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Safety

in Laboratory and Regulatory Services for Herbal Products



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Risk Management of Contaminants in Herbal Substances and Herbal Preparations

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Overview of topics

- Introduction
- Basis of risk management
- Examples from practice
 - pesticides (analytical methods)
 - bromide (regulation)
 - ochratoxin A (reduction of residues)
 - microbiological counts (reduction during processing)
- Summary

Introduction

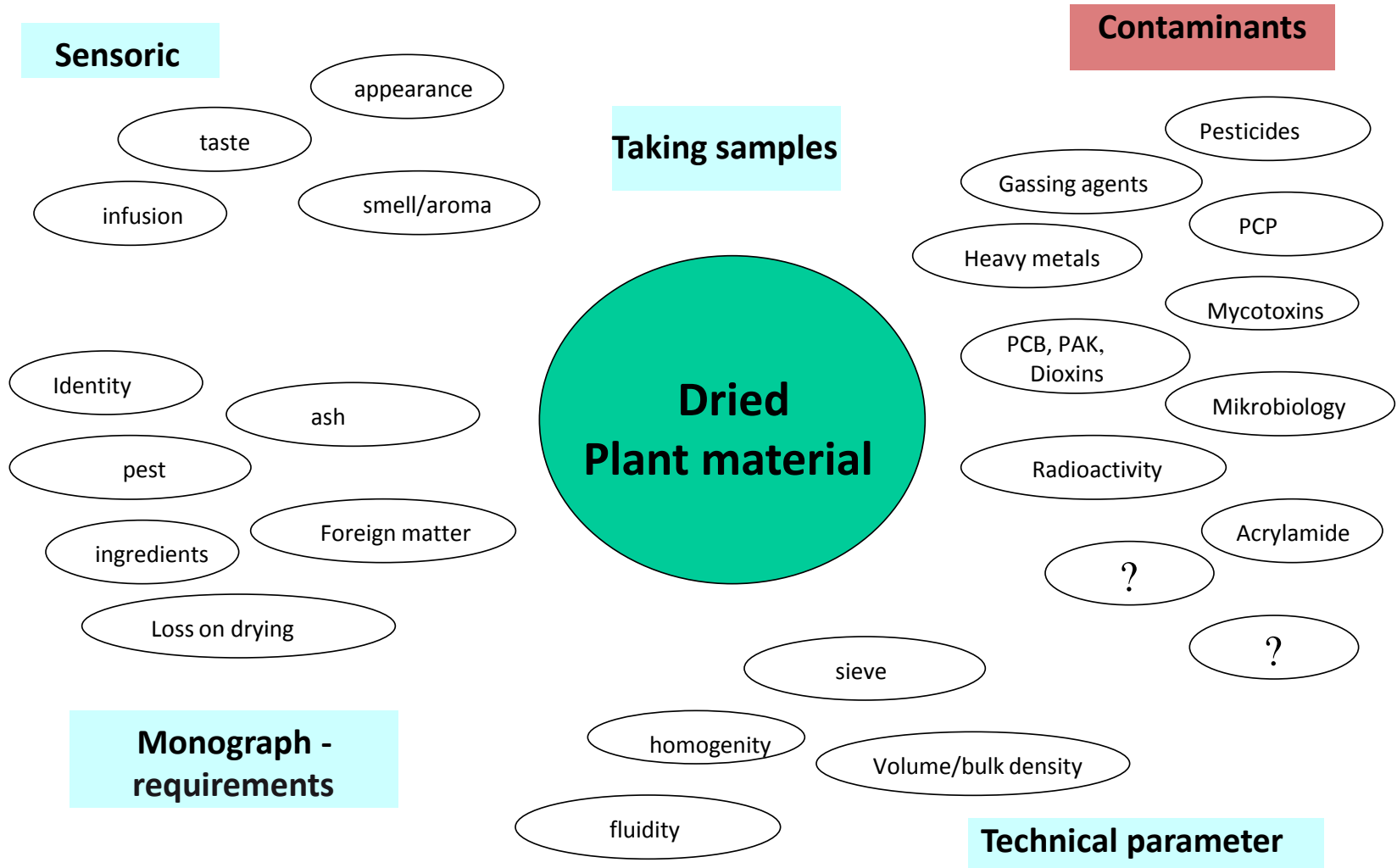
Raw products of herbal origin are naturally subject to considerable variation in terms of their constituents. Of the roughly 400 plants and parts of plants on the herbal market, 30 to 40 plants are cultivated on a large scale. Only about 30 % of the total amount of herbal drugs are gathered from the wild.

Nevertheless, in quantitative terms, the vast majority (80 - 90%) of plant species are collected from the wild.

Introduction

- It must not assumed controlled cultivation conditions only, but should also taken into account the world-wide contamination of the plant world as well as the particular circumstances in the countries of origin, which are mostly outside the EU.

Criteria influencing the quality of herbal material:



BASIS FOR RISKMANAGEMENT OF CONTAMINANTS

Basis for Riskmanagement of Contaminants

- screening of existing regulations, publications
- definition of possible contaminants
- development of suitable methods of analysis
- performing analysis (monitoring products, target analyses)
- evaluation of results (data base)
- risk based schemes of testing (herbal drug is normally tested)

References to Contaminants

- **European Pharmacopoeia** (monograph „Herbal Drugs“, pesticides, heavy metals, aflatoxins, ochratoxin A and microbiology).
- **EC regulations for foodstuffs** (e.g. pesticides, mycotoxins, heavy metals, PAHs, radioactivity, nitrates).
- **National regulations** (e.g. German aflatoxin regulations, German regulation for contaminants).
- **Publications**
(e.g. Codex Alimentarius, Food and Agriculture Organization (FAO/UN);
Compendium: „Worldwide regulations for mycotoxins 1995“;
Current Findings on the Heavy Metal Content in Herbal Drugs, Pharmeuropa Scientific Notes 2009-1).

