganic and organic chemical species of the studied trace metals were thermodynamically calculated. A combined ion-association and ioninteraction model was used for the modeling of inorganic chemical species, which was developed by us for saline and hypersaline waters. For the organic chemical species the Stockholm Humic Model was used. The calculations showed that most harmful for the ecosystem were Mn, Co, and Ni, since they exist in the waters mainly as free Me2+ ions. Fortunately, their concentrations were very low. In 2015 significant for the ecosystem were Zn, Cd and Pb, whose dominant species were the less toxic chloride and organometallic complexes. The least significant ones were Al and Fe, despite their higher concentrations, as well as Cu, which exist as hydroxy or organic-hydroxy complexes.

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Lipid peroxidation and antioxidant vitamins status in tunisian patients with colorectal cancer

P 18

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The aim of the present study was to assess the level of lipid peroxidation such as malondialdehyde (MDA) as an oxidant. Moreover, we analysed the activity of main non-enzymatic antioxidants, Alpha -tocopherol, retinol and ascorbic acid in colorectal cancer patients. In total, 61 blood samples of Colorectal cancer patients and 94 samples from control were collected. Plasma was separated from the blood by centrifugation. Plasma levels malondialdehyde ascorbic acid, retinol and Alpha -tocopherol, were determined. The levels of malondialdehyde were significantly higher in patients with Colorectal cancer (3.79 $\pm$ 1.33 µmol/l) than in controls (2.62 $\pm$ 0,69 µmol/l, $p < 0.0001$). The levels of ascorbic acid in patients with Colorectal cancer (47.47 $\pm$ 14.16 µmol/l) were significantly lower than controls (55.33 $\pm$ 16.81 µmol/l, $p < 0.01$). The retinol concentrations were significantly lower in the group with Colorectal cancer (3.03 $\pm$ 0.58 µmol/l) than in controls (3.56 $\pm$ 0.62 µmol/l, $p < 0.0001$). Alpha-tocopherol levels were significantly lower in the group with Colorectal cancer (18.99 $\pm$ 3.75 µmol/l) than in the controls (22.53 $\pm$ 4.20 µmol/l, $p < 0.0001$). Results obtained in this study indicate significant lower levels of some antioxidants in colorectal cancer patients compared to healthy control, which may lead to enhanced action of oxygen radical, resulting in lipid peroxidation. These findings may be helpful for further studies, as the effect of nutritional supplementation on clinical outcomes.

Element-associated biomarkers in the *MoKaRi* cohort

P 19

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Is it possible to adjust cardiovascular risk factors in adults at increased risk for cardiovascular diseases by a modification of the diet? To answer this question, the Competence Cluster for Nutrition and Cardiovascular Health (nutriCARD) developed a 'cardio-protective' diet within the MoKaRi (modulation of cardiovascular risk factors) study. For 20 weeks (follow-up: 10 and 20 weeks), the study participants (n = 51, male and female, 32 to 76 years old) received daily menu plans which are based on the guidelines of the German Society of Nutrition (DGE) regarding intake of energy, carbohydrate, protein and fat content. Furthermore, the diet was characterized by the limited intake of saturated fatty acids (SFA $\leq$ 7 En%) and the intake of monounsaturated fatty acids (MUFA $\geq$ 10 En%), polyunsaturated fatty acids (PUFA $\geq$ 10 En%) as well as long-chain n-3 fatty acids (LC-PUFA $\geq$ 500 mg/d). In addition, half of the investigated group consumed 3 g EPA + DHA from fish oil/d. All study participants were also encouraged to consume more vegetables and fruits while reducing salt and sugar consumption. The intake of dietary fiber was 40 – 50 g/d, whereas the intake of highly processed food (i.e. fast food and convenience products) was minimized. Blood samples were frequently collected during the whole study (13 visits in total). We analyzed the serum of the MoKaRi cohort via inductively coupled plasma mass spectrometry (ICP-MS/MS) and generated profiles of bulk and trace elements (Mg, Ca, Mn, Fe, Cu, Zn, Se, Cd, As, Mo, and I). In this context, the intervention groups did not differ distinctly from each other. Nonetheless, a (presumably) menu-dependent course regarding the element profiles was found. Moreover, glutathione peroxidase activity (GPx) has been measured using glutathione reductase-coupled test as a function biomarker for Se, while total Se has been additionally determined via total x-ray fluorescence spectroscopy (TXRF). Regarding total Se, the TXRF data correlated well with ICP measurements, but showed no significant differences between the two intervention groups. Compared to baseline, an increase in GPx activity was observed for subsequent visits (weeks 2 to 40).

Amyloid Precursor Protein (APP) Supports Tumorigenesis by Counteracting Iron-mediated Oxidative Stress and p^{38}MAPK-dependent DNA Damage

P 20

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The amyloid precursor protein (APP) plays a pathophysiological key role in Alzheimer disease (AD) due to its neurotoxic proteolytic amyloid-β (Aβ) fragments. However, full-length APP as well as its large Nterminal soluble sAPPα processing product exert neuroprotective features that are intricately involved in iron metabolism. Yet, the functional attributes of APP outside the nervous system and downstream pathways remain unclear. Here, we report that APP functions as iron and redox homeostatic protein that promotes tumorigenesis. On the molecular level, we discovered a previously unrecognized mechanisms by which APP suppresses pro-oxidative iron (Fe^{2+}) accumulation that results in deleterious oxidative stress-induced DNA damage in cancer cells, with p^{38}MAPK stress signaling as a crucial mediator of this process. Consonant with these findings, in human data set analyses of prostate cancers, high levels of APP are significantly associated with a poor event-free survival rate and negatively correlated with tumor suppressive p^{38}MAPK activation. As proof of concept, pharmacological disruption of APP increased levels of redox-active Fe2+ and p^{38}MAPKdependent DNA damage and significantly reduced tumor cell clonogenicity *in vitro* and tumor growth in a xenograft model. Taken together, our results uncover that APP depletion represents a potential novel approach to disrupt iron homeostasis, releasing unchecked pro-oxidative Fe^{2+} that hinders survival and propagation of oncogenic transformed cells and cancer cells.

Metals and essential elements levels in hair and association with Autism severity

P 21

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Previous research suggested that metals and essential elements could contribute to the etiology of autism. The pathophysiological etiology which precipitate autism symptoms remain elusive and controversial in many cases, but both genetic and environmental factors have been implicated. This study investigated the relationship among the levels of toxic metals and essential elements in hair samples and the severity of autism.
We carried out an ambulatory cross-sectional study in the province of Catania (South Italy). The Autistic Spectrum Disorders diagnosis was performed according to DSM IV (Diagnostic and Statistical Manual of Mental Disorders) criteria. Two tools were used to assess the severity and symptoms of autism, namely the Calibrated Severity Score (CSS) and the Autism Diagnostic Observation Schedule (ADOS). Hair samples were collected by single cutting from the occipital region. The samples were cut to lengths of about 1.5–2 cm using clean stainless steel scissors. Approximately 50 mg of hair were used. Metals (Li, Be, Al, Ni, As, Mo, Cd, Hg, U, Pb) and essential trace elements (Cr, Co, Mn, Zn, Cu, Se) were quantified by inductively coupled plasma mass spectrometry analysis. Correlation between the levels of toxic metals/essential elements and severity of autism was analyzed using Spearman correlation by Statistical Package for Social Sciences software (SPSS Inc., Chicago, IL, USA).
The study included 48 cases (70.8% male), their ages ranged from 2 to 17 years with mean of 6 years. We found a positive correlation of Al, As, Cd and Pb (rho of 0.30-0.40, p=0.03) and a negative correlation of Zn (rho of -0.39, p=0.006) with variation in the degree of severity autism for all the severity scale.
This study demonstrates a significant positive association between the severity of autism and the relative body burden of toxic metals and essential elements.

Biological reactions of rats when injecting CuZn alloy of nanoparticles of different sizes P 22

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Using of alloy of nanoparticles is interesting for many areas of science and production by reason of their chemical and physical characteristics arising from the interaction between their components. However, there is insufficient data on biological and, possibly, toxic effects of metal alloys of nanoparticles in scientific literature. The aim of this research was to study biological reactions of the organism of laboratory animals when injecting nanoparticles of brass of various sizes. The study was conducted in Wistar rats (n=48). During the reference period animals were divided into four groups (n=12). The first group was a control group and it was injected with 0.9% sodium chloride solution. The experimental groups were injected once intravenously with nanoparticles of CuZn (pre-suspended in water for injection) whose size was 19 nm, 32 nm and 47 nm, respectively. The entered dose of nanoparticles was 2.3 mg / kg. All nanoparticles were obtained by the method of electrical explosion of a conductor in an argon atmosphere. Biochemical and morphological parameters of blood were determined using the biochemical analyzer CS-T240 and automatic hemanalyzer URIT-2900 Vet Plus. Behavioral reactions were performed using the "Open field test" and the "Light-dark transition test". Results were registered on the 1st, 7th, 14th and 21st days. In the analysis of behavioral reactions throughout the experiment, statistically significant deviations in the "Open field test" from the control values were not observed. There was an increase in the time spent in the light compartment in the "Light-dark transition test". As a result, it can be concluded that the anxiety level of animals, when injecting the dose of nanoparticles, decreased regardless of the size of the nanoparticles. There was a statistically reliable decrease in leukocytes by 51% ($p < 0.01$), erythrocytes by 32% ($p < 0.01$) and hemoglobin by 25% ($p < 0.01$) when injecting CuZn alloy of nanoparticles (19 nm) on the first day. Other changes in the biochemical and morphological parameters of the blood were not detected, the values of all experimental groups were barely different from the control, any changes were within normal values. The results indicate the lack of toxic effect of CuZn alloy of nanoparticles whose size was 32 nm and 47 nm.

Dynamics of trace metals in the system *water-soil-low vegetation-small animals-parasites in the Maglizh area*, Bulgaria

P 23

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Trace metals tracking in the *water-soil-vegetation-animals* food chain provides an opportunity to assess their dynamics and bio-accumulation in the ecosystem. In this study the system *water-soillow vegetation - rattus spp. - hymenoleps spp.* was studied. The experimental area was the industrial zone around Maglizh city situated in the Central Sub-Balkan, Bulgaria, where several machinery construction factories are working. Objects of the survey were surface waters, their nearby soils, grass and low vegetation, and the host-parasite system *Rattus spp. – Hymenolepis spp*. The accumulation of trace metals in the rats was traced to their target tissues, liver and kidney, as well as to their tapeworms. The chemical analyzes of the studied samples showed contamination of the area with Al, Mn, Ni, Zn, Cd, Pb. The contents of Zn, Cu, Mn, Co and Pb in the rats' liver and kidney, as well as in the tapeworms were established. Their bioaccumulation factors were determined. For more precise evaluation of the toxic impact of the trace metals on the bio-systems and on the ecological state of the studied area, chemical species and possible spontaneous precipitations in samples of water and water soil extracts were thermodynamically calculated. The relationship *chemical species – bio-accumulation* was elucidated.
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Synthesis of an ionic imprinted polymer for the speciation of inorganic arsenic in fish samples

P 24

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A novel As (III) ionic imprinted polymer was synthesized as a selective solid phase extraction tool (IIP-SPE) for the separation of As(III) and As(V) from complex matrices such as fish. The IIP was prepared using sodium (meta) arsenite ($NaAsO^2$) as template, 1-vinylimidazole as a functional monomer, divinylbenzene (DVB) as a cross-linker, and 2,2'-azobisisobutyronitrile (AIBN) as an initiator. The polymer was characterized using attenuated total reflection – Fourier transform infrared spectroscopy (ATR-FTIR), scanning electron microscopy (SEM), transmission electron microscopy (TEM), and energy dispersive X-ray spectrometry (EDX). High performance liquid chromatography hyphenated with inductively coupled plasma mass spectrometry (HPLC-ICP-MS) was used for inorganic arsenic species (As(III) and As(V)) determination. Fish samples were previously subjected to a methanol/water extraction procedure for As species isolation, followed to selective As(III) and As(V) separation/pre-concentration from fish methanolic extracts. The following parameters affecting the IIP-SPE procedure were optimized: pH, loading speed and elution speed, and the best conditions were 8.5, 0.25 mL/min, and 0.50 mL/min, respectively. Other factors such as the breakthrough volume, sorption capacity, imprinting effect, selectivity, stability and reusability were also studied.

Combination of trace elements copper, zinc and selenium modulate redox-sensitive transcription factor Nrf-2 in HepG2 cells

P 25

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The transcription factor nuclear factor (erythroid-derived 2)-like 2 (Nrf-2) is known to regulate the expression of antioxidant and detoxification enzymes. By binding to the antioxidant response elements (ARE) in the promoter region of target genes, Nrf-2 initiates the transcription of e.g. thioredoxin reductase 1 (TXNRD1), glutathione peroxidase 2 (GPX2) or NAD(P)H quinone oxidoreductase 1 (NQO1). Those enzymes protect cells against oxidative damage by detoxifying reactive oxygen species and by regulating the intracellular redox state. Some trace elements (TE) have been shown to modulate Nrf-2 activity by changing the cellular redox status. Furthermore, Nrf-2 conversely influences the TE status by altering the expression of TE-specific transport and binding proteins. Based on this, we aimed to establish a screening approach to study Nrf2 activity not only after treatment with single TE but also in response to co-stimulation. To study Nrf-2 activity, we transfected the NQO1 promoter into HepG2 cells and performed a luciferase reporter gene assay. In addition, target gene expression including NQO1, heme oxygenase 1 (HO-1) and GPX2 was analyzed by qPCR. This way, we identified a 2.5-fold increase in NQO1 promoter activity using physiological concentrations of zinc while copper had no stimulatory but even an inhibitory effect. In the next step, the assay will be extended to study effects of TE combinations on Nrf-2 activity.

