TRIBUNAL

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Memo n° 9 : Monika KRUGER

Veterinarian - GERMANY

The Hague, October 15th-16th, 2016

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Monika Kruger is veterinarian, veterinary specialist in microbiology (bacteriology and mycology) and infectious diseases in animals

From 1966-1971, she studied veterinary medicine at the Humboldt University in Berlin (GDR). After graduation, she completed at the National Veterinary Examination Institute of the GDR in Berlin their duty assistant and received in 1972 their approval.

Then inquired Kruger as a research assistant at the Institute of Applied Animal Hygiene in Eberswalde, from there she started to Vietnam in 1974 to found and oversee a pig farm project. In 1976, she returned to Germany and in 1977 at the Institute of Microbiology and Epizootics teaching of HU Berlin Research assistant, later senior assistant.

In 1993 she was appointed to a professorship at the University of Leipzig, where she was the leader of the Institute of Bacteriology and Mycology until her retirement in April 2014.

In her time at the Veterinary Faculty of the University of Leipzig she and her group worked about diseases in pigs, cattle and chickens. Last 15 years, her interests were pathogenic clostridia, especially C. perfringens and C. botulinum as well as the influence of the gastrointestinal microbiota on animal health.

Since 2010 she and her group worked about the influence of the herbicide glyphosate on gastrointestinal microflora of food animals and its role in chronic botulism.

Some papers of the last years:

- **Monika Krüger**: *Clostridium botulinum in animal populations from microbiological point of* view. In:. 9 *Agriculture and veterinary Academy AVA main* meeting.2010.
- **Monika Krüger** and W. Schrödl: *On the importance of the gastrointestinal flora in farm animals, their regulation and possibilities of* influencing. In: *Tierarztl.Umschau* 59. 2008, p 41-46.
- **Monika Krüger**, S. and W. Schrödl: *The gastrointestinal flora (MDF) of the pig - an* overview. In: *Tierarztl. Umschau* 63. 2004, S. 208-211.
  - **Monika Krüger**, Anke Große-Herrenthey , Wieland Schrödl , Achim Gerlach, Arne RodloffVisceral botulism at dairy farms in Schleswig Holstein, Germany e Prevalence of Clostridium botulinum in feces of cows, in animal feeds, in feces of the farmers,and in house dust. doi:10.1016/j.anaerobe.2011.12.013 .
  - Alaa Aldin Alnassan, Awad Ali Shehata, Marianne Kotsch, Wieland Schrodl, **Monika Krüger**, Arwid Daugschies, Berit Bangoura. Efficacy of early treatment with toltrazuril in prevention of
  - coccidiosis and necrotic enteritis in chickens. http://dx.doi.org/10.1080/03079457.2013.823476
  - Monika Krüger, Wieland Schrödl, Jürgen Neuhaus and Awad Ali Shehata. Field Investigations of Glyphosate in Urine of Danish Dairy Cows. http://dx.doi.org/10.4172/2161-0525.1000186.
  - Awad A. Shehata • Wieland Schrödl • Alaa. A. Aldin • Hafez M. Hafez • Monika Krüger. The Effect of Glyphosate on Potential Pathogens and Beneficial Members of Poultry Microbiota In Vitro. Curr Microbiol DOI 10.1007/s00284-012-0277-2.
  - **Monika Krüger,** Marie Skau , Awad Ali Shehata, Wieland Schrödl. Efficacy of Clostridium botulinum types C and D toxoid vaccination
  - in Danish cows. http://dx.doi.org/10.1016/j.anaerobe.2013.06.011.
  - Heidrun Mengel & **Monika Kruger** & Maxie U. Kruger & Bernhard Westphal &
  - Alexander Swidsinski & Sandra Schwarz & Hans-Christian Mundt & Katja Dittmar &
  - Arwid Daugschies. Necrotic enteritis due to simultaneous infection with Isospora suis and clostridia in newborn piglets and its prevention by early treatment with toltrazuril. Parasitol Res (2012) 110:1347–1355.
  - Wagis Ackermann • Manfred Coenen • Wieland Schrödl • Awad A. Shehata • **Monika Krüger**. The Influence of Glyphosate on the Microbiota and Production of Botulinum Neurotoxin During Ruminal Fermentation. Curr Microbio, l DOI 10.1007/s00284-014-0732-3
  - Jürgen Neuhaus & Awad A. Shehata & **Monika Krüger**. Detection of pathogenic clostridia in biogas plant wastes. Folia Microbiol. DOI 10.1007/s12223-014-0334-2.
  - **Monika Krüger**, Wieland Schrödl, Ib Pedersen, Philipp Schledorn and Awad A Shehata. Detection of Glyphosate in Malformed Piglets. http://dx.doi.org/10.4172/2161-0525.1000230.
  - J. R. Aschenbach, T. Seidler, F. Ahrens, W. Schrödl, I. Buchholz, B. Garz, **M. Krüger**
  - & G. Gäbel. Luminal Salmonella Endotoxin Affects Epithelial and Mast Cell Function
  - in the Proximal Colon of Pigs. DOI 10.1080/00365520310003129.
  - Henning Gerlach, Achim Gerlach, Wieland Schrödl, Bernd Schottdorf, Svent Haufe, Hauke Helm, Awad Shehata and **Monika Krüger**. Oral Application of Charcoal and Humic acids to Dairy Cows

