Monitoring of Persistent Organic Pollutants in human milk and blood

Anton Kočan, Jana Klánová

Matrices selected for the GMP

- **The COP has decided that the air monitoring and human exposure through breast milk or maternal blood will be used as core media for the first evaluation planned in 2009.**

- **For future evaluations, the COP has also decided to endeavour to supplement the core data with data from other media such as biota, water, soil, and sediments.**

What is Human Biomonitoring (HBM) ? (1)

- **HBM is the assessment of human exposure to chemicals present in air, water, soil, dust, food or other environmental media via measurement of the chemicals or chemical metabolites present in human specimens, such as blood, urine, hair, saliva, feces, tissues. The result of such measurement is usually called „body burden“.**
- **HBM is a tool to help better understand human exposure to environmental chemicals – both natural and man-made.**
- **If gathered from a representative sample of a population – for instance, children or adults in a particular area – HBM can be used to document whether that subgroup as a whole has been exposed to some chemicals and to what extent.**

What is Human Biomonitoring (HBM) ? (2)

- **HBM is a scientific technique for assessing human exposures to environmental agents and their effects, based on sampling and analysis of an individual´s tissues and fluids. While blood, urine, breast milk and expelled air are most commonly measured, hair, nails, fat, bone and other tissues may also be sampled.**
- **This technique takes advantage of the knowledge that environmental agents that have entered the human body leave markers reflecting this exposure. The marker may be the agent itself or a breakdown product, but it may also be some change in the body resulting from the interaction of the agent or its breakdown product(s) with the individual, such as alterations in the levels of certain enzymes or other proteins which may lead to modifications of normal body processes.**

HBM can be used to :

- **Reinforce regulatory actions by providing actual data about which chemicals get into people and at what levels.**
- **Improve exposure assessment.**
- **Establish baselines or reference ranges.**
- **Facilitate people's right to know what chemicals are in their bodies.**
- **Establish priorities for tackling environmental health-related problems.**

Some variables affecting HBM (2)

- **The precision of chemical analysis is generally believed to constitute only a minor part of the total variance in monitoring time-series of environmental data where sample variation is expected to be large, much larger compared to laboratory precision.**
- **That is true if the same accredited laboratory is used through the whole series. However, if, from year to year, different laboratories carry out the analysis, it could seriously decrease or disable the possibility to evaluate time-series of, for example, POPs.**
- **The same is true if the same laboratory changes its methodology and, for example, co-elutions are resolved leading to a decrease in estimated concentrations unless measures are taken to compensate for them.**
- **If detection limits are improved, i.e. analytes are now found where they were not detected before, that may lead to similar problems depending on how results below the limit of quantification (LOQ) are treated.**

Some variables affecting HBM (1)

- **Fat content and composition in human milk changes dramatically during the first weeks after birth, which leads to variation also in analysed POPs. In order to reduce random variation, sampling should preferably be carried out during a well defined period, e.g. 3 weeks after birth. Also the fat content varies considerably depending on whether sampling is carried out in the beginning or at the end of the feeding session.**
- **The use of narrow sampling unit definition implies that a smaller part of the studied population is represented. Often, this leads to unfounded assumptions of similar trends, e.g., for both sexes or for various age classes. To improve representativity, if economy permits, stratified sampling should be applied rather than allowing for a wider definition of the sampling unit.**

Ethical issues relevant to HBM

- **Ethical issues are relevant in almost every facet of human biomarker research from the design of the studies, the identification and recruitment of subjects, to the handling and use of the data, and interpretation and communication of the results.**
- **The right to monitor chemical pollutants in blood and breast milk is a crucial aspect of community right to know but also brings with it responsibilities to care and support those who are tested. .**
- **Researchers and regulators have to be aware of the potential for biomarker information to affect the lives of subjects and their families. There must be sufficient protection of personally identifiable data and regulation of its use, while ensuring individual subjects right to know their results.**

Number of samples needed

- **More samples provide more precise and reliable estimates of mean concentrations and variance. However, the contributions from additional samples depend to a very high degree on the sampling strategy.**
- **Using pooled samples of several specimens will decrease the number of chemical analyses required to estimate a reliable mean concentrations compared to one or a few individual samples, since a larger proportion of the total population is represented. Disadvantages with pooled samples are that extreme values from single specimens may influence the concentration of the pool without being revealed.**

General considerations pertaining to GMP sampling (1)

- **All sampling should follow established methodological guidelines, which should be agreed upon before the start of any programme activity in a region.**
- **If possible, samples in all programmes should be numbered in the same way.**
- **The sampling window for the initial baseline will be 2003 ± 5 years.**
- **Sample frequency and timing should, as much as possible, be harmonized between matrices.**
  As a rule, samples should be taken at least annually and during the same period every year.
  For some matrices where seasonal influences would be less important (e.g. human breast milk), the sampling frequency and duration might be different.
  For the statistical analysis of the levels it would be preferable to take many samples frequently from one location rather than to take a few samples from many different locations.

General considerations pertaining to GMP sampling (2)

- **Sampling should always include field or trip blanks and, to the extent possible, duplicate samples for the purpose of sample sharing and the analysis of variance.**
- **Sample banking should be considered for all samples. Sample banking is an expensive and resource intensive activity that needs to be sustainable in a long time perspective. However, if properly managed it may yield important insights into exposures over time (e.g for new POPs) and may also be used for retrospective studies.**

A sample bank at the Slovak Medical University in Bratislava

Human milk and maternal blood as matrices for the GMP (1)

- **Human milk and maternal blood are both good sample media for assessing POPs exposure in humans.**
  Furthermore, both these media can be used to demonstrate possible temporal trends and regional variations in levels.
- **Human milk sampling is non-invasive and milk can generally be obtained from lactating mothers in reasonable quantities. In certain populations it may, however, be difficult to obtain human milk samples in the required time period, i.e., 2 to 4 weeks after delivery.**
- **Blood sampling is invasive, but sampling of mothers prior to giving birth may readily be achieved. However, blood sampling may not be acceptable in certain cultures.**
- **Depending on local considerations, biological samples of human origin, including blood and milk, should be considered a potential biohazard. Necessary precaution procedures should be applied to both sampling and handling of all samples, not only in situations where one may expect a problem, e.g. HIV-positive serology and hepatitis.**

Human milk and maternal blood as matrices for the GMP (2)

- **The limit of detection for POPs will in general be lower in milk than in blood.**

  The reason for this is partly the difference in lipids between the media and the fact that larger volumes of milk as compared to blood can normally be obtained.

  When the limit of detection is approached the analytical precision will decrease.

- **An important consideration in the choice of human milk and maternal blood as biological indicators is that we will only obtain information from a specific part of the population both with regard to gender and age.**

  As for future trend studies, a careful evaluation should be done to explore alternative representative groups in a population, e.g. men (specified age groups), youth groups of both gender, school children or infants.

Human milk and maternal blood as matrices for the GMP (3)

- **A population study must be based on sampling and analyses of individual samples; human milk or maternal blood.**

  Pooled samples might be considered for certain contaminants, such as the dioxins which are expensive to analyze and need larger sample volumes.
- **In order to reduce sample variance and facilitate comparability a stratified sample design should be adopted.**

  This should be based on demographic information collected in specific questionnaires, i.e. age, residence, occupational history, smoking habits, current and previous diet, etc.
- **Selection of study groups should be based on known exposure patterns, global or local.**

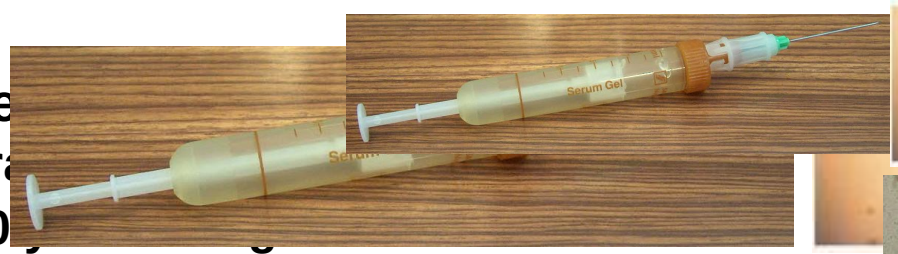
  The groups with known high exposure levels are more sensitive to changes in the environment and will provide better indications in trend analyses. Even in countries with very limited background information one might be able to select population groups of interest, such as rural versus urban; fish eating populations versus rural agricultural populations with high exposures to pesticides; populations living in areas with re-introduction of DDT for malaria prophylaxis etc.

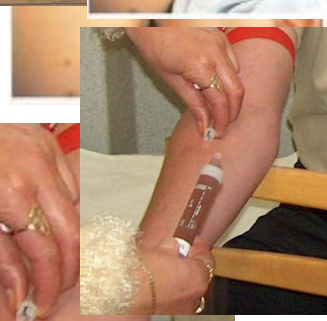
Human milk and maternal blood as matrices for the GMP (4)

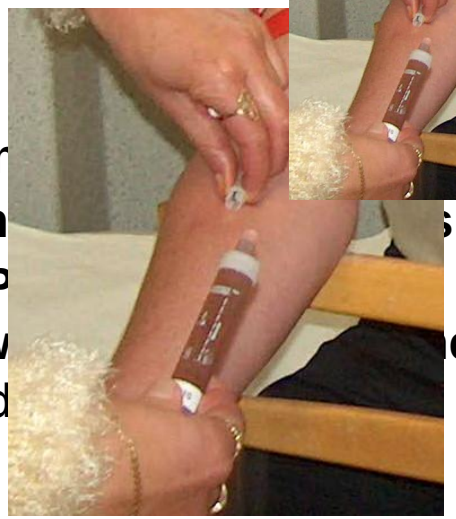
- **Sample size will depend on the circumstances, and to estimate the number of samples needed a number of factors have to be considered to achieve representative samples. For either human milk or blood, 50 individual samples are to be collected. However, new technologies and new, certified laboratories will provide the opportunity to begin epidemiological studies with individual results on a larger scale.**
- **The choice of milk or blood depends very much on the practical implementation regionally or locally. Two examples:**
  - ✓ **In the Arctic many indigenous women deliver their babies and go home to the tundra before they have started their milk production. To collect colostrum provides a very different medium than the fully developed breast milk 2-3 weeks after delivery. It is not possible to trace the women at the right time for breast milk collection. A blood sample will solve that problem.**
  - ✓ **In certain areas of Africa sampling of maternal blood might be problematic. In those cases breast milk is the best matrix.**
- **Trained personnel is crucial at the sampling and analytical stages. Standardized protocols, equipment and education of field personnel as well as laboratory personnel must be implemented.**

Human milk and maternal blood sampling

- **Human milk should be sampled according to a WHO protocol.**
- **Maternal blood should be sampled according to an AMAP protocol.**
- **At least 50 individual milk (blood) samples should be collected. But countries with populations over 50 million should include at least one additional participant per one million population over 50 million. Countries with populations well over 50 million (or with sufficient resources) are encouraged to prepare a second pooled sample (or more) if feasible.**
- **Selection criteria for mothe**
  - ✓ **Mother should be primipara**
  - ✓ **Mother should be under 30**
  - ✓ **Both mother and child should be apparently healthy, normal pregnancy**
  - ✓ **Mother should be breastfeeding one child only (i.e., r**
  - ✓ **Mother should have resided in the area for at least th**
  - ✓ **Mothers who may have unusually high exposure to P vicinity of incinerators, pulp or metal industiries or w chemicals have been produced) should not be includ avoid skewing the results.**







How to identify possible donors (1)

- **Selection before giving birth:**
  - ✓ **Possible donors can be contacted before giving birth In countries with adequate pre-natal coverage.**
  - ✓ **All potential donors should be informed about the benefits of breastfeeding and be encouraged to breastfeed even if they do not intend to or are not selected to participate in the survey.**
  - ✓ **Once a participant indicates a willingness to take part in the survey, she should be invited to complete certain section of the questionnaire.**
    The questionnaire can be completed through a personal interview at the pre-natal clinic or completed by the potential donor at home and returned to the clinic, either in person or by mail.
  - ✓ **Depending on the homogeneity of the population, up to 250 completed questionnaires should be collected. Depending on the country, more than 50 potential and 10 reserve donors should be selected to take into account possible withdrawals. Participants should be notified of their selection and where and when the sample will be collected.**

How to identify possible donors (2)

- **Selection after giving birth:**
  - ✓ **Samples are collected of mothers at postnatal clinics and other venues, e.g., well-baby clinics without pre-selection.**
  - ✓ **Mothers can be contacted directly and interviewed to complete all section of the questionnaire.**
  - ✓ **Samples can then be collected immediately or at another appropriate time.**
  - ✓ **While this method can reduce the time of the survey by up to 3 months, it does not allow for further stratification of the cohort to reduce variability.**
  - ✓ **However, after the cohort selection criteria have been established from the first sample collection, this method offer advantages for the second and subsequent sample collections.**

Collection of samples

- **Sampling can be carried out between 3 to 8 weeks after delivery.**
- **At least 50 ml of milk in total should be collected by hand expression after a feeding or while infant is nursing on the other breast, to take advantage of the let-down reflex of the mother.**

  A human milk pump to facilitate expression can be applied.

  If necessary, the mother may collect the sample at home, in which case manual expression is preferred. If so, she should be given detailed instructions for sampling, storing and transporting of milk samples.

  Mothers should also be given a clean glass jar with a protected screw cap to collect and store the milk sample.

- **The sample sh ectl collected at home, store in t be delivered.**

  Otherwise milk red . °C for a maximum of 72

  If refrigeration let chemically sterilize the mi col ay be placed in the c is g

  CAUTION: The m eep ate away from other childr this

Questionnaire for mothers donating breast milk (1)

QUESTIONNAIRE FOR POTENTIAL HUMAN MILK DONORS

Fourth WHO-Coordinated Survey of Human Milk for Persistent Organic Pollutants

CONFIDENTIAL!