Response of zinc transporter gene expression in various tissues of weaned piglets challenged with subclinical zinc deficiency

P 26

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We investigated changes in the tissue-specific gene expression of all identified mammal zinc (Zn) transporters (solute carrier families 30 (ZnT) and 39 (ZIP)) in weaned piglets challenged with short-term subclinical Zn deficiency (SZD). Forty-eight fully weaned piglets (50% female, 50% male-castrated) were fed ad libitum a corn and soybean meal-based diet with adequate dietary Zn supply (+60 mg Zn/kg diet from $ZnSO_4$ * $7H_2O$ yielding 88 mg total Zn/kg diet) during two weeks of acclimatisation. Subsequently, animals were assigned to eight dietary treatment groups, which were fed restrictively (450 g/d) the same basal diet but with varying dietary Zn concentrations (+0, +5, +10, +15, +20, +30, +40, +60 mg Zn/kg diet from $ZnSO_4$ * $7H_2O$ yielding 28.1, 33.6, 38.8, 42.7, 47.5, 58.2, 67.8, 88.0 mg total Zn/kg diet) for a total experimental period of 8 d. Analyses included quantitative PCR gene expression profiling of ZnT and ZIP mammal Zn transporter genes within jejunum, colon, liver and kidney.
We identified significant differences in the homeostatic adaption to short-term subclinical zinc deficiency compared to earlier studies on clinical (severe) Zn deficiency. This was evident by the identification of gene expression patterns that contradict the present state of knowledge. Many of the investigated Zn transporter transcripts expressed significant breakpoints ($P < 0.0001$) in response to a reduction in dietary Zn. These thresholds either lay close to ~40 or ~60 mg Zn/kg diet. This indicates clear differences in the respective stimuli to which these genes respond. A breakpoint close to 60 mg Zn/kg diet equals the gross Zn requirement threshold under the present experimental conditions. This may highlight a role of certain genes in the regulation of Zn fluxes, to meet the basal requirements and/or compensate for body Zn depletion, respectively. In addition to these genes, a subset of Zn transporters seemed to be involved in the regulation of Zn fluxes for the compensation of stress and inflammatory processes. This was evident by a breakpoint close to ~40 mg Zn/kg diet, which has been earlier related to the response of oxidative stress-associated measures under the present experimental conditions. Interestingly, ZIP4 in the colonic but not the jejunal mucosa was significantly affected by the dietary Zn supply, yielding a high correlation to the apparently-digested amount of Zn. This may point towards a shift of the main side of Zn absorption towards lower parts of the digestive tract during the early stages in the development of Zn deficiency.
Taken together, this study presents the first comparative view on the effects on the gene expression patterns of all known ZnT and ZIP genes in jejunum, colon, liver and kidney of weaned piglets as affected by finelygraded reduction in alimentary zinc supply.

Novel 3D brain barrier model to simultaneously assess toxicokinetics and neurotoxicity P 27

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Neurodegenerative diseases such as dementia, Alzheimer's disease and Parkinson's disease have been increasingly shown to be stronger associated with environmental factors including diet than with genetic factors. In this context, there is an increased occurrence of so-called innovative foodstuffs containing secondary plant products which are postulated to exert neuroprotective effects. Until now, no appropriate *in vitro* model exists to assess the claimed positive impact of these compounds on the brain. Hence, the establishment of such a model is crucial. Therefore, a novel 3D cell culture system will be developed based on the well-characterized primary porcine *in vitro* models of the brain barriers. The validation of this system will be conducted by applying known neuroprotective and neurotoxic compounds. Primary porcine brain cells are cultivated on Transwell® filter inserts between two fluid compartments representing the blood and the brain side. The use of Transwell® units enables the assessment of transfer properties into and out of the CNS. In order to mimic the physiological conditions, the model additionally includes an astrocytoma cellline (CCF-STTG1). The cell viability and barrier integrity will be continuously monitored using the transendothelial/-epithelial electrical resistance (TEER) with the cellZscope® system. In the brain compartment, the human neuronal cell-line LUHMES is cultivated to determine the potential neuroprotective or neurotoxic effects of the test substances by evaluating the neurite outgrowth after exposure. First experiments were conducted with methylmercury (MeHgCl), a known neurotoxicant, and the inorganic mercury chloride ($HgCl_2$) which itself is not able to cross the brain barrier and thus serves as a negative control for the transfer from the blood to the brain side. No substantial transfer of Hg has been observed after 24h incubation of $HgCl_2$ and the neurons showed no change in the neurite outgrowth. In contrast, MeHgCl induced the expected neurite damage, indicating a functional transfer across the *in vitro* barrier. Further administration of other standard neurotoxins such as paraquat as well as neuroprotective substances like dopamine will be used to fully validate the model.

Fluorescent-probe based determination of free zinc in human serum samples P 28

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Zinc is an essential trace element with numerous functions for a myriad of biological processes. [1-3] The total serum zinc concentration is the most commonly used biomarker for zinc. It has been recommended for the zinc status of populations, but it has its limitations when it comes to individuals and a good biomarker for this application area is still missing. [4] The term free zinc describes the zinc pool that is loosely bound to low molecular weight fractions. It seems to represent the zinc that is biologically active and available for interaction with cells and therefore has been suggested as a possible biomarker for an individual's zinc status. [5-7]
We established a 96-well plate format-assay to measure the free zinc concentration in human serum samples, allowing the quantification of multiple samples simultaneously in a microplate reader-based approach. The fluorescent probe Zinpyr-1 is used to detect free zinc in the serum samples. For the quantification of the free zinc concentration the formula [Zn] = KD • ((F-F_{min})/(F_{max}-F) by Grynkiewicz et al. is applied. [8] Because the values for F (fluorescence of the probe induced by the sample), F_{min} (autofluorescence of the probe in the absence of zinc) and F_{max} (maximum fluorescence of the zinc-saturated probe) are detected successively in the same well, a sample volume of only 10 µL is required.
The established method has been applied on 154 commercial human serum samples. The measured free zinc concentrations ranged from 0.09 to 0.42 nM and did not correlate with the total serum zinc concentration. Also no correlation for the serum free zinc concentration and age has been found. However, the free zinc concentration of the sera from females (0.21 $\pm$ 0.05 nM) was significantly lower than that of males (0.23 $\pm$ 0.06 nM) (unpaired t-test, $p \leq 0.05$).
Further information is needed about an individual's free zinc status and factors that might influence it in order to evaluate the free zinc concentration in serum as a possible biomarker for the zinc status. The established method can be applied to generate this data.

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Global DNA (hydroxy)methylation in murine liver: impact of age, gender and trace element status

P 29

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Within the research project TraceAge, interactions of essential trace elements and changes of trace element (TE) levels during aging are studied. Trace elements of interest are copper, iodine, iron, manganese, selenium and zinc. Among others, endpoints of genomic stability are investigated in mice of different age and TE status. Epigenetics are studied in the murine liver as a factor important for both genomic stability as well as aging. According to literature, aging is linked to epigenetic alterations, especially to DNA methylation. Furthermore, it was shown that dietary deficiencies of essential trace elements such as selenium and zinc can have an effect on the DNA methylation. DNA methylation is defined as enzymatic methylation of cytosine and is thereby affecting gene expression. Methylcytosine can be further transformed to hydroxymethylcytosine which is thought to be a first intermediate in active demethylation and might have direct epigenetic effects.
Global DNA (hydroxy)methylation is measured in murine liver via HPLC-MS/MS based on a previous publication (Müller, Finke, Ebert, Kopp, Schumacher, Kleuser, Francesconi, Raber, Schwerdtle; Archives of Toxicology 2018, 92, 1751-1765). In the present work, the method was adapted to *in vivo* usage. For that purpose, DNA was isolated from murine liver and enzymatically hydrolyzed to nucleoside level followed by HPLC-MS/MS measurement of cytidine, methylcytidine and hydroxymethylcytidine. Subsequently, the ratios of methylcytidine and hydroxymethylcytidine to cytidine are calculated. Quantification is performed by use of isotope-labelled internal standards. Method performance was tested concerning interday precision of DNA isolation and hydrolysis. The epigenetic status of mice in relation to their TE levels, gender and age is currently determined applying this method to murine liver tissue.

Role of zinc in tryptophan-kynurenine pathways activation during obesity

P 30

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Introduction: Several works report a relationship between zinc (Zn) deficiency and obesity in humans. Obesity denotes disproportionate white adipose tissue extensions with adipocyte hypertrophy, inflammation and increase of circulating levels of proinflammatory cytokines. An excessive production of proinflammatory cytokines can result in greater enzyme activity of indoleamine 2,3 dioxygenase (IDO) in the tryptophan pathway, leading to tryptophan depletion and increased kynurenine levels. The current study examines the role of zinc in adipose tissue hypertrophy of high-fat treated mice with certain aspects of metabolic tryptophan conversion. In addition a cell culture model was developed to depict the interrelation between zinc and the tryptophankynurenine pathway under proinflammatory conditions.
Methods: *In vivo* studies were performed with mice fed with either standard diet (SD); high - fat diet (HFD); and zinc deficient high-fat diet (HFD-ZD). At the end of the experiment, the animal serums were analyzed for total zinc as well as tryptophan and kynurenine levels. In addition, adipose tissues were evaluated for total weight as well as adipocyte size and numbers. *In vitro* model was established to study tryptophan metabolism upon treatment with proinflammatory cytokines in the presence or absence of Zn. The human glioma cell line T98G was chosen due to its high expression levels of IDO.
Results: *In vivo* studies depict a strong decrease of serum zinc levels of HFD-ZD mice. High-fat diet induced obesity, evaluated by animal weight and adipose tissue parameters. Regarding zinc deprivation the most interesting aspect was that animals on HFD-ZD showed a significant decrease in the volume of adipocytes compared to HFD. Serum of animals subjected to HFD-diet showed a strong decrease of both tryptophan and kynurenine levels when compared with SD animals. The results from the cell culture experiments led us conclude that IDO activity as well as kynurenine levels were significantly up-regulated in T98G cells by stimulation with proinflammatory cytokines compared to unstimulated glioma. However, no significant differences in IDO activity and kynurenine levels were observed compared to zinc deficient conditions, indicating that zinc is ancillary in that part of the tryptophan kynurenine pathway. In perspective it‘s worth to deepen the investigations- both in mice as well as in the cell culture model- whether zinc is regulatory involved in other parts of the tryptophan kynurenine pathway but also to unravel the effect of zinc deficiency on formation of proinflammatory cytokines by macrophages.

Speciation analysis of trace arsenic in surface waters using polymeric ionic liquid sorbent

P 31

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Arsenic is a toxic element for humans and the toxicity of As is highly dependent on its chemical forms. Due to their trace concentration and the influences of coexisting substances in real water samples, development of analytical procedures for reliable and accurate determination and speciation of As is a difficult analytical task. Widely used approaches in routine analytical practice are based on preliminary separation/preconcentration of As species by solid phase extraction with suitable sorbent.

In this study we present new polymeric ionic liquid (PIL) used as a sorbent for separation/ preconcentration and speciation of trace inorganic arsenic species in water samples. PIL was synthesized via chemical modification of polymeric precursor. In the first step, polymer nanoparticles were prepared by cross-linking dispersion copolymerization of glycidyl methacrylate and trimethylolpropane trimethacrylate in acetonitrile. These polymer nanoparticles were further modified by chemical binding with 1-methylimidazole (MIA) to obtain PIL material – P(MIA). The synthesized P(MIA) was characterized using elemental analysis, Fourier transform infrared spectroscopy, scanning electron microscopy, and nitrogen adsorption–desorption measurements. The adsorption properties of P(MIA) toward As(III) and As(V) were studied by batch procedure. Laboratory experiments performed showed that P(MIA) is characterized with high selectivity, permiting quantitative sorption (> 95%) of As(V) at pH 2-8 and unsignificant sorption (less than 3%) for As(III) in this pH range. The adsorbed As(V) was quantitatively eluted from the P(MIA) using using nitric acid and measured by ETAAS under optimal instrumental parameters. Total arsenic content was determined as As(V) after oxidation of As(III) to As(V) using $KMnO_4$. Finally, the concentration of As(III) was calculated by subtracting the As(V) content from total As. The sorbent showed high capacity toward As(V) and good mechanical and chemical stability. The P(MIA) was successfully applied in analytical procedure for determination and speciation of As in surface water samples.

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Trace element and mineral status of children with trisomy 21 (Down's syndrome)

P 32

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The existing studies demonstrate that patients with Down's syndrome are characterized by a significant alteration of micronutrient status and trace element and mineral metabolism in particular. At the same time, the existing data are contradictory. Therefore, the objective of the study was to assess the levels of minerals and trace elements in Down's syndrome patients. A total of 40 children with Down's syndrome and 40 age- and gender-matched controls were enrolled in the present study. Hair analysis was performed using inductively-coupled plasma mass-spectrometry at NexION 300D (PerkinElmer, USA) equipped with ESI SC-2 DX4 autosampler (Elemental Scientific Inc., USA). Preliminary preparation of the hair samples included washing with acetone and distilled deionized water with subsequent digestion for 20 minutes at 170-180°C using Berghof Speedwave 4 (Berghof Products & Instruments, Germany). Laboratory quality control was performed using the certified reference material of human hair GBW09101 (Shanghai Institute of Nuclear Research, Academia Sinica, China). The obtained data demonstrate that Down's syndrome patients were characterized by a significant increase in hair P and Zn content, whereas hair Hg levels were reduced both in girls and boys as compared to the neurotypical controls. At the same time, hair Mg levels were significantly higher in boys, whereas Cr and Si content was found to be elevated in girls with Down's syndrome when compared to the controls. Hair content of lead and arsenic was elevated in boys and girls, respectively. Similarly, hair Ag levels were significantly increased only in female examinees, whereas significantly lower hair Pt levels were observed only in boys with Down's syndrome. Further analysis also demonstrated that hair trace element content in patients with Down's syndrome was not only gender-specific, but also depended on the age and body weight of the examinees. Particularly, a significant correlation between body weight and hair trace element content was revealed. Generally, the data demonstrate that patients with Down's syndrome are characterized by significant alteration of trace element status. Hypothetically, these impairments may at least partially mediate the observed metabolic disturbances.
The study is supported by RFBR (18-013-01026).