Influences Clostridium botulinum Blood Serum Antibody Level and Glyphosate Excretion in Urine. http://dx.doi.org/10.4172/2161-0495.186.

- Henning Gerlach, Achim Gerlach, Wieland Schrödl, Svent Haufe, Bernd Schottdorf, Awad A. Shehata and **Monika Krüger**. Oral Application of Charcoal and Humic Acids Influence Selected Gastrointestinal Microbiota, Enzymes, Electrolytes, and Substrates in the
- Blood of Dairy Cows Challenged with Glyphosate in GMO Feeds. http://dx.doi.org/10.4172/2161-0525.1000256.
- **Monika Krüger** , Awad Ali Shehata, Wieland Schrödl , Arne Rodloff. Glyphosate suppresses the antagonistic effect of Enterococcus spp. on Clostridium botulinum. Anaerobe 20 (2013) 74-78.
- **Monika Krüger**, Philipp Schledorn, Wieland Schrödl, Hans-Wolfgang Hoppe, Walburga Lutz and Awad A. Shehata. Detection of Glyphosate Residues in Animals and Humans. http://dx.doi.org/10.4172/2161-0525.1000210.
- R. Rulff, W. Schrodl, S. Basiouni, J. Neuhaus, M. Krüger. Is downer cow syndrome related to chronic botulism? Polish Journal of Veterinary Sciences Vol. 18, No. 4 (2015), 759–765. http://doi 10.1515/pjvs-2015-0098.
- Ramón Rulff, Shereen Basiouni, **Monika Krüger.** Biochemical Investigations and Glyphosate Detection in Downer Cow Syndrome. International Journal of Advancements in Research & Technology, Volume 5, Issue 1, January -2016 ISSN 2278-7763.

Prof. Dr. em. Monika Krüger, Veterinary Faculty, University of Leipzig, Institute of Bacteriology and Mycology - Gerichshain, October 6, 2016

Glyphosate detection in urines, organs and muscles of food animal and in urines of humans

Introduction

Since 2010 epidemiological investigations were done in my formerly institute. In 2010 we finished the scientific project "*Botulinom*". This project was done with 8 other scientific institutions to discover the causes of the increasing cases of chronic botulism in Germany. The aim of my institute was to investigate the relationship of gut microbiota to the detection of *Clostridium (C.) botulinum* and/or botulinum neuro toxin (BoNT) in rumen fluids and feces of cows of diseased (5) and unsuspicious (2) farms . Our results showed that only in cases of microbial dysbiosis of rumen fluids or feces C. botulinum and/or BoNT were detected. The causes of these results were unknown. During a telephone call in 2010 I heard the first time from the herbicide glyphosate. The next time my coworker and I informed us about glyphosate and its activities. It was very interesting that increase of chronic botulism cases of dairy cows in Germany started in the middle of 1990 and was accompanied by feeding of GMO soy contaminated with glyphosate.

