Fresenius Z Anal Chem (1988) 332:561–564

Fresenius Zeitschrift für Analytische Chemie

Problems in choosing the organs of roe deer (*Capreolus capreolus*) as reference material*

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Probleme bei der Organauswahl von Rehen (*Capreolus capreolus*) als Referenzmaterial

Summary. Within a research programme of the specimen bank the standard operation procedures have to be elaborated for roe deer. The liver seems to be one of the important organs to be collected for this task. Most of the chlorinated hydrocarbons and elements accumulate preferably in this organ. The distribution of the contaminants is very homogeneous in this tissue. The mean weights of liver from roe deer vary from ca. 280–500 g. At least 100–180 animals have to be collected from certain groups with distinct parameters such as age, sex, living space and time of the year in order to get 50 kg reference material. There seem to exist certain levels of contamination for the different groups. On the completion of further data for proving these levels the livers could be pooled.

Introduction

Since wildlife accumulates pollutants all the year in its homerange, it is interesting to choose them as bioindicators [1]. Roe deer especially has certain advantages as a bioindicator within the terrestrial food chain for certain tasks in the environmental specimen bank [2]. Therefore it seems to be only a question of time until we have to prepare the organs of roe deer for reference material.

Table 1 may give an impression of the advantages of choosing roe deer as a bioindicator, from which we could collect certain organs for reference material. We observe a wide distribution in the Eurasian continent, a limited homerange up to 40 ha, a familiar food chain and enough animals for specimen collection. The weight of an organ, such as for instance the liver is high enough for subsampling, which may be important for the analyses to be performed. Under what circumstances these livers should be pooled will be outlined later.

Results and discussion

Specimen from roe deer were taken in the last three years in three forestry districts in the east of Lower Saxony. The areas of the districts are nearly 8,000 ha each, and are planted mainly with oak, beech and pine.

In a first step we investigated the organotropy of the different chlorinated hydrocarbons and elements.

In Tables 2 and 3 the relation of the main contaminants was calculated from the mean values of the contaminants by taking kidney fat and kidney equal to 1. The results were calculated for organics on extracted fat and for anorganics on fresh matter basis. Preferably young roebucks from one forest district were chosen to limit influencing factors. Predominantly α, β-BCH, HCB, DDE accumulate in the liver, the PCB components mainly in kidney fat.

Some elements such as Mo, Al, Mn, Cu, Fe are predominantly concentrated in the liver, while the toxic metals Pb and Cd have higher concentrations in the kidney. Consequently, a part of the contaminants seems to have a high accumulation in the liver.

In a first step in the sample procedures we eleborated the distribution of the contaminants in the liver. Three livers were cut into 7 pieces each and the samples were analysed separately. Tables 4 and 5 show a comparison of 7 analyses of the organ with the combined extraction solution measured 7 times. The calculated mean values of the chlorinated hydrocarbons nearly correspond. The coefficients of variation are slightly higher in the organs than in the combined extraction solution (α, γ-BCH, HCB, 4,4′-DDE, Dieldrin, PCB 138, 180). Comparing the elements in parallel we observe only higher coefficients of variation for Pb, Cd, Al, Ca, Fe. There seem to exist certain irregularities in determining V and Al by ICP, as we may conclude from the high coefficients for the combined digestion solution.

Thus the contaminants have an excellent equal distribution in the liver. Parts of a liver to be taken for analysis should therefore be representative for the whole organ.

One main requirement for preparing reference material is a homogeneous distribution of the contaminants in the matrix. Therefore we marked out a plan how to collect the organs in order to get at least a total amount of 50 kg [3].

The samples are combined under aspects of different parameters such as forest district, sex, time of the year and age of the animals (Table 6).

