GV-SOLAS
Gesellschaft für Versuchstierkunde
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Specialist information

From the Committee for Nutrition of Laboratory Animals

Guidelines for the Quality assured Manufacturing of Laboratory Animal Diets

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Content

1. Introduction

These guidelines are intended to provide manufacturers with binding recommendations for the production, storage, and transport of feed for laboratory animals in order to achieve a high degree of standardization and to meet quality requirements from the areas of Good Manufacturing Practice (GMP) and Good Laboratory Practice (GLP). The guidelines should therefore also be integrated into existing quality assurance and management systems. This enables both manufacturers and users to compare the described procedures with the processes implemented in their facilities and to optimize them where necessary. In addition to compliance with feed law regulations and consideration of animal welfare aspects, further principles must be observed, including restricted raw material selection, specific features of processing and handling, extensive elimination of undesirable substances, and continuous verification of product quality. The following sections address only special requirements that deviate from the relevant ISO 9001 standards or from the requirements for conventional compound feed manufacturers.

These guidelines apply to all feeds for breeding and maintenance and, where applicable, to special diets for laboratory animals; however, the tolerances (Chapter 7.2) refer exclusively to complete feeds for breeding and maintenance.

The Nutrition Committee of GV-SOLAS intends, with these “Guidelines for the Quality-Assured Manufacture of Feed for Laboratory Animals,” to provide binding guidance for both manufacturers and users. These guidelines are subject to a limited revision service. Therefore, especially with regard to EU regulations, it is recommended in cases of doubt to consult the relevant official links or contact the feed supplier.

2. Quality Standards and Quality Assurance

Certification should now be considered a basic requirement for the production of feed for laboratory animals. ISO 9001ff with HACCP or comparable standards are particularly recommended; these are certified by accredited private companies and monitored at least once a year. An HACCP concept is mandatory for feed business operators in the EU (Regulation (EC) No. 183/2005) and is reviewed by the competent feed authority. GLP certification does not currently appear to be possible for feed manufacturers, although GLP compliance is required in certain areas (substance mixing, etc.). Manufacturers of feed for laboratory animals must operate a functioning quality management system with the active participation of management and staff.

3. Formulations and Specifications

Knowledge from various fields is applied to the development and production of feed for laboratory animals: animal nutrition (e.g., physiology, nutritional requirements, acceptance), veterinary medicine (e.g., maintaining health, prophylaxis, therapy), feed science (e.g., raw materials, ingredients, additives, processing, technological properties, hygiene), feed and drug law (e.g., production, storage, distribution), laboratory animal science (e.g., experimental design, standardization, repeatability, biometrics). These should be kept up to date with the latest scientific and technical developments.

3.1. Feed Ingredients / Feed Materials

In general, only feed materials and additives approved under the feed law should be used; special diets for use in animal experiments may represent an exception. In addition, the quality requirements specific to the laboratory animal sector with regard to consistent nutrient content, hygiene, and avoidance of contamination with undesirable substances must be considered.

Feed for the breeding and maintenance of laboratory animals is mainly composed of raw materials of plant origin (e.g., grain, soy products). For non-carnivorous species (rodents and rabbits), feed materials of animal origin and their processed products, in particular animal and fish meal, should not be used if possible due to the high variability of these raw materials and for sustainability reasons (overfishing, bycatch).

Raw materials should only be purchased from certified suppliers who guarantee compliance with the relevant specifications. It is recommended that suppliers be assessed regularly and that the main suppliers also be inspected or audited regularly. Raw materials are procured only by qualified personnel. Purchasing is based on specific procurement documents that contain clear descriptions and specifications of the products and other quality-related information. Particular attention must be paid to ensuring consistent nutrient contents and high hygienic quality (microbiological contamination), and to potential contamination with undesirable substances.

The incoming goods are inspected in accordance with the procurement documents; in particular, sensory and/or chemical analyses must be performed to ensure conformity with the specifications, whereby only approved raw materials may be processed.

In general, higher standards for the storage of raw materials are implemented than in the compound feed industry; for example, sensitive raw materials (vitamins, certain fats) should only be stored in a controlled environment, refrigerated if necessary, during prolonged storage. Continuous monitoring of temperature and relative humidity is a prerequisite.

3.2. Development

Product development typically consists of planning, specifications, results, testing, verification, and validation. National and international scientific recommendations from the field of animal nutrition must be considered when designing new formulations.

3.3. Manufacturing and Testing instructions

As part of product development, both manufacturing instructions and testing instructions must be defined. A consistent product quality is only ensured by specifying the raw materials (feed mate-rials, additives, premixes), processing steps, and parameters to be tested (via SOPs).

3.4. Fixed Formula / Optimized Formulation

Defined, so-called fixed formulations (“fixed formula”) must not be modified per se. Accordingly, even minor changes, such as further development based on scientific data, require prior notification of customers and their consent. In such cases, a new product code or name should be introduced to clearly indicate that the formulation has been modified. Although

raw materials are subject to natural variations in nutrient content, fixed formulations largely maintain a constant effect originating from the raw material itself or its constituents, thereby achieving a high degree of standardization. Consequently, fixed formulations are predominantly used for the laboratory animal feed.