After studying the glyphosate literature we investigated urines of cows with HPLC (ca. 40 urines, 1 specimen 180.00 €) to get more epidemiological information. Without financial support we had to look for another detection system and we found an ELISA (Enzyme- linked- Immuno- Assay) of an American company (Abraxis). This test had to validate in all investigated materials (urines of different animals and humans, organs, meat) with official recognized tests (gas chromatography and atom adsorption spectroscopy). After this epidemiological investigations started.

1. Investigation of 240 urines of Danish cows of 8 farms (15 fresh cows and 15 high yielding cows per farm) with chronic botulism

Monika Krüger, Wieland Schrödl, Jürgen Neuhaus and Awad Ali Shehata (2013): Field Investigations of Glyphosate in Urine of Danish Dairy Cows. J Environ Anal Toxicol 2013, 3:5

Farm	Total number of cows	Average milk yields kg/cow
W	150	9.1
K	180	10.3
R	175	10.2
V	200	8.6
S	140	10.7
T	180	11.2
B	400	10.8
E	300	8.8

Table 1: Characterization of Danish dairy farms investigated in this study.

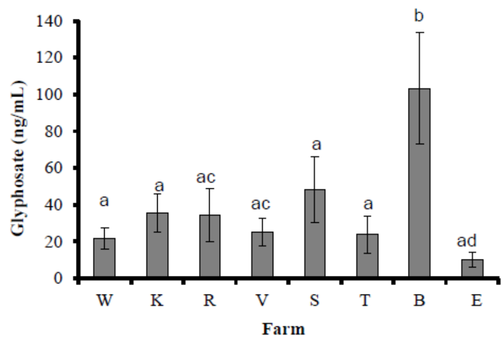
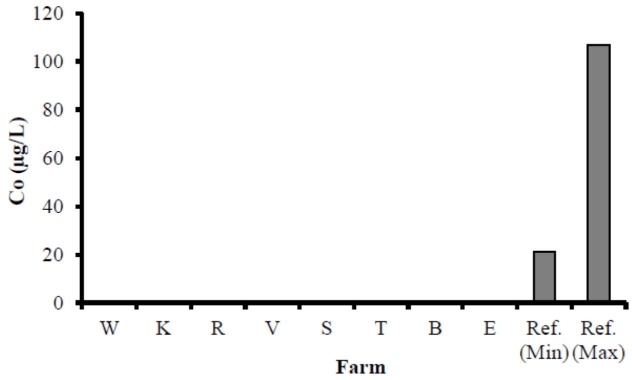
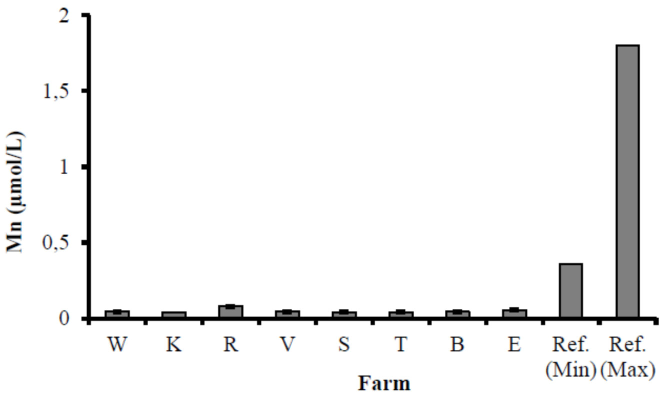


Figure 1: Glyphosate in urine of Danish cows. Significant glyphosate differences were seen between different farms (ab: p< 0.001, cd: p< 0.001) using t-test.





It was very important that in all herds manganese and cobalt were very low. All cows of the eight Danish dairy farms excreted glyphosate in their urine.at significant different amounts between the farms. We found increase blood serum levels of parameters indicative for cytotoxicity like GLDH, GOT, and CK and lipid profile marker cholesterol in cows at all farms and high urea levels in half of the farm animals. Correlations between glyphosate and some of the measured blood serum parameters to CK (R=0.135), Se (R=0.188) Co (R=-0.403) and Zn (R=0.175) demonstrate that glyphosate is toxic to the normal metabolism of dairy cows. This study gives the first documentation to which extent Danish dairy cattle are exposed to glyphosate and its impact on different parameters.