Optimization of the single-element-analysis for copper by Total Reflection X-ray Fluorescence (TXRF) in cell lysates

P 33

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The trace element copper is an important co-factor for the activity of enzymes such as superoxide dismutase in cells. The cellular amount of copper can be determined by using the Total Reflection X-ray Fluorescence (TXRF), a method for multi element analysis. The main advantage of TXRF over element analysis by ICPMS/MS is the low sample volume of e.g. plasma or serum samples which is needed for the measurements. On the contrary, TXRF provides a less accurate quantification compared to ICP-MS/MS and thus there is the need of carful validation and external calibration to produce proper results. We aimed to apply the TXRF technique to analyse the copper content of cultured cells.
For the establishment of an external calibration, lysates of untreated hepatocytes (HepG2) were used. The cell lysates were adjusted to a protein concentration of 5 mg/ml, aliquoted and spiked with copper sulphate (0.1; 2; 4; 6; 8 mg/L). The samples were analysed without previous digestion. The measurement time was 500 s. As internal standard gallium (0.5 mg/L) was used for quantification. The copper signal was higher when copper was spiked into cell lysates than into pure water. Thus, also the slope of linear regression was higher in the cell lysates than in the water matrix. For the quantification of single elements by TXRF in cell lysates it appears to be necessary to generate a calibration curve for these elements to eliminate potential matrix effects, which, however, needs to be further optimized.

Influence of selenium supplementation on cellular stress responses and metabolism P 34

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The essential trace element selenium is important for the antioxidant capacity of cells and exerts its function mainly through selenoproteins, e.g. glutathione peroxidases. A dysregulation of the antioxidative response and increased oxidative stress can contribute to obesity, insulin resistance and type 2 diabetes. Therefore, maintaining sufficient antioxidative capacity is crucial for cellular metabolism. With a subadequate selenium status in western European citizens, a selenium supplementation has been proposed to be beneficial for human health. However, available data regarding its effect on energy metabolism and insulin sensitivity remain largely controversial.
We aim to investigate whether a non-toxic (200nM) selenite supplementation affects the mitochondrial stress response, specifically the integrated stress response and subsequently cell metabolism in metabolically important rodent cell culture models: hepatic Hepa1c1c7, hypothalamic CLU183 and pancreatic INS1 cells.
Selenite supplementation induces the expression of integrated stress response (ISR) genes, e.g. the transcription factors ATF4 and CHOP in a time-dependent manner. This upregulation is accompanied by reduced mitochondrial respiration with a mild concomitant increase in carbonylated proteins as a marker for oxidative stress and an enhanced GPx1 expression. As a result, selenite supplementation impairs ATPproduction in CLU183 and Hepa1c1c7 cells as well as β-oxidation. In CLU183 cells this associates with reduced gene expression of acyl CoA dehydrogenases SCAD and LCAD.
As mitochondrial function is crucial for glucose-stimulated insulin secretion (GSIS) of beta cells, we also stimulated INS1 cells with selenite followed by glucose stimulation. This analysis revealed that GSIS is completely blunted in in selenite-treated cells.

Cobalt accumulation and iron redistribution in blood and organs of mice after long-term chronic exposure to cobalt chloride

P 35

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The wide use of cobalt (Co) in food, industry and medical devices requires full elucidation of its biological effects on tissues and organs. The aim of the study was to assess cobalt accumulation and iron (Fe) redistribution profile in blood serum, erythrocytes (RBC) and target organs (spleen and liver) of mice after long-term chronic exposure. Pregnant ICR mice were subjected to daily dose of 75 or 125 mg/kg body weight cobalt chloride ($CoCl_2x6H_2O$) 2–3 days before they gave birth. The compound was dissolved in regular tap water. After birth, the mothers continued to obtain the same doses. On postnatal day 25, the newborn mice were separated into individual cages to ensure that all experimental animals obtained the required daily doses. The mice were weighed weekly and Co concentration was adjusted accordingly. Significant gender differences neither in body weight nor in hematological parameters were found and the experimental groups consisted of equal number male and female mice. Animals were fed a standard diet and had access to food ad libitum. The treated mice were sacrificed by decapitation after etherization on postnatal day 90. Results were compared with age-matched control groups consisting of mice obtaining regular tap water. Chronic exposure to $CoCl_2$ induced significant accumulation of the metal ions in RBC and blood serum. Its content in the sera was ~2-fold higher compared to that in RBC. In the spleens and livers of the exposed mice the metal ions increased ~2-fold and more than 25-fold, respectively. Co concentration was higher in all samples of mice obtaining the higher dose, suggesting a dose-dependent effect. The results also indicate that liver is more sensitive to Co exposure than the spleen. Simultaneously, Fe content increased in samples of Co-exposed mice. It was ~2-fold higher in the spleens and livers of mice exposed to 125 mg/kg $CoCl_2$ compared to the untreated controls. The results suggest a good relationship between cobalt exposure and iron tissue distribution after long-term treatment.

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Comparative study on the effects of salinomycin, monensin and meso-2,3-dimercaptosuccinic acid on the concentrations of lead, calcium, copper, iron and zinc in the lungs and heart in lead-exposed mice

P 36

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Humans can be exposed to lead (Pb) through inhalation or ingestion. 40 % of the inhaled Pb dust accumulates in the lungs in adults. It has been found that environmental lead (Pb) exposure reduces the pulmonary function test values in Pb-exposed workers compared to non-exposed control group. Pb is one of main causes for cardiovascular disease mortality. Prolonged environmental exposure to Pb elevates the frequency of hypertension incidents. Meso-2,3-dimercaptosuccinic acid (DMSA) is a chelating agent of first choice for treatment of Pb intoxication. This chelating agent however is distributed in the extracellular space and therefore is not suitable for mobilizing aged Pb deposits. Lipophilic chelating agents are tested for treatment of Pb intoxications. In this study we present new data on the effects of two lipophilic polyether ionophorous antibiotics - monensin and salinomycin on Pb, calcium (Ca), copper (Cu), iron (Fe) and zinc (Zn) concentrations in the lungs and heart of Pb-exposed mice. The effects of both antibiotics were compared with those of DMSA. The data revealed that Pb-exposure of mice for 14 days significantly increased the concentrations of Pb in both organs compared to the untreated controls. Treatment of Pb intoxicated mice with monensin, salinomycin or DMSA significantly reduced the Pb contents in the heart and lungs compared to the toxic control group. Lower endogenous levels of Ca, Cu and Zn in the lungs of Pb-exposed mice compared to the untreated controls were observed. The endogenous Fe concentration in the lung was not affected by Pb intoxication. Cu and Fe concentrations in the heart were elevated in compared to untreated mice. The tested chelating agents did not recover the Pb-induced alterations in the essential metal ions concentrations in both organs. These results should be taken into account when DMSA, monensin and salinomycin are applied as antidotes to Pb poisoning.

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Effect of acute sodium nitrite intoxication on some essential biometals in mouse spleen P 37

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Sodium nitrite ($NaNO_2$) is an inorganic salt with various industrial and medicinal applications. However, toxicity to humans and animals is well documented in nitrite overexposure. Sodium nitrite is highly reactive with blood hemoglobin, converting it to methemoglobin and thus reducing the oxygen-carrying capacity of the blood. $NaNO_2$ intoxication is shown to decrease the activity of major antioxidant defence enzymes. Literature data also demonstrate a close relationship between the activity of these enzymes and the concentration of various biometals, usually serving as their cofactors. Considering the above, the present study was undertaken to investigate the effect of $NaNO_2$ intoxication on the content of Ca, Zn and Fe in mouse spleen. Mature male ICR mice were subjected to acute NaNO2 exposure by a single intraperitoneal injection of 120 mg/kg body weight. The animals were sacrificed at different time intervals (5 hours, 1 and 2 days) after treatment. Tissue homogenates from spleens were prepared and processed for atomic absorption analysis of Ca, Zn and Fe content. Five hours after $NaNO_2$ administration, statistically significant decrease in Ca and Fe levels in the spleens of treated mice was observed, compared to the untreated controls. One and two days after intoxication, lower Ca and Zn concentrations were measured, statistically significant for Zn on the 1^{st} day. In contrast, Fe levels in the spleen were elevated, although not statistically significant. The results of the present study demonstrate that acute $NaNO_2$ intoxication provokes changes in the endogenous levels of Ca, Zn and Fe in mouse spleen. These findings suggest disruption of the ionic balance and impact on the activity of antioxidant defence enzymes.

Systematic review of dietary lithium intake and suicide in man and lithium intake via beer

P 38

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World health organization (WHO) documents 800.000 reports on suicide each year[1]. Concerning the age group between 15 and 29, suicide is the second most common cause of death. According to WHO, bad health care, war, abuse or discrimination are important factors influencing suicide rates[1]. By now, little attention has been paid for lithium intake via drinking water as a possible hazard factor. Although lithium is widely used in drug therapy to treat bipolar disorders, obsession and depression, respectively, there is rather limited knowledge regarding its dietary sources or even essentiality.
Although, the suicide preventing properties of lithium and its relation with bipolar disorders are known for a long time, the role of an inadequate dietary lithium, like low levels of lithium in drinking water have yet not been systematically studied. Various studies show an inverse associations of regional lithium levels in drinking water and suicide rates. Due to a landmark study in 1990, using crime rate data for 1978-1987, statistically significant inverse correlations were observed between water lithium levels and the rates of homicide, suicide and forcible rape[2].
An observational study with 199 participants assumed a causal connection between lithium levels in drinking water and the incidence of suicide. This study observed significant lower serum lithium concentrations of people who attempted suicide compared to a control group[3].
The provisional recommended daily lithium intake for an adult (70 kg body weight) is 1000 µg/l per day 2. Especially drinking water and other beverages produced by drinking water like tea and coffee may have a major impact on lithium intake. Besides drinking water, locally brewed beer might influence lithium intake, especially in men. Importantly, beer is the only locally produced beverage with water pre-treatment during the production process. Therefore, within this study lithium was determined in 42 representative beers available on the German market. Lithium analysis by ICP-MS revealed an arithmetic mean of 8.5 µg/l, a median of 8.5 µg/l with a standard derivation of 4.98 µg/l. Since beer consumption accounts for 10% of water intake in man, we suggest to monitor dietary lithium intake via beer in future studies. Also it may interesting to optimize production processes to ensure high lithium levels in different beverages including beer thereby improving lithium status in humans.

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Alkaline comet assay protocol to assess DNA damage in human cells and mouse tissue P 39

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The aim of the DFG research unit TraceAge (#2558) is to elucidate interactions of essential trace elements (TE) and the impact of their specific age-related profiles on the aging process. It is estimated, that an altered trace element homeostasis contributes to aging by disturbing DNA repair mechanisms, thus leading to genomic instability. Due to its sensitivity, simplicity and reproducibility, the comet assay (single cell gel electrophoresis) is one of the most frequently used tests among the variety of available DNA damage detection techniques. Nevertheless, interexperimental variability as well as variability between different laboratories evaluating identical samples is still a serious issue. To limit this variation, numerous approaches have been made and the critical steps in the procedure have been identified. Especially agarose concentration, duration of alkaline incubation, electrophoresis conditions and the different analyzing methods are of particular interest.
In this work an in vitro alkaline protocol of the comet assay was adapted regarding electrophoresis conditions and quantifying methods (visual and semi-automated scoring with the software programs Komet 5® (BFI Optilas, Germany) and Comet assay IV® (Perceptive Instruments, UK)), with the aim to reduce variability.
In addition, different substances including Hydrogenperoxide and tert-Butylhydroxyperoxide, as well as UVC exposure were tested in human liver carcinoma (HepG2) and/or human lung carcinoma (A549) cells. By the determination of the dose-response relationship suitable conditions for a treatment with a positive control could be adjusted.
As a next step, the protocol should be modified by integrating a digestion of DNA with a lesion-specific endonuclease to determine the amount of oxidized bases. Additionally we plan to adapt the assay to mouse tissue and blood samples, among others to examine the genomic stability of young and old mice supplied with different TE profiles.