With semi-fixed formulations, limited optimization of the mixture using the same feed materials is permitted in order to keep the constituents in the final product as constant as possible. Cost-optimized ("least cost") formulations are used in the conventional compound feed industry, where raw materials may be substituted based on current market prices.

Purified, semi-purified, and synthetic (chemically defined) diets (purified diets, 'experimental diets') are based on highly refined and specifically defined components (e.g. casein, starch, cellulose, soybean oil). These raw materials show only minimal variation in their nutrients. The corresponding formulations are exclusively fixed formulations. Such fixed formulations can serve as the basis for customer-specific diets and may be adjusted accordingly.

3.5. Intended Use

Feeds for laboratory animals can be divided into the following categories:

- Complete feed: means compound feed which, by reason of its composition, is sufficient for a daily ration (Regulation (EC) No 767/2009).
- Complementary feed for breeding and/or maintenance: laboratory animal feed that contain higher levels of certain substances, particularly ingredients or additives, than complete feeds for the respective animal category and which, due to their composition, are intended to supplement other feeds to meet the animals' nutritional requirements. Some complementary feeds may also be used as enrichment, e.g. hay-based treats for rats.
- Special or experimental diets: feeds with modified or precisely adjusted nutrient contents (e.g. high-fat diets, diets with certain nutrient deficits) and their corresponding controls.
- Compound feeds with added substances (generally chemicals or medicinal products); these are used for specific research areas and testing (e.g. toxicology).
- Feeds for particular nutritional purposes (dietetic feeds); this category is of minor importance for laboratory animals.

3.6. Nutrients ('Analytical Constituents') and Additives

The specifications of the respective laboratory animal diets should be based on current nutrition-al recommendations for laboratory animals. A corresponding data sheet should include, in addition to the product name and instructions for use, information on nutrients (e.g. crude protein, crude fat, crude fibre, crude ash, calcium, phosphorus, sodium, amino acids) and additives (vitamins and trace elements).

Note: Data sheets often differ from additive declarations on labels. This is the case when the label indicates the added amount of additive (which was the obligatory declaration form in the past) rather than the total content of the respective additive.

3.7. Undesirable Substances / Contaminants

Analysis for undesirable substances (residues and contaminants) is mandatory for specific research areas and studies with defined GLP requirements, as well as for regular internal monitoring (risk assessment). These substances must be analysed using standardized and validated methods by accredited laboratories and testing institutions. The legally established maximum levels are binding.

4. Manufacturing

Consistent quality is based on clear and concise process descriptions, the use of trained personnel and suitable production equipment, monitoring and control of process parameters and product characteristics, and process verification. The layout of production and storage areas and the use of appropriate equipment should minimize contamination, dust or dirt accumulation, and any effects that could impair the product quality.

4.1. Premises and Equipment

Storage and production areas must be separated to avoid contamination, and process workflows and cleaning procedures must be validated. Critical points in the production process must be defined in the manufacturing and testing instructions, monitored, and documented; this is also part of HACCP system. Documentation includes, among other things, the raw material batches used, weighed quantities and permitted deviations (tolerances), process parameters, and the persons responsible for the process.

The manufacturing of standard feeds (breeding, maintenance) shall be strictly separated from production areas for experimental diets, especially feeds containing test substances. The same applies to equipment (mixers, scales, shovels, cleaning equipment, etc.) in order to prevent contamination.

4.2. Hygiene Management

Hygiene rules are intended to ensure that products are stored and delivered in a hygienically sound condition. Regular measures and controls also include personnel, sanitary facilities, buildings, machines, and equipment. In particular, pest control measures shall be defined, document-ed, and implemented with regard to type, scope, and frequency. The EC Regulation No 183/2005[1] on feed hygiene and the „European Feed Manufacturers Guide“[2] should be considered as minimum requirements.

[1] Regulation (EC) No 183/2005 laying down requirements for feed hygiene https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32005R0183&from=EN

[2] https://food.ec.europa.eu/system/files/2020-08/animal-feed_fh_good-practice_eu-guide_efmc_v1-2.pdf

4.3. Cleaning and Maintenance

Machines and equipment should be designed to allow effective cleaning; they must be inspected, maintained, and repaired at regular intervals and as necessary. Records must be kept for these activities.

When manufacturing special diets (e.g. with substance incorporation), machines and equipment must be clearly labelled (‘in use’ / ‘cleaned’) to prevent the use of contaminated equipment and thus avoid cross-contamination.

5. Packaging, Storage, Transport

The products are packaged in accordance with defined packaging instructions using materials that preserve their quality, including protection against contamination and damage. The packaged goods have to be labelled in compliance with legal requirements. Measures to ensure traceability are implemented.

The storage areas (designated storage spaces) should be clearly organized and designed in such a way that thorough cleaning and maintenance are possible and the risk of errors is minimized. In general, stock rotation should follow the “first in / first out” (FIFO) principle, unless otherwise required by customer specifications. Furthermore, separate areas should be available for raw materials, finished products, quarantine storage, and blocked goods. During regular monitoring of the warehouse, the hygienic status of storage areas and products is checked, and expired products are removed.