Section 1: Personal Information

Name	Phone number	Today's Date (dd/mm/yyyy)
	e-mail	
Address		

Section for National Coordinator

Individual Identification Code		Pool Identification Code	

Is the participant eligible? Yes ☐ No ☐

What is the status of donor in regard to the survey?

Selected ☐ Alternate ☐ Not Selected ☐

If this mother has been pre-selected to donate a sample (or is designated as an alternate), the top of Section 4 should be completed and detached from this questionnaire. Section 4 should be sent to the clinic to be completed at the time of sample collection.

Section 2: Screening Questionnaire

Name of Interviewer:	Date of interview (dd/mm/yyyy):

Place of interview:

1. Are you planning to breastfeed your child? Yes ☐ No ☐

2. Is this your first child? Yes ☐ No ☐

3. Are you expecting a single child? (not twins) Yes ☐ No ☐

4. Are you having a normal healthy pregnancy? Yes ☐ No ☐

5. Have you lived in your current area for 10 years? Yes ☐ No ☐ Actual _____

6. Are you under 30 years of age? Yes ☐ No ☐

If yes, date of birth _____

7. Do you live near incinerators, pulp and paper industries, metal industries or where chemicals are produced Yes ☐ No ☐

Questionnaire for mothers donating breast milk (2)

Section 3: Health History Questionnaire

Date of Birth (dd/mm/yyyy)		Age	
Height (cm)		Weight before pregnancy	

1. What is your expected delivery date (dd/mm/yyyy)?

2. Where have you been residing during last 10 years:

urban (city) ☐ rural (countryside) ☐

3. How would you describe your dietary habits before pregnancy?

Mixed diet ☐ Vegetarian but with milk and eggs ☐

Strictly vegetarian ☐ Other ☐

4. How often, on average, did you eat following foods before pregnancy?

	Fish and fish products (e.g. tuna salad)	Marine mammals (e.g. whales, dolphins)	Seafood other than fish and marine mammals (e.g. shrimps, mussels)	Milk and milk products (e.g. cheese, butter, cream, yogurt)	Meat and poultry and derived products (e.g. sausage)	Eggs
Never						
Less than once a week						
Once a week						
Twice a week						
More than twice a week but not every day						
Every day						

4.1 What types of fish do you consume most often?

Fish from the sea ☐ Freshwater fish ☐ Both ☐

Please state the species if known :

5. Was your mother born in this country?

Yes ☐ No ☐

6. Were you breastfed?

Yes ☐ No ☐ Do not know ☐

If you know, for how long? _____

7. Were you engaged in work other than housework before pregnancy?

Yes ☐ No ☐

If yes, please state the duration and describe type of work :

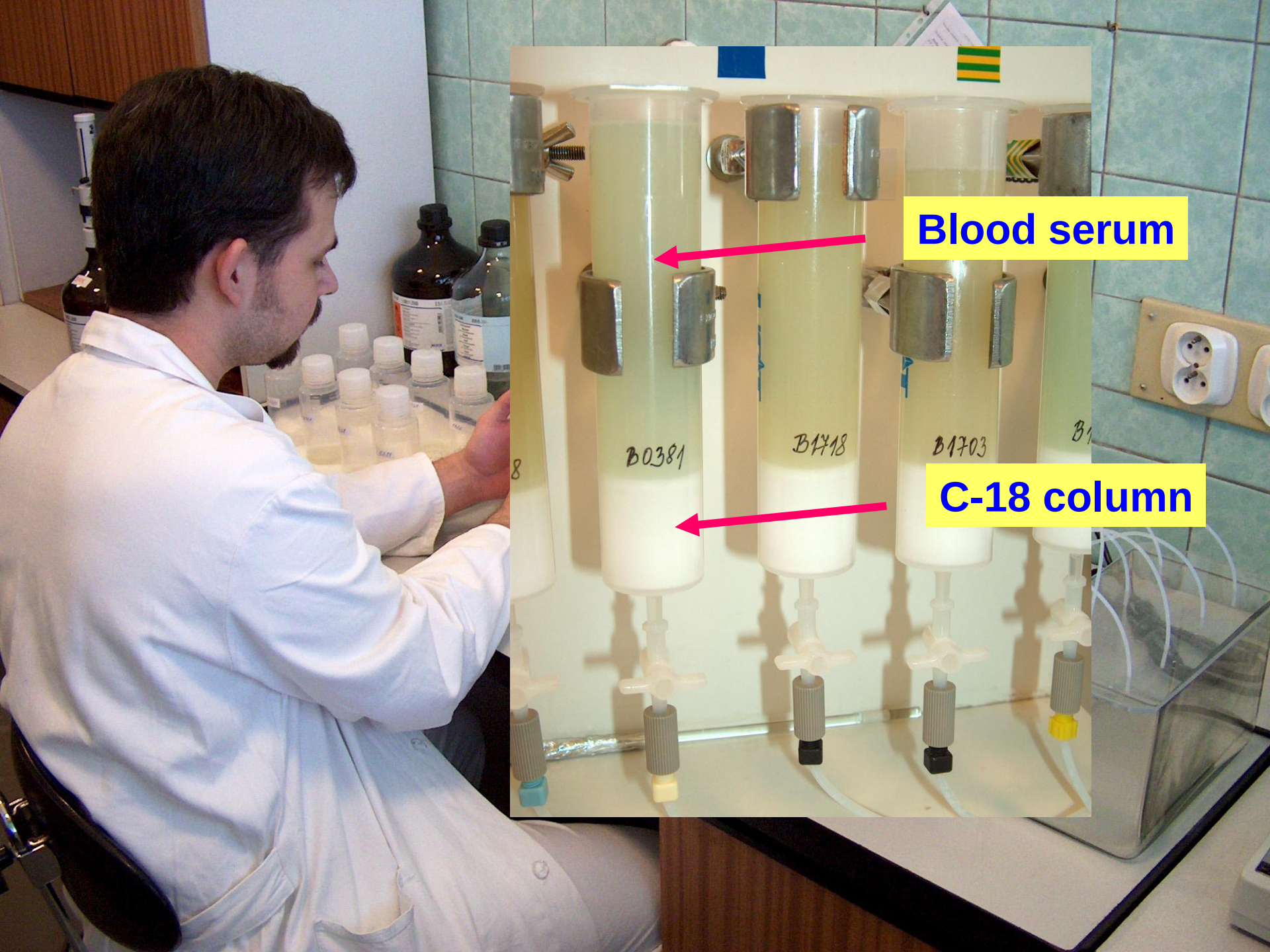
8. Has the inside of your house been sprayed with DDT in order to prevent mosquitoes?

Yes ☐ No ☐ Do not know ☐

If yes, when? _____

PCDD/PCDF/PCB analysis (1)

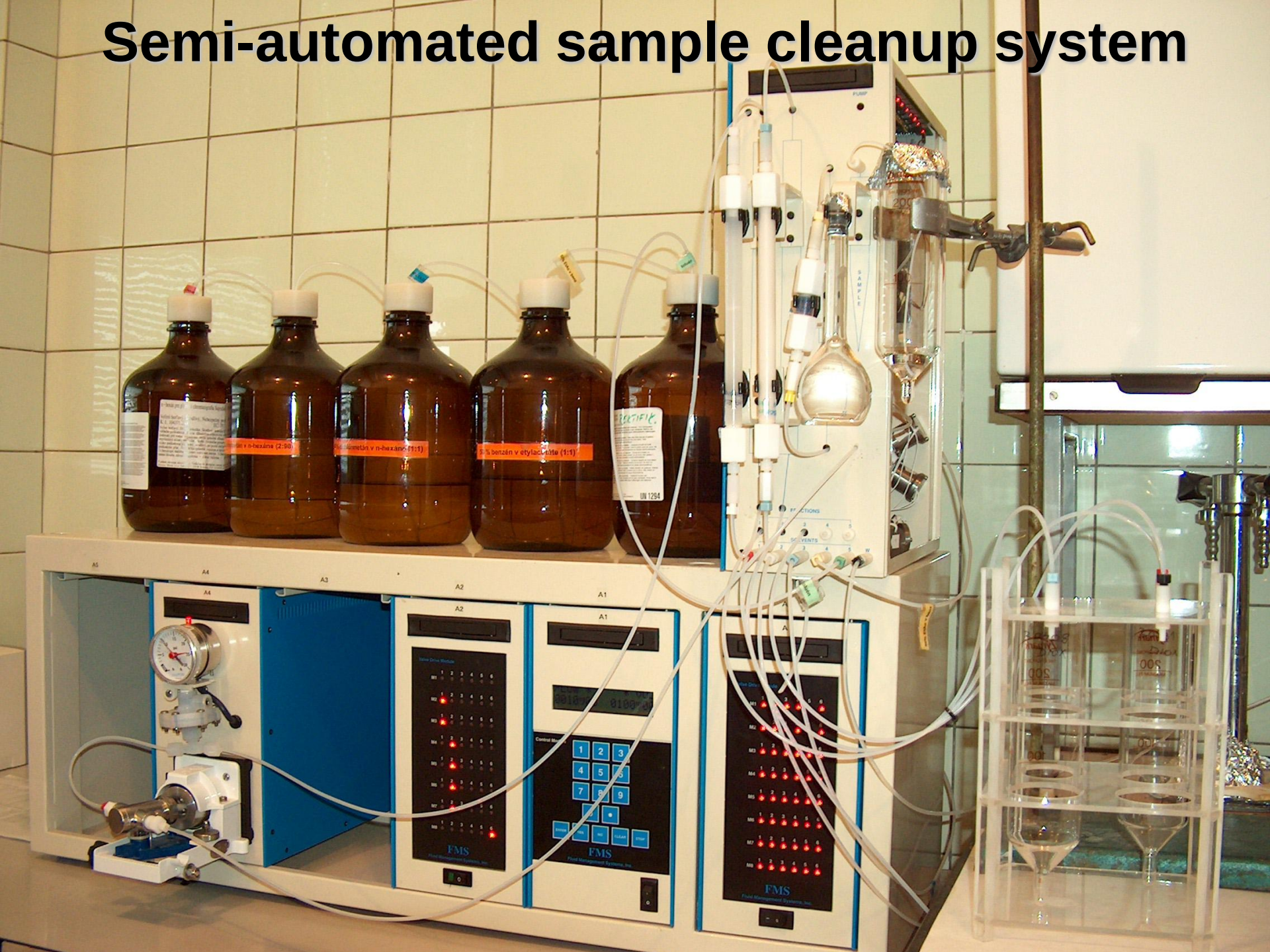
- **SPE (10 g C-18 column), 10 - 30 mL serum**

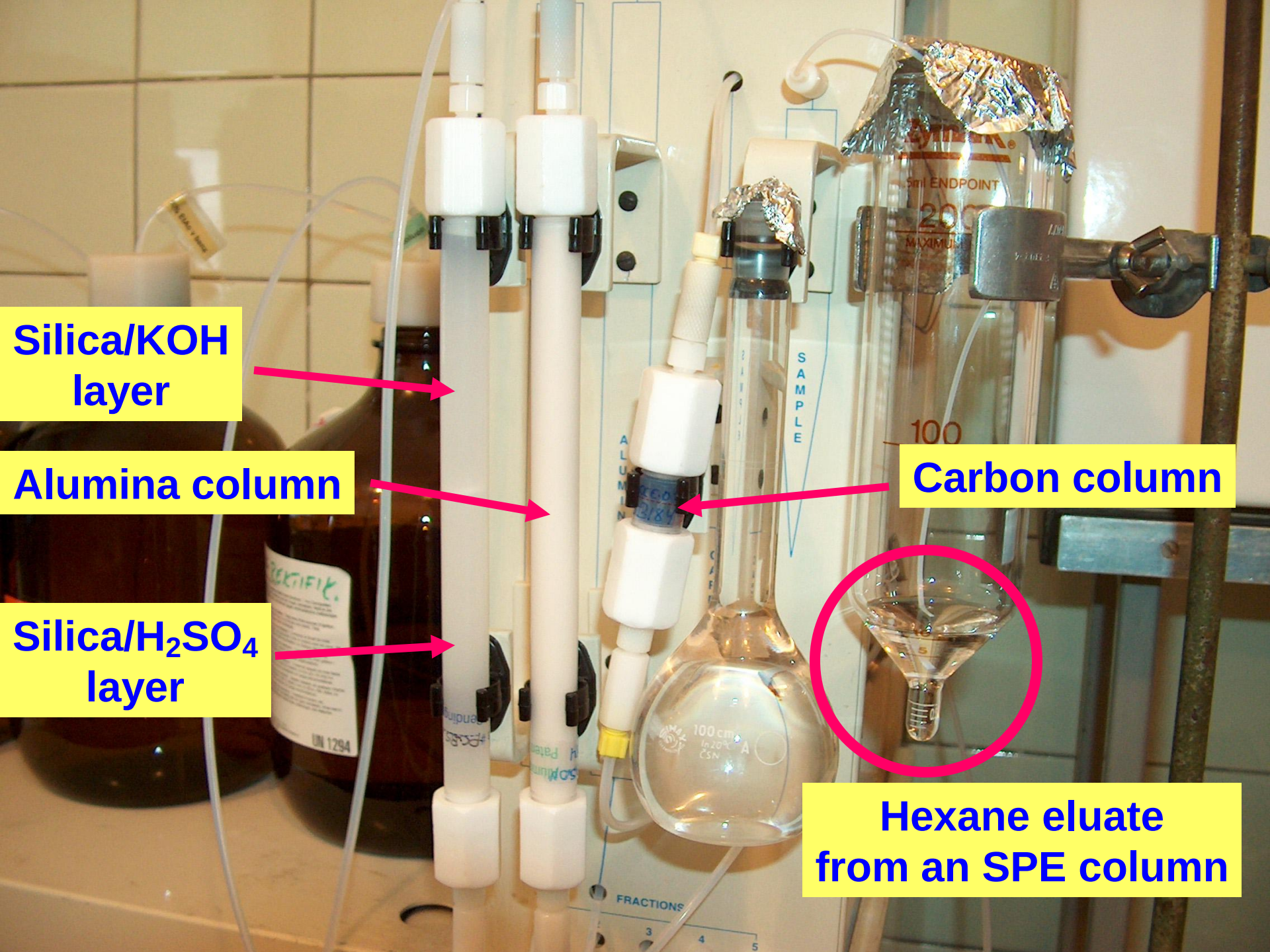

Blood serum
C-18 column
B0381
B1718
B1703

PCDD/PCDF/PCB analysis (1)