Serum and hair levels of essential trace elements in preschool children with attention deficit hyperactivity disorder (ADHD): a case-control study

P 40

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Attention deficit hyperactivity disorder (ADHD) is a neurodevelopmental disorder characterized by impaired attention, excessive activity, and behavioral problems. Trace element metabolism was shown to play a significant role in ADHD development. Particularly, decreased serum Zn was shown to be associated with the inattention score (Arnold et al., 2005). Certain studies demonstrated that Zn, Fe, and Mg supplementation significantly improved behavior in ADHD, although the data are rather contradictory (Hariri, Azabdakht, 2015). Moreover, serious contradictions on trace element status of ADHD children exist. Therefore, the objective of the present study was to assess serum and hair levels of essential trace elements (Cu, Fe, Mn, Se, and Zn) in children with ADHD using ICP-MS technique. A total of 50 ADHD children and 50 neurotypical controls were involved in the present study. The protocol was approved by the local ethics committee. The obtained data demonstrate that ADHD girls were characterized by a significant increase in hair Fe (12.4(9.616.9) vs 10.31(6.8-14.7) μg/g) content in association with decreased Mn levels (0.180(0.130-0.302) vs 0.257(0.154-0.408) μg/g) as compared to the respective control values. At the same time, in boys suffering from ASD only a significant increase in hair Fe was observed compared to the controls (13.89(9.72-18.01) vs 11.13(8.33-16.71) μg/g). Similarly, serum Fe concentration was found to be decreased in relation to the controls both in girls (1.07±0.38 vs 1.34±0.20 μg/ml) and boys (1.23±0.51 vs 1.57±0.34 μg/ml). In contrast to hair, serum Mn was elevated in serum from females with ADHD by 14% when compared to the controls (0.0024±0.0008 vs 0.0021±0.0006 μg/ml). No significant group difference in both hair and serum Cu, Se, and Zn levels were observed between ADHD and neurotypical examinees irrespectively of the age of children. Therefore, the obtained data indicate that alterations of Fe and Mn metabolism may be at least partially related to ADHD pathogenesis. However, further studies are required to estimate the intimate mechanisms of this interaction as well as associations between trace element status and school performance in ADHD children.

The project was supported by RFBR (19-013-00528).

Trends of hair lead (Pb) accumulation in 3-6-year old children in 2004-2010 (Moscow, Russia)

P 41

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Lead (Pb) is a toxic metal being characterized by high neurotoxicity especially in children. Even chronic low-dose Pb exposure is associated with mental, behavioral, memory, mood disorders, altered language and motor skills, as well as higher risk of autism spectrum disorder (ASD), attention deficit hyperactivity disorder (ADHD), and violence. The existing data from ESPREME project demonstrated that Pb-induced IQ loss in Europe accounts for annual €44.3 bln loss (ESPREME, 2012). The use of leaded gasoline was banned in order to reduce environmental emissions (since 2003 in Russia). Certain studies have demonstrated the efficiency of these efforts. Particularly, the contribution of gasoline to environmental lead levels in China was significantly reduced by 30%. At the same time, no data on the efficiency of these efforts in Russia exist. Therefore, the objective of the present study was to assess the dynamics of hair lead levels in 3-6-yearold children living in Moscow in 2004-2006 and 2008-2010. A total of 300 children per each period were involved. Hair Pb content was assessed using inductively-coupled plasma mass-spectrometry at NexION 300D (PerkinElmer, USA). Laboratory quality control was performed using the certified reference materials of human hair GBW09101. The obtained data demonstrate that hair lead content in boys aged 3-6 years in 2008-2010 was significantly reduced by 35% as compared to the respective values from 2004-2006 (0.836(0.502-1.562)(CV=126%) vs 1.279(0.697-2.042)(CV=106%) μg/g, $p < 0.001$). Similarly, hair Pb levels in 3-6 y.o. girls were 20% lower when comparing 2008-2010 values to 2004-2006 (0.726(0.3771.372)(CV=124%) vs 0.904(0.540-1.729)(CV=112%) μg/g, $p < 0.001$). At both time points hair Pb was significantly higher in boys than in girls ($p < 0.001$ and $p = 0.003$, respectively). Using a cut-off for hair Pb as 1.9 μg/g, it has been demonstrated that the percentage of children exposed to lead was also characterized by a significant reduction (17% vs 24%, $p < 0.001$). Generally, the existing data demonstrate that leaded gasoline ban in 2003 was effective in reduction of children Pb exposure as assessed by hair analysis. However, additional legislative and technical efforts are required for further reduction of Pb emissions and exposure. The project was supported by RFBR (19-013-00738).

Risk of osteoporosis among vegetarians and vegans

P 42

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One of the most common pathology associated with a predominantly plant diet is osteopenia and osteoporosis. An analysis of a number of studies has shown that vegans are at a greater risk of reducing of bone mineral density, thereby increasing the incidence of fractures.
The higher risk of decrease in bone mineral density (BMD) is probably due to the fact, that vegans often have nutrient deficiencies, necessary to maintain consistency of bone homeostasis, such as calcium, zinc, vitamins D and B12, ω3-PUFA. In addition, vegans are also more likely to have protein, especially methionine, deficiency.
At the same time, vegan diet is rich in potassium and magnesium; it has less saturated fat and cholesterol. A significant role in maintaining the BMD is acid-base balance. Lowering plasma pH contributes to bone resorption. Omnivorous diets in Western countries often have an acidifying effect on blood plasma. In contrast, the plant diet causes blood alkalization, which leads to preservation of calcium in bone tissue.
Research data in this area shows that, in compliance with a number of conditions (sufficient consumption of protein, calcium, zinc, vitamins D and B12, ω3-PUFA with food or dietary supplements), vegan diet can have significant benefits, thus reducing potential risks of osteoporosis and pathological fractures.
Nowadays the Institute of Nutrition in Moscow is conducting a comprehensive survey of vegetarians and vegans in order to identify disorders of bone tissue and other metabolic disorders that can lead to a decrease in BMD and an increase of the risk of osteoporosis.

Speciation analysis of inorganic antimony in water samples assisted by vinylimidazole-based polymeric sorbent

P 43

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Antimony is a chemical element that occurs in the environment as a result of natural and anthropogenic processes and its biogeochemical cycle is nowadays sufficiently characterized. Toxicological profiles and bioaccumulative properties of particular Sb species are well known – the inorganic chemical forms are much more harmful than organic species, especially the lower oxidation state Sb^{III}. In this sense, reliable knowledge about Sb speciation is essential taking into account significant differences between toxicity and bioavailability of trivalent and pentavalent forms.

This work is focused on the preparation of vinylimidazole-based polymeric material and its further application as a sorbent for non-chromatographic speciation analysis of inorganic antimony in water samples. The polymer was synthesized by radical polymerization of vinylimidazole as a functional monomer and trimethylolpropane trimethacrylate as a cross-linking agent. Extraction efficiency and selectivity of the prepared material toward Sb species were studied by batch method. Several parameters such as acidity of the aqueous media, amount of sorbent, time of sorption, type and concentration of the eluent were investigated in order to define the optimal chemical conditions for selective separation and enrichment of both Sb^{III} and Sb^{V}. Inorganic Sb species at trace levels were separated by quantitative retention of SbV at pH 4-5 for 15 minutes followed by elution with 0.5 M HNO_3 and ETAAS measurement. In a parallel sample, after conversion of Sb^{III} to Sb^{V} by oxidation, total concentration of antimony was determined in the eluate solution by the same extraction procedure. Finally, the amount of SbIII in the analyzed samples was calculated by subtraction. Additionally, a simple analytical scheme for speciation and determination of Sb in natural and bottled waters based on two sequential extraction steps was proposed.

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ICP-DRC-MS analysis of the levels of zinc (Zn) and its antagonists cadmium (Cd) and lead (Pb) in leaves of four medicinal plants

P 44

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The existing data demonstrate that essential trace elements may at least partially mediate the physiological effects of various medicinal plants and their preparations in association with other phytochemicals. At the same time, heavy metal contamination may significantly reduce activity of phytomedicines or even cause adverse health effects. However, data on the level of trace elements in medicinal plants are limited. Therefore, the objective of the present study was to assess the levels of zinc (Zn) and its antagonists cadmium (Cd) and lead (Pb) in leaves of four medicinal plants widely used in Russia. Samples of oregano (Origanum vulgare), sage (Salvia officinalis), yarrow (Achillea millefolium), and plantain (Plantago major) were collected in 2018. The leaves were dried on air and subjected to microwave digestion. Zn, Cd, and Pb content was assessed using inductively-coupled plasma mass-spectrometry at NexION 300D (PerkinElmer, USA). The obtained data demonstrate that the highest Zn content was revealed in O. vulgare leaves (32.98±4.11 μg/g), exceeding the respective values for P. major and S. officinalis by 76% and 20% ($p < 0.05$). At the same time, no significant group difference between leaf Zn content in O. vulgare and A. millefolium was observed. Leaf Pb content was also found to be the highest in O. vulgare (0.68±0.29 μg/g), followed by S. officinalis (0.43±0.15 μg/g), A. millefolium (0.28±0.05 μg/g), and the lowest values in P. major (0.12±0.04 μg/g). In turn, the highest and lowest levels of cadmium were detected in O. vulgare (0.19±0.04 μg/g) and S. officinalis (0.03±0.01 μg/g). Therefore, despite the highest Zn content, O. vulgare is also characterized by the maximal levels of Cd and Ob, being Zn antagonists, whereas A. millefolium is characterized by more than twofold lower Cd/Zn and Pb/Zn ratios although having nearly similar Zn content. The obtained data underline the necessity of toxic metal assessment in plant-based pharmaceuticals instead of analysis of only essential elements in order to take into account potential interactions that may affect biological effects.
The project was supported by RFBR (19-016-00103).

Zinc status of children with chronic inflammatory diseases of the upper respiratory tract

P 45

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Zinc plays an essential role in immune system functioning regulating both cellular and humoral immunity (Haaze, Rink, 2014). Therefore, zinc deficiency is known to be associated with altered immunity and higher susceptibility to infectious diseases. In turn, Zn supplementation was shown to be effective in improvement of immune deficiency and increased recovery from infectious diseases. Particularly, it has been demonstrated that Zn losenges significantly reduce the period of common cold (Hemilä et al., 2016). At the same time, the existing data on the role of Zn imbalance in children with chronic inflammatory diseases of the upper respiratory tract are insufficient. Therefore, the objective of the study was assessment of serum and hair Zn levels in children with chronic inflammatory diseases of the upper respiratory tract being also characterized by high frequency of infectious disease episodes ("frequently ill children") in relation to a particular diagnosis. A total of 200 children were involved in the present study. Hair and serum Zn levels were assessed using inductively-coupled plasma mass-spectrometry at NexION300D (PerkinElmer, USA) and continuous laboratory quality control using the certified reference materials of human hair (GBW09010, PRC) and serum (ClinChek, Recipe, Germany). The obtained data demonstrate that "frequently ill children" are characterized by a significant decrease in both hair and serum Zn levels as compared to the healthy controls. Further stratification by an ICD-10 diagnosis demonstrated a significant effect of the diagnosis (J31, J32, J35, J37) with the lowest values of both hair and serum Zn observed for chronic laryngotracheitis (J37). At the same time, chronic sinusitis was associated with a significantly lower serum Zn levels, but not hair Zn content. In addition, clinical features of the chronic sinusitis (allergy, polyposis, etc.) also had a significant effect on Zn metabolism. The obtained data demonstrate that Zn deficiency may be involved in altered immune response in children with chronic inflammatory diseases of the upper respiratory tract ("frequently ill children") and Zn supplementation may be potentially beneficial. However, the particular diagnosis has a significant effect on altered Zn handling and the dose for Zn supplementation should be based on personalized assessment of Zn status.

The impact of high-Se crop processing on flour selenium content

P 46

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Crop enrichment with Se is proposed to be the one of the most beneficial strategies in overcoming Se deficiency. One of the ways for enrichment of edible crops is cultivating on Se-rich soils. Crops are characterized by the higher Se accumulation rates as compared to other agricultural species including potato, vegetables, beans, melon, and fruits. At the same time, nutritional value of Se enriched crops and its products is of particular interest. Therefore, the objective of the present study was to assess the impact of high-Se crop processing on flour selenium content. The samples of wheat, rice, and maize, as well as the flour and bread were collected from the seleniferous area of Punjab (India). Se levels were assessed using ICP-DRC-MS at NexION 300D (PerkinElmer, USA). The obtained data demonstrate that growing on seleniferous soils results in a significant increase in Se content in wheat (105.0 (103.0-109.0) vs 0.22 (0.18-0.77) μg/g, $p < 0.001$), rice (21.20 (17.73-21.48) vs 0.225(0.142-0.250) μg/g, $p < 0.001$), and maize (24.68 (23.83-25.22) vs 0.360 (0.058-0.385) μg/g) as compared to the normal soil Se content. Similarly, Se content was found to be higher in flour from wheat (117 (99.3-118 vs 2.34 (2.33-2.64) μg/g, $p < 0.001$), rice (24.73 (18.6-24.79) vs 0.287 (0.267-0.288) μg/g, $p < 0.001$), and maize (23.14 (22.99-25.76) vs 0.087 (0.076-0.095, $p < 0.001$) μg/g) from seleniferous regions. At the same time, crop processing during flour production had a significant impact on Se content. Although flour Se content from Se-rich crops was nearly similar to crude crops Se levels, flour from wheat, rice, and maize growing on normal Se soils contained significantly higher levels of selenium. Bread baked from Se-rich flour was also characterized by a significant increase in total Se content irrespectively of the crop type. However, bakery also had a significant effect on Se levels. Therefore, the obtained data demonstrate that flour and bread from Se-rich crops may be successfully used as the tool for increasing dietary Se intake. However, further studies are required in order to assess Se bioavailability from different sources as well as Se speciation.
The project was supported by RFBR (17-55-45027).