Possible Contaminants

1. Pesticides
2. Gassing agents (Methyl bromide, Ethylene oxid, PH_3)
3. Heavy metals, especially toxic metals
4. Mycotoxins (aflatoxin, ochratoxin A and others)
5. Radioactive contamination (Cs 134/137)
6. Microbiological contamination
7. Other contaminants (dioxins, PAH`s, acrylamide, ...)

PESTICIDES (SUITABILITY OF ANALYTICAL METHODS)

- scope of testing
- analytical methods
- matrix effects
- risk assessment

Development of methods for pesticide testing

There are **550 compounds and metabolites** listed in EU 396/2005, but totally existing **about 1.650 chemical pesticides** (Pesticide Manual 2003).

In analytical laboratories **400-600** analytes could be detected with:

- multi methods
- group specific methods
- single residue methods.

There is an analytical gap of more than 1000 substances.

Development of methods for pesticide testing

Multi methods:

GC-methods (e.g. DFG S19)

→about 200-300 substances

LC-MS/MS (e.g. Quechers)

→about 200-300 substances

Group specific methods:

Dithiocarbamates, Phenoxyalkancarboxylic acids, Phenylureas, Carbamates, ...

→about 60 substances

Single methods:

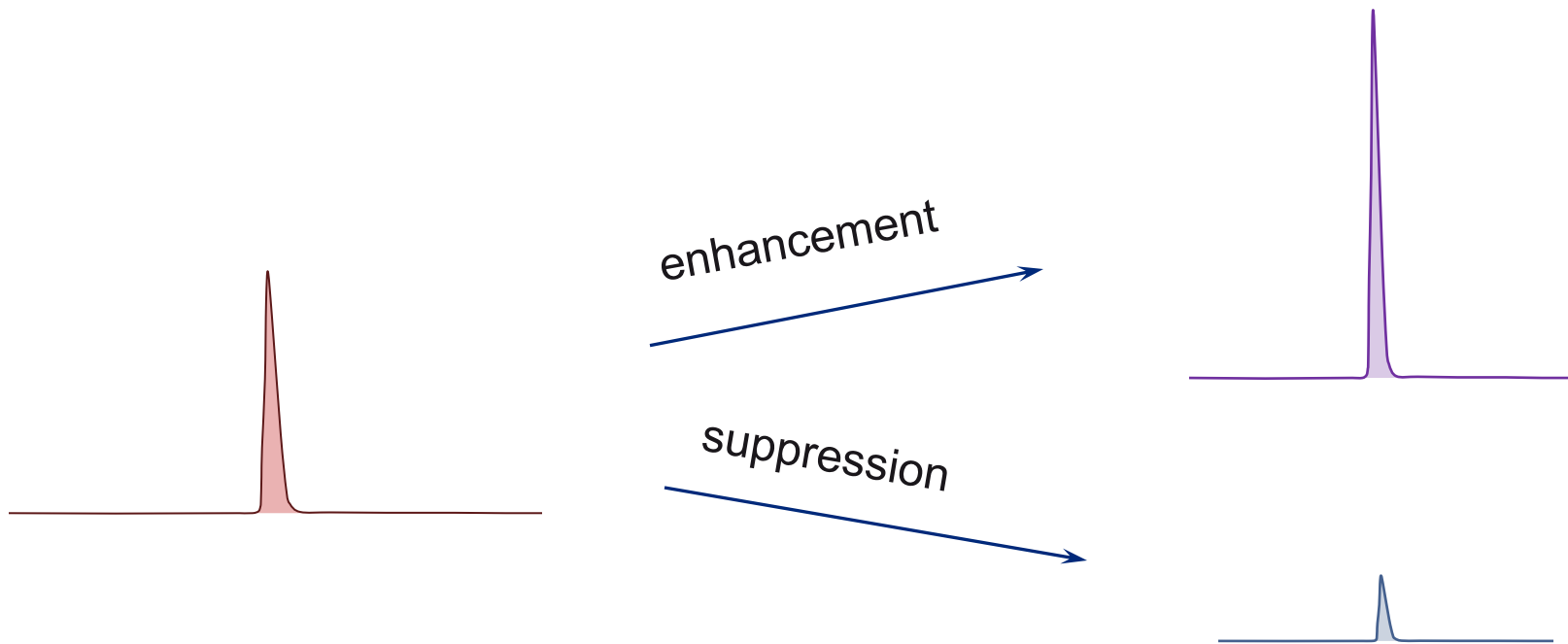
Pyridat, Chlormequat, Glyphosate, Paraquat, Nicotine ...

→about 20 substances

Matrix effects

In dried herbal material a strong matrix effect has been observed in several cases.

