NutraSteward
Providing regulatory support for food and feed ingredients

August 31, 2023

Dr. David Edwards
Director, Division of Animal Feeds
Center for Veterinary Medicine
U.S. Food and Drug Administration
1225 Wilkins Avenue
Rockville
Maryland 20852

Confidential

Dear Dr. Edwards,

Re: GRAS Notice for Purified Yeast Cell Wall as a Source of Fermentable Fiber for Animals (Previous Ref: AGRN No. 56)

A GRAS notice was submitted to the CVM by Phileo, a Division of S.I.Lesaffre (hereafter referred to as "Phileo") in September 2021. The CVM raised questions on the GRAS notice, and on November 10, 2022, Phileo requested that the CVM cease to evaluate the notice. A meeting was held with the CVM on December 12, 2022 in order to discuss the questions raised by the CVM in its evaluation. Following on from the meeting, Phileo has revised the GRAS notice for purified yeast cell wall and an updated version is attached. To aid the CVM's review, the amendments made to the original GRAS notice in order to address the questions raised during the first review are summarized below:

Manufacturing Process

- The control parameters in the manufacturing process are more specifically defined [Appendix 01 - CONFIDENTIAL]
- Post-fermentation separation is described in further detail [Appendix 01 - CONFIDENTIAL]

Methods to Analyze Mannans and Beta-Glucans

- Phileo has provided copies of the confidential internal methods of analysis that are used for the analysis of mannans and beta-glucans [Appendices 02A and 02B]. In the original GRAS notice, the contents were also verified by an external laboratory using the method described in Appendix 07B. Mannans and beta-glucans are routinely measured using Phileo's internal methods and the analytical data in Section 2.4.1 reflects these results. However, the stability study analyses was sent for external analysis and was conducted using the method in Appendix 07B. Phileo confirms that the methods yield comparable results.

Stability

- Additional stability study results are provided to support the 24-month shelf-life of purified yeast cell wall [Appendix 07A – CONFIDENTIAL]. The microbial content was analyzed at 24 months and demonstrated to comply with the product specifications.
- Over the 24-month storage period, the beta-glucans content was not observed to decrease and variations in the values reported fall within normal analytical variation
- Phileo confirms that an error was made in reporting the manufacturing date of Batch (b) (4) and that the correct date is 03/15/2018.
- Further explanation for the timing of the stability study and suitability of batches produced over 2018 and 2019 for inclusion in the study is provided in Section 2.5.

Identity of Purified Yeast Cell Wall

- The molecular weight range of beta-glucans in purified yeast cell wall is reported in Appendix 03S.

Raw Materials and Manufacturing Process

- Details of enzymes and defoamer which comply the AAFCO ingredient definitions and the Enzyme Technical Association list, respectively are provided and confirmed to be used in the manufacturing process [Appendices 01E and 01K – CONFIDENTIAL]
- The conditions of the laboratory-scale propagation are clarified further in Appendix 01 – CONFIDENTIAL.
- The proposed product specifications have been adjusted to reflect the analytical data [Table 2.2]
- The results of analysis for lead are updated in Table 2.3.
- English translations are provided for the microbiological testing and minerals

Analytical Methods

- An English translation of the coliforms method has been provided
- As mentioned above, Phileo uses the internal methods described in Appendices 02A and 02B [CONFIDENTIAL] to analyze for mannans and beta-glucans. Samples are also sent out to an external laboratory for verification or as an alternative, and the method employed is that described in Appendix 07B. The methods yield comparable results and can both be relied on.

Utility

- The intended use of purified yeast cell wall is as a source of beta-glucans. The intended use is referred to as such throughout the GRAS notice. Beta-glucans act as fermentable fibers and are intended to supplement but not replace nutrients in the diet. Thus, the technical effect of purified yeast cell wall does not have any bearing on safety and no further evaluation of utility is warranted in accordance with 21 CFR §570.230(d) and likewise, the use levels intended for addition to feed do not require further justification.
- Phileo recognizes that there are numerous reports in the published literature in which the function of beta-glucans as fermentable fibers are linked to indirect effects of this

nutritional property, such as positive benefits on digestive health and the immune system. However, Phileo confirms that the only intended use of beta-glucans in animal food in the U.S. is as a source of fermentable fiber, not dissimilar to existing counterparts such as FOS and inulin. *In vitro* assays and ADME data are provided within the GRAS notice supporting the fermentable fiber function of beta-glucans to support this statement.