Nutritional deficiency of trace element in Tajik schoolchildren: experience from UN WFP program

P 47

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Nutritional intake is the major determinant of trace element status of the population. National traditions and food habits therefore play a significant role in the intake of trace elements in each particular country that may have an impact on population health. The specific features of trace element status of children living in the Central Asia being characterized by strong national dietary traditions are not estimated. Therefore, the aim of the study was comparative analysis of the levels of trace elements in hair of children living in Tajikistan, Kazakhstan, Turkmenistan, Azerbaijan, and central Russia (Ryazan region) (control). The study was performed within the United Nations World Food Program (UN WFP). The schools were divided into three types: I – schools not involved in the WFP program; II – schools involved in the WFP program and the pilot project on school infrastructure improvement and hot meals provision; III – schools involved in the WFP program only (provision of fortified flour, peas, vegetable oil and iodized salt) during the recent 5-6 years. Hair trace element content was assessed using inductively-coupled plasma mass-spectrometry after microwave digestion. The obtained data demonstrate that children from Tajikistan are characterized by the lowest hair copper, iodine, chromium, and vanadium content as compared to other countries. No significant country difference was observed for hair cobalt, selenium, and zinc. Oppositely, hair iron content tended to be higher in children from Tajikistan as compared to other countries. At the same time, a pilot project of food enrichment had a significant impact on hair trace element content. Particularly, children from Type II and III schools were characterized by significantly higher levels of Co, Fe, Mg, Mn, V, and Zn, whereas the level of mercury was significantly reduced as compared to Type I schools. The obtained data the significant positive correlation between the anthropometric parameters, nutritional and element status of the examined schoolchildren. Therefore, the obtained data demonstrate that Tajik schoolchildren are characterized by higher risk of micronutrient deficiency, whereas food enrichment program is an effective tool to counteract trace element deficiency.

Selenite supplementation alleviates diet-induced insulin resistance in C57Bl/6N mice P 48

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The worldwide rise of obesity is a central issue of our healthcare systems and gives rise to associated pathologies, such as insulin resistance and type 2 diabetes. Mechanistically, obesity leads to chronic inflammation and oxidative stress in various tissues, which is a major contributor to the development of insulin resistance. The cell´s capacity to counteract oxidative stress relies mainly on the correct function of antioxidant protein like the selenoprotein Glutathione-Peroxidase 1 (Gpx1). These proteins show lower expression in insulin-sensitive tissues of obese mice, contributing to obese pathologies. Additionally, there are indications of a sub-optimal selenium status in Western Europe. Therefore, we aim to investigate whether a selenium supplementation can counter-act the pathological effects of diet-induced obesity.
We discovered that feeding C57Bl/6N mice a selenite-rich high fat diet (676 ng selenite/g food) led, as expected, to an increase in Gpx1 expression in peripheral tissues. Interestingly, we observed a decreased fat accumulation in males using NMR without changes in total bodyweight, as well as a decrease in total adipose tissue weight. Females showed unaltered body composition. Both females and males under selenium supplementation show a significantly increased early response glucose uptake after insulin stimulus and mild improved insulin tolerance compared to HFD-treated mice. Unexpectedly, the liver reveals higher oxidative stress, as well as higher expression of the cellular stress marker SOCS3, both signs of decreased hepatic insulin sensitivity. In line, hepatic expression of insulin-responsive fat metabolism genes, Stearoyl-CoA desaturase-1 (SCD1) and Fatty acid synthase (FASN) are decreased thus possibly contributing to the decreased fat accumulation in these animals.
In summary we can show a sex specific decrease in fat accumulation in mice fed selenite supplemented high fat diet. Additionally, feeding a selenite-rich high fat diet leads to tissue-specific alterations of cellular stress responses. However, it also seems to overall improve the metabolism, highlighting a disassociation between hepatic and whole-body insulin sensitivity in obese mice upon selenite treatment.

Characterization of the toxicity of arsenolipids in pre-differentiated and differentiated human neurons

P 49

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Lipid-soluble organoarsenicals, so-called arsenolipids, are found in marine organisms. They can be subdivided into several distinct groups including arsenic-containing hydrocarbons (AsHCs) or their thioxoanalogs (thioxo-AsHCs). An *in vitro* study in liver cells indicated, that AsHC 332 is metabolized to thioxo-AsHC 348 (Müller et al., 2018). In addition, first *in vivo* feeding studies with AsHC 332 in *Drosophila melanogaster*, showed an accumulation of AsHC 332 in various organs and the brain (Niehoff et al., 2016). This result indicates that AsHCs can easily cross the brain barrier and enter the brain.
LUHMES (Lund human mesencephalic) cells were chosen as a model for investigation of neurodevelopmental effects in pre-differentiated and neurotoxic effects in fully differentiated neurons. Thioxo-AsHC 348, compared to iAs^{III} and AsHC 332, exerted cytotoxicity at lower concentration in fully differentiated and at similar concentration in pre-differentiated neurons (Witt et al. 2017a, b). Neurite mass was used as an indicator for neurite outgrowth in pre-differentiated neurons, which was more affected by thioxo-AsHC 348 than neurite degeneration in fully differentiated neurons. Cellular bioavailability of thioxo-AsHC 348 was measured *via* ICP-MS/MS. Cellular arsenic concentrations in fully differentiated neurons were higher for thioxo-AsHC 348 than for iAs^{III} but comparable to AsHC 332. Oxidative stress was assessed by using the dihydroethidium assay after 48 h of incubation with iAs^{III}, AsHC 332 and thioxo-AsHC 348, but no change in the level of oxygen and nitrogen species was detected. Furthermore, the formation of DNA single strand breaks (SSB) was investigated *via* alkaline unwinding. Here, low micromolar concentrations of iAs^{III} led to an increase in SSB, whereas AsHC 332 and thioxo-AsHC 348 showed no induction of SSB.
These results indicate that the toxic mode of action differs between inorganic iAs^{III} and arsenolipids. Future studies will be directed at obtaining a better understanding of the influence of toxic mode of action on general markers for oxidative stress (e.g. glutathione). Furthermore, a possible metabolism of thioxo-AsHC 348 in neurons should be investigated.

Müller et al. (2018), Arch Toxicol, 92, 1751-1765
Niehoff, et al. (2016), Anal Chem 88
Witt, et al. (2017a), Mol Nutr Food Res 61
Witt, et al. (2017b), Arch Toxicol 91

Dietary exposure estimates to fifteen trace elements in an adult population of Emilia Romagna region, Northern Italy

P 50

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The health effects and the exposure levels of trace elements in humans are important public health topics. Assessing their dietary intake is fundamental to evaluate the long-term risks for public health and for food safety assessment. Since a priority of food safety regulatory agencies is to ensure the protection of consumers and to assess the health risks for the general population, to estimate the actual dietary intake of trace elements for comparison with the upper and lower tolerable levels is very important. In this study, we aimed to evaluate the dietary intake of several trace elements in an Italian community, namely of antimony, barium, beryllium, boron, cobalt, lithium, molybdenum, nickel, silver, strontium, tellurium, thallium, titanium, uranium, and vanadium. To do that, in 2016-2017 we collected a total of 908 food samples available in Italian markets and groceries from two Northern Italy provinces (Modena and Reggio Emilia), and we measured their trace element content through inductively-coupled plasma mass spectrometry. We also administered a validated semi-quantitative food frequency questionnaire to 708 residents (300 men and 48 women) in the Emilia-Romagna Region, to assess their dietary habits and eventually to estimate their dietary intake of the aforementioned trace elements. Overall, study results showed that in our population the dietary exposure levels to selected trace elements could be considered similar to that observed in other European and non-European populations. Though we cannot rule out the possibility that the dietary exposure estimates in the present study may not be representative of the population as a whole, our results provide a good and updated assessment of trace elements far frequently evaluated in a sample of Italian adult consumers from the Emilia Romagna region. Our findings finally suggest that our population should not be at risk of adverse health effects in relation to excess or deficiency of the investigated trace elements since the estimated dietary intake generally point out exposure levels within the safe range as far as indicated by recommendations of international agencies.

Red blood cell's copper in Wilson disease

P 51

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Copper in red blood cells (RBC) is not involved in copper transport. Copper transporters ATPase 7A and 7B are not involved in RBC copper metabolism, which is then expected to have a normal function in copper metabolic diseases such as Wilson's disease (WD). In some Wilsonian patients, following episodes of acute haemolysis, a transient and important increase in serum total (CuT) and exchangeable (CuEX) copper has been described, suggesting that RBC may be a reservoir of free copper. Discrepancies in biological results are observed: CuT, CuEx and urinary copper are very elevated while ceruloplasmin remains low.
We studied RBC copper in WD patients (n = 40) and non-diseased controls (Non WD) (n = 48). RBC copper bounds are studied by determination of RBC exchangeable copper (RBC-CuEX) in 14 patients and 9 controls. CuEx determination involves an incubation step (v/v, EDTA 3 g/L, 1 hour) followed by ultrafiltration on filters with a 30 kDa cut-off. Other cut-off filters (50 and 100 kDa) were also tested. Results show that despite a significant difference ($p < 0.0001$) in mean serum copper between WD patients (4.0 µmol/L) and controls (12.5 µmol/L), RBC copper concentrations are equivalent ($p = 0.19$) in the two groups (WD = 10.9 µmol/L, non-WD = 11.7 µmol/L). Surprisingly, RBC-CuEx shows very slight variation in both groups (0.32 - 0.41 µmol/L) and mean results are similar between patients (0.36 µmol/L) and controls (0.36 µmol/L). It also appears that RBC-CuEx is very low and does not exceed 3% of total RBC copper. Various attempts to mobilize RBC-CuEX have been conducted without ever significantly increasing its concentrations. Ultrafiltration of RBC using different cut-off filters permitted to estimate molecular mass of copper-binding molecules. With 30 kDa filters, only 2% of total RBC Copper is found in ultrafiltrate. This value increased to 7% and 61% when using filters of 50 and 100 kDa respectively. We then demonstrated that RBC copper is not in balance with serum copper and that RBC constitutes an independent pool of copper strongly bound to variable ligands which remains to be determined.

Characterizing selenoneine by the use of in vitro models

P 52

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Selenium (Se) is generally known as an essential element and plays an important role in a variety of metabolic functions. Se is present in most of human tissues as both small and high molecular mass species. On the other hand, Se has a very narrow range of beneficial uptake and shows toxic properties, strongly depending on the chemical form present. The aim of this study is to assess the antioxidative potential as well as toxic effects of the recently discovered Se species selenoneine by the use of *in vitro* models. Selenoneine is a Se analogue of ergothioneine, a naturally occurring antioxidant. Originally, it was isolated from bluefin tuna (*Thunnus thynnus*) and it was shown to be mainly responsible for the non-protein Se pool in blood of fish-eating populations in Japan and Canada. However, up to now there is only limited information on this compound: it has been postulated that selenoneine has greater antioxidant activity than ergothioneine and possibly promotes methylmercury detoxification. In a first step we assessed cytotoxicity of selenoneine in cultured human liver cancer cells (HepG2) and primary porcine brain capillary endothelial cells (PBCEC) by dehydrogenase activity and lysosomal integrity. Here selenoneine failed to affect the viability markers, indicating no cytotoxic effects in both cellular models. Selenoneine did not substantially decrease the transendothelial electrical resistance of an *in vitro* blood brain barrier (BBB), but showed some transfer towards the brain facing compartment. Thus, in a next step we will focus on the potential protective but also toxic effects of selenoneine in human neurons.

Essential trace elements in *Vespa crabro* and *Vespa velutina* P 53

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Wasps are at the top of the invertebrate food chain and therefore they are crucial in maintaining the equilibrium of terrestrial ecosystems. Unfortunately, due to human trades, some species have colonized areas outside of their natural range, posing a serious threat to biodiversity. Among them, concern is raised by an Asian wasp, *Vespa velutina*. Since there was no coevolution with this predator, its effects on honey bee colonies are much more severe than those posed by the native European hornet (*Vespa crabro*). In the frame of our comparative studies in unconventional animals, this research investigated the concentrations of selected trace elements in these two species.
Specimens of *V. crabro* (n= 20) and *V. velutina* (n=20) were collected from the wild in Northern Italy, immediately frozen and stored at -20°C. Samples (0.5 g) were digested in 2 mL 65% HNO_3 and 0.5 mL 30% H_2O_2 in a microwave oven (Milestone 1200). The acid solutions were directly aspirated into the flame of an atomic absorption spectrophotometer (Perkin Elmer Analyst 100) for Fe, Zn, Cu and Cd analysis.
In both species, essential metal concentrations presented the following decreasing order: Fe > Zn > Cu. In all the analysed samples, Cd concentrations were lower than the LOD. Metal levels determined in this research are of the same order of magnitude as those reported by other authors in *Vespa affinis*. European hornets presented significantly higher concentrations of Fe and Zn than *V. velutina*; this difference might be related to species-specific diet and life-style. Data on trace metal concentrations in *Hymenoptera* are fragmentary and mainly related to Fe and magnetoreception in honey bees, therefore this research, though preliminary, can shed more light on the biochemical complexity of these insects.

Determination of arsenic species in the BfR MEAL Study

P 54

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Total Diet Study (TDS) denotes an internationally recognized method to establish the average concentration in which substances are contained in prepared foods. The established data set provides a basis for exposure assessment substances from foods. According to the TDS design, the BfR MEAL Study (meals for exposure estimation and analysis of foods) samples foods based on a previously established food list (covering at least 90% of the German diet). Foods are representatively purchased, prepared as consumed and similar foods (subsamples) are pooled to a composite sample prior to analysis. The investigated analytes include both substances that are beneficial to health and potentially harmful. The core module of the BfR MEAL Study contains elements and environmental contaminants such as arsenic. Arsenic occurs in different forms in food. Inorganic arsenic (iAs) is found as arsenite As(III) and arsenate As(V) and can be metabolized into organic species such as monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA). Inorganic arsenic is toxic and classified as carcinogenic to humans by international authorities. Determination of total arsenic was performed by ICP–MS/MS after microwave digestion. Arsenic species were first extracted with trifluoroacetic acid/H_2O_2 and subsequently measured by anion-exchange HPLC-ICP-MS/MS using aqueous malonic acid as mobile phase. Chromatographic separation of water-based arsenic species was achieved within 6 minutes. To assess extraction efficiency, the content of total arsenic in the extracts was determined. The most prevalent arsenic species in seafood is arsenobetaine, which is known to be non-toxic to humans. The anion exchange method is not capable of reliably resolving arsenobetaine from other early eluting species. Therefore, samples that showed signals at low retention times were subjected to cation-exchange HPLC-ICP-MS/MS using aqueous pyridine as mobile phase. Seafood, mushrooms, rice and rice products as well as all other foods that contain quantifiable amounts of total arsenic will be analyzed using this speciation technique.