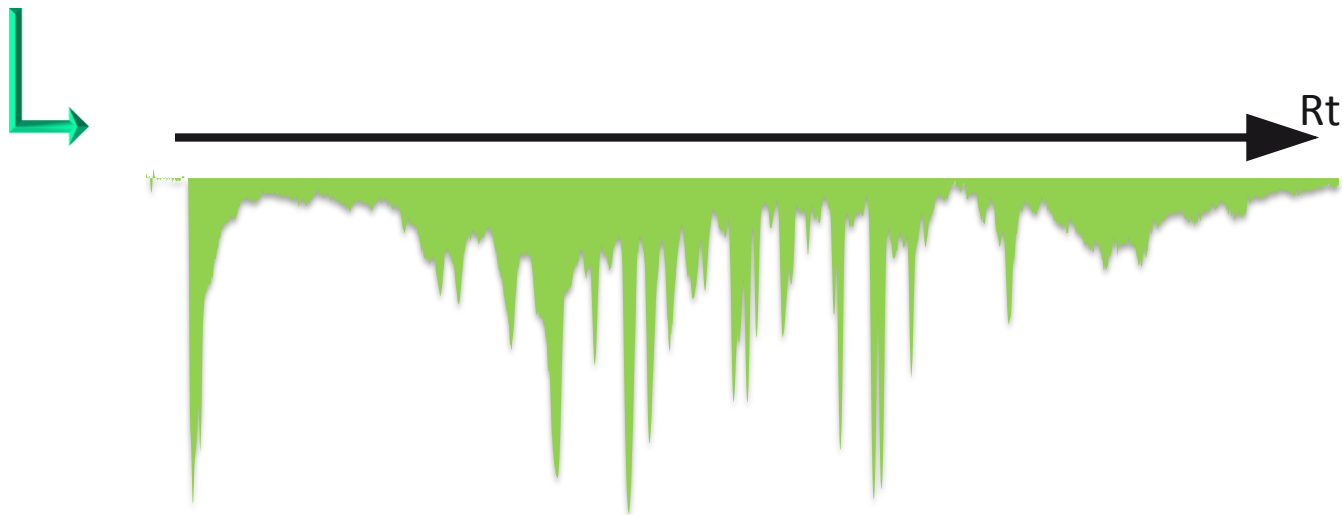
→ Matrix effects cause changes in signal intensity



Measurement of matrix effects in LC-MS/MS

Determination of matrix effect profiles with **postcolumn infusion**

(G. Kempe, 6th International Fresenius conference, 24th May 2011)



→ with continuous measurement of matrix effects the matrix load in the entire chromatogram could be made visible.

Requirements for calibration

- matrix calibration (or internal labelled standard) is required
- in LC-MS/MS there are mainly **peak suppressions** observed:
 - in LC-MS/MS each sample has to be calibrated necessarily in the same matrix
- in the GC-MS-MS **suppression or fortification** of the signal are possible
- unclear findings should be confirmed by standard addition; but false positive results caused by matrix (same product ions, same RT)
 - change chromatography or **HRMS**

Criteria for pesticide residues

- persistence of substances
- country of origin (worldwide, EU), supplier
- harvested or wild collected
- cross contamination (*field to field, warehouses, processing, packing, etc.*)
- treatment on the field, in the warehouses, transport
- kind of product

Requirements for a effective control of pesticides in herbal products:

- detailed informations about the **legal requirements**
- expericence and knowledge **which pesticides are used** on the different plants and in which country respectively
- Expericence and knowledge about **risks of contamination** (data base)
- Choice of the **most suitable test method(s)**
- monitoring and target analyses with herbal drug

Pesticides - assessment

- it is not possible to guarantee products without any pesticide residue
- people are very sensitive to any pesticide residues
- in quality control not all pesticides could be tested every time because of the number of substances (analytical gap) and the number of different methods used (→ **risk based analysis**).

BROMIDE (REGULATIONS)

- origin of bromide residues
- data base
- risk assessment

Bromide

The bromide content in a plant material can occur of natural origin (maritime organisms, like fucus or seaweed) or taken from soil (salts; close to sea) and accumulated (e.g. chamomile).

A high bromide content is also caused by a methyl bromide treatment (soil, products).

Methyl bromide

A very reactive and high toxic substance that can be detected for only a very short period after treatment.

→ *Maximum residue limit: 0.01 mg/kg*

Bromide

There is only an indirect determination via „total inorganic bromide“ discribed. In this case a specific detection of methyl bromide treatment is not possible.

Maximum residue limits (MRLs) of „total inorganic bromide“ (EC 396/2005) :

Spices	400 mg/kg
Chamomile flowers	250 mg/kg
Hibiscus flowers	100 mg/kg
Tea (camelia s.)	70 mg/kg
Fresh herbs	50 mg/kg (→ 250 mg/kg)
Other Herbal Infusions	50 mg/kg

Bromide

Maximum residue limit (MRL) of „total inorganic bromide“ (Ph.Eur.) :

Herbal drugs

50 mg/kg (threshold ?)

Proposed maximum residue level of „total inorganic bromide“ in organic foodstuff (BNN) :

Organic foodstuff

5 mg/kg (*)

(*) If the use of methyl bromide could be excluded higher levels are allowed.

Bromide

In herbal drugs bromide values from 1 up to 400mg/kg (chamomile) have been found; maritime plants contain organic bond bromine in higher concentrations (fucus contains values up to 865mg/kg). (*)

But only a few results are above 50mg/kg:

→ chamomile, fucus, hibiscus, lemon verbena, lemongrass, mate, some herbal drugs for homoeopathic use.

(*) Data base of the German manufacturers` Association`s (BAH) Working group on Contaminants; containing datas of 1330 samples analysed from 2002 until 2010.

Bromide: assessment of risk

Toxicological relevance

The ADI of 0.4 mg/kg body weight yields an acceptable total daily intake of 24 mg bromide / person for a 60 kg person and 4 mg bromide / person for a 10 kg child.

Assuming a maximum residual level of 250 µg bromide per g herbal tea (250 ppm) and regarding an ADI of 0.4 mg/kg body weight

•an adult with 60 kg is allowed to consume $24,000 \mu\text{g} / 250 (\mu\text{g} / \text{g}) = 96 \text{ g}$ herbal tea per day (= 9,6 liter herbal infusion)

•a child with 10 kg is allowed to consume $4,000 \mu\text{g} / 250 (\mu\text{g} / \text{g}) = 16 \text{ g}$ herbal tea per day (= 1,6 liter herbal infusion).

Bromide: assessment of risk

- there is only a low risk concerning toxicity
- for some herbal drugs (pharmaceutical or homoepathic use) exceptional MRLs should be set
- there is a high risk for an objection of herbal products of organic origin (low limit, methyl bromide treatment must be excluded).