Before loading, transport vehicles should be checked for their hygienic condition and cleaned again if required; feed must not be loaded into damp cargo spaces.

External Storage

External warehouses or interim storage facilities have to be included in the manufacturer’s hygiene management system. This includes regular and documented inspections of the premises as well as checks of stored goods and storage management processes.

6. Quality Control and Analytics

Quality control includes, but is not limited to, sampling, specification, and testing, and ensures that the required tests are carried out. The documentation (test records) should indicate the test method, results, the resulting assessment and decision, as well as the responsible persons for testing. The records shall be archived.

6.1. Product Inspection and Testing

Both raw materials and finished products are subject to inspection and analyses. Based on testing (analysis) plans, samples are taken and compliance with the defined specifications for individual physical, chemical, and microbiological characteristics is verified and documented. Tolerances are defined in the relevant EU regulations. Tested and released goods should be labelled accordingly; rejected products must be blocked and protected against unintended access.

6.2. Sampling

Sampling should preferably be carried out in accordance with Commission Regulation (EC) No 152/2009 "laying down the methods of sampling and analysis for the official control of feed," in particular with regard to the number of individual samples, sample quantity, and preparation of a final sample. The retained samples should be stored separately and inspected at regular intervals. After the expiration date plus a specified period, the retained samples should be reevaluated upon disposal and any deviations documented.

6.3. Analytical Methods

Analytical testing may only be performed using standardized and validated methods in accredited laboratories. It is recommended that methods and limits of detection be stated in the test reports.

For internal testing of raw materials and compound feeds, near-infrared spectroscopy (NIRS – Near-Infrared Reflectance Spectroscopy) may also be used.

6.4. Test Results and Release

If test results are within the defined tolerances, the goods are released; only released goods may be processed or placed on the market. If test results fall outside the specification, the goods must be blocked and, where appropriate, re-testing, reprocessing with renewed testing, or disposal must be initiated. If test results are missing, the goods shall be labelled accordingly (e.g. “Analysis in Progress”). Test results should be documented. The statistical evaluation of the analytical results is recommended in order to identify trends.

7. Legal Standards and Regulations (Status: 2023)

7.1. Labelling of Feed for Laboratory Animals (Declaration)

Considering Regulation (EC) No 767/2009 the following information is required at a minimum for feed for laboratory animals (whereby “Member States may apply national provisions for feed intended animals kept for scientific or experimental purposes“):

- Type of feed: The designation shall indicate whether the feed is a “feed material,” “complete feed,” or “complementary feed,” and for which animal species or category it is intended.
- Content of constituents: Crude protein, crude fat and oils, crude fibre, crude ash
- Additives for which a maximum content is set for any kind of target species (vitamins A and D; all added trace elements)
- Net weight
- Minimum storage life: stated as “Best before … (month and year)”, or alternatively, the date of manufacture is indicated on the label “… time period in days or months after the date of manufacture “
- Composition: List of feed materials or corresponding categories of feed material in descending order by weight or in percentage by weight
- Batch identification number (lot number)
- Intended use and instructions for proper use

- Name and address or company name and address of the feed business operator responsible for labelling
- Approval number

Thus, the following declaration of analytical constituents is mandatory for complete feeds and complementary feeds (with the exception of mineral feeds): crude protein, crude fibre, crude fat, crude ash.

7.2. Tolerances

7.2.1. Analytical Constituents ("Nutrients")

The tolerances should apply exclusively to the declared contents of analytical constituents (labelled by the manufacturer). The (overall) tolerance covers technical and analytical deviations; i.e. process-related sources of error of the manufacturing process (raw material variability, technological processing inaccuracy, segregation) as well as those of chemical analysis (including sampling and sample preparation).

The definition of tolerances for feed is set out in Annex IV of Regulation (EC) No 767/2009, last amended by Regulations (EC) No 939/2010 and 2017/2279. Information on constituent contents in compound feeds for laboratory animals is still considered correct if the determined values do not deviate from the declared values by more than the limits specified in Table 1. These values include process-related deviations for sampling and analysis.

The regulations mentioned are subject to continuous revision or are supplemented or replaced by new regulations. In cases of doubt, the most recent EU regulation (English version) shall apply.

Table 1: Contents of analytical constituents ("nutrients") and their tolerances in compound feed for laboratory animals
Commission Regulation (EU) 2017/2279

		Tolerance	
Constituent	**Declared content [%]**	**Below labelled value**	**Above labelled value**
Crude protein	< 16 ≥ 16 to < 24 ≥ 24	2,0 Units 12,5 % (relative) 3,0 Units	2,0 Units 12,5 % (relative) 3,0 Units
Crude fat	< 16 ≥ 16 to < 24 ≥ 24	2,0 Units 12,5 % (relative) 3,0 Units	4,0 Units 25 % (relative) 6,0 Units
Crude ash	< 8 ≥ 8 to < 32 ≥ 32	2,0 Units 12,5 % (relative) 8,0 Units	1,0 Units 12,5 % (relative) 4,0 Units
Crude fibre	< 10 ≥ 10 to < 20 ≥ 20	1,75 Units 17,5 % (relative) 3,5 Units	1,75 Units 17,5 % (relative) 3,5 Units