- **SPE (10 g C-18 column), 10 - 30 mL serum**
- **Clean-up using semi-automated equipment (H_2SO_4/silica, alumina, carbon chromatography) :**

Semi-automated sample cleanup system




Silica/KOH layer
Alumina column
Silica/H_2SO_4 layer
Carbon column
Hexane eluate from an SPE column

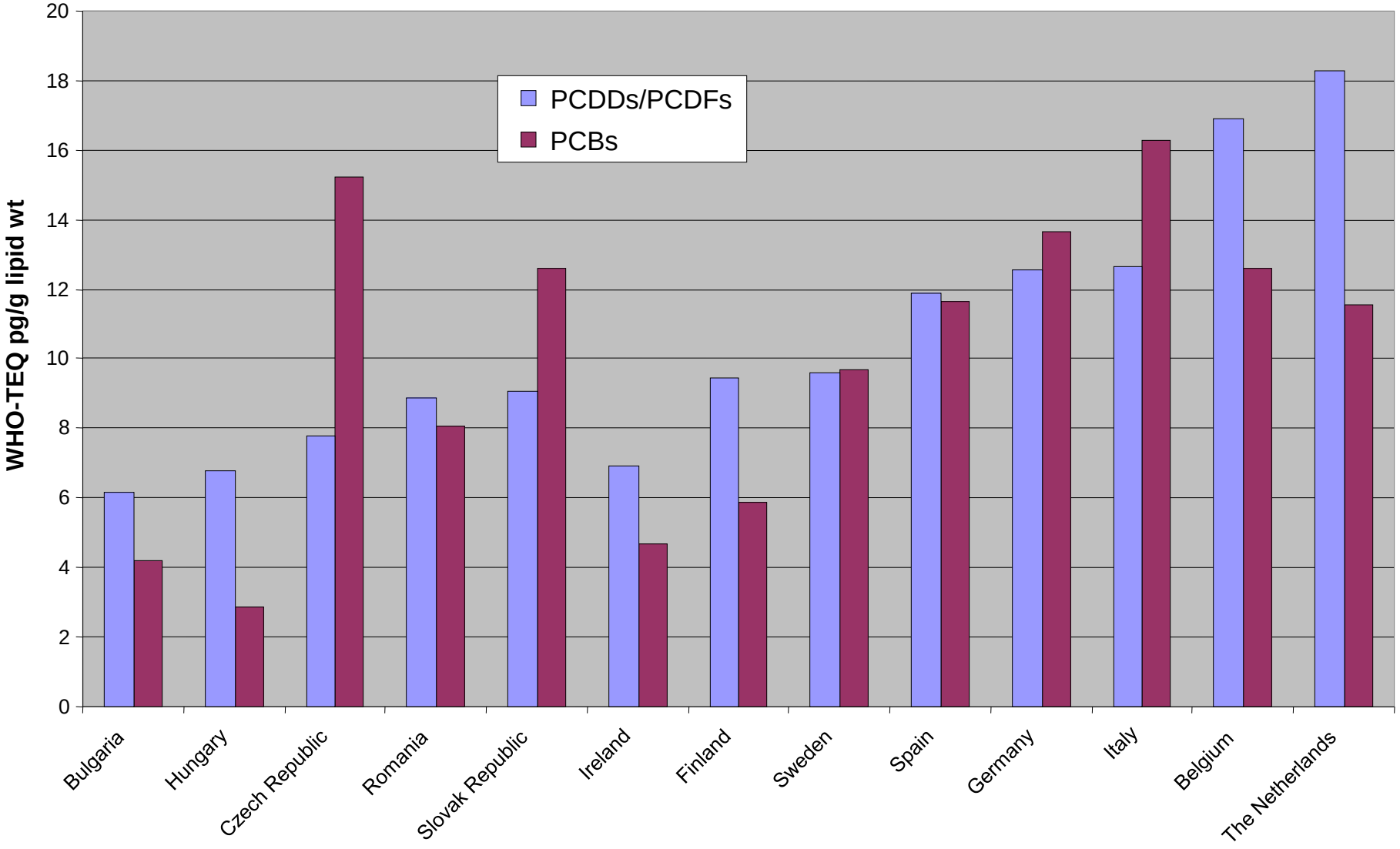
PCDD/PCDF/PCB analysis (1)

- **SPE (10 g C-18 column), 10 - 30 mL serum**
- **Clean-up using semi-automated equipment (H_2SO_4/silica, alumina, carbon chromatography) :**
  - **Fraction 1:**
    - **WHO mono-*ortho*-PCBs (105, 114, 118, 123, 156, 157, 167, 189)**
    - **other ortho PCBs (18, 28, 44, 49, 52, 66, 74, 87, 99, 101, 110, 128, 138, 146, 149, 151, 153, 170, 172, 177, 178, 180, 183, 187, 194, 195, 196/203, 201, 206, 209)**
  - **Fraction 2:**
    - **Seven 2378-PCDDs**
    - **Ten 2378-PCDFs**
    - **Four coplanar PCBs (77, 81, 126, 169)**

PCDD/PCDF/PCB analysis (2)

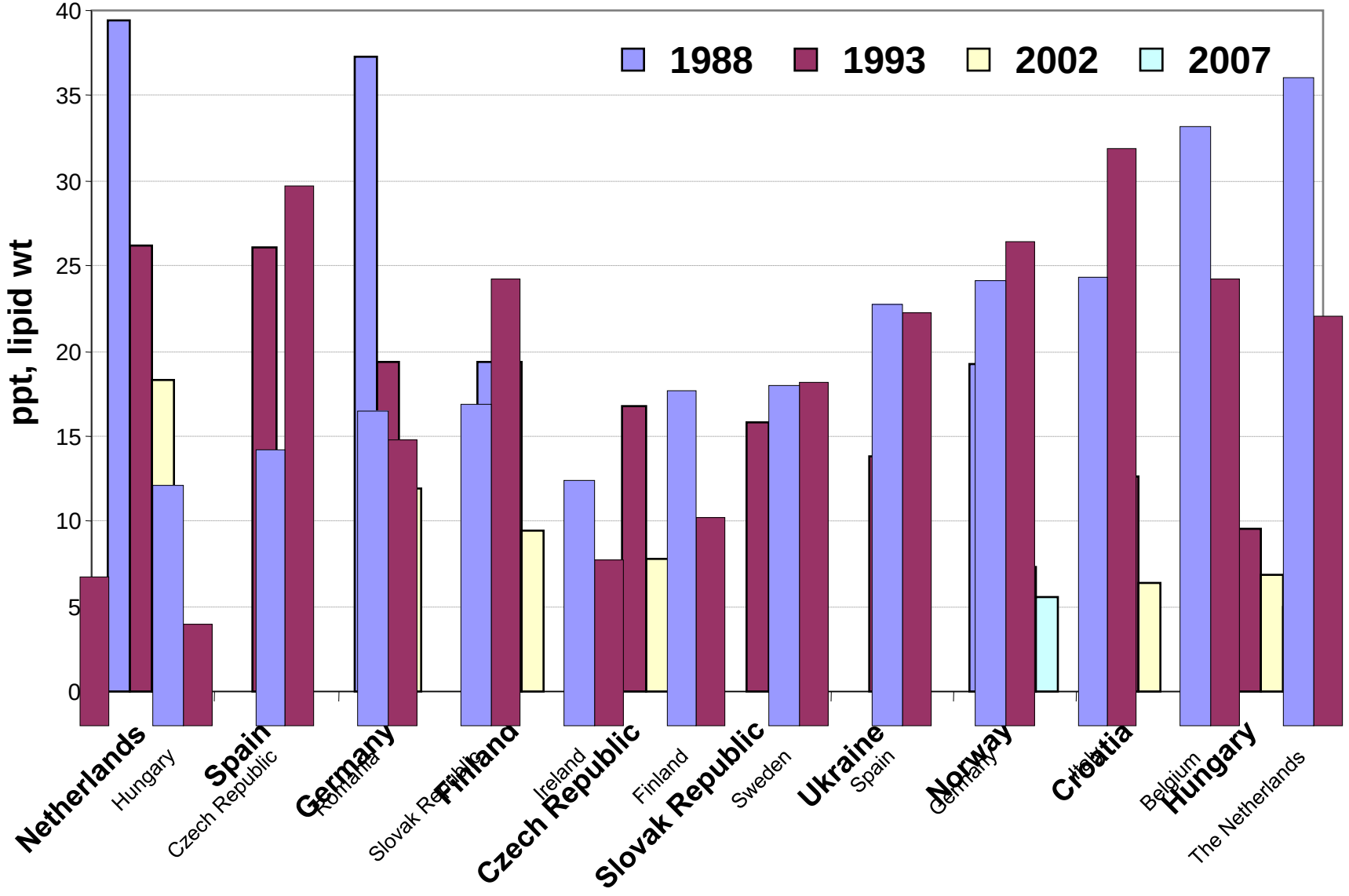
- **1 analysis batch is composed of:**
  - **14 serum samples**
  - **1 in-house RM (spiked porcine serum)**
  - **1 blank (no serum)**
  - **occasionally CRM (serum)**
- **HRGC (DB5-ms) separation**
- **HRMS (10000 res) quantification**
- **Isotope dilution method based on EPA 1613 and 1668**

European comparison of PCDD/F and PCB levels in Human milk (WHO Exposure Study 2001/2002)



Source: Van Leuwen, R. Malish 2002 "WHO exposure study on the levels of PCBs, PCDDs and PCDFs in Human Milk" 3rd round

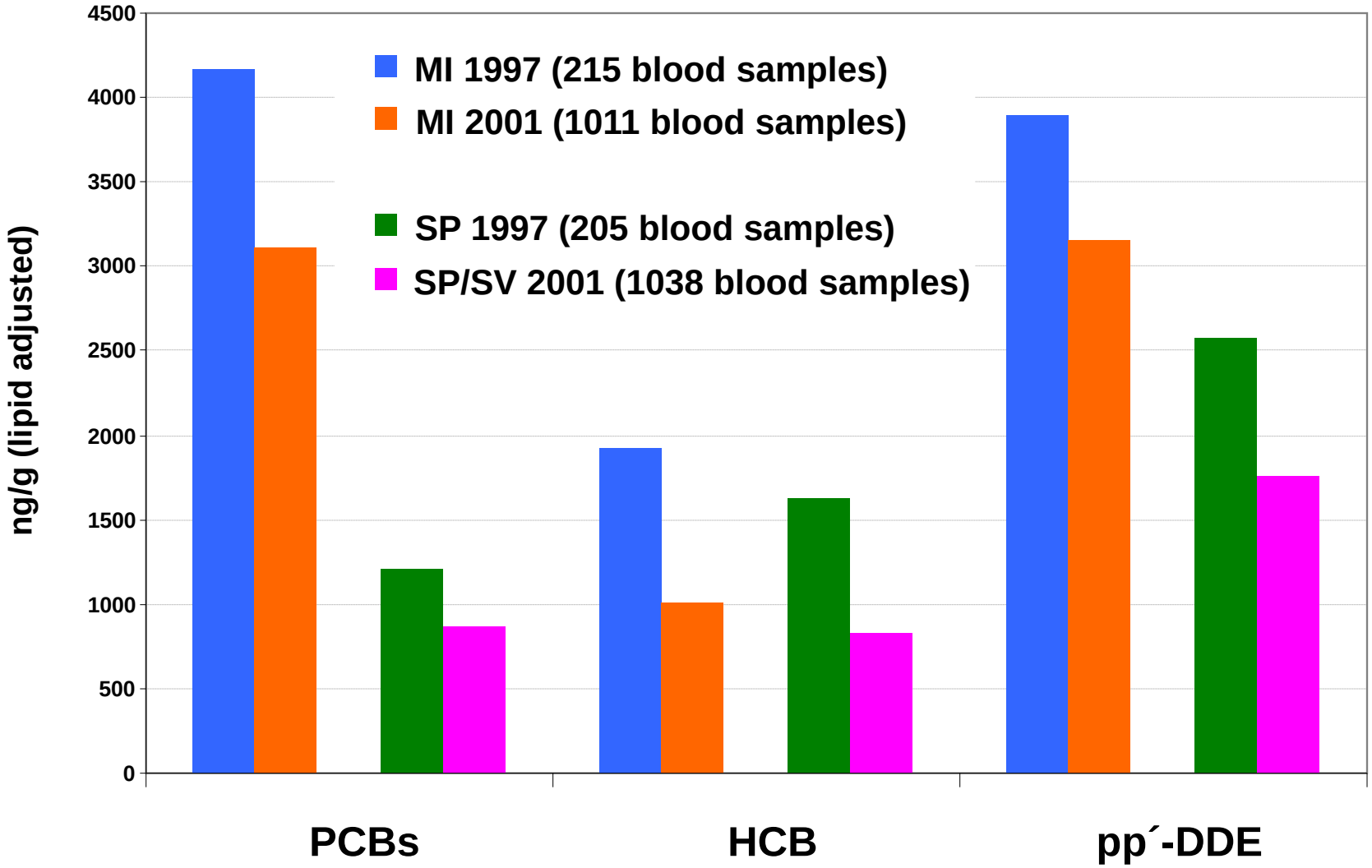
Time trends in PCDD/F levels in human milk (WHO Exposure Studies)



Source: WHO-Leuwen, R. Malish 2002 "WHO exposure study on the levels of PCBs, PCDDs and PCDFs in Human Milk" 3rd round

Slovakia‘s districts chosen for exposure studies

Time trends in PCB levels in human blood (2 districts in Slovakia)



“Cons” - Why is Public Health Biomonitoring Controversial?