Concentrations of trace elements in mature breast milk from Italian women

P 55

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Breastfeeding is recommended for infants worldwide, as human milk is the best source of nutrients. Nevertheless, human milk is also a pathway of maternal excretion of toxic substances including toxic metals and thus a source of infant exposure.
There is a need for reliable and updated data about mother's milk which can be used as background data for setting adequate daily intakes of many elements for infants. Indeed, nutrient levels in infant formulas, the most common substitutes for human milk, are generally modeled on the composition of breast milk with the aim to make them similar.
The aim of this study is to provide updated information on concentrations of a range of essential (Fe, Mn, Zn, Cu and Se), non-essential/toxic elements (Cr, Ni, Cd and Pb) in human milk collected from a significant group (n.195) of healthy lactating women living in Modena-Italy, together with information on maternal characteristics and dietary habits. Samples were collected between day 30 and 40 after childbirth (mature milk), and the trace element concentrations were determined by inductively coupled plasma mass spectrometry (ICP-MS) and atomic absorption spectroscopy (AAS) after microwave digestion.
Breast milk contains adequate levels of essential trace elements, despite the inter-individual variability which was not influenced by dietary habits. No difference was detected according to the country of origin, whereas milk from mothers living in the industrial area contained a higher Fe content ($p<0.02$).
Pb and Cd were detected in 75.6% and 80.6% of milk samples respectively, although none in amounts that could represent a health hazard, being well below the maximum tolerable limits set by EFSA.
The levels of essential micronutrients in lactating women living in Modena are suitable for the proper development of infants and no risk for excessive toxic elements intake was detected. Understanding the range of variation of essential elements in human milk will help to determine the most physiologically relevant concentrations to inform guidelines for supplementation and the production of infant formulae.

Safety assessment of using FeCo alloy of nanoparticles

P 56

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Increasing use of alloy of nanoparticles in various industries is an important toxicological problem. Supermagnetic nanoparticles of FeCo are an alternative to the iron oxide nanoparticles for cancer diagnosis and targeted therapy due to their larger anisotropy and coercivity. The aim of this research was to assess the safety of using FeCo alloy of nanoparticles when injecting intravenously. The study was conducted in Wistar rats (n=24). The control group was injected once intravenously with 0.9% sodium chloride solution. The experimental group was injected once intravenously with nanoparticles of FeCo (pre-suspended in water for injection) in dose 2.3 mg / kg (45 nm). Nanoparticles were obtained by the gas-phase method. Behavioral reactions were performed using the "Open field test" and the "Light-dark transition test". Biochemical and morphological parameters of blood were determined using the biochemical analyzer CS-T240 and the automatic hemanalyzer URIT-2900 Vet Plus. The results were registered on the 1st, 7th and 14th days. There was a statistically reliable decrease of the grooming by 75% ($p < 0.01$), an increase of the investigatory reaction of the organism by 60% ($p < 0.05$) comparing to the control group in the "Open field test" on the first day. There was the increase in the number of entries into the bright chamber by 100% ($p < 0.01$) and 28.6% ($p < 0.01$) in the "Light-dark transition test" on the 1st and 7th days, respectively. The time spent in the light compartment was longer throughout the experiment in the test group ($p < 0.05$). Nanoparticles have a neurotropic influence and are likely to have an anxiolytic effect. There was an increase in leukocytes by 21.6% ($p < 0.01$), lymphocytes by 12.4% ($p < 0.01$), hemoglobin by 3% ($p < 0.05$) and erythrocytes by 5% ($p < 0.01$) when injecting nanoparticles on the first day. The level of leukocytes and lymphocytes continued to increase on day 7th – by 43% ($p < 0.05$) and 18% ($p < 0.05$) higher than in the control group. The analysis of biochemical parameters showed an increase in the level of creatine by 33% ($p < 0.01$), a decrease in urea by 36% ($p < 0.05$) on the first day; a decrease in the activity of ALT by 36.6% ($p < 0.05$) on the first day, however, this indicator was higher than the control values by 35% ($p < 0.01$) by 14 days; an increase in the activity of creatinekinase by 36.6% ($p < 0.05$) on the first day. According to the data obtained earlier on the toxicity study of Fe_3O^4 nanoparticles, FeCo nanoparticles are less toxic nanomaterial (NP-Fe3O4 > NPFeCo). However, with the introduction of this dose, there were changes in the behavioral reactions; in blood tests that characterize the inflammatory reaction of the body. The results of the pilot study indicate the prospects of using FeCo alloy of nanoparticles for medical purposes.

Copper chelators. Can they also chelate zinc?

P 57

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Zinc is one of the most abundant metals in our body and is essential for many enzymes and transcription factors. Its deficiency, manifested e.g. by growth retardation, immune dysfunction and skin disorders, is caused mostly by insufficient zinc content in the diet or derangement in GIT absorption. Less is known, that zinc deficiency can follow long-life treatment by metal chelators which are mostly non-selective.
Hence, the aim of this work was to study possible capacity of clinically and experimentally used copper chelators to chelate zinc ions by using a competitive spectrophotometric method based on dithizone. The tested compounds included D-penicillamine, trientine and ammonium tetrathiomolybdate /ATTM/ which have been clinically used or tested in the treatment of Wilson's disease, and experimentally tested series of four 8-quinolinoles (8-quinolinol, 5-chloro-7-iodo-8-quinolinol, 5,7-dichloro-8-quinolinol /chloroxine/ and 5-nitro-8-quinolinol /nitroxoline/). Various physiologically relevant pH levels ranging from 4.5 to 7.5 were simulated.
All tested compounds showed the ability to bind zinc. The most potent clinically used chelator was trientine with approximately 65% zinc binding activity in the molar ratio 1:1 at pH 7.5. However, experimentally used 8-quinolinoles showed comparable or even higher binding capacity at lower pH levels. Particularly nitroxoline was a very potent zinc chelator at all pH levels. Surprisingly all of the tested compounds showed higher affinity for Zn ion in comparison with ability of D-penicillamine to bind Cu ions.
In conclusion we can assume that clinically used copper-chelators as well as ATTM and 8-quinolinoles are also relatively potent zinc chelators and hence their longer administration use can possibly result in side effects associated with zinc deficiency.

Cognitive disorders in a child population exposed to lead in the Marrakech region

P 58

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Like deficits affecting overall measures in children, lead exposure leads to more specific deficits suggestive of attention deficit disorder.
Objective: To evaluate the visual attention function among schoolchildren living in a mining area compared to a control area in Marrakech.
METHOD: We used the Bell Test (Gauthier et al., 1989) or Bell Dam Test (CBT), for the assessment of visual attention and unilateral spatial neglect (NSU) among children in school at the first and second level of basic education. Interviews with parents were conducted to identify co-variables significantly related to lead exposure.
Results: We deduced that attention is affected in these children. The omission of six bells or more to the right or left of the TBC page was found in 88.5% of children with a profile of unilateral negligence on the right side (2.5%). The scanning method chosen was disorganized in 32.5% of them. Our results are similar to those recently presented by EL Azmy et al. (2014) in children in the Mrirt area and agree with Gauthier et al. (1989) who showed that the strategy of scanning hemiplegic left is disorganized in comparison with that of normal.
Conclusion: It should be noted that a visual attention deficit could be related to a dysfunction in the posterior associative cortex in the temporal and parietal lobes of these children following exposure to lead.
Key words: visual attention, visuospatial negligence, environment, lead, child, brain dysfunction.

Trace element status of women with contraceptive procedure ESSURE®

P 59

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Background: ESSURE® (Bayer) is a permanent contraceptive procedure that uses a small implant inserted through the vagina and the cervix (by hysteroscopy) into the opening of each of the fallopian tube. The ESSURE® coils show a complex metal composition, including iron, nickel, chromium, titanium, silver, tin and platinum. The device has been linked to several serious health complications, including persistent pain, bleeding, allergic reactions possibly leading to removal surgery. Bayer stopped sales of ESSURE® in September 2018.
Objectives: The aims of this study were (1) to measure metal levels in urines, peritoneal fluid and fallopian tissues from women who underwent surgical removal of ESSURE® devices and (2) to evaluate the decrease of urinary toxic metals levels during follow-up of the patients.
Materials and Methods: This study was a sub-protocol of the single-center prospective cohort ABLIMCO study: Evaluation of symptom resolution after surgical removal of ESSURE® sterilization devices. Metal levels in urine and peritoneal fluid were compared to levels obtained from patients undergoing gynecological surgery for other indications. Metal levels in fallopian tissues close to ESSURE® device (fibrotic tissues) were compared to levels in non-fibrotic fallopian tissues. Toxic metals concentrations were determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) analysis in a PerkinElmer NexION 350.
Results: A total of 55 women with symptoms attributed to the device underwent removal surgery. The median delay between ESSURE® sterilization and removal surgery was 5.4 years. The preliminary results suggest higher levels of Cr, and to a lesser extent, of Ni in women who underwent surgical removal of ESSURE® devices.
Conclusions: The results of this study bring new aspects of the physiopathology of the symptoms associated with the ESSURE® device.

Establishment of a novel analytical sandwich assay for human Ceruloplasmin P 60

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There are several important physiological activities associated with the hepatically-derived Copper (Cu) transport protein Ceruloplasmin (CP), including iron metabolism, multi-copper oxidase, defense against oxidative stress and participation as positive acute-phase reactant. CP constitutes a secretory protein of 1046 amino acids, and accounts for 60% to 95% of total serum copper. It may thus be considered as the major Cubinding protein in blood and a suitable biomarker of Cu status.
In order to enable large scale analyses on the physiological role of CP and its relevance for health and disease, we decided to establish a robust, reliable and readily available quantification assay that shall be suitable for high-throughput CP analysis from clinical or cell culture samples. To this end, we have generated a set of monoclonal antibodies (mAb). A suitable pair for sandwich ELISA detection was selected. Using these mAb, a 96-well sandwich ELISA was set up.
The newly generated assay performance parameters were determined with a commercially available human CP standard with a purity of $\geq$ 95%. In replicates of 4, the non-linear curve-fitting yielded a goodness-of-fit measure of $r^2 \geq 0.9943$. The lower limit of quantification (LLOQ) was determined at a CP concentration of 0.10 mg/L, and the upper limit of quantification (ULOQ) at 6.78 mg/L, thereby defining the working range at CP concentrations between 0.10 and 6.78 mg/L. Typical CP serum levels in healthy or diseased subjects are in the range of 100-1000 mg/L, and thereby the assay is suitable for CP quantification form human serum or plasma. Specificity of the ELISA and the mAb pair used was verified by testing a set human sera (n=15). The data indicated a significant correlation between the CP levels determined and the total Cu concentrations, yielding a correlation with r=0.5 and P=0.0025 of CP to Cu concentrations. This result supports the notion that CP concentrations are related to total serum Cu, and that Cu concentrations and CP levels are not identical but complementary biomarkers of Cu status. In view of the low volume requirement of the newly established CP-ELISA (5 µl per sample), this ELISA is suited for large-scale cohort studies.

Autoimmunity to Selenoprotein P in thyroid patients P 61

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Autoimmunity is the major cause for two thyroid diseases, Hashimoto thyroiditis and Graves' disease, which are associated with decreased quality of life. Antibodies to thyroglobulin (Tg), thyroperoxidase (TPO), and TSH receptor (TSH-R) are of diagnostic importance in these autoimmune thyroid diseases (AITD). The thyroid gland is rich in the trace elements iodine and selenium (Se). Selenoproteins contribute to thyroid metabolism by catalyzing thyroid hormone turnover and protecting the tissues against oxidative damage. Se deficiency constitutes a risk factor for AITD. Se transport and metabolism are mediated by selenoprotein P (SELENOP), which is synthesized by hepatocytes and supplies other tissues with the essential trace element. Potential autoimmunity to SELENOP may interfere with the Se supply and impair thyroid function. We hypothesize that autoimmunity to SELENOP may contribute to the development of AITD.

A novel assay for the quantification of SELENOP-autoantibodies (aAB) was established and used to analyze serum samples from a cohort of thyroid patients (n = 257). The Se level in serum was assessed by total reflection X-ray fluorescence, serum SELENOP by a sandwich ELISA, and serum glutathione peroxidase (GPX)-activity by a photometric assay.

Prevalence of SELENOP-aAB in the thyroid patients was 9%. The SELENOP-aAB positive patients displayed higher serum Se concentrations as compared to SELENOP-aAB negative patients (94.2 $\pm$ 36.9 µg/L vs. 75.1 $\pm$ 18.9 µg/L). In parallel, serum GPX activity was also higher (128.7 $\pm$ 32.4 U/L vs. 106.4 $\pm$ 31.6 U/L), but SELENOP concentrations were not different (4.4 $\pm$ 1.4 mg/L vs. 4.2 $\pm$ 2.1 mg/L).

These results indicate that SELENOP-aAB affect biomarkers of Se status, and may be of pathophysiological relevance in diseases that are shown to be SELENOP-dependent like AITD, cancer or cardiovascular disease. This hypothesis needs to be tested in large clinical studies.