MYCOTOXINS (REDUCTION OF OCHRATOXINE A RESIDUES)

- occurrence
- reduction
- risk management

Significance and occurrence of mycotoxins

Metabolic products of moulds, characteristic of each species, for the most part heat-stable

Acute toxicity: damage to liver, kidneys, nervous system, skin, mucous membranes, immune systems

Chronic toxicity: may provoke cancer, may cause congenital abnormalities and malformations in the embryo

UN Food and Agriculture Organisation (FAO):

- 25% of the world production of foodstuffs
- 20% of the cereal harvest in the EU

is contaminated with mycotoxins.

Moulds forming Mycotoxins

Micro-organism

Aspergillus spec.

Aspergillus spec., Penicillium spec.

Fusarium spec.

Mycotoxin

Aflatoxin B1, B2, G1, G2,
(Metabolite in mammals: Aflatoxin M1)

Patulin

Ochratoxin A

Fumonisin

Zearalenon

Trichothecenes

(Deoxynivalenol, Nivalenol, T2 Toxin and HT2 Toxin)

Origin and occurrence of Ochratoxin A

Origin

- *Aspergillus ochraceus* on foodstuffs of plant origin, Ochratoxins principally Ochratoxin A,
- in warm regions of the earth

Occurrence

- in harvest products worldwide (toxins generated during storage)
 - Maize, oats, barley, wheat, rye
 - Rice, millet, buckwheat
 - Soya beans
 - Ground nuts, Brazil nuts, pepper
 - Coffee, cocoa (chocolate), wine, beer
 - **Dried fruit, vegetables, spices**

Ochratoxin A limits

Product	Limit µg/kg	Regulation
Raw cereal grains	5,0	(EC) No 1881/2006
Dried wine fruit	10,0	(EC) No 1881/2006
Soluble coffee	10,0	(EC) No 1881/2006
Roasted coffee beans	5,0	(EC) No 1881/2006
Wine, grape must, grape juice	2,0	(EC) No 1881/2006
Several spices	30 (15)	(EC) No 1881/2006
Baby food	0,50	(EC) No 1881/2006
Liquorice root	20	(EC) No 1881/2006

Ochratoxin A positive samples

Analysis of 1978 samples show:

products with positive results in many cases ($>20\mu\text{g}/\text{kg}$):

cocoa, currants, **dandelion root**, ginger, **liquorice root**,
marshmallow root, **orange flowers**

products with sporadical positive results:

linden flowers, orange peels

Effect of improved GACP on Ochratoxin A levels in Liquorice root (ppb)

Year	n	max. value	90 th percentile
1999	50	425	234
2000	159	904	145
2001	192	337	41
2002	152	423	34
2003	60	72	20
2010	257	117	26

Risk management of ochratoxin A

- target analyses of ochratoxin A (based on risk assessment)
- ochratoxin A is soluble in alcoholic solvents: analyses of the extracts have to be performed if possible contamination of raw material is known
- in quality control not all mycotoxins have to be tested every time, because only a few herbal drugs are contaminated
- monitoring of other mycotoxins
- providing contamination (e.g. drying conditions; GACP)

MICROBIOLOGICAL COUNTS (REDUCTION DURING PROCESSING)

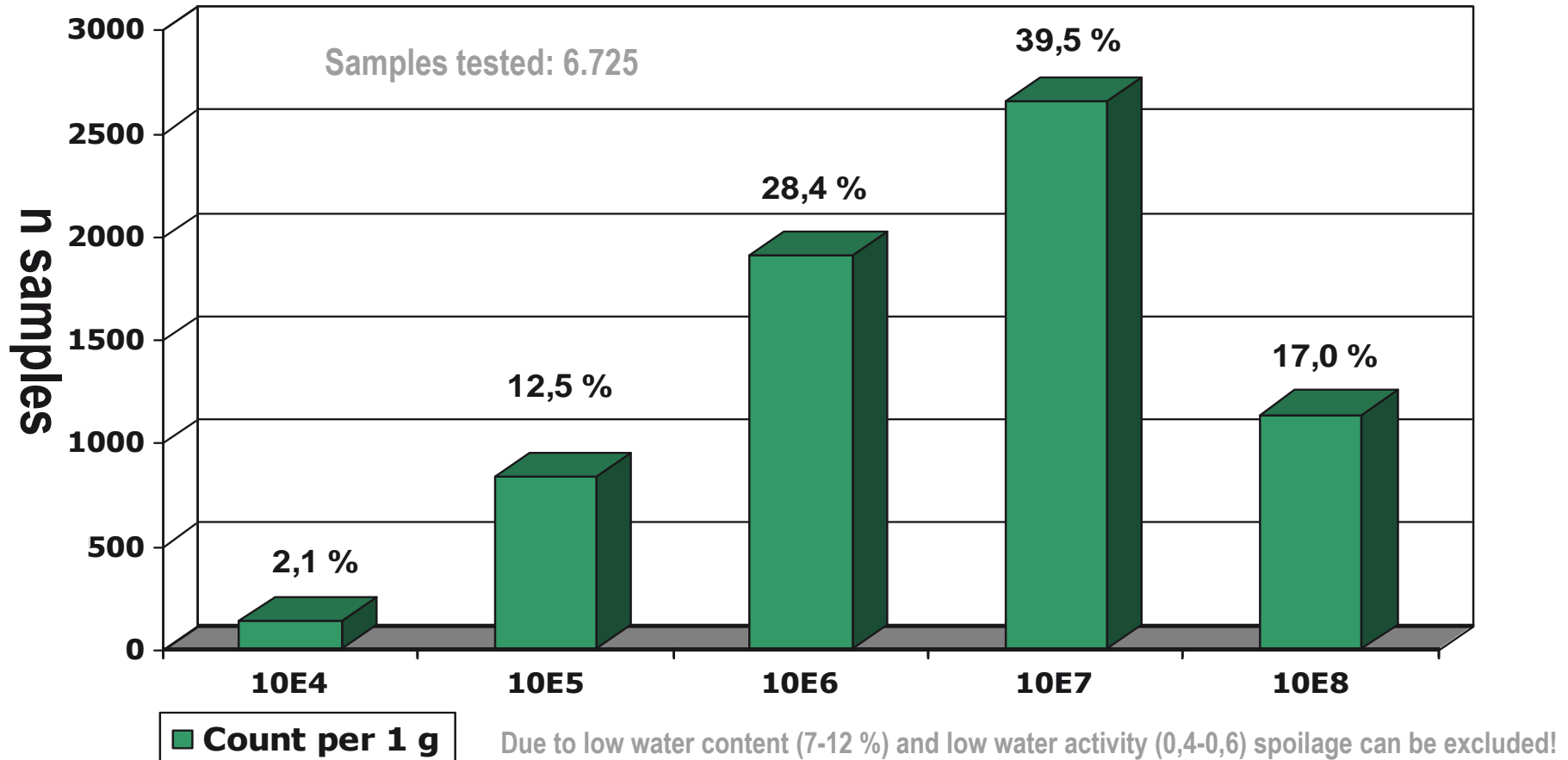
- microbiological counts
- acceptance criteria
- procedures for reduction