		Tolerance	
Constituent	**Declared content [%]**	**Below labelled value**	**Above labelled value**
Sugar	< 10	1,75 Units	3,5 Units
	≥ 10 to < 20	17,5 % (relative)	35 % (relative)
	≥ 20	3,5 Units	7 Units
Starch	< 10	3,5 Units	3,5 Units
	≥ 10 to < 20	35 % (relative)	35 % (relative)
	≥ 20	7 Units	7 Units
Calcium, Magnesium, Sodium	< 1	0,3 Units	0,6 Units
	≥ 1 to < 5	30,0 % (relative)	60,0 % (relative)
	≥ 5	1,5 Units	3,0 Units
Phosphor, total	< 1	0,3 Units	0,3 Units
	≥ 1 to < 5	30,0 % (relative)	30,0 % (relative)
	≥ 5	1,5 Units	1,5 Units
Potassium	< 1	0,2 Units	0,4 Units
	≥ 1 to < 5	20,0 % (relative)	40,0 % (relative)
	≥ 5	1,0 Units	2,0 Units
Moisture (water) *	< 2	*	0,4 Units
	≥ 2 to < 5		20,0 % (relative)
	≥ 5 to < 12,5		1,0 Units
	≥ 12,5		8 % (relative)

* Deviation applies only for values above the labelled value, deviations below the labelled value are permissible

Example:

Complete feed: 20 % Crude protein declared (label; Regulation (EC) 767/2009).

Analysis: 18 % Crude protein

Tolerance: ±12,5 % (relative)
= min. 17,5 % crude protein (maximum 22,5 %).

The analytical value of 18 % protein is above the minimum. The feed is therefore compliant. Figure 1 shows examples of the declared values for protein (15, 20, and 25 % in green), the corresponding tolerance limits (dark green), and values outside these tolerances (red).

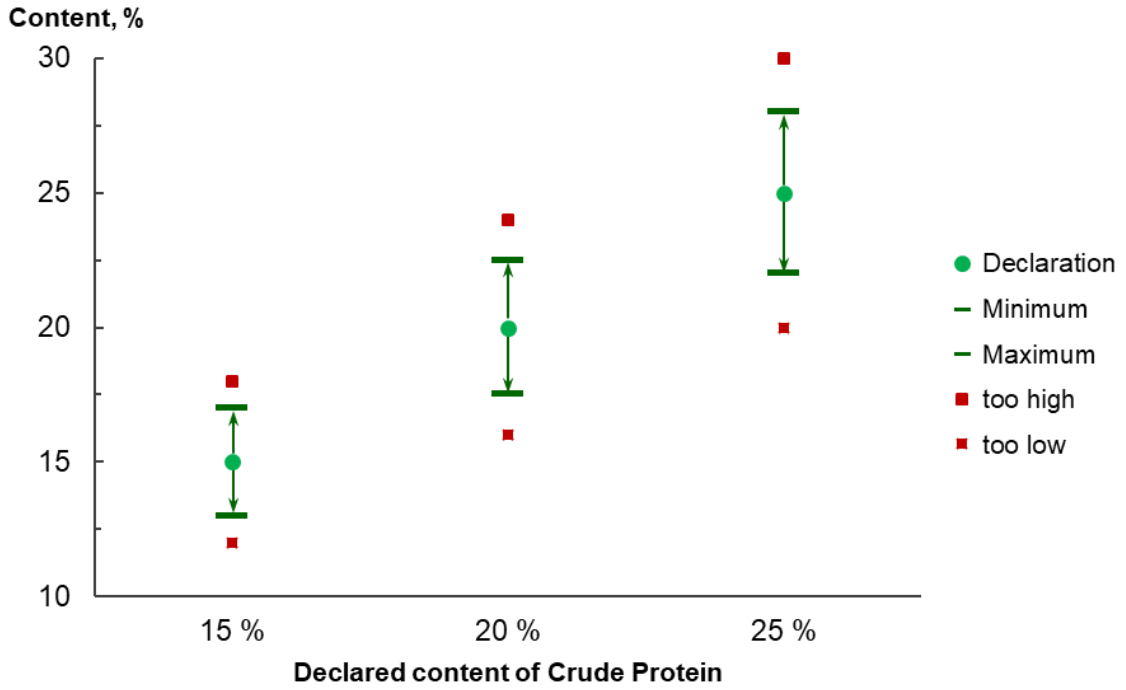

Figure 1: Tolerances (example) for crude protein

Note: For analytical constituents (nutrients), the total tolerance is currently specified. Thus, no analytical tolerance shall be applied (already included). The EU is planning to adopt the same approach as for additives (see below), once analytical tolerances covering measurement uncertainties and procedural variations are fixed at Union level.

7.2.2. Tolerances for Additives and Analytical Uncertainty

The tolerance rules in Part B of Regulation (EC) No 939/2010[3] (and Regulation (EC) No 767/2009[4]) refer only to technical tolerances (mixing inaccuracies and errors). These shall be considered when assessing compliance of the additive contents declared by manufacturers in accordance with Annexes I, V, VI, and VII of Regulation (EC) No. 767/2009. In addition, the analytical uncertainty for the respective additive has to be considered.