The mean weights were calculated of liver and left kidney of 298 animals. The relation of the two organs seems to be approx, 10:1. Older animals as the female groups in the winter time have the highest weights. Since a collection of the kidney organs would require 5 times more animals than for the liver the last organ should have the preference in preparing reference material. Groups with more than 50

* This paper is granted by the "Umweltbundesamt" in a research programme

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Table 1
Advantages of choosing roe deer as reference material

Criteria	Roe deer	Valuation
Distribution	West Europe Near East Iran	Part of a continent
Homerange	25–40 ha	Controlled area
Bioindicator	Herbivore in forest and agricultural areas	Clear food chain
Number of shot animals	700,000/Year in FRG	Enough for sampling
Weight of liver	$\bar{X}=372$ g $N=298$	High enough
Weight of kidney	$\bar{X}=$ 33 g $N=258$	Too low

Table 2. Relation[a] of the chlorinated hydrocarbons in the organs of <18 months old roebucks

CH	Liver	Kidney	Kidney fat[a]	Brain
α-BCH	28	3	1	1
β-BCH	5	4	1	0.3
γ-BCH	0.7	0.7	1	0.2
HCB	3	2	1	0.1
4,4′-DDE	2	0.7	1	0.1
PCB 138	0.7	0.3	1	0.1
PCB 153	0.6	0.3	1	0.1
PCB 180	0.6	0.3	1	0.1

[a] The relation was calculated from the mean values of the contaminants by taking kidney fat and kidney = 1, $N=43$

Table 3. Relation[a] of the elements in the organs of <18 months old roebucks

Elements	Liver	Kidney[a]
Pb	0.6	1
Cd	0.1	1
V	0.9	1
Cr	1.3	1
Mo	3.8	1
Al	4.9	1
Mn	2.6	1
Cu	1.5	1
Zn	0.8	1
Ca	0.6	1
Fe	2.3	1
Mg	1.0	1
Ni	1.2	1
Tl	0.8	1
Sb	1.2	1

[a] The relation was calculated from the mean values of the contaminants by taking kidney fat and kidney = 1, $N=43$

animals were marked with a circle. According to the calculation we have prepared only 17–20 kg reference material from one group. Therefore, a further requirement is to pool more animals with a certain level of contamination.

In Figs. 1 and 2 we present first results of the contamination level for the chlorinated hydrocarbons and elements in different groups of roe deer. We have chosen a small group of young roebucks from the forest district near Braunschweig with distinct parameters such as sex, age and living space. This group is compared with all the animals so far collected and analysed. Only those results were calculated where median and range were over the detection limit. The smaller group is always mentioned in the first line of the different components.

Table 4. Distribution of chlorinated hydrocarbons in the liver of roe deer

CH	7 samples prepared separately		Combined extraction solution – 7 analyses	
	$\bar{X}$	± CV	$\bar{X}$	± CV
α-BCH	0.115	*13.9*	0.122	4.9
β-BCH	0.028	17.9	0.029	17.2
γ-BCH	0.017	*17.6*	0.025	8.0
HCB	0.020	*10.0*	0.021	3.3
4,4′-DDE	0.057	*12.3*	0.061	4.9
4,4′-DDD	0.030	10.0	0.030	10
4,4′-DDT	0.004	50	0.006	50
Dieldrin	0.035	*8.6*	0.037	2.2
PCB 101	0.010	10.0	0.011	27.3
PCB 138	0.030	*16.7*	0.037	5.4
PCB 153	0.044	9.1	0.045	4.4
PCB 180	0.030	*10.0*	0.030	3.3

Mean values mg/kg fat

Table 5. Distribution of 15 elements in the liver of roe deer

Elements	7 samples prepared separately		Combined digestion solution – 7 analyses	
	$\bar{X}$	± CV	$\bar{X}$	± CV
Pb[a]	0.021	*19.0*	0.018	5.56
Cd	0.138	*5.80*	0.139	2.16
V	0.047	23.4	0.060	*26.7*
Cr	0.329	3.04	0.351	1.99
Mo	0.699	2.72	0.765	1.70
Al	1.99	*36.6*	1.94	*8.04*
Mn	5.35	1.72	5.55	1.15
Cu	25.9	2.04	26.6	1.29
Zn	33.3	1.82	35.2	1.27
Ca	38.4	*3.59*	39.9	1.27
Fe	95.7	*5.76*	99.0	1.26
Mg	173	1.57	177	1.24
Ni	<0.050	–	<0.050	–
Tl	<0.075	–	<0.075	–
Sb	<0.125	–	<0.125	–