Regulations for the Microbiological Quality

- European Pharmacopeia Ph.Eur. 7th edition, 5.1.8: Microbiological quality of herbal medicinal products for oral use
- EHIA`S (European Herbal Infusion Association) recommended microbiological specifications for Herbal infusion (dry) and Herbal infusion raw materials (dry), (EHIA) 4, May 2011
- Recommendations of the working group on Food - Microbiology and Hygiene of the German Society for Hygiene and Microbiology (DGHM, May 2011)
- USP 34, <2023> : Microbiological Attributes of nonsterile nutritional and dietary supplements.

Microbiological Count of Herbal Drugs

Total Aerobic Count



Total Aerobic Count

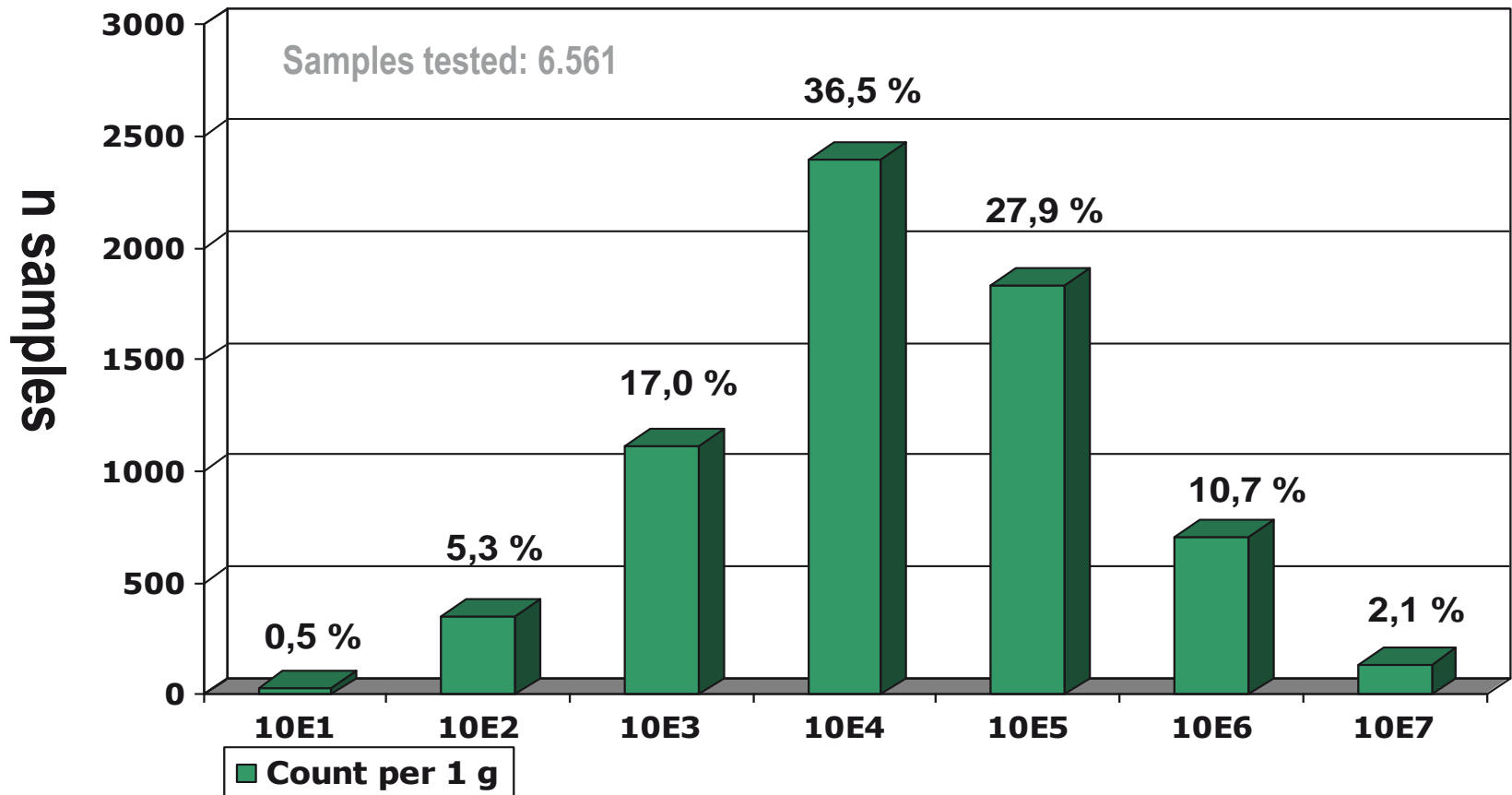
Produkt	Limit	Problem
Dry soups, DGHM	10^6	-
Herbal infusion raw material, EHIA	10^8	-
Herbal medicinal teas, Ph. Eur.	10^7	+
Botanicals to be treated with boiling water before use, USP 34	10^5	++
Spices for direct ingestion, DGHM	-	-
Medicinal drugs, direct intake, Ph. Eur.	10^5	++
Instant products, DGHM	10^5	-
Extracts and tinctures, Ph. Eur.	10^4	-