- **May cause unfounded fear. Because it can be measured, doesn’t mean it causes harm.**
- **Hard to tell how much is too much. No “meaning” without reference ranges.**
- **Provides no information about sources.**
- **May modify behavior in negative ways.**
- **May cause valuable products to be banned.**

“Pros” - Biomonitoring helps answer Important Public Health Questions

- What are we exposed to and how much?
- Do public health policies and regulatory programs reduce exposures over time?
- Are some groups more highly exposed than the general population?
- What are the relationships between exposure and disease?

Source: P. Flessel, EHL, California DHS

Why to breastfeed

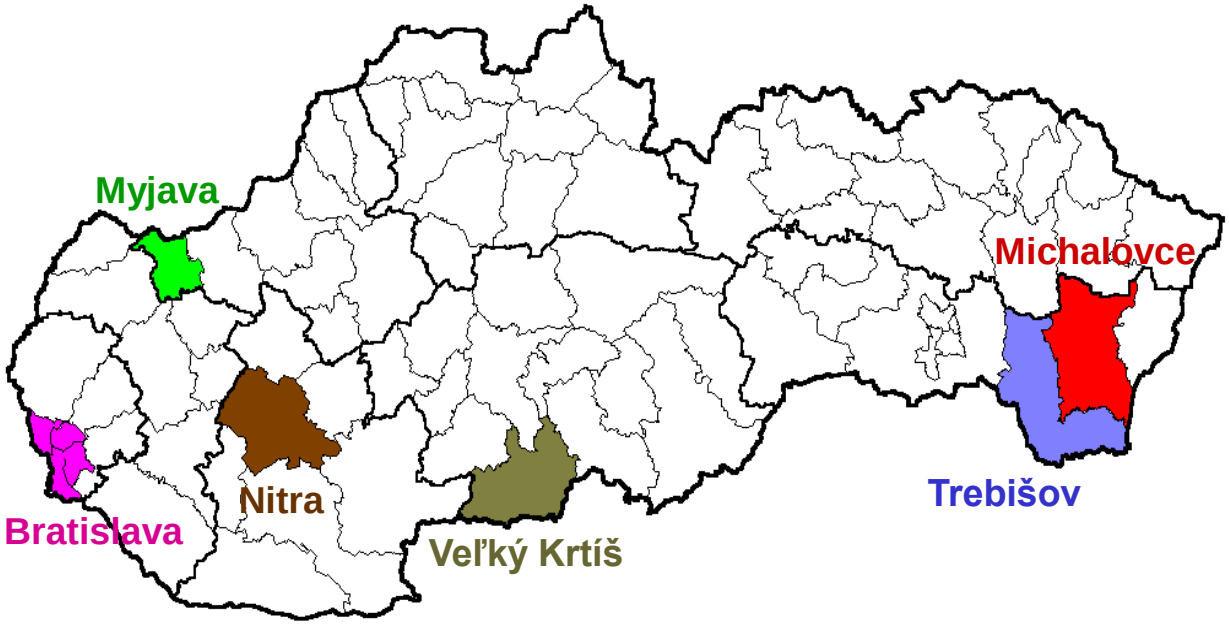
- **Nutrition:**
  - ✓ **Breast milk provides, in an easily digested form, all the nutrients an infant needs for the first six months of life. Exclusive breastfeeding (i.e., no other food or drinks given, not even water) for the first six months offers maximum protection to infants against pneumonia, diarrhoea and other common infections of childhood.**
  - ✓ **Up to 2 years of age or more, breast milk continues to provide high-quality nutrients and helps protect against infection.**

    From 6 to 12 months, breast milk usually provides 60–80% of all energy, protein and other nutritional requirements – e.g., vitamins and other micronutrients.

    From 12 to 23 months, breastfeeding can provide up to 35–40% of these requirements.
- **Family planning / child spacing:**
  - ✓ **Breastfeeding delays the return of a woman's fertility. A woman who does not breastfeed is at increased risk of becoming pregnant again as early as six weeks after the birth of the child.**
- **Psychosocial development:**
  - ✓ **Breastfeeding promotes the emotional relationship, or bonding, between mother and child.**

Slovakia‘s Districts Chosen for POPs Exposure Studies:

in 1992 - 94

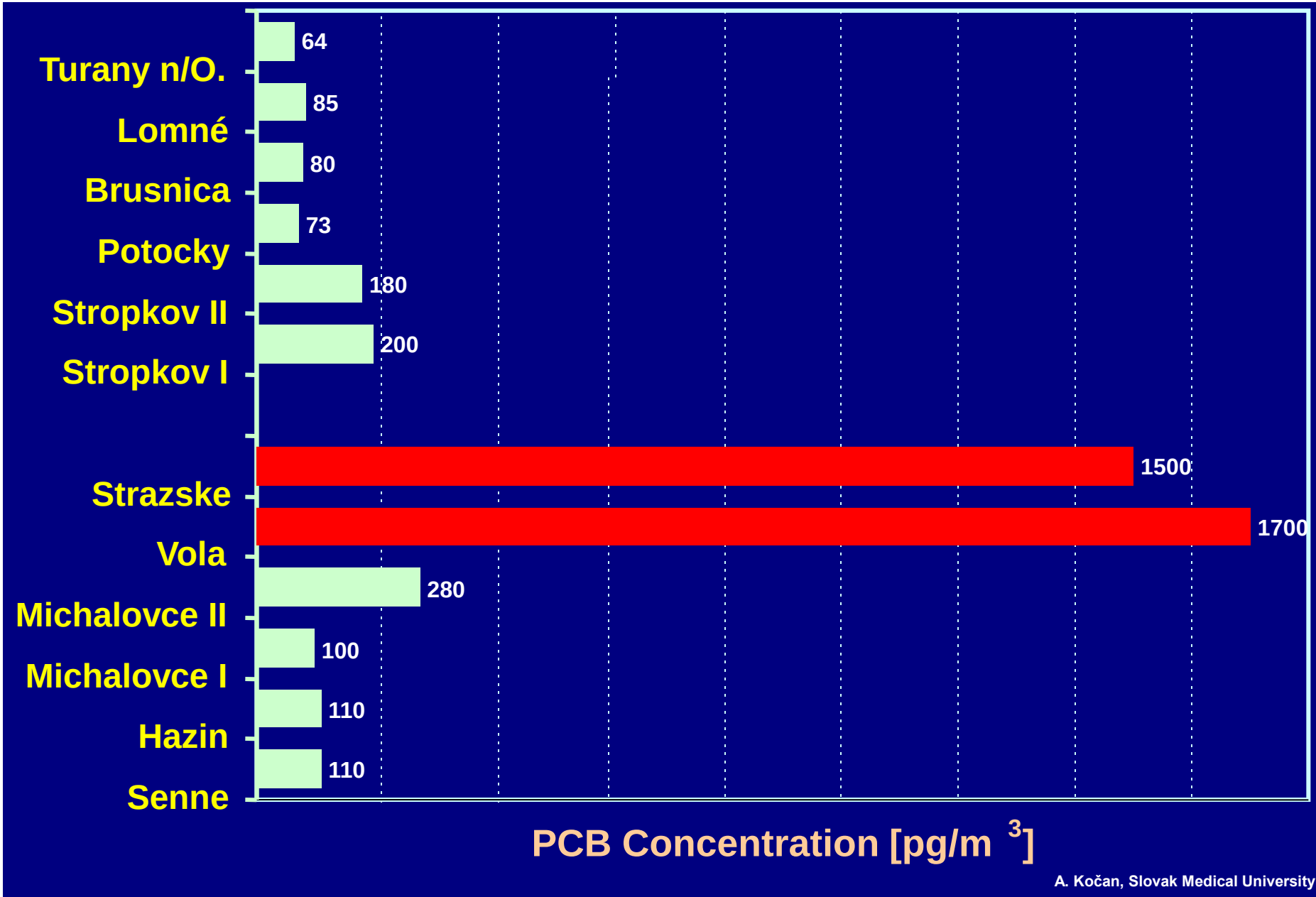


in 1997

in 2001 & 2003-05

PCB Concentrations in Ambient Air

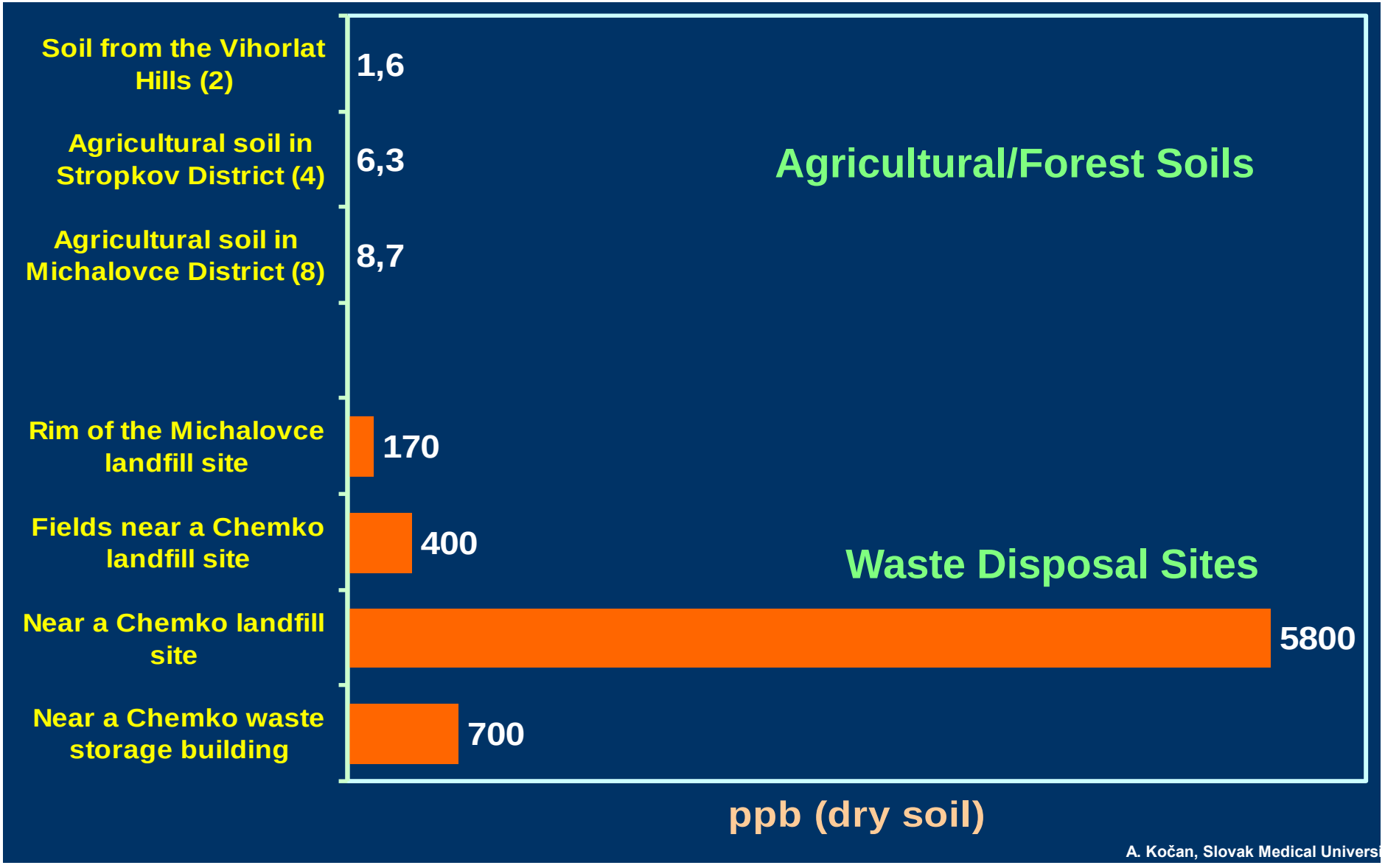
Michalovce (polluted district) vs Stropkov (control district)