[a] Pb determined by AAS, other elements by ICP
Mean values in mg/kg

There seem to be different levels of concentration concerning the components BCH, DDE, PCB 138 and 153. They have higher concentrations than the other chlorinated hydrocarbons. The medians alter with lower contents for the BCH and higher contents for the DDE and the main PCB components in young roebucks, respectively. Although

Table 6. Mean weights[a] of liver and kidney from roe deer[b]

Forest district	Organ	Roebuck				Female roe deer			
		16. 05. – 30. 11.		01. 12. – 31. 01.		01. 09. – 30. 11.		01. 12. – 31. 01.	
		<18 months	>18 months	<18 months	>18 months	<18 months	>18 months	<18 months	>18 months
Braunschweig	L	○ 348	–	281	–	○ 342	489	○ 370	○ 408
	K	36	–	47	–	35	53	47	87
Fallersleben	L	372	–	–	–	309	426	361	436
	K	35	–	–	–	43	52	48	68
Lappwald	L	412	–	323	–	–	–	314	380
	K	35	–	31	–	–	–	36	63

[a] In g freshweight from the 16. 05. 1985 – 31. 01. 1987
[b] *N* 298, groups below 5 were not calculated; *L* liver; *K* kidney; ○ more than 50 animals

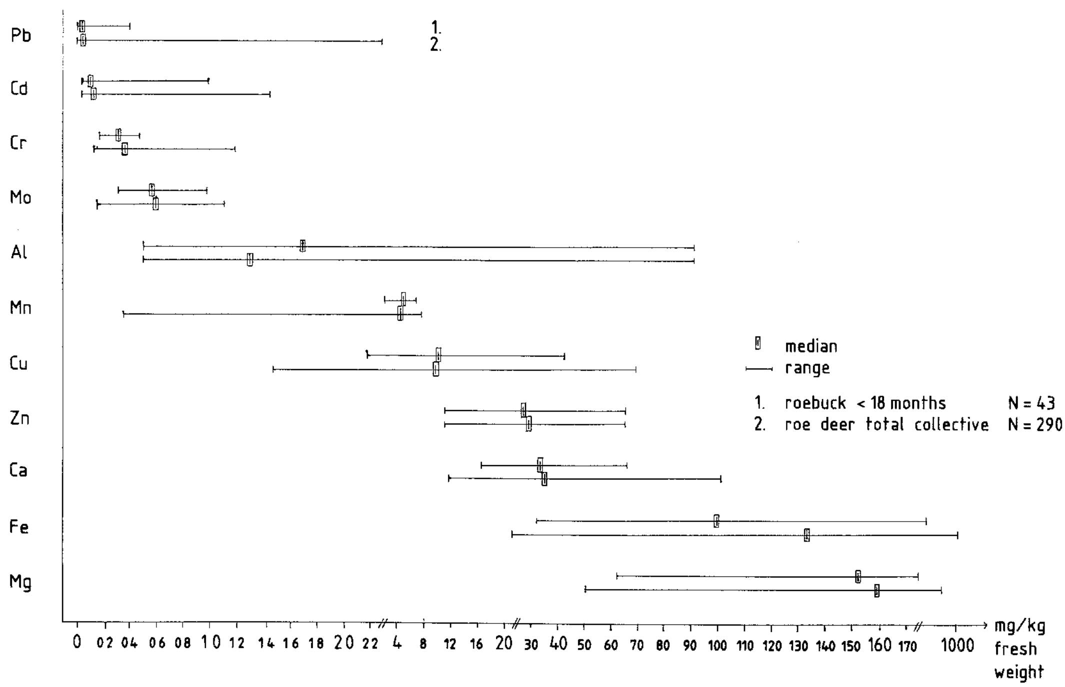

Fig. 1 Comparison of element contents in the liver of different collectives

we have selected a young group of roebucks with certain parameters, the ranges are still fairly high compared with the total group. Generally the roebuck group covers 3/4 of the ranges of all the animals, except the higher chlorinated PCB with only 1/3.