Russian Requirements

500.000

++

Microbiological Count of Herbal Drugs Moulds and Yeast's



Moulds and Yeast`s

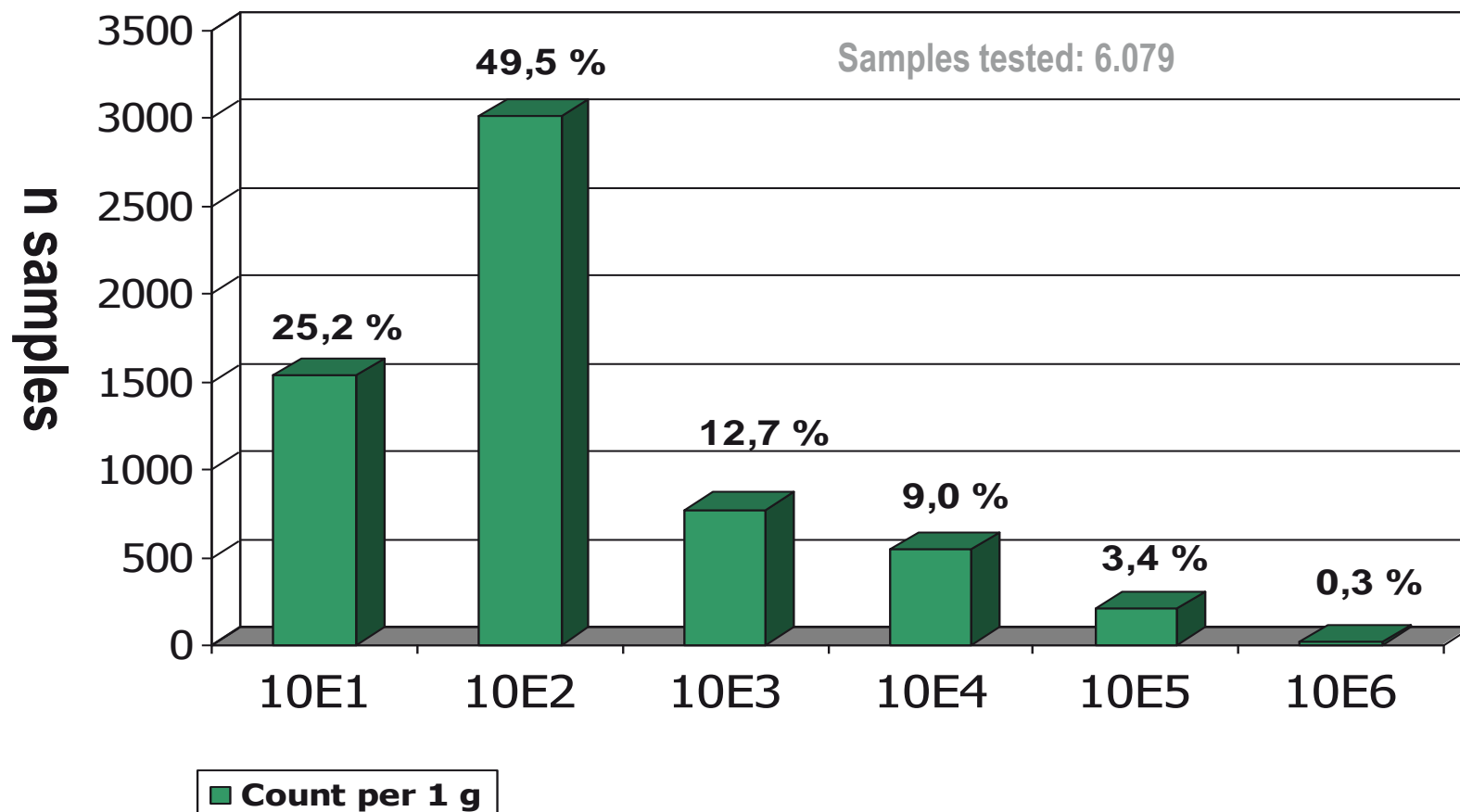
Produkt	Limit	Problem
Dry soups, DGHM	10^4	+
Herbal infusion raw material, EHIA	10^5	+
Herbal medicinal teas, Ph. Eur.	10^5	+
Botanicals to be treated with boiling water before use, USP 34	10^3	++
Spices for direct ingestion, DGHM	10^5	+
Medicinal drugs, direct intake, Ph. Eur.	10^4	++
Instant products, DGHM	10^4	-
Extracts and tinctures, Ph. Eur.	10^2	-