- Phileo notes that the website language for Safglucan® refers to a range of yeast-derived products that are marketed globally and that purified yeast wall (min. 50% beta-glucans) as intended for use in the U.S., is not well-described having not yet been marketed in this jurisdiction. To reflect the planned entry into the U.S. market and the intended function of beta-glucans, Phileo has revised the website and a copy is enclosed herein. This language has replaced the previous website as of September 2023.

Target Animal Safety

- Endotoxins and lipoproteins are generally associated with bacteria and not Saccharomyces cerevisiae. These potential inherent constituents are addressed in Section 2.4.5 and 6.1.3.
- Phileo recognizes that the intestinal barrier in calves is not well-developed in the hours after birth. Thus, the conditions of use of purified yeast wall have been updated: maximum 0.05 g/head/day of purified yeast cell wall for calves that are 2 days of age or older.
- The intended use and use levels have been expanded to clarify the scope of use in smaller ruminants and calves (Sections 1.3 and 2.6).

Regulatory Pathway

- Phileo welcomes the potential introduction of a new pathway for zootechnical additives in the U.S. Although this pathway may be applicable in the future, Phileo currently only wishes to market purified yeast cell wall as a source of beta-glucans where the beta-glucans act as a fermentable fiber in the diet of animals. Further work may be undertaken in the future, to further understand the potential indirect benefits of the fermentable fiber (e.g., immune effects) but the current scope of use is only nutritional.

We trust the enclosed GRAS notice for purified yeast cell wall meets your current requirements and remain at your disposal to address any questions which arise.

Yours sincerely,

(b)(6)

Dr. Elizabeth Lewis
Scientific & Regulatory Advisor
NutraSteward

Enclosure: Website language to replace the existing information in September 2023.

Content of Phileo Safglucan webpage – to be implemented September 2023

Safglucan® purified yeast fraction **concentrated in 1.3/1.6 beta-glucans**

β-Glucans are a class of polysaccharides consisting of D-glucose units that are polymerized primarily via the β-1,3 glycosidic bonds, in addition to the β-1,4 and/or β-1,6 bonds. They are present in various food products such as cereals, mushrooms, and seaweeds and are known for their numerous effects on the human body, depending on their structures, which are diverse. The major physicochemical properties of β-glucans include their antioxidant property, which is responsible for the scavenging of reactive oxygen species, and their role as dietary fiber for preventing the absorption of cholesterol, for promoting egestion, and for producing short-chain fatty acids in the intestine. Dietary β-glucans also support the immune system through their structural as indigestible carbohydrates helping to activate cells of the mucosal immune system via β-glucan receptors, such as dectin-1.

Safglucan® is one of the most promising solution to maintain animal performance & wellbeing in many species, including pets, cattle, swine, poultry, and farmed fish.

Composition and mode of action of Safglucan®

Phileo's innovative nutritional solution Safglucan® is composed of a purified fraction with a minimum of 50% of 1.3/1.6 beta-glucans extracted from a primary grown probiotic yeast strain Saccharomyces cerevisiae in a controlled process. Beta-glucans can be extracted from several natural sources, such as yeasts, algae, cereals, and fungi. Safglucan® has been analyzed in our laboratories to determine the length and the frequency of 1.3/1.6 beta-glucans ratio branching and its physicochemical properties.

Phileo's selection of Safglucan® is based on its mode of action on in vitro cell models and the results of animal trials to confirm its benefits.

The information provided in this document is to the best of our knowledge, true and accurate. Not all products are available in all regions nor are the associated intended uses and/or claims the same in all regions. Products must only be used in compliance with local laws and regulations and we cannot guarantee freedom for every intended use or country.

Responding to the needs of our customers

Packaging

- 10 kg cardboard box containing 2 individual multilayer bags of 5 kg

Shelflife

- 2 years from production date, in original packaging.