If a minimum and/or maximum content of an additive in a feed has been established in the relevant legal act authorizing that additive, the technical tolerances listed in Table 2 apply only to values above the minimum or below the maximum content.

[3] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32010R0939&from=en
[4] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32009R0767&from=en

Table 2: Specifications on the content of additives in feed for laboratory animals are still considered correct if the determined contents deviate from the declared values by no more than (technical deviations)

	Range		Per kg feed	Deviation
1.	< 0,5	Units	= < 0,5 mg; 500 IU; 0,5x10^9 KBE	40 %
2.	≥ 0,5 to <1,0	Units	= < 1 mg; 1.000 IU; 1,0x10^9 KBE	0,2 Units
3.	≥ 1,0 to <500	Units	= < 500 mg; 500.000 IU; 500x10^9 KBE	20 %
4.	≥ 500 to <1000	Units	= < 1.000 mg; 1.000.000 IU	100 Units
5.	≥ 1.000	Units	= ≥ 1.000 mg; 1.000.000 IU	10 %

* The legal provisions are subject to change. The values listed are subject to limited revisions service. Current values can be found under Regulation (EC) No 767/2009 and (EC) No 939/2010.

Original text Reg. (EC) No 939/2010: *"As long as the fixed maximum content of an additive as referred to in point 2 is not exceeded, the deviation above the declared content may go up to three times the tolerance laid down in point 1. However, if for feed additives belonging to the group of microorganisms a maximum content is laid down in the respective authorising act for that feed additive, the maximum content shall constitute the upper permitted value."*

Table 3: Examples of uncertainties of measurement for feed additives VDLUFA Version 13, 2022 [5]

Additives	Determined content	Analytic range
Amino acids: Lysine, Methionine, Cysteine, Threonine, Tryptophan	0,08 - <0,30 % 0,30 - <0,46 % 0,46 - <2,83 % 2,83 - <3,36 % 3,36 - <10,30 %	20 % (relative) 0,06 Units 13 % (relative) 0,37 Units 11 % (relative)
Iron	113 - <371 mg/kg 371 - <510 mg/kg 510 - <10.000 mg/kg	22 % (relative) 82 Units 16 % (relative)
Copper	5,0 - <500 mg/kg 500 - <915 mg/kg 915 - <4.900 mg/kg	22 % (relative) 110 Units 12 % (relative)
Selenium	0,10 - <0,50 mg/kg 0,50 - <0,75 mg/kg 0,75 - <13,5 mg/kg 13,5 - <20,5 mg/kg 20,5 - <76,0 mg/kg	50 % (relative) 0,25 Units 33,3 % (relative) 4,5 Units 22 % (relative)

[5] https://www.vdlufa.de/ & https://www.vdlufa.de/wp-content/uploads/2022/04/ASR-eASR-Version-13_2022.pdf

Manganese	22,0	-	3.200 mg/kg	19	% (relative)
Zinc	18	-	10.000 mg/kg	16	% (relative)
Iodine	0,40	-	<46 mg/kg	37	% (relative)
	46	-	<113 mg/kg	17	Units
	113	-	<149 mg/kg	15	% (relative)
Cobalt	0,08	-	26,9 mg/kg	39	% (relative)
Vitamin A	7.800	-	<100.000 IE/kg	30	% (relative)
	100.000	-	<125.000 IE/kg	30.000	Units
	125.000	-	<375.000 IE/kg	24	% (relative)
	375.000	-	<450.000 IE/kg	90.000	Units
	450.000	-	<1.020.000 IE/kg	20	% (relative)
Vitamin E	22,4	-	<120 mg/kg	25	% (relative)
	120	-	<188 mg/kg	30	Units)
	188	-	<10.000 mg/kg	16	% (relative)
Vitamin D_3	1.000	-	<3.080 IE/kg	50	% (relative)
	3.080	-	<5.100 IE/kg	1.540	Units
	5.100	-	<6.150.000 IE/kg	30	% (relative)

Example:

Complete feed: 0,35 mg/kg Selenium declared (label; Regulation (EC) 767/2009)

Analysis Example 1: 0,6 mg/kg Selenium

Tolerance: ±40 % (Regulation (EC) 939/2010)
= min. 0,21 mg/kg Se / max. 0,49 mg/kg Se *

Analytical uncertainty: ±0,25 mg/kg Se (applied to the analytical value)
= min. 0,35 mg/kg Se / max. 0,85 mg/kg Se

* The maximum value may even be up to 3 × 40% (= +120%), provided the legal maximum content for selenium (0.5 mg/kg) is not exceeded.

With 0.6 mg selenium per kg feed, the analytical value is above the technical tolerance range and also above the legal maximum. However, when applying the analytical uncertainty according to VDLUFA (Edition 13; 02/2022), it is below the upper limit. The feed is therefore compliant and may be released. If the analyzed content is 0.8 mg/kg, however, it remains outside the tolerance range even when considering analytical uncertainty. The feed contains too much selenium and must be blocked.