2. Epidemiological investigations of animals and humans for glyphosate residues

Monika Krüger, Philipp Schledorn, Wieland Schrödl, Hans-Wolfgang Hoppe, Walburga Lutz and Awad A. Shehata (2014): Detection of Glyphosate Residues in Animals and Humans. J Environ Anal Toxicol 2014, 4:2.

In the study glyphosate residues were tested in urine and different organs of dairy cows as well as in urine of hares, rabbits and humans. Glyphosate excretion in German dairy cows was significantly lower than Danish cows. Cows kept in genetically modified free area had significantly lower glyphosate concentrations in urine than conventional husbandry cows. Also glyphosate was detected in different organs of slaughtered cows as intestine, liver, muscles, spleen and kidney. Fattening rabbits showed significantly higher glyphosate residues in urine than hares. Moreover, glyphosate was significantly higher in urine of humans with conventional feeding. Furthermore, chronically ill humans showed significantly higher glyphosate residues in urine than healthy population. The presence of glyphosate residues in both humans and animals could haul the entire population towards numerous health hazards, studying the impact of glyphosate residues on health is warranted and the global regulations for the use of glyphosate may have to be re-evaluated.

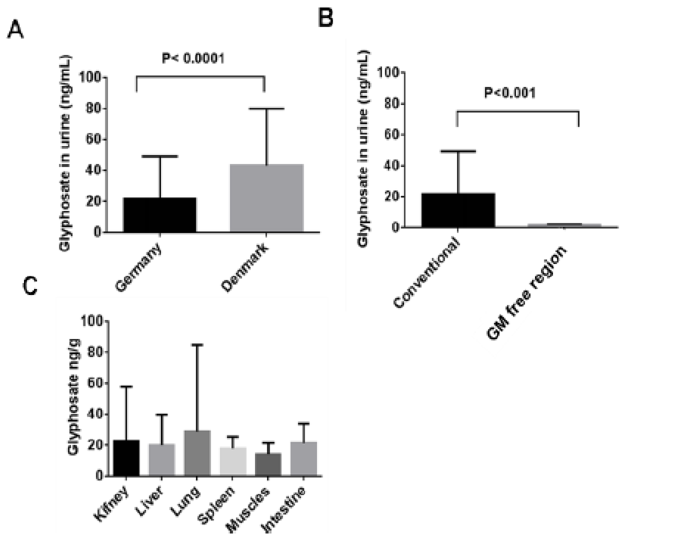


Figure 1: Glyphosate excretion in urine of cows. A) Comparison of glyphosate excretion in urine of German (343) and Danish (N=242) cows. B) Glyphosate in urine of conventional (N=343) and organic (N=32) livestock husbandry in Germany. C: Glyphosate accumulation in different organs.

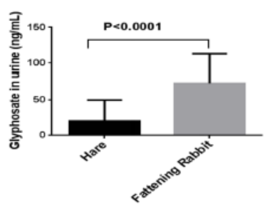


Figure 2: Glyphosate excretion in urine hares (N=193) and fattening rabbits (N=77).

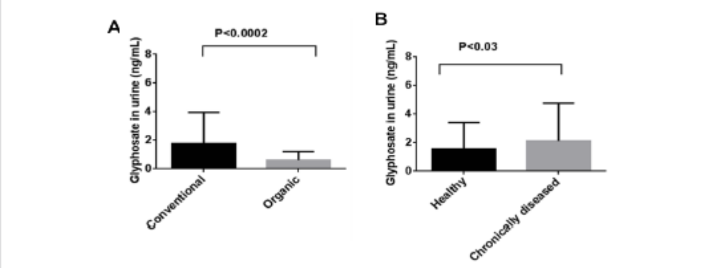


Figure 3: Glyphosate in humans. A) Comparison of glyphosate excretion with urine of humans with conventional (N=99) and predominantly organic (N=41) feeding. B) Glyphosate in healthy (N=102) and chronically (N=199) diseased humans.