A. Kočan, Slovak Medical University

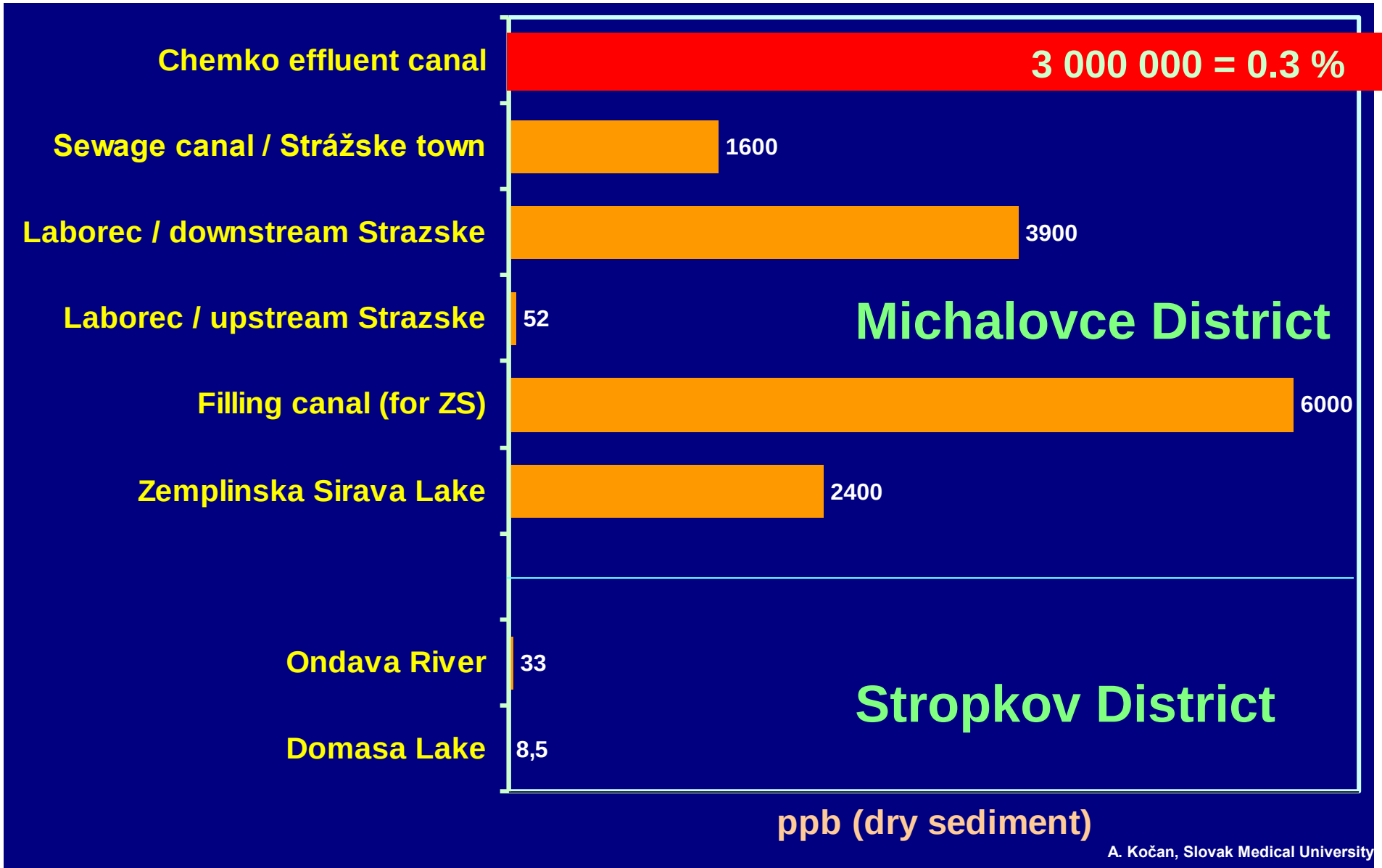
PCB Concentrations in Soil

Vicinity of Chemko disposal sites and agricultural fields



PCB Levels in Sediment Samples

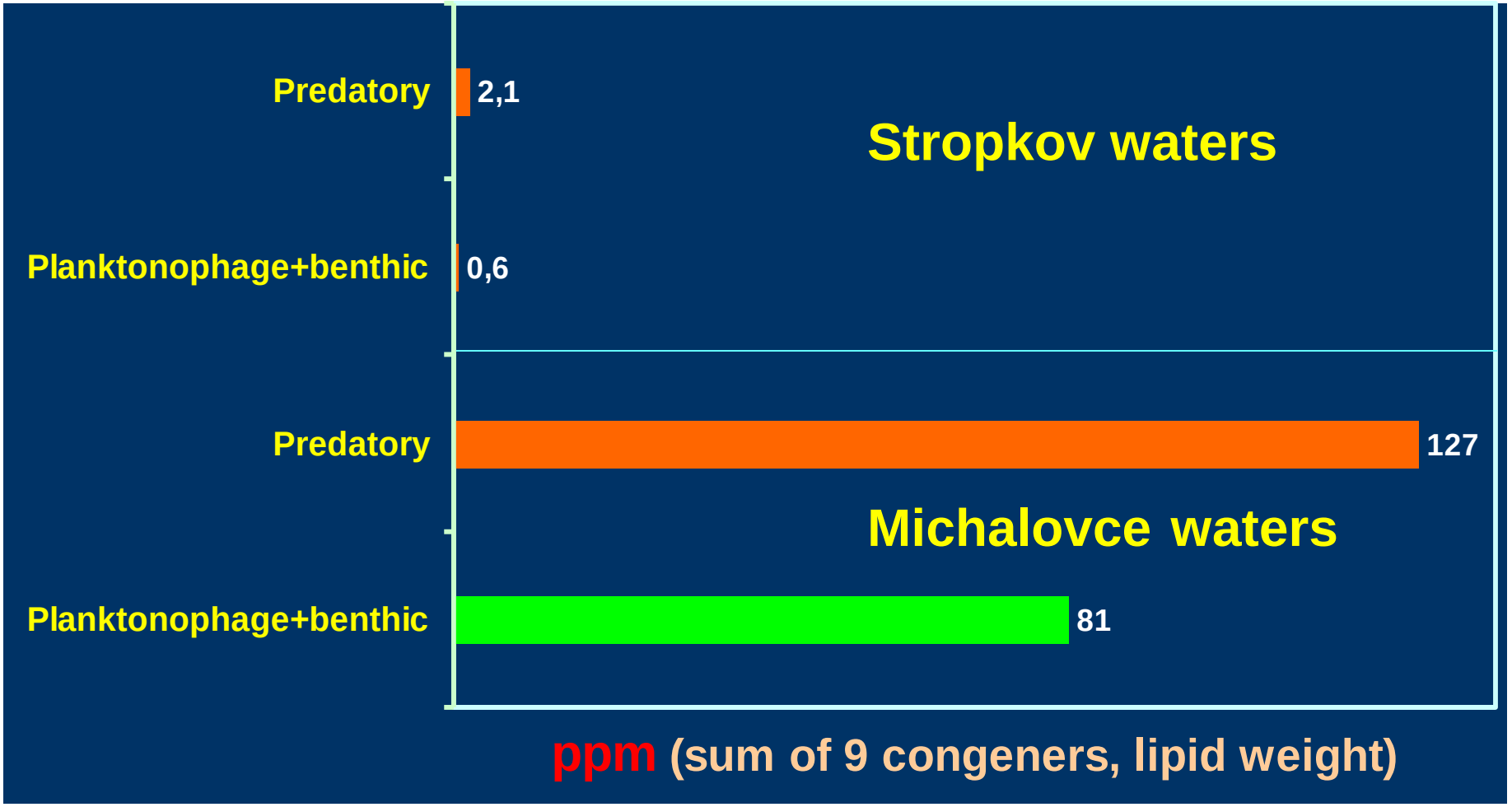
Michalovce (polluted district) vs Stropkov (control district)



A. Kočan, Slovak Medical University

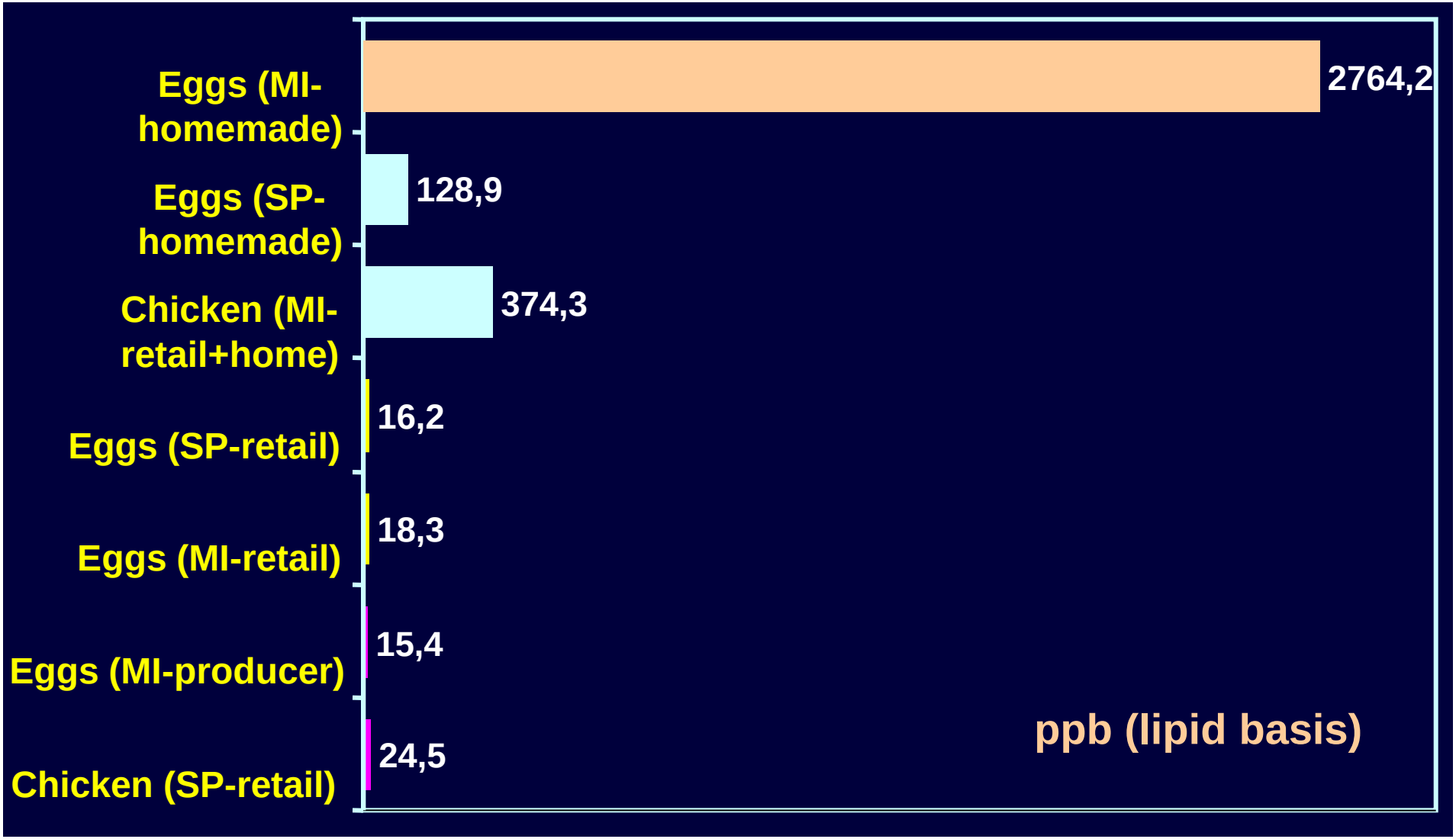
PCB Levels in Fish

Michalovce (polluted district) vs Stropkov (control district)

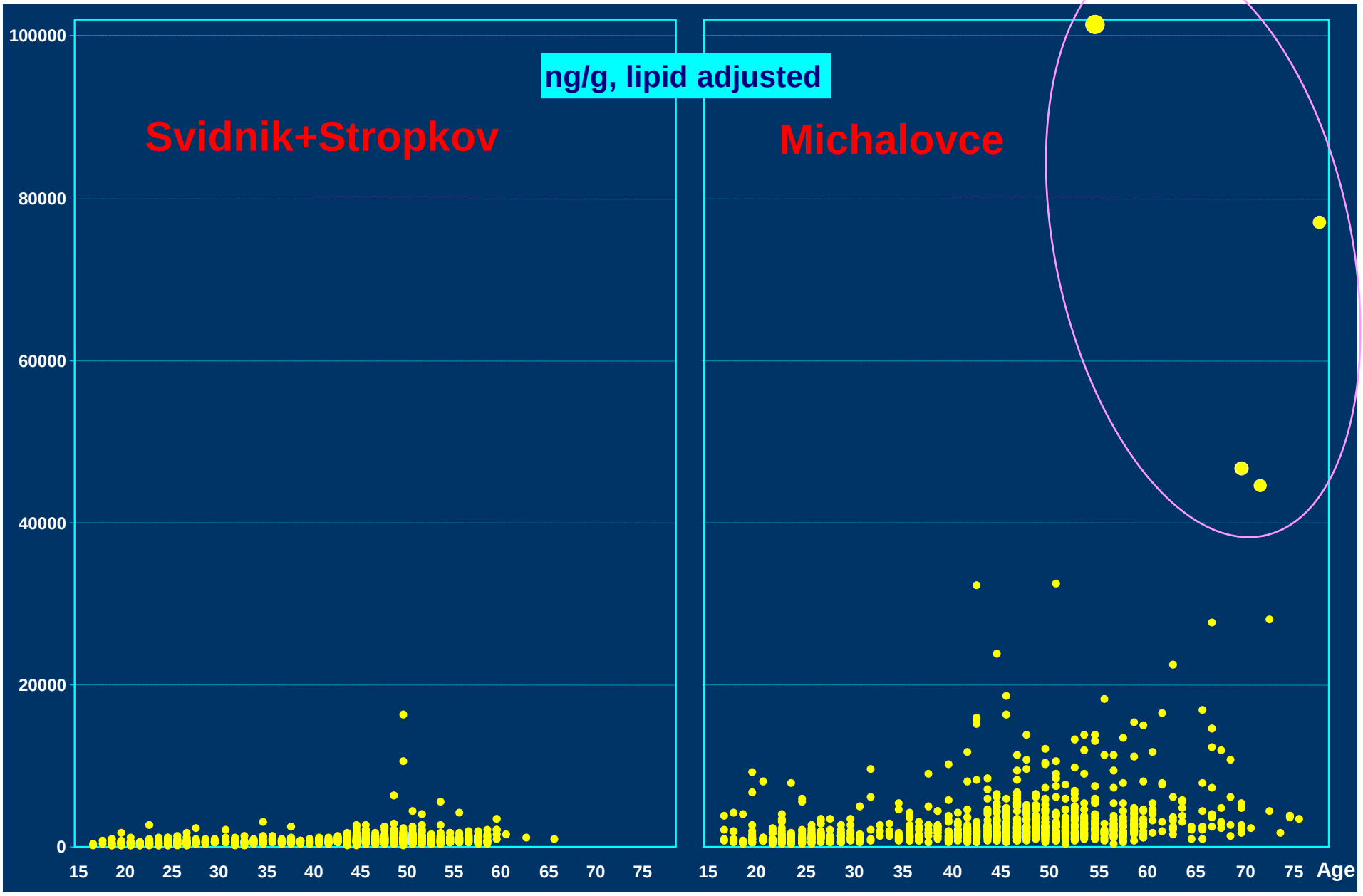


Average PCB Levels in Eggs and Chicken Collected in Michalovce and Stropkov Districts