Storage

- keep in a dry and cool place for optimum preservation

Product category

- EU Feed material – Yeast product
- USA Purified Yeast Cell Wall – GRAS for feed

GRAS Notice for Purified Yeast Cell Wall for Use as a Source of Beta-Glucans in Animal Food

Phileo, Division of S.I.Lesaffre
137 Rue Gabriel Péri – BP 3029
59703 Marcq-en-Baroeul Cedex
France

August, 2023

GRAS Notice for Purified Yeast Cell Wall for Use as a Source of Beta-Glucans in Animal Food

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

NOMENCLATURE

Phileo is a Division of S.I.Lesaffre and in this respect, benefits from access to its expertise, technologies, laboratories and manufacturing plants. For this reason, although purified yeast cell wall will be marketed by Phileo, reference is also made herein as relevant, to Lesaffre (e.g., culture collection) and manufacturing plants that fall under Lesaffre (see Appendix 01 and accompanying sub-appendices).

Beta-glucans can derive from a variety of sources and reference is made as relevant within the GRAS notice to the source, e.g., yeast beta-glucans, mushroom beta-glucans and cereal (oat, barley etc.) beta-glucans.

GRAS Notice for Purified Yeast Cell Wall for Use as a Source of Beta-Glucans in Animal Food

PART 1. §570.225. SIGNED STATEMENTS AND CERTIFICATION

In accordance with 21 CFR §570 Subpart E consisting of §570.203 to 280, Phileo, Division of S.I.Lesaffre (hereafter referred to as "Phileo"), herby informs the U.S. Food and Drug Administration (FDA) that they are submitting a Generally Recognized As Safe (GRAS) notice for purified yeast cell wall.

1.1 NAME AND ADDRESS OF ORGANIZATION

Phileo, Division of S.I.Lesaffre
137 Rue Gabriel Péri – BP 3029
59703 Marcq-en-Baroeul Cedex
France

1.2 NAME OF THE NOTIFIED SUBSTANCE

The notified substance is purified yeast cell wall.

1.3 INTENDED CONDITIONS OF USE

Purified yeast cell wall is intended for use as a source of beta-glucans in the food of all categories and species of animals in the United States (U.S.). The amount to be incorporated into food will vary depending on the species and life stage of the animal. Typical use-levels of purified yeast cell wall in animal food are as follows:

Animal Species	**Typical Use Levels**
Poultry	0.125 to 0.15 g/kg complete feed – Equivalent to min. 62.5 to 75 mg beta-glucans/kg complete feed
Swine	0.5 g/kg complete feed – Equivalent to min. 250 mg beta-glucans/kg complete feed
Aquaculture	0.4 to 1.2 g/kg complete feed Equivalent to 200 to 600 mg beta-glucans/kg complete feed
Pets (cats and dogs)	0.25 to 1.0 g/kg complete feed – Equivalent to 125 to 500 mg beta-glucans/kg complete feed
Ruminants	10 g/head/day – larger ruminants (dairy and beef cattle, bison) – Equivalent to min. 5,000 mg beta-glucans/head/day 1 g/head/day – smaller ruminants (goats, sheep) – Equivalent to min. 500 mg beta-glucans/head/day 0.05 g/head/day – milk replacer (calves; min. 2 days of age) – Equivalent to min. 25 mg beta-glucans/head/day
Equine species	0.25 to 5 g/head/day – Equivalent to 125 to 2,500 mg beta-glucans/head/day

1.4 STATUTORY BASIS FOR THE CONCLUSION OF GRAS STATUS

Pursuant to 21 CFR §570.30(a) and (b), purified yeast cell wall as manufactured for Phileo, has been concluded to have GRAS status for use as a source of beta-glucans in the food of all categories and species of animal under the conditions described in Part 1.3, on the basis of scientific procedures.

1.5 PREMARKET EXCEPTION STATUS

Phileo herby informs the U.S. FDA of the view that purified yeast cell wall is not subject to the premarket approval requirements of the Federal Food, Drug and Cosmetic Act (FFDCA) based on Phileo's conclusion that the notified substance is GRAS under the conditions of intended use as described in Part 1.3 above.