Russian Requirements

100

+++

Microbiological Contamination of Herbal Drugs Escherichia coli



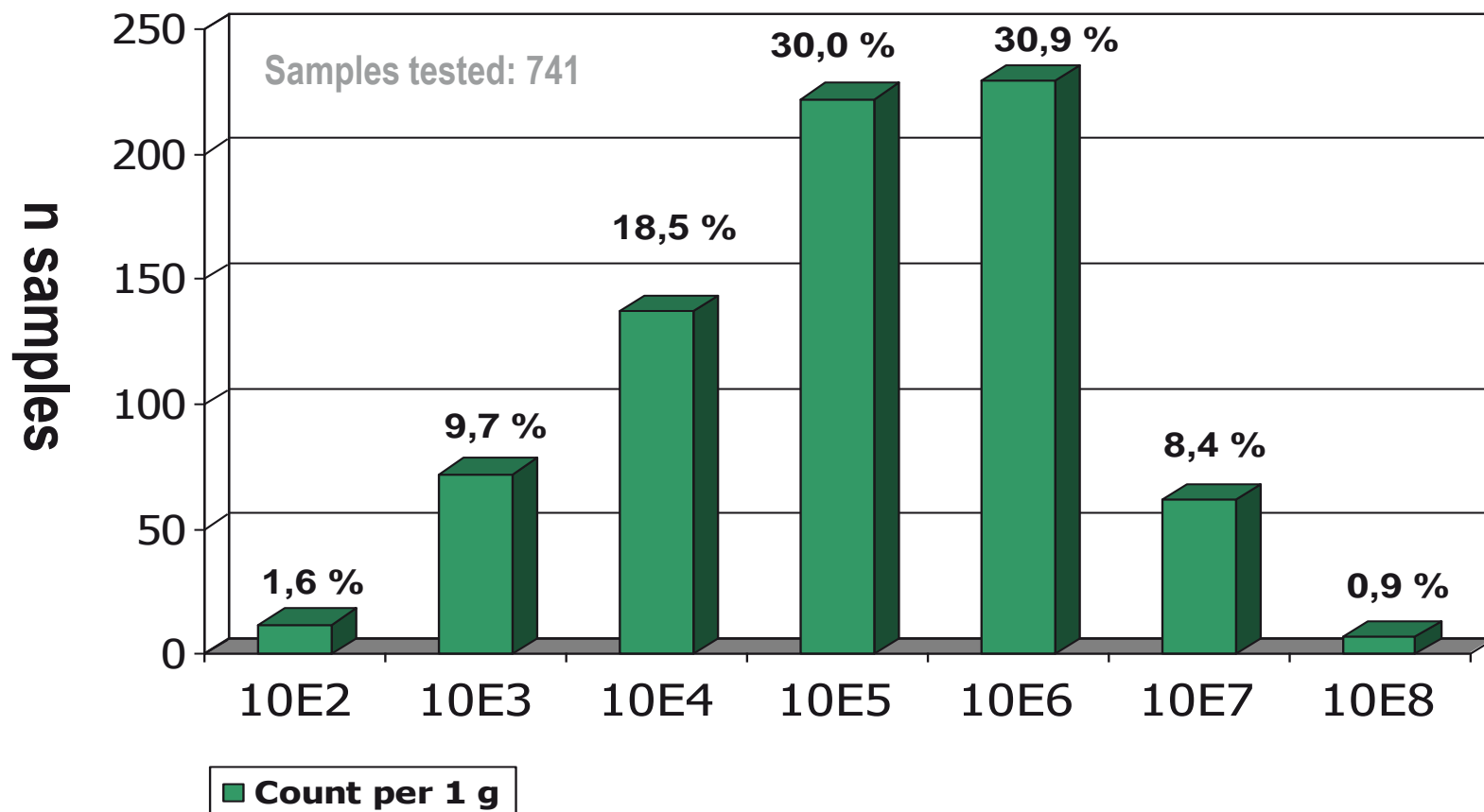
E.coli

Produkt	Limit	Problem
Dry soups, DGHM	10^2	-
Herbal infusion raw material, EHIA	10^4	+
Herbal medicinal teas, Ph. Eur.	10^3	++
Botanicals to be treated with boiling water before use, USP 34	nd	+++
Spices for direct ingestion, DGHM	10^3	++
Medicinal drugs, direct intake Ph. Eur.	nd	+++
Instant products DGHM	10^1	-
Extracts and tinctures Ph. Eur.	nd	-