3. Detection of Glyphosate in urines of 2009 German people and 48 EU parliamentarians

Monika Krüger, Andrea Lindner, Johannes Heimrath (2016): Report “Urinal 2015 Campaign” information on glyphosate contamination of German people.

The citizen`s initiative “Landwende” (Heimrath and coworkers, 2014) developed the idea to investigate glyphosate in the urine of people throughout Germany. With logistical help of the Biotest Institute in Munich and the ‘Basic’ organic food trade chain, 11,000 urine test kits with a questionnaire were distributed to interested people at 26 locations and in ‘Basic’ supermarkets in Germany in 2015. The name of this campaign was “Urinal 2015”. Altogether, 2011 filled urine sets were sent to the BioCheck Holzhausen laboratory by post for glyphosate analysis. Note for sampling was following: Please fill the sample container provided with central ray urine until half full! Afterwards, put the sample in boiling water for 10 min to prevent the transport of possible pathogens.

The results of these investigations show that glyphosate was in 2001 urines. Only 8 urines out of 2009 were below the glyphosate detection limit of the method. All other persons were contaminated. The glyphosate amounts in urines were corrected by generating the glyphosate-creatinine quotient to consider the amount of incorporated liquids. Under this correction only the difference between organic and non-organic eaters (Fig. 6 and 7) was significant.

The results show that correction of glyphosate results (Fig. 1) by glyphosate-creatinine quotient (Fig. 2) is important because of more water incorporation of females.

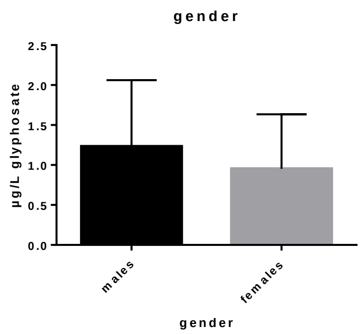


Fig 1.Glyphosate detection in urines of males (n=888) and females (n=1113).The genders differ highly significant, p<0.0001[Mann-Witney-Test]. Males: 1.3 ± 0.83 µg/L, females: 0.95 ± 0.68 µg/L.

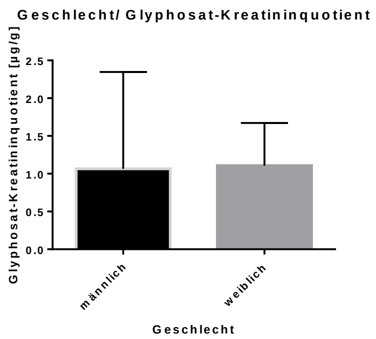


Fig 2.Glyphosate-creatinine quotient in urines of males (n=888) and females (n=1113). **No significant differences** between genders.

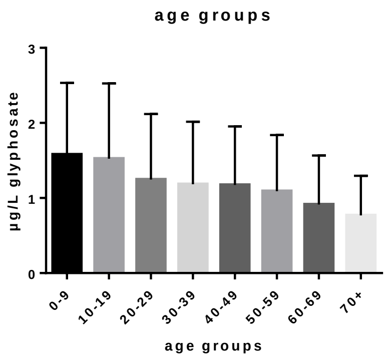


Fig. 3. Glyphosate detection in urines of participants in relation to age. Group „70+“differ from all age groups between 0 and 59 years p<0.0001,group 60-69 years differ significantly from age groups (AG) 10 – 19 years p< 0.01, 20 – 29 years p<0.001 , 30-39 years p< 0.0001, 50-59 years p<0.01 [one-way-ANOVA; Tukey´s multiple comparisons Test]. AG 0-9 years: N=24 (1.58 ± 0.93 ng/ml), AG 10-19 years: N=27 (1.52 ± 0.98 µg/L), AG 20-29 years: N=117 (1.25 ± 0.87 µg/L), AG 30-39 years: N=306 (1.18 ± 0.83 µg/L), AG 40-49 years: N=376 (1.17 ± 0.78 µg/L), AG 50-59 years: N=542 (1.09 ± 0.75 µg/L), AG 60-69 years: N=442 r (0.91 ± 0.65 µg/L), AG 70+ years: N=154 (0.77 ± 0.53 µg/L), without information N=23.