1.6 AVAILABILITY OF INFORMATION

The data and information that serve as the basis for this GRAS notification will be made available to the U.S. FDA for review and copying upon request during customary business hours at the offices of:

Phileo, Division of S.I.Lesaffre
137 Rue Gabriel Péri – BP 3029
59703 Marcq-en-Baroeul Cedex
France

In addition, upon request, Phileo will supply the U.S. FDA with a complete copy of the data and information either in an electronic format that is accessible for the Agency's evaluation or on paper.

1.7 FREEDOM OF INFORMATION ACT, 5 U.S.C. 552

In Phileo's view, all data and information presented in Parts 2 through 7 of this notice do not contain any trade secret, commercial or financial information that is privileged or confidential, and therefore, all data and information presented herein are not exempt from the Freedom of Information Act, 5 U.S.C. Section 552. The exception is the information in Appendices 01, 01A to 01M, 02A and 02B, and 07A and 07C which is highly proprietary commercial information and confidential.

1.8 CERTIFICATION

Eric Auclair hereby certifies that to the best of his knowledge, all data and information presented in this notice constitutes a complete, representative and balanced submission, which includes all unfavorable as well as favorable information known to Phileo and pertinent to the evaluation of the safety and GRAS status of purified yeast cell wall.

Signed, (b)(6)

_____ _____ _____30.08.2023_____

Eric Auclair Date

PART 2. §570.230. IDENTITY, METHOD OF MANUFACTURE, SPECIFICATIONS AND PHYSICAL OR TECHNICAL EFFECT

2.1 IDENTITY

2.1.1 Common or Usual Names

Purified yeast cell wall; purified yeast fraction concentrated in 1,3/1,6 beta-glucans; yeast cell wall extract; cell wall extract of *Saccharomyces cerevisiae*; yeast beta-glucans.

2.1.2 Trade Names

Phileo currently markets purified yeast cell wall for use as a source of beta-glucans in the European Union (EU) and other countries under the trade name "Safglucan®".

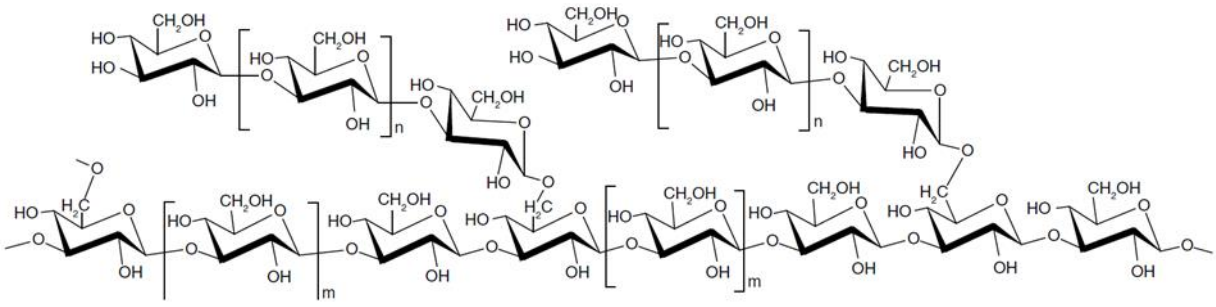
2.1.3 Identity

Purified yeast cell wall is the product obtained by extraction and purification of the structural components of the cell wall of *Saccharomyces cerevisiae* containing not less than 50% beta-glucans. The other components of the purified yeast cell wall are primarily fat (not more than 20%), protein (not more than 8%), ash (not more than 10%) and small amounts of mannans (not more than 5%). The ingredient is a light-beige free-flowing powder.

2.1.4 Description of the Yeast Beta-Glucans Component

Fungal beta-glucans, including yeast beta-glucans, are primarily composed of a backbone chain of 1,3-linked β-glucopyranosyl units with randomly dispersed side chains of β-D-glucopyranosyl units attached by 1,6-linkages. Yeast beta-glucans generally exhibit moderate branching with the exact degree of branching likely to depend on growth conditions (Klis *et al*., 2002; Lam & Cheung., 2013; Zhu *et al*., 2016). The structure of yeast beta-glucans is depicted in Figure 2.1. Analysis of purified yeast cell wall by Phileo indicates that the beta-glucans components contain a 1,6-linkage to a side chain every 8 to 10 D-glucose units (i.e., m = 8 to 10).