Russian Requirements

no limit

Microbiological Count of Herbal Drugs Enterobacteria



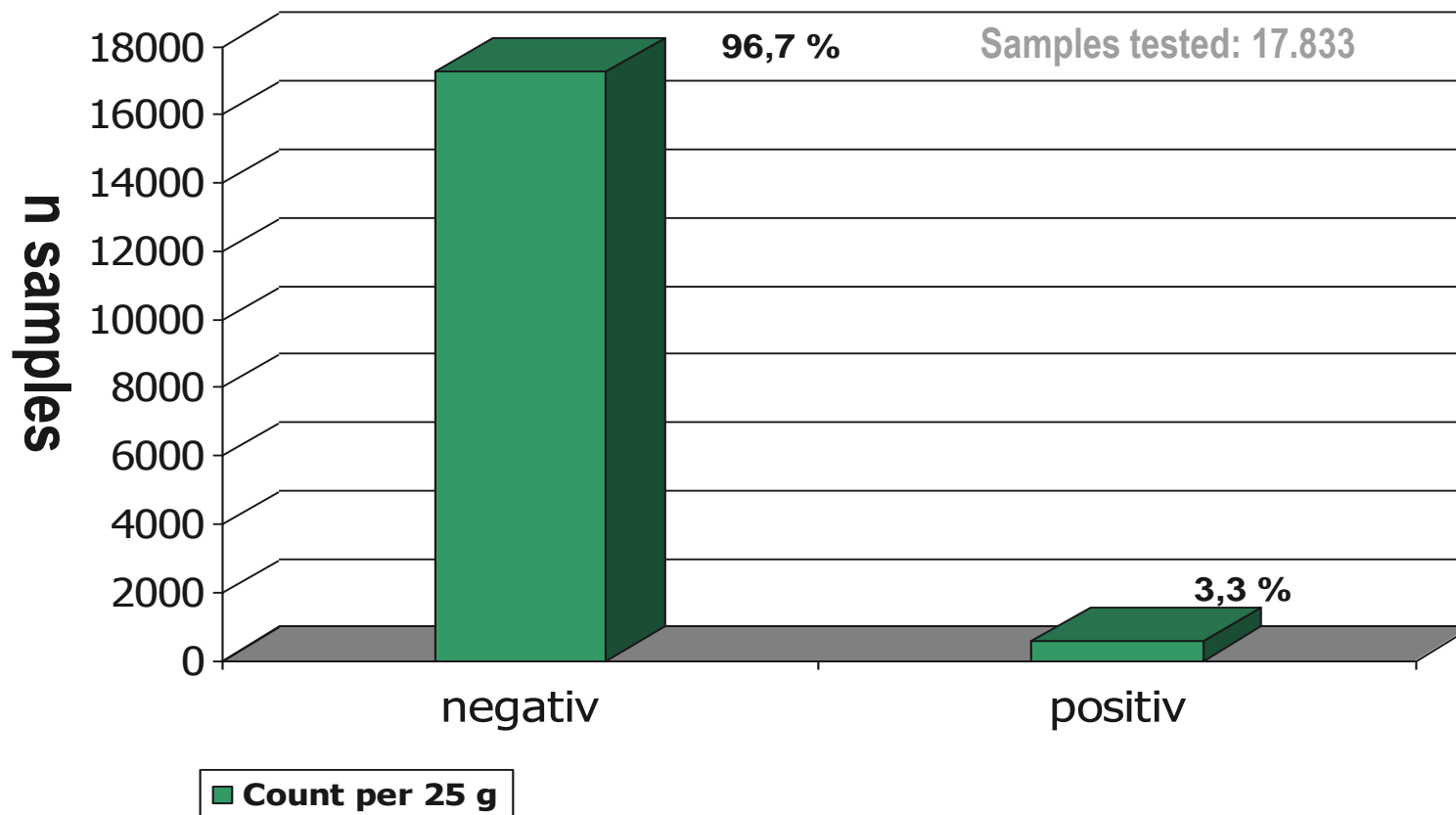
Enterobacteria

Produkt	Limit	Problem
Dry soups, DGHM	-	-
Herbal infusion raw material, EHIA	-	-
Herbal medicinal teas, Ph.Eur	-	-
Botanicals to be treated with boiling water before use, USP 34	-	-
Spices for direct ingestion, DGHM	-	-
Medicinal drugs, direct intake, Ph.Eur.	10^4	++
Instant products, DGHM	-	-
Extracts and tinctures, Ph. Eur	10^2	-

Russian Requirements (Coliforms) absent/g +++

Microbiological Contamination of Herbal Drugs

Salmonella



Salmonella

Produkt	Limit	Problem
Dry soups, DGHM	nd/25g	+/-
Herbal infusion raw material, EHIA	nd/125g	++
Herbal medicinal teas, Ph. Eur.	nd/25g	++
Botanicals to be treated with boiling water before use, USP 34	-	-
Spices for direct ingestion, DGHM	nd/25g	++
Medicinal drugs, direct intake, Ph. Eur.	nd/25g	++
Instant products, DGHM	nd/25g	-
Extracts and tinctures, Ph. Eur.	nd/25g	-

Russian Requirements

absent/25g

++

Drugs Frequently Contaminated with Salmonella (Findings Referred to Samples of 5x25g)

Product	number of lots	% positive lots sample (5 x 25 g)
Lemon Balm	71	24
Verbena	32	9
Fennel	102	10
Red currant leaves	8	38
Camomile	316	40
Linden	46	44
Silver Linden	63	13
Peppermint	413	31
Lemon grass	84	18

Problems of Salmonella positiv results

- A special problem in view to the assessment of the microbiological quality of herbal products is their homogeneity
- Contamination with Salmonella species results from spot contamination
- Statistically seen it is almost impossible to confirm that Salmonella is absent in a herbal product.

Procedures for the reduction of microbiological counts

Ethylene oxide treatment has been banned in the EC.

The treatment with **ionising rays** is effective

- ❑ for medicinal herbal drugs, it is allowable, but it requires a very costly licensing procedure, at least in Germany,
- ❑ for nutritional products, it is allowed under certain conditions (aromatic plants with appropriate labelling)

Water vapour treatment may lead to technologically poorly applicable products, possibly impaired in terms of their physico-chemical quality and sensory properties.

Other procedures, available, are not universally applicable for herbal drugs (e.g. high frequency treatment).

Reduction of the Microbiological Count by Water Vapour Treatment

Water vapour treatment can be a suitable procedure for the reduction of microbial counts under the following conditions:

- Continuous procedures to minimise the negative impact of heat on the herbal product
- High temperatures with short exposure time.

→ Reduction of the microbial count (total count by factor 10^1 to 10^2 ; enterobacteria, e.coli by factor 10^3 to 10^5)

→ Complete destruction of Salmonella

→ No authorisation or labelling required

Decrease of the microbiological counts in infusions prepared with boiling water

(Tests of PhytoLab)

The microbiological counts of 20 lots of **Senna leaves and pods** were compared with the counts of infusions prepared with these drugs and boiling water.

Micro-organism	count/ g drug	count/ g infusion
Total aerobic count	$1,8 \times 10^7$ - $3,0 \times 10^5$ ↑	$6,5 \times 10^5$ - 1×10^3
Moulds and yeast's	$8,0 \times 10^5$ - $1,0 \times 10^3$ ↑	$1,0 \times 10^1$ - n.d.
Enterobacter	$2,5 \times 10^6$ - $4,0 \times 10^3$ ↑	n.d.
Escherichia coli	$5,0 \times 10^5$ - $2,0 \times 10^2$ ↑	n.d.
n.d. = not detectable		

SUMMARY

Basis for Riskmanagement of Contaminants

- literature research and update of existing regulations, publications
- definition of possible contaminants
- development of suitable methods
- performing analyses (monitoring products, target analyses)
- evaluation of results (data base) and risk assessment
- risk based schemes of testing (herbal drug is normally tested)
- improve quality (GACP, processing)



Thank you for
your
interest!

Vielen Dank!

Ihr Ansprechpartner
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