By using the glyphosate-creatinine quotient the differences between age groups were not significant. The younger participants drink more than the older one.

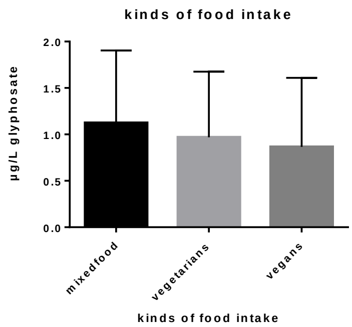
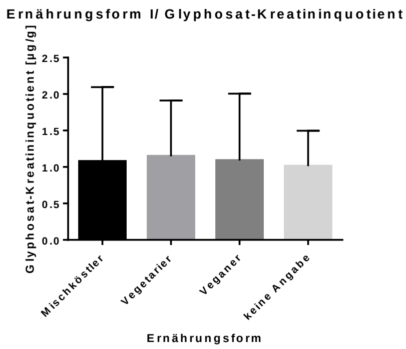


Fig. 4. Glyphosate concentrations in urines of participants in relation to kinds of food intake. Participants with mixed food **highly significant** differ from vegetarians p< 0.001 and significant from vegans p<0.01 [one-way-ANOVA; Tukey´s multiple comparisons Test]. Mixed food: N=1431 (1.13 ± 0.78 µg/L), vegetarians: N=421 (0.97 ± 0.71 µg/L), vegans: N=105 (0.87 ± 0.74 µg/L), without information: n=62.



Tab. 5. Glyphosate-creatinine quotient of mixed food eaters, vegetarians, vegans and without information**. No significant differences.**

The glyphosate-creatinine quotient was not **significant different** between mixed food and the others.

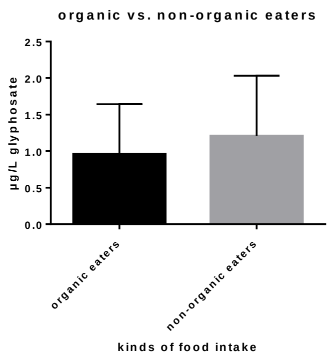


Fig. 6. Glyphosate concentrations in urines of participants in relation to incorporation of organic or not organic foods. **Highly significant differences** between organic and non- organic eaters: p <0.0001

[Mann-Whitney-Test]. Organic eaters: N=963 (0.958±0.68 µg/L), non-organic eaters: N=926 (1.21±0.83 µg/L), without information: N=122.

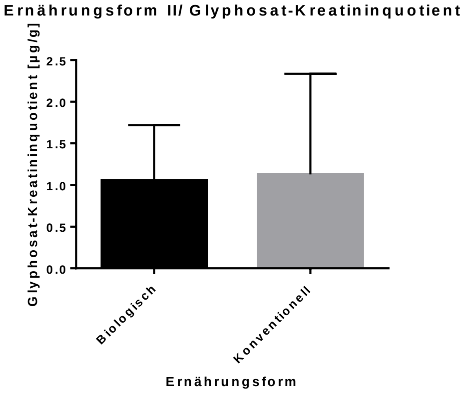


Fig. 7. Glyphosate-creatinine quotient of organic and non-organic eaters. S**ignificant differences** between organic and non- organic eaters: p <0.0032.

48 urines of members of the EU-parliament were investigated for glyphosate and creatinine. No urine was zero for glyphosate. The participants belonged to 13 countries. The relatively small numbers of participants were the cause of no significant results between the groups. All participants excreted glyphosate by urine. This means that glyphosate could be also a health problem of EU-parliament members.