Total, bioaccessible and bioavailable concentrations of iron and manganese in açaí (Euterpe oleracea Mart.) pulps

P 62

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In the last years, açaí pulp has received much attention as one of the new "superfruits" due to its remarkable features and potential benefits. Besides, the consumption of açaí and related products like drinks and smoothies has increased in Brazil and around the world. Therefore, it is of utmost relevance to expand the knowledge about the açaí chemical composition and consumption safety. A previous work of our group observed relatively high average content of Mn (45 mg/100 g dry weight) in açaí pulps compared to the contents commonly found in traditional food sources of this element. In this work, the total, bioaccessible and bioavailable concentrations of Fe and Mn were assessed in açaí pulps. In 9 samples analyzed, Fe and Mn total contents ranged from 27.6 to 73 and from 145 to 1197 mg kg^{-1}, respectively. Fe and Mn bioaccessibilities represented from 29 to 40 and from 39 to 55% of total amounts in pulps. Fe bioavailabilities were lower than LOQ and those of Mn varied from 8 to 17% of total. A daily consumption of 100 g of açaí pulp exceeds by at least 1.5-fold the recommended Mn daily intakes for adults whereas poorly contributes to Fe intakes. Since the lowest Mn bioaccessible and bioavailable fraction corresponded to a Mn intake value higher than the tolerable upper intakes for children and that high amounts of Mn intake may impair Fe absorption, the higher açaí consumption by the Brazilian northern population may be worrisome. Future nutritional and toxicological studies on this issue must be undertaken. Moreover, more studies to separate and identify the Mn species in açaí pulp and in its bioaccessible and bioavailable fractions are required since the chemical speciation of Mn may explain how the chemical forms of this element change and/or modulate its bioaccessibility and bioavailability as well as its toxicity or benecial health effects.

Evaluation of mercury levels in blood in gestant population in the Community of Madrid, Spain

P 63

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Methyl Mercury, a potent neurotoxic, can produce harmful effects particularly affecting the development of the fetus and newborn nervous system. The main exposure route to methylmercury (MeHg) is is by eating fish and shellfish containing methylmercury. Since 2004, women of reproductive age in Spain have been urged to not eat some species (tuna, shark and swordfish...), and eat, as part of a healthy diet, fish and shellfish that contain lower levels MeHg. However, few studies have examined trends in fish consumption and Hg exposure over time in gestant women with respect to information received.
Objetives: To provide levels of Mercury and Selenium in blood and serum in a sample of gestant women of Spanish population (Community of Madrid).
To determine if the concentration of mercury varies according to dietary habits, the degree of information received by pregnant women and their age and cultural level.
Material and methods: We studied 160 voluntaries women of childbearing age (16-45 years).The subjects were interviewed about eating habits and seafood consumption (demographics variables). Mercury and Selenium were tested using Cold Vapor-Atomic Absorption Spectrometry (CVAAS) and Electrothermal Atomic Absorption Spectrometry (ETAAS).
Results: The blood mercury concentration in pregnant women was, on average, 2.89 μg/L (geometric mean, GM, 2.19 μg/L). The GM of the blood mercury concentration is positively associated with the the fish intake. There were 16 (12%) cases of pregnant women with a concentration higher than the level recommended by the EPA (6.4 μg/L THg). In addition, we have found a positively association between blood Mercury and serum Selenium.
Conclusion: Mercury total levels observed in women of childbearing age in Spain were higher than those observed in other occidental studies. These data might also be a preliminary tool for screening and assessing mercury exposure. This information is necessary for developing better prevention strategies and the need for tailored fish consumption advisories.
In the present study, we observed that 12% THg results exceeded the security limit set by Environmental Protection Agency (6.4 THg μg/L) and 32% results greater than the relevant benchmark level of 3.5 μg/L suggested by differents researchers.

Selenium Status in pediatric epilepsy Patients in Germany

P 64

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Due to the relatively poor content of selenium (Se) in European soil, the population in Germany might be Sedeficient. Se is essential for the biosynthesis of selenoproteins. Genetic disorders in selenoprotein biosynthesis may lead to developmental defects, delayed bone maturation, high sensitivity to oxidative noxae and neurological symptoms. The selenoprotein P (SELENOP) knockout mouse model displayed an impaired development and epileptic seizures under limiting dietary Se supply. This finding is supported by a few case reports of children with intractable but Se-responsive epileptic seizures. We thus hypothesize that Se deficiency might be a common finding in epileptic children.
In order to test our hypothesis, serum samples were collected from a set of 100 consecutive pediatric patients. The project was approved by the ethical committee of Charité Berlin. Serum Se concentrations were determined by total reflection X-ray fluorescence, the Se transporter SELENOP was analyzed by a sandwich ELISA, and serum glutathione peroxidase (GPX)-activity was measured by a photometric test.
Children with epilepsy (n=28) displayed a similar Se status as compared to the non-epilepsy group (n= 72) (61.3 $\pm$ 14.8 µg/L vs. 57.9 $\pm$ 12.8 µg/L). The group of pediatric patients with phacomatosis (n=25) showed a particular low Se status in comparison to the other children (n = 75). This relative deficit was mirrored in other Se status biomarkers (mean $\pm$ SD; serum Se: 53.7 $\pm$ 13.2 µg/L vs. 60.6 $\pm$ 13.1 µg/L, serum SELENOP: 3.8 $\pm$ 1.0 mg/L vs. 4.4 $\pm$ 1.1 mg/L, and serum GPX-activity: 273.2 $\pm$ 90.7 U/L vs. 319.5 $\pm$ 93.8 U/L). These results indicate that on average, pediatric patients with epilepsy do not display a marked Se deficit, and that other groups of pediatric patients may display disease-specific abnormalities in their Se profile. Future studies are needed to test whether the differences observed are relevant for the disease risk, and if a correction of the deranged trace element profile is required to positively influence the course of the disease.

Cross-sectional analysis of trace element status in thyroid patients

P 65

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Trace elements (TE) are minerals present in small amounts in living tissues. Lack of essential TE may cause deficiency symptoms, such as Keshan disease and hypothyroidism (Se and iodine deficiency, respectively). On the other hand, TE in excessive amounts can lead to toxic effects. European populations are characterized by mild to moderate iodine and Se deficiency. The TE Se is essential for selenoproteins, which protect against oxidative damage, regulate the immune system and control the activity of thyroid hormones. The thyroid gland contains high Se levels and expresses a variety of selenoproteins, implicated in antioxidant function and metabolism of thyroid hormones. An inadequate Se supply may contribute to thyroid disease. To understand whether patients with thyroid diseases have a specific TE profile and if there is a particular TE deficiency in different thyroid disorders, cross-sectional samples of serum from thyroid patients were collected and evaluated for the TE status. Serum concentrations were determined by total reflection X-ray fluorescence, the Se transporter (SELENOP) was analyzed by sandwich ELISA, and serum glutathione peroxidase (GPX)-activity was measured by a photometric test. The thyroid patients (n=257) displayed lower serum Se concentrations than a cohort of healthy subjects (n=200) (76.8 $\pm$ 21.6 µg/L vs. 85.1 $\pm$ 17.4 µg/L). In addition, patients had lower serum calcium (Ca) concentrations (111.7 $\pm$ 10.9 mg/L vs. 98.4 $\pm$ 7.1 mg/L). There were no significant differences in serum Cu or zinc concentrations. In comparison to a reference cohort of healthy Europeans, the thyroid patients displayed slightly lower serum SELENOP concentrations (4.2 $\pm$ 2.0 mg/L). Average serum GPX-activity was 108.3 $\pm$ 32.3 U/L. As expected, both serum Se and SELENOP displayed a positive correlation ($p < 0.0001$), as well as serum Se and serum GPX activity ($p < 0.0001$). The results suggest that serum SELENOP and GPX activity both are suitable biomarkers of Se status, in addition to total Se concentrations. Next, we intend to analyze whether the different thyroid diseases display particular TE profiles, and whether disease course and severity is mirrored in the circulating TE patterns.

Toxic (Mercury, Lead and Cadmium) and Essential (Selenium, Copper, Zinc and Iron) Trace Elements and their Relationship with Glomerular Filtration in Children

P 66

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BACKGROUND. Toxic trace elements and alterations of essentials have been associated with nephrotoxicity and cardiovascular disease in the general population. The aim of this study is to evaluate the concentration of these elements in children that have routine health check-ups and their association with glomerular filtration. METHODS. Blood lead and cadmium, as well as selenium in serum, were measured by atomic absorption spectrometry with electrothermal atomization with a Zeeman background correction system in an AAnalyst 800 spectrometer from Perkin Elmer. The mercury in blood was analyzed by atomic absorption spectroscopy by thermal decomposition and amalgamation in a Perkin-Elmer SMS-100 spectrophotometer. Copper and zinc were measured by flame spectrometry on a Perkin Elmer AAnalyst 200 spectrometer and the iron was analyzed by colorimetry. The glomerular filtrate was estimated by formula CKD-EPI. RESULTS. 85 children were recruited (42 boys and 43 girls) with an average age of 10.5 (SD: 2.3) years, range 7-15. The mean GF was 142.81 (SD: 12.5) ml / min. Boys had higher GF than girls (150.6 (SD: 8.5) vs 136.2 (11.6), $p < 0.001$). The median of mercury in blood was 2.2 (IQR 1.09-4.49 µg/l), of lead 1.1 (IQR 0.7-1.6 µg/dl); the mean of cadmium was 0.07 (SD: 0.05) µg/l. Cadmium was below the limit of detection in 94% of the sample. The mean concentration of selenium was 69.5 (SD:13.9) µg/l; the copper, zinc and iron concentrations were 124.7 (SD: 29.6) µg/dl, 90.1 (SD: 12.7) µg/dl and 73.4 (SD: 31.9) mg/dl respectively. No association was found between levels of trace elements and GF. However, we found that for each year of increase in age, there was a decrease in GF of 2.7 ml/min ($p < 0.001$). CONCLUSIONS. No association has been observed between toxic and / or essential trace elements and GF in the studied population of children.

Association of genetic polymorphism in biotransformation system genes and the content of essential elements in the hair of children living in industrial region in Russia

P 67

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Introduction: Modern biomonitoring content of trace elements in biological samples need to go the identification of options for genetic determination of metabolism, but there are many unknown aspects in it.
Aim: To study the relationship between the presence of allelic variants of biotransformation system genes and the content of essential trace elements in the hair of children living in industrial regions of Russia.
Methods: 86 DNA samples of children, living on the territory Chelyabinsk region were analyzed. The genotyping was performed using PCR and allele-specific hybridization on biochip, which allowed to identify 17 allelic variants in the genes CYP2D6, CYP2C9, CYP2C19, NAT2, GSTT1, GSTM1, ABCB1 (MDR1). To estimate the rate of acetylation process in the body the algorithm NAT2PRED was used. The ratio of the elemental composition of hair was carried out by the method of ISP-MS.
Results: It was found that the A allele of rs3892097 (1934G > A, c.506-1G > A) in CYP2D6 gene was more frequent in children with increased level of As compared with normal control (25% vs 13.54%, p=0.075) and in children with decreased level of Ca compared with normal group (29.55% vs. 14.84%, p=0.042). The T allele of rs1799853 (c.430C > T, p.Arg144Cys) in CYP2C9 gene was more frequent in children with increased level of Pb compared with normal control (16.07% vs. 6.03%, p=0.0482). Also the A allele of rs1799930 (c.590G > A, p.Arg197Gln) in NAT2 gene was more frequent in children with increased level of Pb compared with children having normal level (44.54% vs. 23.28%, p=0.007) and in children with decreased level of Zn compared with normal level group (50% vs. 27%, p=0.031.
Conclusion: The A allele of CYP2D6 gene (rs3892097) and the T allele of CYP2C9 gene (rs1799853) code the cytochrome variants with reduced enzymatic activity. The A allele of NAT2 gene codes the enzyme variant with slow acetylating rate. In our study, the presence of allelic variants with reduced enzymatic activities was associated with accumulation of heavy metals (As and Pb) and loss of essential microelements (Ca and Zn) in the hair of the children subjected to heavy metals exposure in industrial zones of Chelyabinsk region.

Erythrocyte/Plasma Ratio as a Biological Indicator for Exposure to Methylmercury

P 68

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Background. The benefits of fish consumption for both children and adults are well-known. However, the main source of exposure to methylmercury (MeHg) is the consumption of contaminated fish. The Environmental Protection Agency (EPA) has set limits of 5.8 µg/L for blood MeHg. MeHg is estimated to make up about 90% of total blood mercury. Thus, the corresponding level of total mercury in blood is 6.4 µg/L. Approximately 95% of MeHg is absorbed in the gastrointestinal tract. Once absorbed, MeHg enters the bloodstream in a proportion of about 20 (erythrocytes)/1 (plasma).
The aim of this study is to evaluate mercury concentration in blood, plasma and the erythrocyte/plasma rate for the exposure to MeHg from fish consumption.
Methods. We recruited 24 volunteer employees from the department of laboratory medicine from the hospital Clinico San Carlos of Madrid (5 men and 19 women) with a mean age of 39.42 (SD 14.06). A risk exposure and standardized questionnaire for fish consumption was administered to the participants in the study. Blood and plasma mercury concentrations were measured by atomic absorption spectrometry and thermal decomposition amalgamation in a Perkin Elmer SMS100. Hematocrit was calculated by an automated analyzer Coulter LH 750.
Results. The median of whole blood mercury was 6.04 µg/l (IQR=4.56-10.65); median plasma mercury: 0.94 µg/l (IQR= 0.60-1.30); median erythrocyte/plasma rate: 17.29 µg/l (IQR=14.57-19.32). Fish was consumed on an average of 9 times per month (IQR= 8-16). No statistically significant correlations between overall frequency of fish consumption and blood mercury (r=0.269 p=0.203) and plasma (r=0.226 p=0.312) were obtained. However, we found a statistically significant correlation between the frequency of fish consumption per month and the erythrocyte/plasma rate (r=0.453 p=0.034). Considering fish consumption based on groups, i.e. white, fat, cephalopods, shellfish and canned tuna, no statistically significant correlations with any of the analyzed biological indicators were observed.
Conclusions. In the present study the erythrocyte/plasma rate is a better biological indicator for exposure to mercury from fish consumption than mercury in whole blood. Taking into account that vulnerable populations, such as pregnant women and children, have low hematocrit in general, the use of this rate in these groups would be interesting.