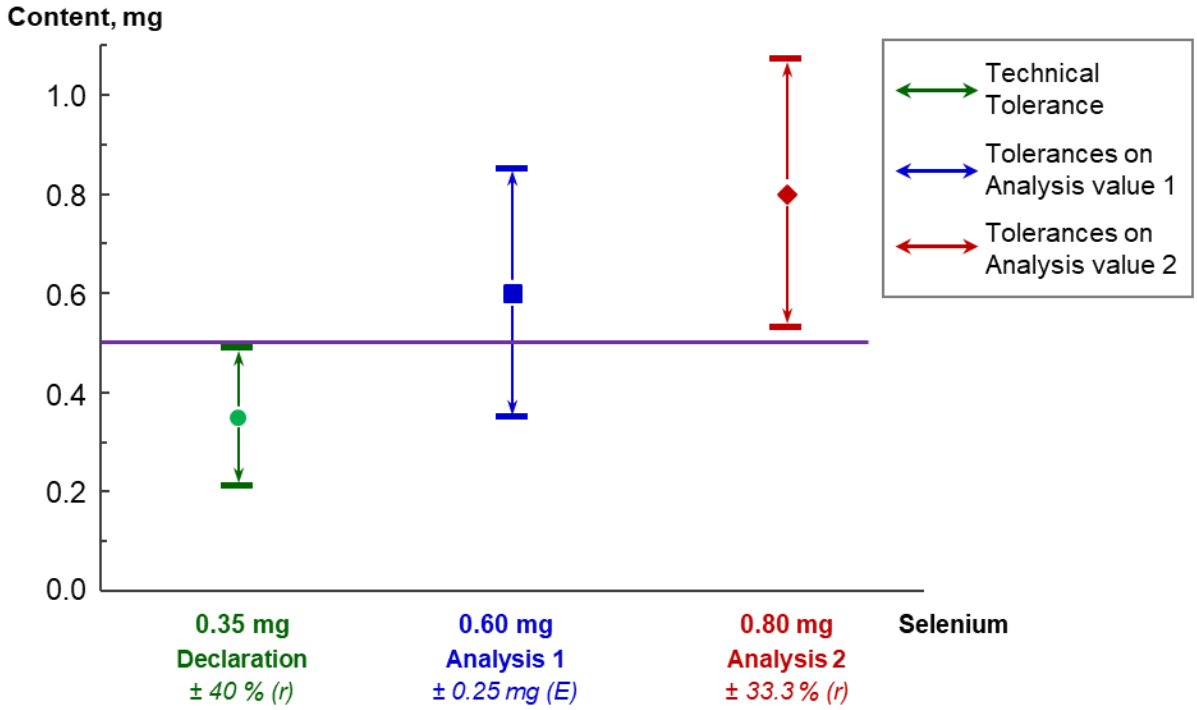

Figure 2: Technical and analytical tolerances for a declared selenium content and the corresponding analysis values.

Note: The analytical tolerance always has to be considered for additives and undesirable substances (see Section 7.2.3). As these substances generally occur at low concentrations, the analytical tolerance is correspondingly high.

7.2.3. Undesirable Substances and Maximum Limits

According to feed legislation, undesirable substances are contaminants in feed that enter the feed unintentionally and uncontrollably. For these substances, maximum levels have been established that are binding for all EU Member States (Directive 2002/32/EC[6], , Commission Recommendation on the maximum levels for Fusarium toxins 2006/576/EC) and 2016/1319[7]. Table 4 provides only a brief overview of the contaminants; the list is continuously being expanded and improved by the EU based on current data from research.

The following table contains only a small selection of contaminants. Which contaminants are analyzed may be adapted to the specific purpose of the corresponding study (toxicity/GLP). It applies in particular to undesirable substances that the regulations mentioned above are subject to continuous revision; they can be supplemented or replaced by new regulations. In case of doubt, the latest EU regulation (English version) is therefore valid.

[6] Consolidated Version 2002/32/EC: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02002L0032-20191128&from=EN

[7] Commission Recommendation 2006/576/EC & Commission Recommendation (EU) 2013/1319: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32006H0576&from=EN https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32016H1319&from=EN

Table 4: Upper limits for undesirable substances (extract) according to Directive 2002/32/EC and Commission Recommendations for Fusarium toxins 2006/576/EC and (EU) 2016/1319.
Examples for the permissible limits. The analysis of these contaminants is not mandatory; it can be adapted to the respective study (see text).