The medians and ranges of the elements in the same groups are demonstrated in Fig. 1. The elements are listed according to the contents to be expected, beginning with the lowest and ending with the highest. The scale for the contents in mg/kg fresh weight is partly interrupted to demonstrate also the higher contents. The medians show differences for Cd, Cr, Mo, Zn, Ca, Fe, Mg with higher contents in the group of all animals. Aluminium contaminations seem to have higher levels in the younger group.

The ranges for the young roebuck group seem to be rather limited for the elements Pb, Cr, Mn and increase for Cd, Mo, Cu, Ca. Nearly equal ranges are to be observed in both groups for Al, Zn, Fe, Mg.

A further discussion of the results might be too early, because the analytical work is not yet finished and the statistical evaluation is still in progress. So far we have observed similar results in other regions [4–12]. We only wanted to demonstrate what differences are to be expected for the contaminants under the aspects we have chosen such as age, sex and living space. Most probably we will have to accept a certain individuality of each animal resorbing the contaminants via the digestion tract quite differently although we have tried to limit the influencing parameters. This phenomen has already been discussed for cattle and to some degree for other wildlife [6, 7].

Conclusion

1. The present stage of our work defining roebuck for specimen banking demonstrates that liver will be the main organ for specimen collection. Nearly half of the chlorinated hydrocarbons and elements determined accumulate to a higher level in the liver compared with the kidney and, to some extent, kidney fat and brain.

2. First results concerning the distribution of the contaminants in two groups show a different accumulation

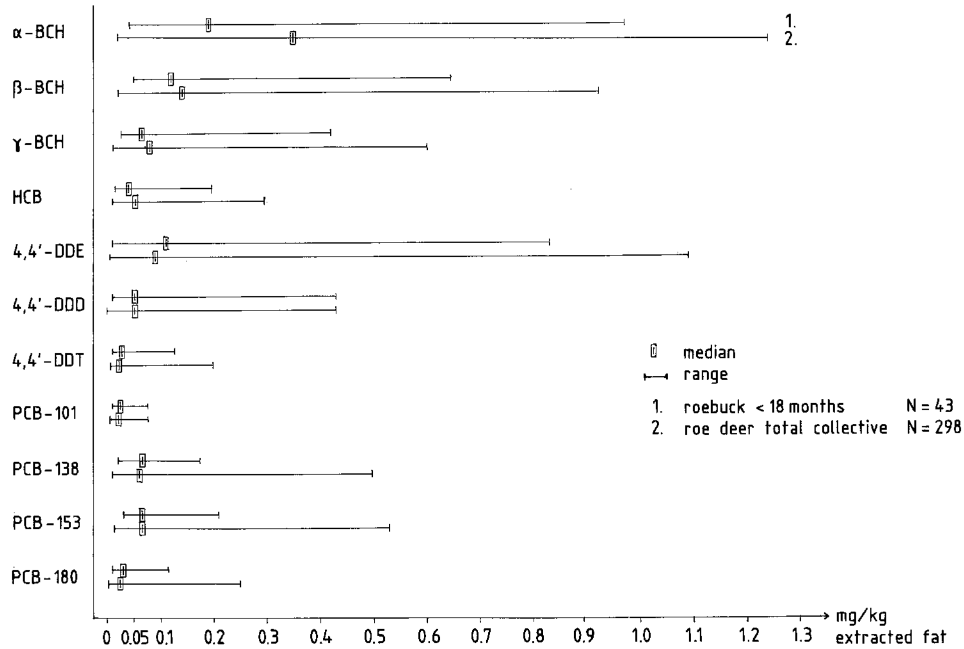

Fig. 2 Comparison of chlorinated hydrocarbon contents in the liver of different collectives

of the components analysed so far. The assumption seems to be correct that certain levels of contamination are to be expected in the different groups to be formed under aspects such as age, sex, living space and time of the year.

3. The liver weights allow an estimation of the numbers of roe deer to be collected to get at least 50 kg reference material. Nearly 100–180 animals would be necessary for the different groups, except that an equal contamination level of certain groups permits pooling of the samples.

Acknowledgement. The authors would like to thank Mrs. B. Wolfsteller for technical assistance.

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Received July 1, 1988