Biological Indicators for Exposure to Mercury from Dental Amalgam

P 69

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BACKGROUND. Dental amalgam has been widely used and consists of approximately 50% of metallic mercury. Mercury can be released from amalgam as mercury vapor and inhaled or in a particulate form and swallowed. There are some factors that influence the rate of release of mercury vapor from amalgam, being stimulated, among others, by hot liquids, the number of amalgam surfaces, nicotine-containing chewing gum, bruxism and bleaching agents. The aim of this study is to evaluate the concentration of total mercury in whole blood, plasma, erythrocyte/plasma ratio and urine as well as their suitability for mercury exposure assessment in dental amalgam.
METHODS. We recruited 24 volunteer employees from the department of laboratory medicine from the hospital Clinico San Carlos of Madrid (5 men and 19 women) with a mean age of 39.42 (SD 14.06). A risk exposure and standardized questionnaire was administered to the participants. Blood mercury concentrations (µg/l) were measured by atomic absorption spectrometry and thermal decomposition amalgamation in a Perkin Elmer SMS100. Hematocrit was calculated by an automated analyzer Coulter LH 750.
RESULTS. The median of whole blood mercury was 6.04 µg/l (IQR=4.56-10.65); median plasma mercury: 0.94 µg/l (IQR= 0.60-1.30); median erythrocyte/plasma ratio: 17.29 µg/l (IQR=14.57- 19.32) and the median of urine mercury was 1.10 µg/l (IQR=0.91-1.43). Overall, 10 subjects had dental amalgam with a median of 3.5 amalgam fillings (IQR= 2.8- 5.5). Median of urine mercury was higher in the group with dental amalgams, but not statistically significant (1.39 µg/l IQR= 0.91 – 2.22 vs 0.99 µg/l IQR= 0.76 – 1.21 p=0.061). In spite of the small sample size of this preliminary study, we observed a positive correlation between the number of dental fillings and the concentration of mercury in urine referred as per creatinine (r= 0.419 p= 0.041).
CONCLUSIONS. In this study, concentration of mercury in urine referred as per creatinine is the best biological indicator for exposure to mercury from dental amalgam.

Bioaccessibility and total content of ultra-trace elements in Brassica rapa lines cultivated in southern Spain

P 70

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Introduction: Nowadays, the adaptation of vegetable varieties to new climatic conditions is one of the main challenges facing agriculture. Vegetable species belonging to Brassiceae genus (among them *Brassica rapa*) are considered as one of the first cultivated and domesticated plant groups. Brassica vegetables can be a proper source for some nutritional relevant ultra-trace elements with a high bioaccessibility due to its low content of chelanting agents such as oxalic acid and phytic acid.
Material and methods: Four *Brassica rapa* lines selected with respect to their production capacity of turnip tops production and the different length of their productive cycle have been cultivated in the edaphoclimatic conditions of the South of Spain. Cr, Ni and Co contents in *Brassica rapa* were analyzed by ET - AAS. Ultra-trace element bioaccessibility was determined by a process based on mimicking the physiological conditions of the gastrointestinal tract. Results and discussion: Cr content ranged between 561 – 1555 µg/Kg. Regarding Ni, concentrations of this trace element ranged between 245 – 1232 µg/Kg. Statistically significant differences were found between the four varieties for both trace elements studied. Finally, Co contents ranged between 46 – 120 µg/Kg. Cr bioaccessible concentrations ranged between 73 – 131 µg/Kg with a higher uniformity among analyzed samples. Cr bioaccessibility in *Brassica rapa* varieties showed medium percentages around 12%. It was also observed a high bioaccessibility of Ni present in Brassica rapa with percentages close to 100% for the four lines. Co bioaccessible concentrations were negligible. A simulation software (@Risk) was used to determine the contributions of Brassica rapa lines to Dietary References Intakes (DRI) for these elements. It was found that intake between 15 – 20 g of these dehydrated vegetables provided for 50th percentile, 22.2 µg of Cr (widely covering the DRI of this micronutrient) 11.1 µg of Ni or 1.47 µg of Co (covering DRI of vitamin B12).

CE-ICP-MS enables accurate quantification of metal-glycinates-sulphate complexes in feed

P 71

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Traceability of metal-glycinate-sulphate complexes in feed requires specific analysis to differentiate complexes from inorganic forms. The method using CE-ICP-MS developed by Vacchina et al. (2013) focused of the quantification of metal-glycinate-sulphate at one single concentration but did not focus on the quantification of inorganic form. The objective of this work was to extend the method to quantify both organic and inorganic forms of various metals at low inclusion levels. A 50/50 w/w mix of corn flour and soybean meal was used as feed. Copper-glycinate-sulphate (CU-GLY), Manganese-glycinate-sulphate (MN-GLY) and Zinc-glycinate-sulphate (ZN-GLY) complexes (provided by Pancosma SA) were used for in-feed inclusions. The feed metal background concentrations and species repartitions were assessed. CU-GLY was spiked on feed at levels matching 5, 15 and 45 ppm, corresponding to metal concentrations of 1.2, 3.6 and 10.8 ppm. MN-GLY and ZN-GLY were spiked at 15, 45 and 100 ppm corresponding to 3.3, 9.9, 22 ppm Mn and 3.9, 11.7, 26 ppm Zn, respectively. The un-supplemented feed contained 0.06 ppm Cu, 0.05 ppm Mn and 0.12 ppm Zn, where 69.9% of Cu, 100% of Mn and 1.77% of Zn were present under inorganic metal species and where 30.1% of Cu was present under CU-GLY and 98.23% of Zn was present under ZN-GLY. The supplemented feeds at the 3 tested doses, from the lowest to the highest inclusion levels, contained in total respectively: 1.1, 3.05 and 9.06 ppm Cu; 2.99, 8.9 and 18.2 ppm Mn; 3.72, 10.9 and 23.4 ppm Zn. The metal-glycinates species recovered by analysis within the different supplemented feeds ranged from 76.26 to 89.32% for CU-GLY, form 94.5 to 98.51% for MN-GLY and from 76.05 to 98.96% for ZN-GLY. These results showed that CE-ICP-MS technique can be used to quantify low doses and to measure metal-species repartition between metal-glycinate and inorganic metals, when included in feeds. For the first time, this study highlighted that the raw materials used contain metal-glycinate compounds. This raises the question of the occurrence of these compounds within the different raw materials used in feed production that could dramatically affect the way to supplement minerals in animal feed.

Can solubility be used to predict availability? The example of zinc, selenium and manganese in salmon diets

P 72

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Zinc (Zn), selenium (Se) and manganese (Mn) are naturally present in ingredients of salmon diets. However, the amount present is not always enough to cover requirements and some compounds in the ingredients can reduce mineral availability. Thus, Zn, Se and Mn are usually supplemented to diets as inorganic or organic forms to ensure that the nutritional requirements of Atlantic salmon are met. Choosing mineral sources with higher availability can reduce the amounts added to the diets. Consequently, there is an increasing interest of investigating the availability of inorganic and organic mineral forms. Dietary mineral availability is usually studied in vivo but having a fast and reliable method for estimating dietary mineral availability can greatly facilitate the diet composition process and evaluation of new feed ingredients. Mineral availability depends in part on their solubility at the point of contact with the absorbing sites. This study proposes an *in vitro* digestion method (acidic and alkaline hydrolysis). The method was applied to evaluate the solubility of inorganic and organic forms of Zn, Se and Mn in diets for Atlantic salmon. An inorganic mineral (IM) diet was supplemented with zinc sulphate, sodium selenite and manganous sulphate and an organic mineral (OM) diet was supplemented with zinc chelate of glycine, L-selenomethionine and manganese chelate of glycine. The results showed that for both diets, solubility of Zn was low in both the acidic hydrolysis and in the alkaline hydrolysis. For Se, the solubility was higher in the OM diet compared with the IM diet. Whereas for Mn, the solubility in the acidic hydrolysis was higher in the IM diet than the OM diet. The solubility of Zn, Se and Mn was influenced both from the chemical form of the mineral supplemented in diet and the gastrointestinal environment. The solubility results were compared with availability measurements of Zn, Se and Mn from a trial with Atlantic salmon. The results showed a correlation between solubility and availability for Mn but not for Zn or Se. Still, the effect of the chemical form of the minerals was similar for Zn, Se and Mn solubility and Zn, Se and Mn availability.

Homeostasis of age associated trace elements in the nematode *C. elegans*

P 73

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Trace elements (TE) such as manganese (Mn), iron (Fe), copper (Cu), zinc (Zn) and selenium (Se) are involved in several physiological systems like enzymatic reactions, signaling pathways and immune response. Therefore, excessive exposure can lead to health issues. Accumulation of metals, such as Mn in the human brain, may result in a type of neurodegeneration, resembling the symptomatology of Parkinson's disease (PD). Since humans generally do not consume isolated nutrients, interactions between TE, such as Mn and Zn under physiological conditions, are of central importance. Therefore, the impact of an altered homeostasis of these TE in the context of PD was investigated in this study.
Due to its fast life cycle, highly conserved signaling pathways and a complete characterized genome, the nematode *Caenorhabditis elegans (C. elegans)* is a fitting model organism for investigations in the homeostasis of Mn and Zn. Here, eggs of wild type (WT) and genetically modified PD mutants were hatched on and fed by Mn and Zn enriched *Escherichia coli (E. coli)* covered agar plates. To investigate changes in the bioavailability of TE during aging, we compared L4 larvae that were hatched on TE enriched conditions. In addition, chronically Mn- and Zn-exposed larvae, well as the combination of both TE via nutrition, were raised until they reached day 5 of adulthood. Inductively coupled plasma tandem mass spectrometry (ICP-MS/MS) analysis showed increased TE concentrations in middle-aged worms (day 5 of adulthood) compared to young ones (L4) following TE exposure. There is also evidence that exposing worms to Mn and Zn alters the homeostasis of Mn as well as the homeostasis of Zn to different extends. It can be assumed that PD mutants differ partly, as TE intake seems to be higher in worms exposed to Mn and Zn as compared to WT worms. An altered homeostasis in PD mutants is also addressed in current studies. These results highlight the lack of knowledge in this field and thus the need for further mechanistical studies to clarify the relationship of Mn and Zn homeostasis in PD's etiology. Therefore, endpoints such as oxidative stress will be evaluated in future studies.

Syngeneity of metallic elements in biomass and its impact on the adsorptive properties of lignocelluliosic biomass-derived biochar

P 74

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Despite considerable reduction in hydrologic and atmospheric emissions, the consumption of metallic elements (MEs) is increasing and, therefore, their amount in the products used and in the waste streams is also growing. Therefore, the level of the environmental pollution of MEs and their bioaccumulation in the living matter, have increased. This is a new syngenetic mechanism of MEs penetration into biomass. The increase in the amount of MEs in biomass and its products, which can be used for decreasing the level of pollution, causes the changes in their properties. The growing demand for organic products in the market promotes their use in various fields. One of such products is biochar. Among the innovative environmental applications, biochar has the potential as an adsorbent for retaining contaminants in environmental engineering and agrotechnical systems. Natural lignocellulosic and biochar composition variations would lead to a new field of application of biochar and reduce resources for biochar modifications. The aim of this study was to determine influence of syngenetic MEs on the adsorptive properties and adsorption performance of lignocellulosic biochar. Evaluating biochar as a potential adsorbent of inorganic pollutants, its valuable adsorbing properties and the influence of syngenetic MEs on their strengthening will be discussed.

Selenium speciation in the aquaculture feed chain

P 75

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Selenium (Se) is an essential micronutrient for both vertebrate and fish. For farmed fish, such as Atlantic salmon (*Salmo salar*), the major source of Se is the feed. Today's fish feed (in Norway) for Atlantic salmon contains an average of 0.8 mg/kg, ranging from 0.3 to 1.8 mg/kg [1]. Selenium can be present in different chemical forms, or Se species, that have different physiochemical properties affecting the uptake and the toxicity of the element.
The levels of Se in fish feed depends on the levels of Se in the feed ingredients, e.g. natural high levels of Se in fish meal or plant meal cause high levels of Se in the complete feed. Supplementation of of Se is regulated through European Union regulations with a maximum level of 0.5 mg/kg feed [2], and the addition of organic selenium (Se-yeast) is limited to a maximum level of 0.2 mg/kg feed [3,4]. To control whether feed comply with the legislation there is a need for an analytical method that can discriminate between organic and inorganic selenium species in feed. Furthermore, Se speciation analysis of feed ingredients, feed and salmon tissue can contribute to a better understanding of the species related transfer of Se in the aquaculture feed chain.
Selenium species were determined using High Pressure Liquid Chromatography coupled to Inductively Coupled Plasma Mass Spectrometry (HPLC-ICPMS). Two analytical methods, using anion-exchange for the determination of inorganic Se species, and cation-exchange for the determination of organic Se species, were applied. Different procedures were evaluated for the extraction of inorganic and organic Se species. Results from the analysis of feed ingredients, fish feed and muscle of Atlantic salmon will be presented, and discussed with regards to analytical challenges.

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