Organochlorine compounds	**mg/kg**		**Dioxins** [5]	**ng WHO-PCDD/ F-TEQ/kg**
Hexachlorobenzene, HCB	0,01		[Σ PCDDs & PCDFs]	1,75
α-HCH	0,02			
β-HCH	0,01		**Dioxins & Dioxin-like PCBs** [6]	**ng WHO-PCDD/ F-TEQ/kg**
γ-HCH (Lindane)	0,20			
Heptachlor und -epoxide	0,01		[Σ PCDDs, PCDFs & PCBs]	5,5
α, γ -Chlordan, oxy-	0,02			
Aldrien und Dieldrin	0,01			
Endrin	0,01		**Non-Dioxin-like PCBs** [7]	**mg/kg**
DDT + DDE + DDD	0,05		[Σ PCBs 28, 52, 101, 138, 153, 180]	0,4
α, β Endosulfan und -sulphate	0,10			
Heavy metals	**mg/kg**		**Mycotoxins**	**mg/kg**
Arsenic, As [1]	2,0		Aflatoxin B_1 [8]	0,010
Lead/Plumb, Pb	5,0		Aflatoxin B_2	0,005
Cadmium, Cd [2]	2,0		Aflatoxin G_1	0,005
Mercury [3]	0,1		Aflatoxin G_2	0,005
Fluorine [4]	150			
(Selenium	0,5)		**Fusarium toxins** [9]	**mg/kg**
			Deoxynivalenol (DON)	5,00
Nitrite (as Na nitrite), mg/kg	15		Ochratoxin A *	0,05
Nitrosamines			Zearalenon *	0,10
N-Nitrosodiethylamine (NDEA)	0,01		Fumonisin B_1 + B_2	5,00
N-Nitrosodimethylamine (NDMA)	0,01			

The maximum contents of the contaminants are related to a feed with 88 % dry matter.

1) Total arsenic. Complete feed for fish and fur animals: 10 mg/kg and complete feed for pet animals containing fish, aquatic animals and similar products: 10 mg/kg
2) Complete feed for cattle (except calves), sheep (except lambs), goats (except kids), fish: 1 mg/kg; for other farm animals: 0.5 mg/kg
3) Feed for fish: 0.2 mg/kg; for dogs, cats, fur animals and ornamental fish: 0.3 mg/kg
4) Complete feed for pigs: 100 mg/kg; fish and poultry (except chicks): 350 mg/kg; chicks: 250 mg/kg; cattle, sheep and goats during lactation: 30 mg/kg, other 50 mg/kg
5) Feed for farm animals, except fish: 0.75 ng/kg (fish 1.75 ng/kg)
6) Feed for farm animals, except fish: 1.5 ng/kg (fish 5.5 ng/kg)
7) Feed for farm animals, except fish: 0.01 mg/kg (fish 0.04 mg/kg)
8) Compound feed for pigs, cattle, sheep, goats and poultry: 0.02 mg/kg; compound feed for dairy cattle, goats, sheep and calves, kids, lambs, piglets, young poultry (0.005 mg/kg)
9) The recommended upper limits apply to complete feed for monogastric animals.

* taken from recommendations for feed for pigs/piglets, because recommendations for feed for pets do not exist

Also the uncertainty of measurement has to be considered (Table 5), when assessing the maximum permissible levels. These values are also continuously revised and adapted to new or improved methods.

Table 5: Examples of Uncertainties of Measurements for Undesirable Substances VDLUFA Version 13, 2022 [8]

Contaminant	Determined content				Analytical range	
Arsenic	0,125	–	1,0	mg/kg	50	% (relative)
	1,0	–	2,5	mg/kg	0,5	Units
	2,5	–	3,7	mg/kg	20	% (relative)
Lead / Plumb	0,5	–	3,0	mg/kg	50	% (relative)
	3,0	–	5,0	mg/kg	1,5	Units
	5,0	–	10,0	mg/kg	30	% (relative)
Cadmium	0,05	–	0,20	mg /kg	50	% (relative)
	0,20	–	0,40	mg /kg	0,10	Units
	0,40	–	1,00	mg /kg	25	% (relative)
	1,00	–	1,40	mg /kg	0,25	Units
Mercury	0,05	–	0,06	mg /kg	50	% (relative)
	0,06	–	0,10	mg /kg	0,03	Units
	0,10	–	0,20	mg /kg	30	% (relative)
	0,20	–	0,30	mg /kg	0,06	Units
	0,30	–	2,00	mg /kg	20	% (relative)
Aflatoxin B_1	1	–	4	µg/kg	50	% (relative)
	4	–	10	µg/kg	2	Units
			> 10	mg/kg	20	% (relative)
Deoxynivalenol, DON	140	–	32.000	µg/kg	40	% (relative)
Zearalenone, ZEA	11	–	2.800	µg/kg	60	% (relative)
Organochlorine compounds *	6	–	106	µg/kg	55	% (relative)

* If maximum levels are set for the sum of several chlorinated hydrocarbons, the information in the column “determined content” refers to the sum (e.g. DDT + DDE + DDD, calculated as DDT).

Antibiotic Activity

Antibiotics must not be added to feed for laboratory animals. Compliance is verified by qualitative or quantitative testing for the most important antibiotics (residue analysis).

7.3. Microbiology

This section of the guidelines was partially adopted from the previous version and was originally developed in cooperation with the Hygiene Committee of the GV-SOLAS.

[8] https://www.vdlufa.de/ & https://www.vdlufa.de/wp-content/uploads/2022/04/ASR-eASR-Version-13_2022.pdf

The purpose of the recommendations for microbiological feed testing and evaluation is to ensure systematic testing of feed samples for defined microorganisms using validated methods.