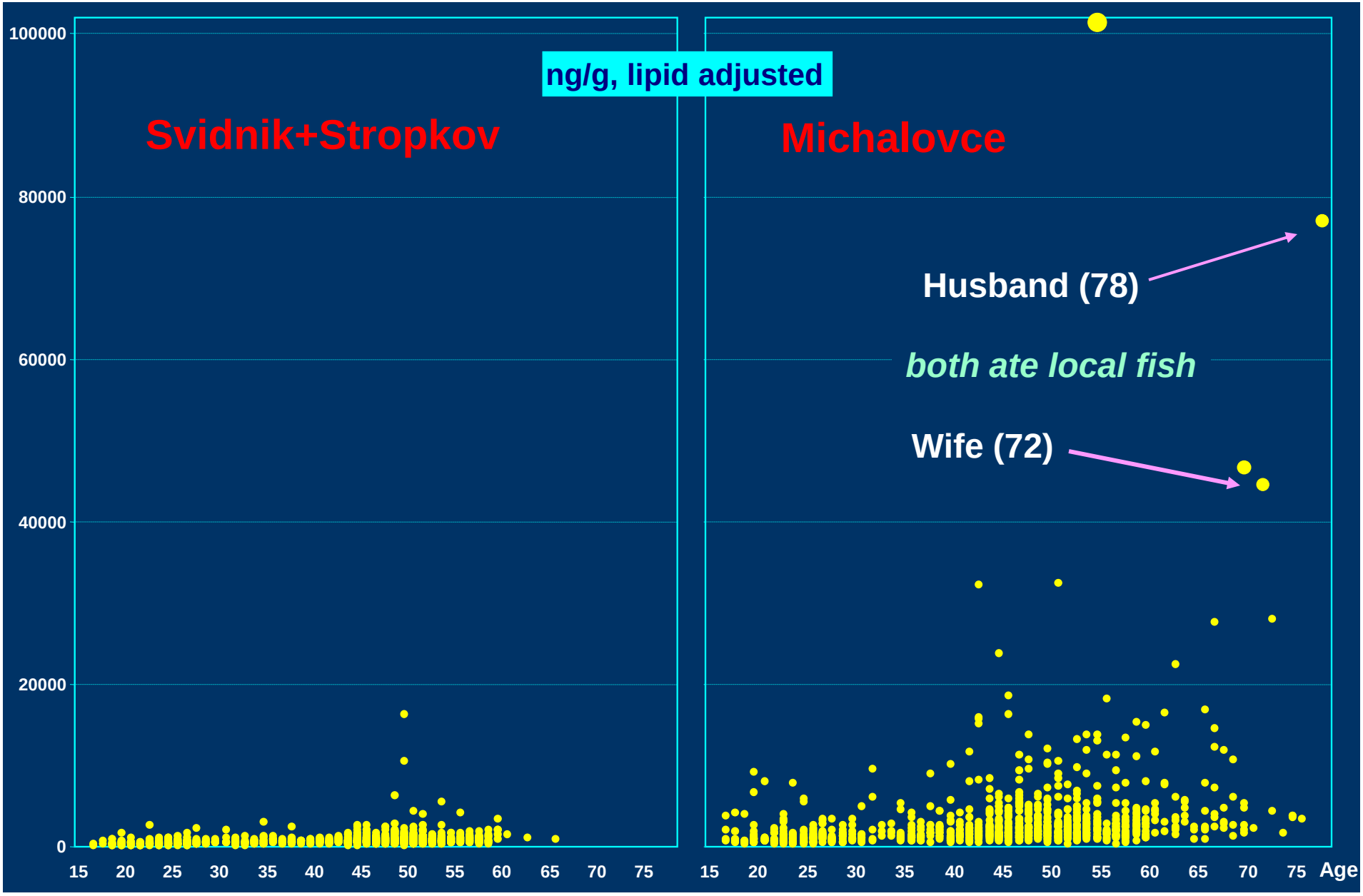
Sum of 28, 52, 101, 118, 138, 153, 156, 170 and 180 congeners