Tab. 1. Glyphosate and creatinine concentrations in urines of 48 members of the EU parliament in relation to their nationality

		µg/L glyphosate				creatinine (g/l)		Gly (µg)/Crea (g)
country	**n**	**average**	**standard deviation**	**maximum**	**minimum**	**average**	**standard deviation**	
BELGIUM	20	1,63	0,90	3,57	0,56	1,15	0,51	1,41
CZECH REPUBLIC	1	0,17	0,00	0,17		0,11	0,00	1,49
FRANCE	7	2,10	0,70	2,45	0,39	1,27	0,48	1,65
GERMANY	7	1,87	0,72	2,59	0,68	1,31	0,40	1,43
HUNGARY	1	2,63	0,00	2,63		1,25	0,00	2,10
ITALY	4	2,62	0,13	2,84	2,53	1,25	0,43	2,09
LITHUANIA	1	0,98	0,00			1,23	0,00	0,80
NETHERLANDS	1	1,18	0,00			0,95	0,00	1,25
SPAIN	1	2,40	0,00			2,28	0,00	1,05
UNITED KINGDOM	2	0,56	0,11	0,67	0,45	0,63	0,22	0,88
CROATIA	1	2,46	0,00			0,85	0,00	2,90
FINLAND	1	0,62	0,00			0,67	0,00	0,92
IRELAND	1	0,80	0,00			0,79	0,00	1,02

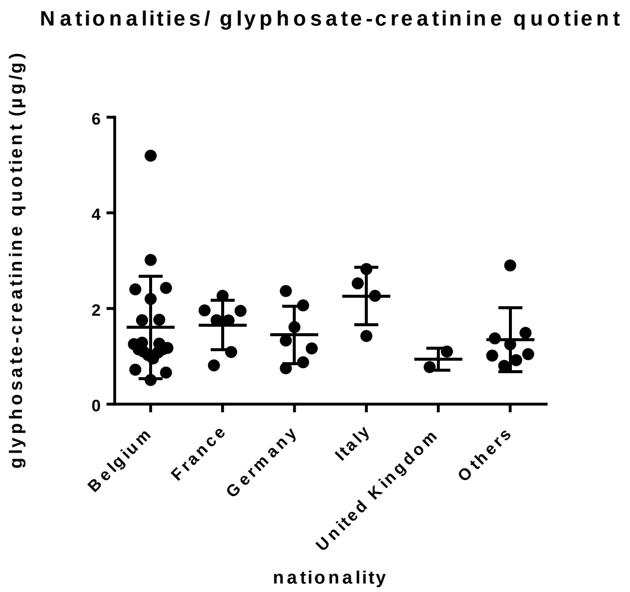


Fig. 8a. Distribution of glyphosate amounts in urines of members of the EU parliament in relation to nationality. **No significant differences** between the participants of the different countries

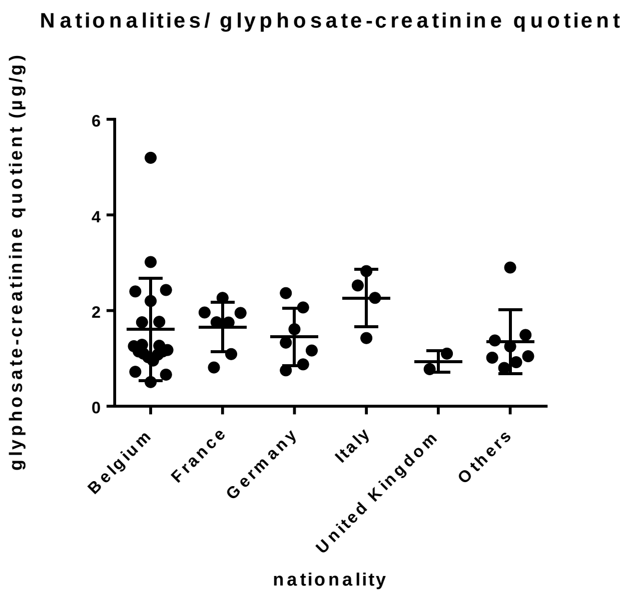


Fig. 8b. Distribution of glyphosate amounts in urines of members of the EU parliament in relation to creatinine concentration and nationality. **No significant differences** between the participants of the different countries