These recommendations exclusively consider routine testing for pathogens or groups of pathogens that are necessary for assessing the hygienic quality of feed for laboratory animals. In specific cases and in the event of exceeding the specified limits, further analyses may be required.

7.3.1. Sampling, Storage, and Transport of Feed Samples

The number of batch-related individual samples for microbiological testing should be based on the methods of sampling and analysis for the official control of feed (Regulation (EC) No 152/2009).

Sampling shall be carried out under hygienic conditions. For irradiated feed, a separate sample for microbiological testing has to be taken prior to sterilization; this sample should be packaged identically to the feed batch and placed at the center of the pallet or of the packaging.

Sample packaging should be selected to ensure adequate protection of feed samples during transport. Transport times to the laboratory should be kept as short as possible.

7.3.2. Test Methods

The preparation of feed samples submitted for testing and subsequent analysis may only be carried out in accredited institutions (e.g. according to DIN EN ISO/IEC 17025) and only using current, official, and validated methods.

7.3.3. Recommendations to the Maximum Permissible Contamination

The following table (Table 6) provides recommendations on the maximum permissible microbial contamination of compound feeds (complete feeds and complementary feeds) for laboratory animals.

Table 6: Recommendation on the maximum permissible limits for microorganisms in compound feed for laboratory animals

Microorganisms	**Compound feed for laboratory animals**	
	Crude fibre content < 7 %	**Crude fibre content > 7 %**
Aerobic mesophilic bacteria	$< 1 \times 10^5$ CFU/g	$< 5 \times 10^5$ CFU/g
Escherichia coli	$< 1 \times 10^1$ CFU/g	$< 1 \times 10^1$ CFU/g
Yeast/Molds	$< 1 \times 10^3$ CFU/g	$< 5 \times 10^3$ CFU/g
Salmonella	not detectable	not detectable

CFU: Colony forming units

8. Important Links to Regulatory Frameworks

The links to EU regulations or directives included in these guidelines always refer to the English pages, as only the original English text is legally valid in case of doubt.

1. European Commission > Food Safety > Food > Animal feed: https://food.ec.europa.eu/safety/animal-feed_en
2. European Food Safety Authority, EFSA: https://www.efsa.europa.eu/en
3. Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, BVL: https://www.bvl.bund.de/DE/Home/home_node.html
   Futtermittel: https://www.bvl.bund.de/DE/Arbeitsbereiche/02_Futtermittel/futtermittel_node.html
   Tierarzneimittel: https://www.bvl.bund.de/DE/Arbeitsbereiche/05_Tierarzneimittel/tierarzneimittel_node.html
4. Regulation (EC) No 183/2005 laying down requirements for feed hygiene https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32005R0183&from=EN
5. Commission Regulation (EC) No 152/2009 laying down the methods of sampling and analysis for the official control of feed https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32009R0152&from=EN
6. Regulation (EC) No 767/2009 on the placing on the market and use of feed https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32009R0767&from=en
7. Commission Regulation (EU) No 939/2010 amending Annex IV to Regulation (EC) No 767/2009 on permitted tolerances for the compositional labelling of feed materials or compound feed https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32010R0939&from=en
8. Commission Regulation (EU) 2017/2279amending Annexes II, IV, VI, VII and VIII to Regulation (EC) No 767/2009 of the European Parliament and of the Council on the placing on the market and use of feed https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32017R2279&from=EN
9. VDLUFA 2022, Analysenspielräume (ASR) für Futtermitteluntersuchungen; VDLUFA Version 13, 01.02.2022 (perhaps European Union Reference Laboratories) https://www.vdlufa.de/wp-content/uploads/2022/04/ASR-eASR-Version-13_2022.pdf & https://www.vdlufa.de/
10. Directive 2002/32/EC on undesirable substances in animal feed; Consolidated Version (2019) https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02002L0032-20191128&from=EN
11. Commission Recommendation 2006/576/EC on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32006H0576&from=EN
12. Commission Recommendation (EU) 2016/1319 amending Recommendation 2006/576/EC as regards deoxynivalenol, zearalenone and ochratoxin A in pet food https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32016H1319&from=EN
13. 13.FEFAC (Federation Europeenne des Fabricants D'Aliments Composes) European Feed Manufacturers Guide 2014: "A Community Guide to good practice for the EU industrial compound feed and premixtures manufacturing sector for food producing animals" https://food.ec.europa.eu/system/files/2020-08/animal-feed_fh_good-practice_eu-guide_efmc_v1-2.pdf

14. Good Manufacturing Practice (GMP) guidelines
https://health.ec.europa.eu/medicinal-products/eudralex/eudralex-volume-4_en &
https://www.bundesgesundheitsministerium.de/service/gesetze-und-verordnungen/bekanntmachungen.html

15. 15.OECD Series on Principles of GLP and Compliance Monitoring Number 5 (Revised): No 5: Compliance of Laboratory Suppliers with GLP Principles
https://www.oecd.org/content/dam/oecd/en/publications/reports/2000/09/compliance-of-laboratory-suppliers-with-glp-principles_g1gh32f0/9789264078611-en.pdf
[https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?doclanguage=en&cote=env/jm/mono(99)21]

16. DIN EN ISO 9001 ff (Beuth)

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