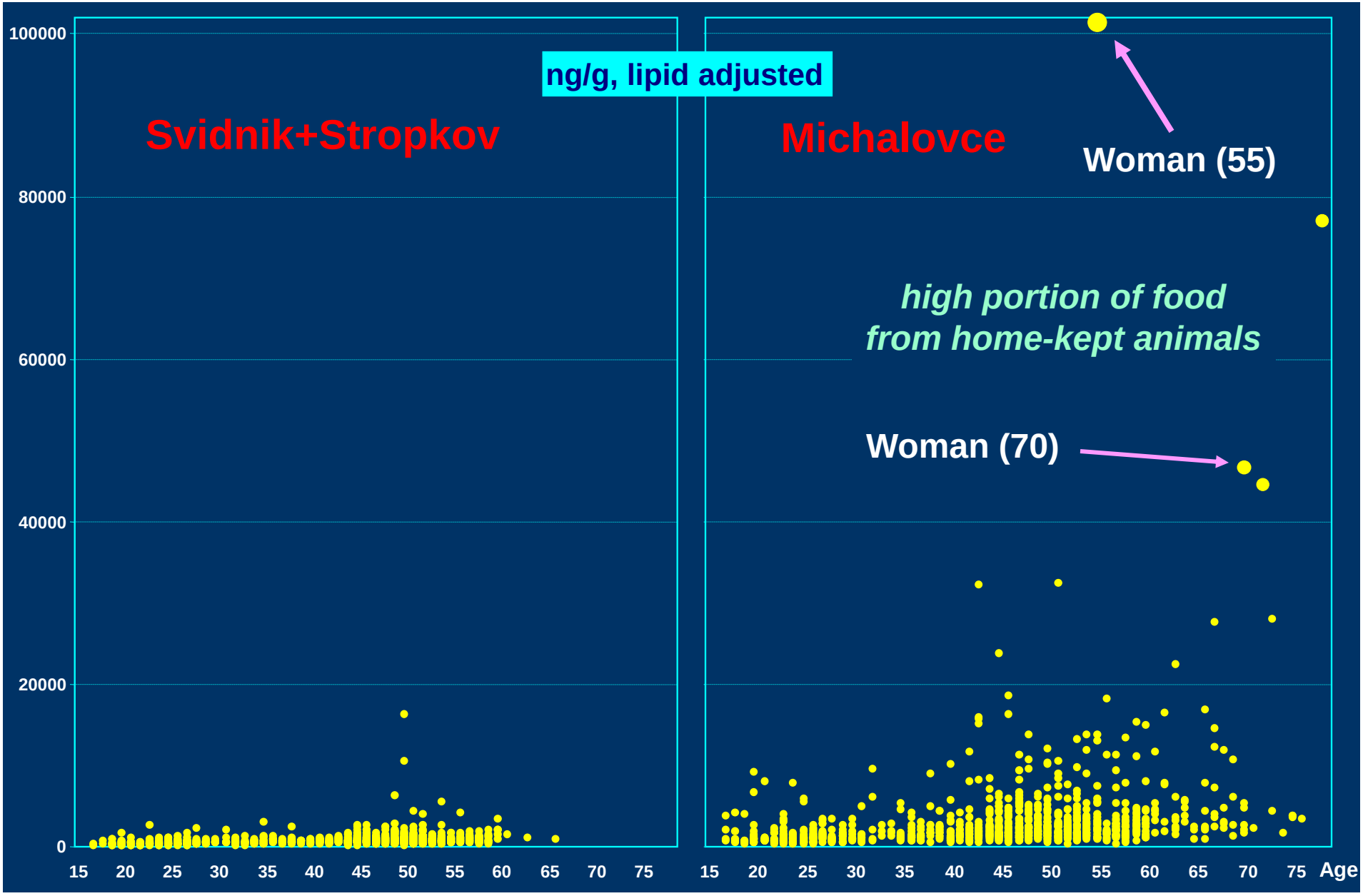
PCB Levels - Adults



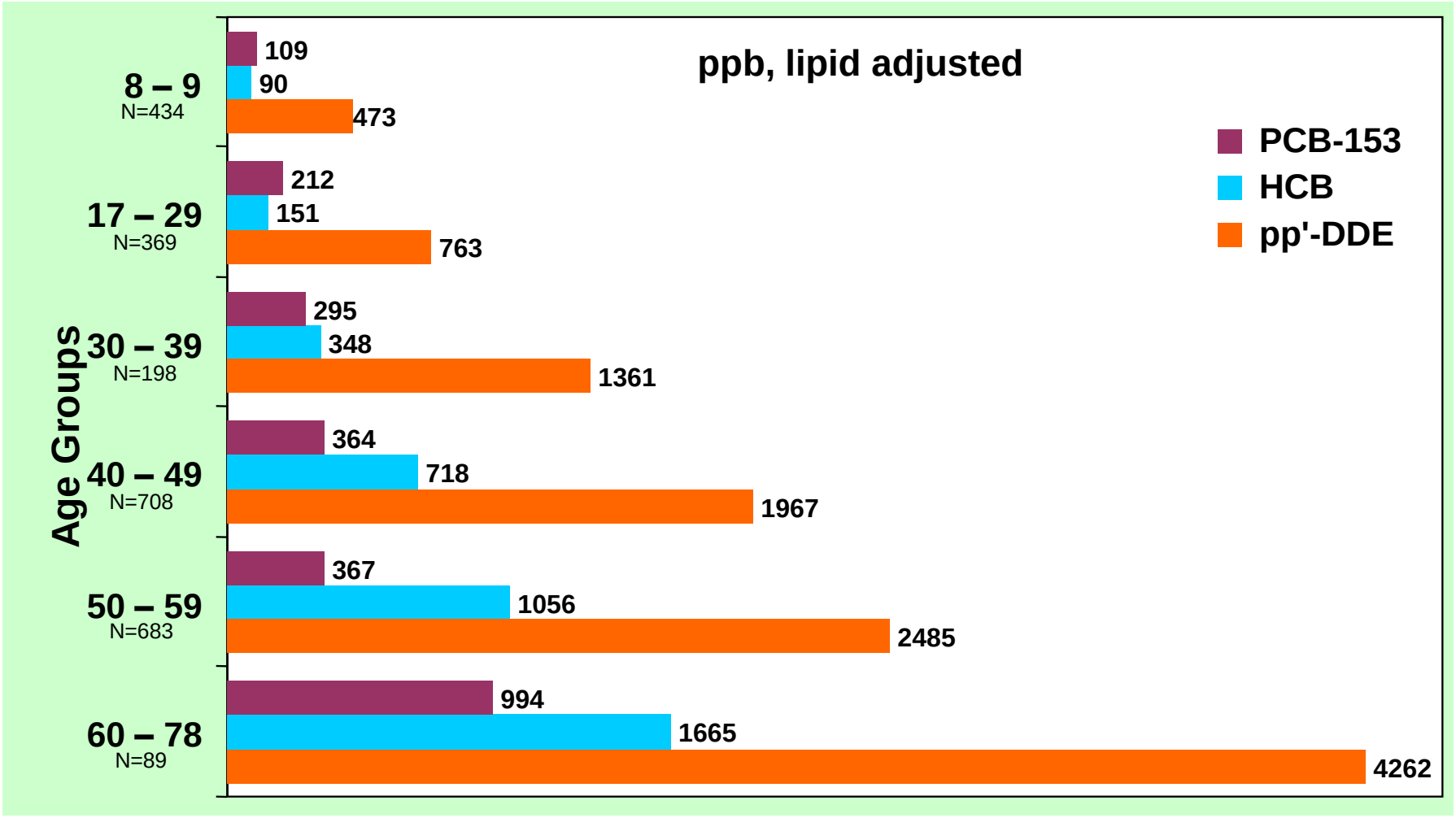
PCB Levels - Adults



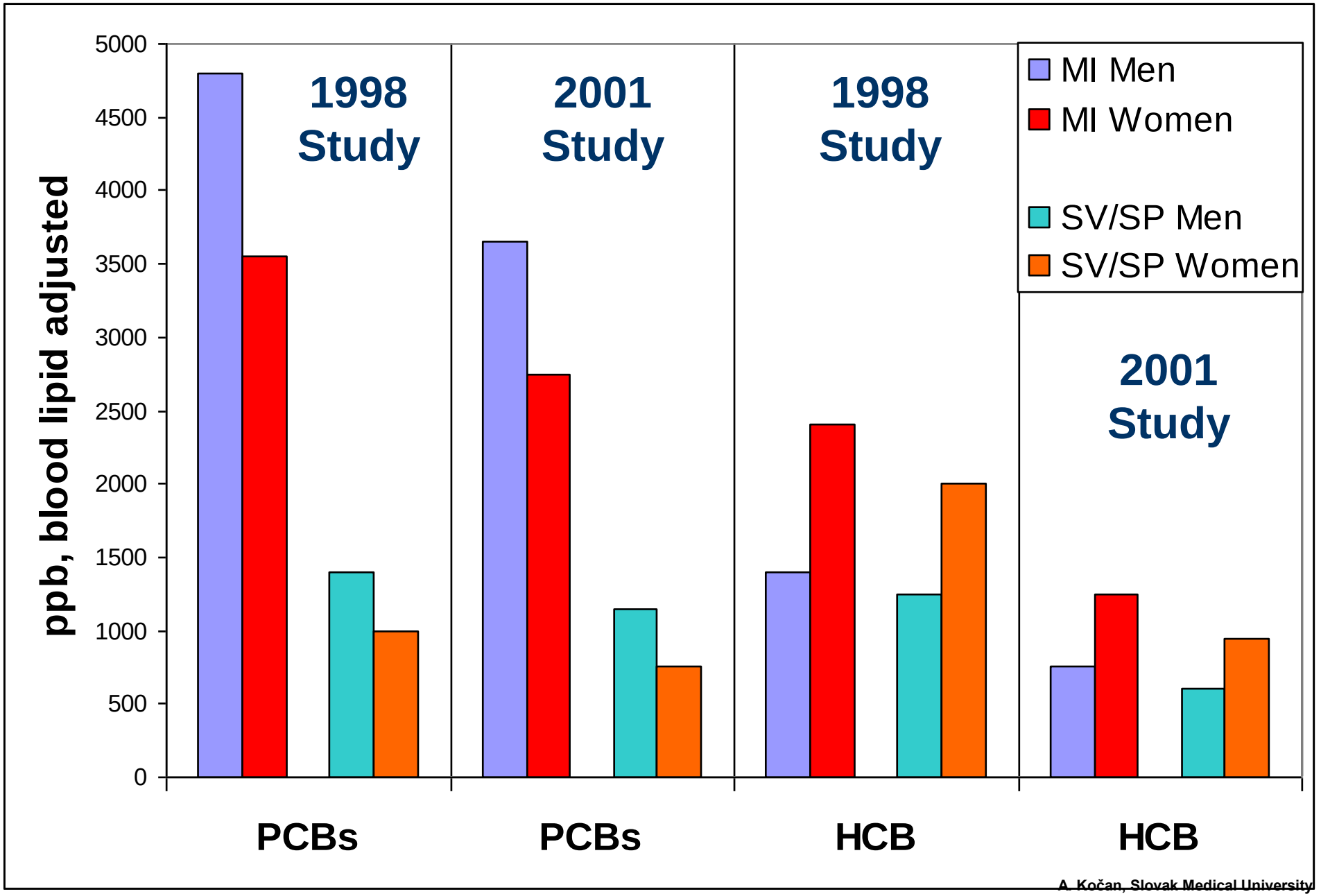
PCB Levels - Adults



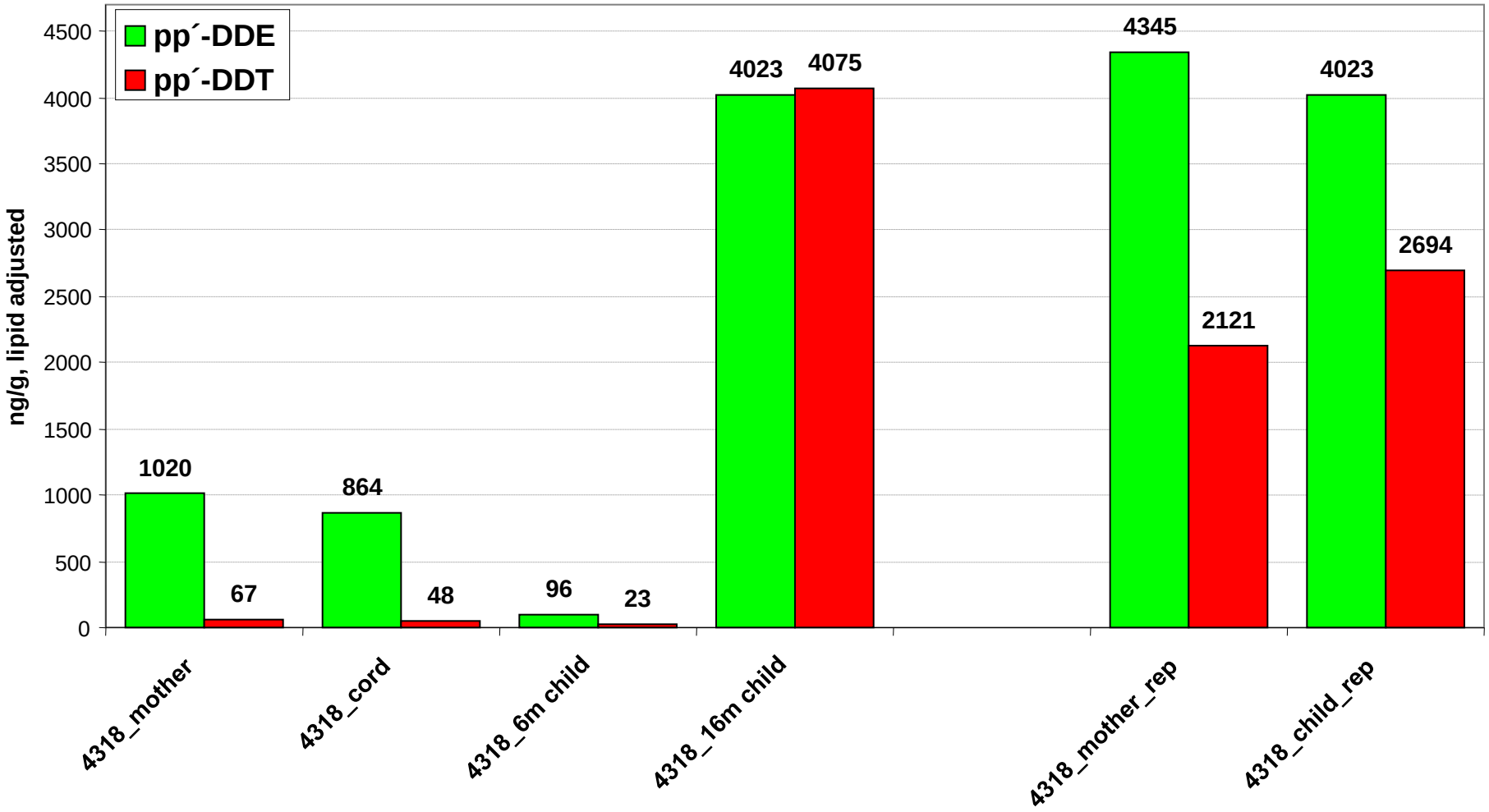
Median PCB-153, HCB, and p,p'-DDE levels in blood serum vs age groups (specimens collected within the PCBRISK project in 2001



Comparison of POP levels in men and women and time trends



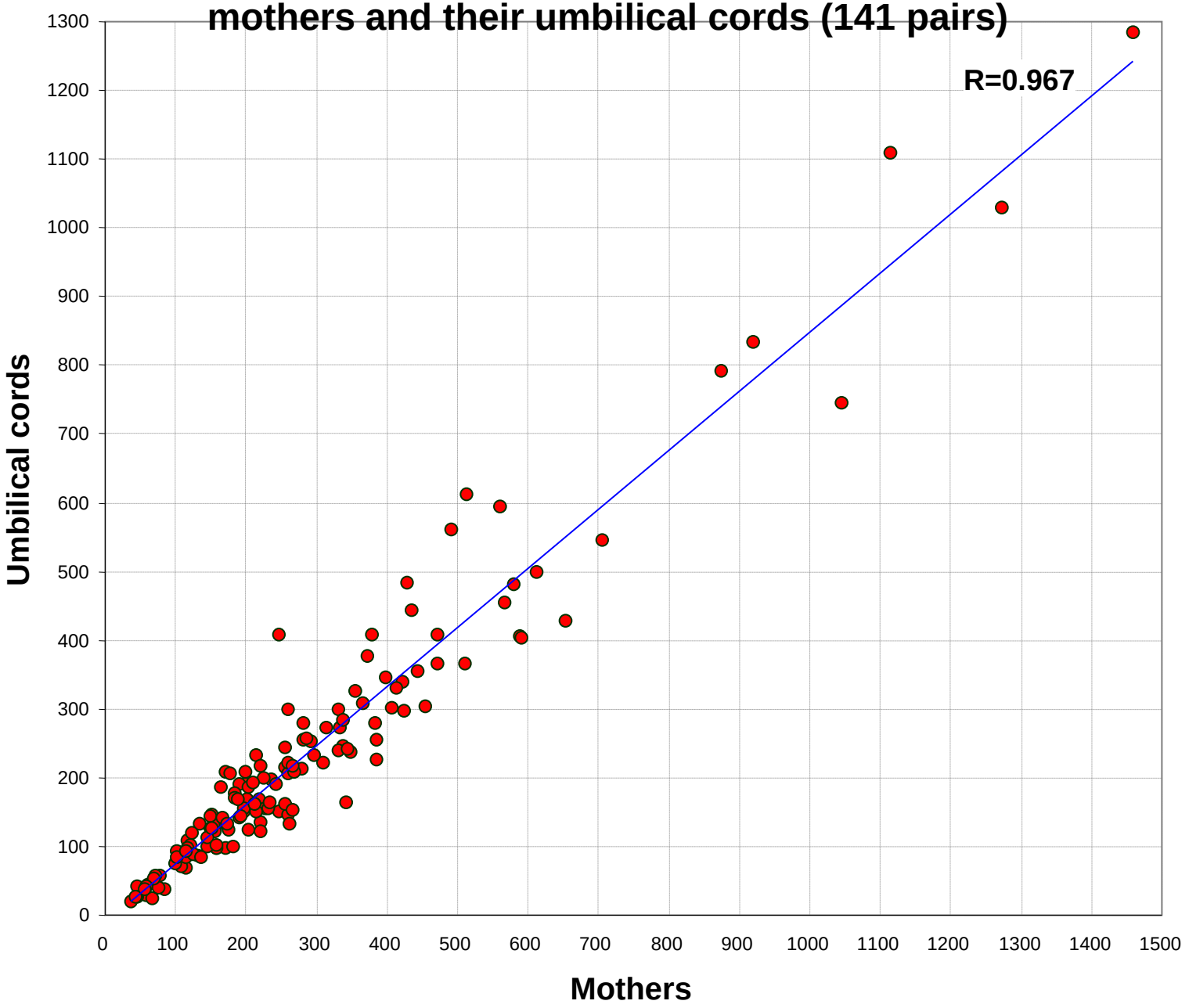
pp'-DDE and pp'-DDT levels in the blood of a mother and her child (#4318)

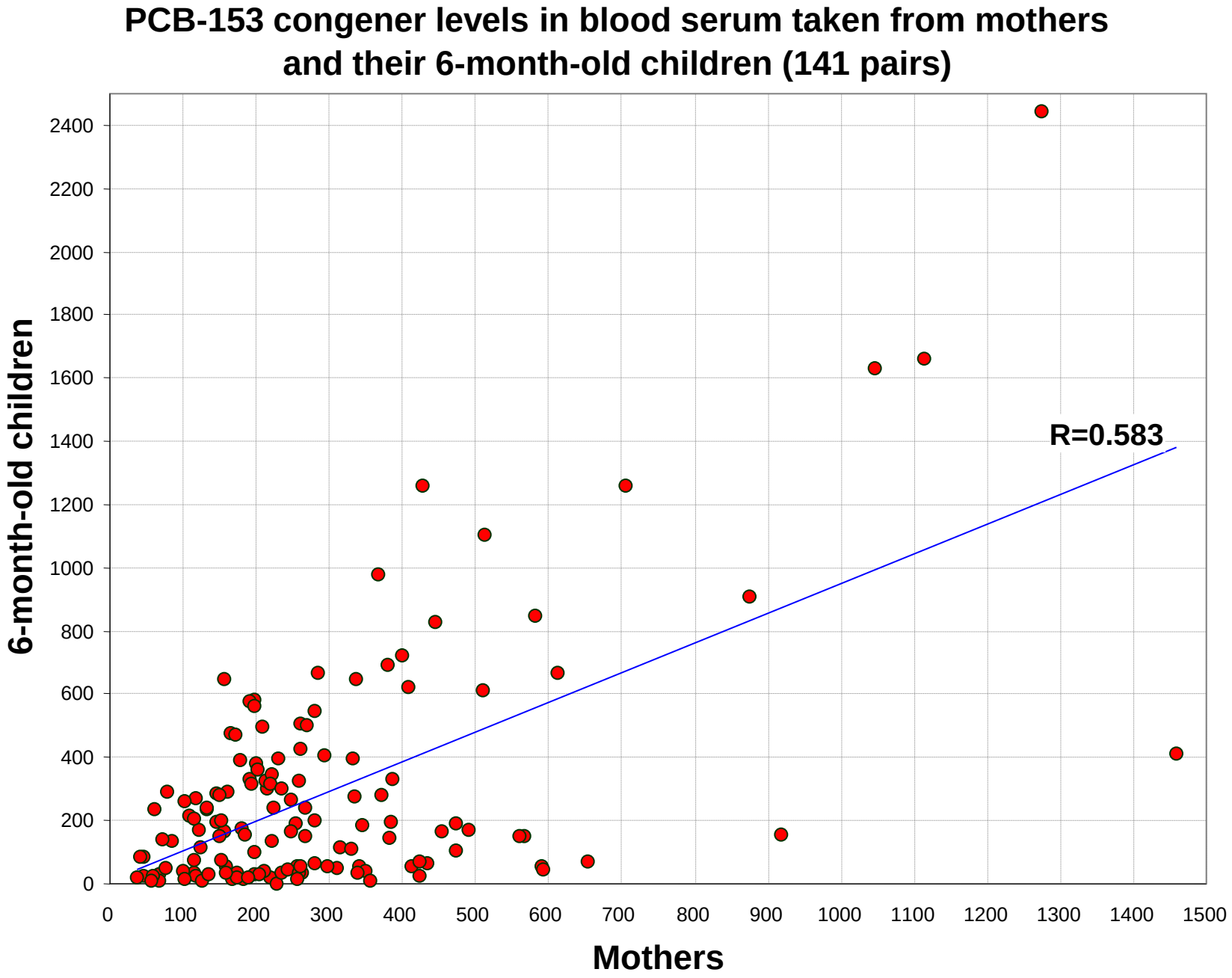


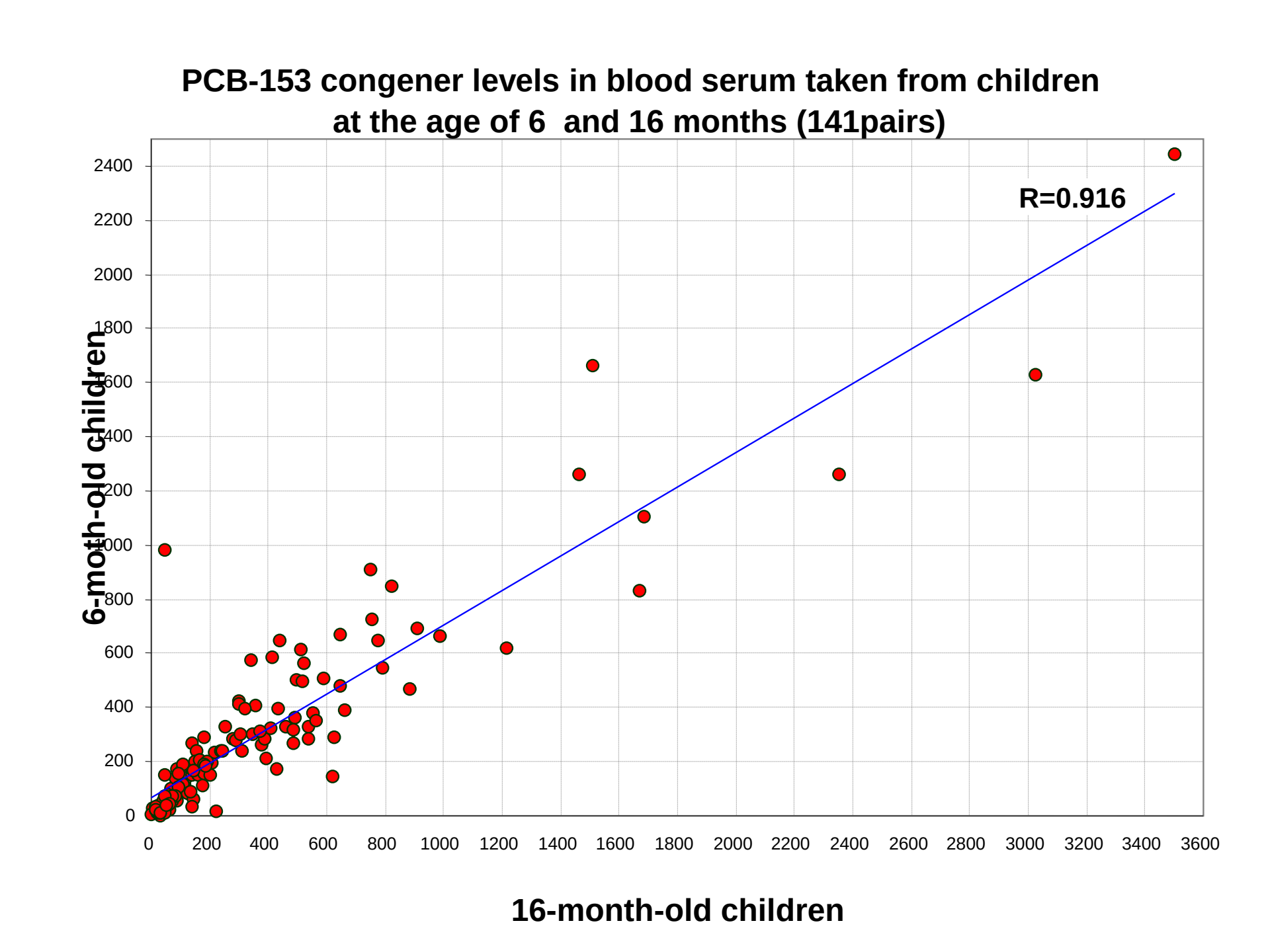
Mother‘s and child‘s blood was taken when the child was 21 months old

- **32- and 4.3-fold increase of DDT and DDE respectively in a mother before delivery vs the mother 21 months later**
- **180- and 42-fold increase of DDT and DDE respectively in her 16-m child vs the 6-m child**
- **35-% decrease of DDT in the child at the age of 21 moths while DDE unchanged**

A. Kočan, Slovak Medical University

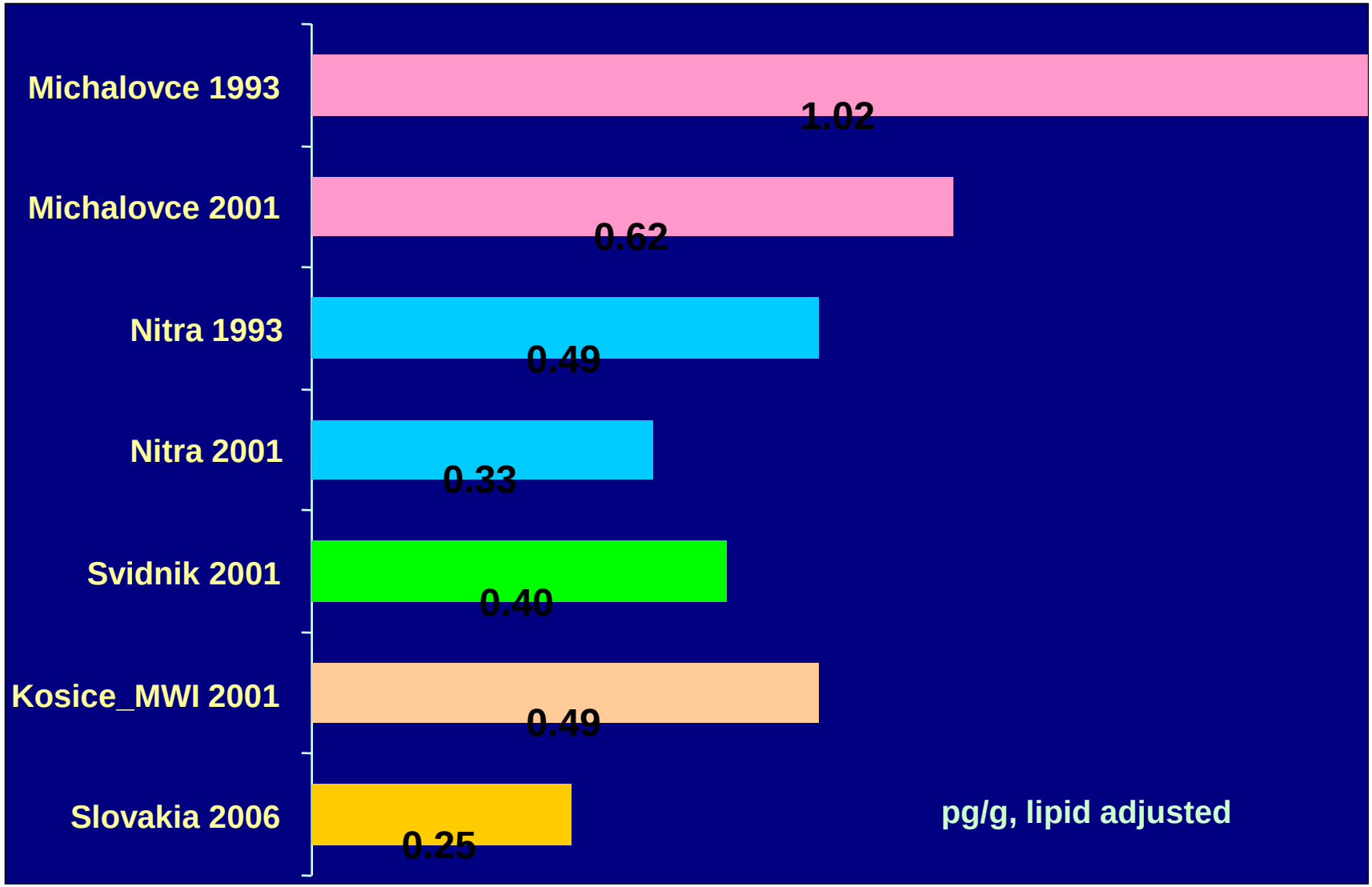

PCB-153 congener levels in blood serum taken from mothers and their umbilical cords (141 pairs)
R=0.967
Umbilical cords
Mothers
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500


PCB-153 congener levels in blood serum taken from mothers and their 6-month-old children (141 pairs)
6-month-old children
Mothers
R=0.583
0
200
400
600
800
1000
1200
1400
1600
1800
2000
2200
2400
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500

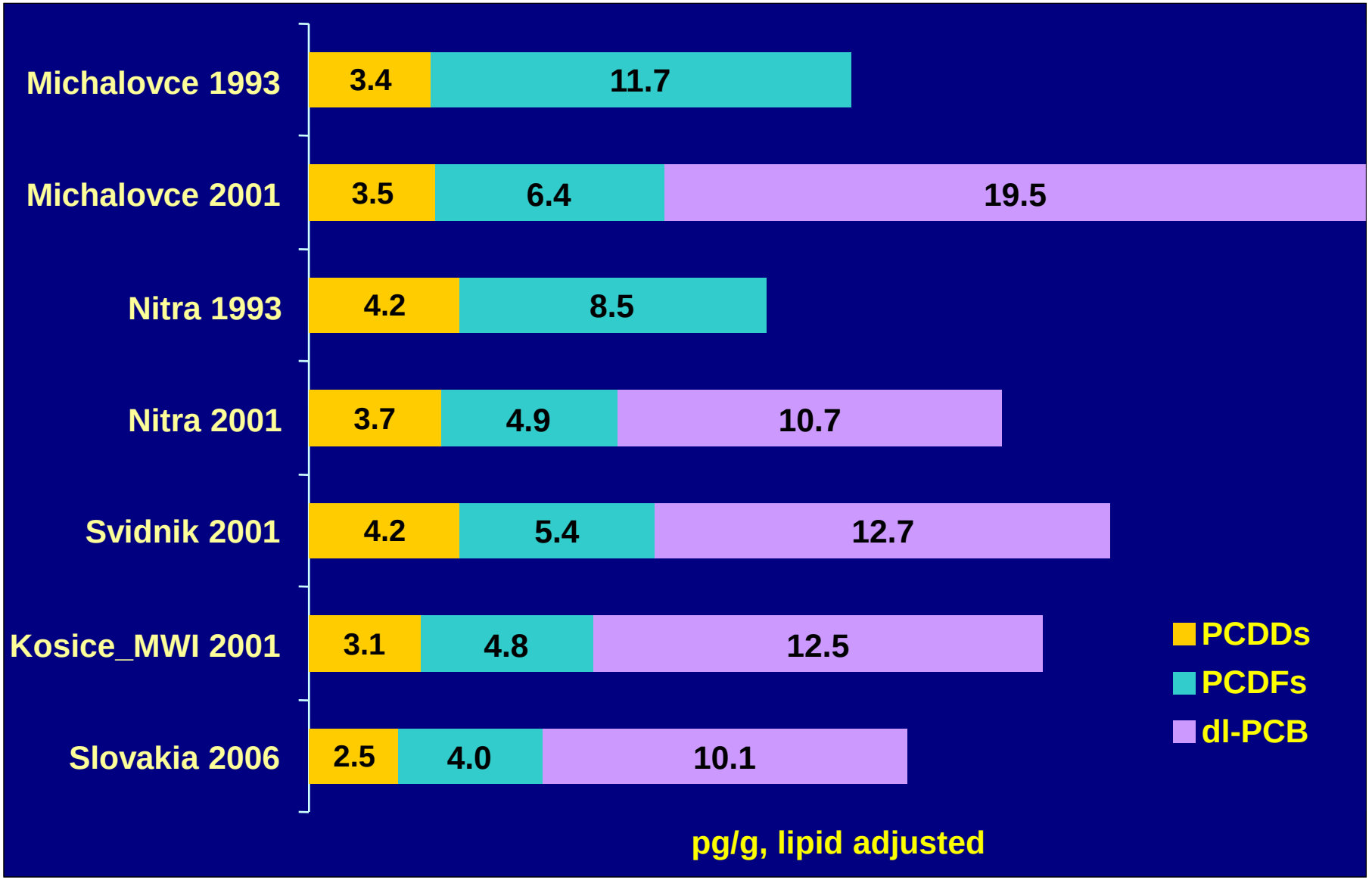

PCB-153 congener levels in blood serum taken from children at the age of 6 and 16 months (141pairs)
R=0.916
6-moth-old children
16-month-old children
0
200
400
600
800
1000
1200
1400
1600
1800
2000
2200
2400
2600
2800
3000
3200
3400
3600

PCB Levels in Human Milk Samples / WHO

Sum of 28, 52, 101, 138, 153, and 180 congeners



PCDD/F-TEQ Levels in Human Milk Samples / WHO



Recommended Literature & Links Pertaining to Human POPs Monitoring

- **Guidance on the Global Monitoring Plan for Persistent Organic Pollutants. UNEP. February 2007 (preliminary version).**
- **Guidance for Analysis of Persistent Organic Pollutants (POPs). UNEP. March 2007. http://www.chem.unep.ch/pops/laboratory/analytical_guidance_en.pdf**
- **Guidelines for Developing a National Protocol. WHO. March 2007 (revised). http://www.who.int/foodsafety/chem/POPprotocol.pdf**
- **http://www.pops.int**
- **http://www.chem.unep.ch/pops/default.html**
- **http://www.cdc.gov/biomonitoring**
- **http://www.aphl.org/programs/environmental_health/biomonitoring/Pages/default.aspx**
- **http://www.who.int/foodsafety/chem/pops/en/**