Figure 2.1: Structure of Yeast Beta-Glucans (Raa, 2015)



2.1.5 Taxonomic Classification of the Source

The taxonomic classification of *S. cerevisiae* is provided in Table 2.1. Purified yeast cell wall will be manufactured from non-genetically modified *S. cerevisiae strains* that form part of Lesaffre's industrial strain collection.

Table 2.1: Taxonomic Classification of *S. cerevisiae*	
Taxonomy	**Taxonomic Assignment**
Kingdom	Fungi
Phylum	Ascomycota
Class	Hemiascomycetes
Order	Saccharomycetales
Family	Saccharomycetaceae
Genus	*Saccharomyces*
Species	*Saccharomyces cerevisiae*

2.2 METHOD OF MANUFACTURE

2.2.1 Overview of the Manufacturing Process

The manufacturing process to purified yeast cell wall consists of three stages: (a) (b) (4) Further details of the manufacturing method are provided in Appendix 01 (CONFIDENTIAL).

2.2.2 Production Controls

(b) (4)

2.3 PRODUCT SPECIFICATIONS

Appropriate feed-grade specifications have been established for purified yeast cell wall and are presented in Table 2.2. Copies of the methods of analyses are provided in Appendices 02A and B (CONFIDENTIAL), and Appendices 02C to L. Purified yeast cell wall is composed of not less than 50% beta-glucans along with not more than 20% fat, 8% crude protein (CP) and 10% crude ash. The mannans component of the yeast cell wall is removed during processing and represents not more than 5% of the purified yeast cell wall ingredient.

Microbial specifications are set for purified yeast cell wall which include relevant indicators of the control of the fermentation and processing processes (i.e., total plate count, yeast and molds, coliforms, *Escherichia coli* and *Salmonella*).

Heavy metal specifications are set for purified yeast cell wall which reflect the maximum limits specified by the EC as well as the analyzed levels in production batches of the ingredient. In the EU, limits are set for lead of 5 mg/kg in yeasts, and for arsenic, cadmium and mercury of 2, 1 and 0.1 mg/kg in feed materials of non-animal or non-mineral origin. By comparison, the levels in purified yeast cell wall are

specified to not exceed 1, 1, 0.5 and 0.1 mg/kg (as-is) for lead, arsenic, cadmium and mercury, respectively which are either consistent with, or lower than, the EU maximum limits.

Table 2.2: Proposed Product Specifications for Purified Yeast Cell Wall		
Parameter	**Specification**	**Method of Analysis**
Appearance	Light beige powder	Visual inspection
Composition		
Mannans	≤5%	Internal method (Appendix 02A - CONFIDENTIAL)
Beta-glucans	≥50%	Internal method (Appendix 02B - CONFIDENTIAL)
Crude protein	≤8%	Kjeldahl method (Appendix 02C)
Fat	≤20%	Internal method (Appendix 02D)
Crude ash	≤10%	Internal method (Appendix 02E)
Moisture	≤6%	Internal method (Appendix 02F)
Microbiology		
Mesophilic aerobic flora	<5,000 CFU/g	NF EN ISO 4833-1 (Appendix 02G)
Yeast and molds	<100 CFU/g	Method derived from ISO 6611 (Appendix 02H)
Coliforms	<100 CFU/g	NF V08-050 (Appendix 02I)
Escherichia coli	<10 CFU/g	NF ISO 16649-2 (Appendix 02J)
Salmonella	Absent/25g	NF EN ISO 6579-1 (Appendix 02K)
Heavy Metals		
Lead	<1 mg/kg	DIN EN 15763:2010 (2010-04) mod (Appendix 02L)
Arsenic	<1 mg/kg	DIN EN 15763:2010 (2010-04) mod (Appendix 02L)
Cadmium	<0.5 mg/kg	DIN EN 15763:2010 (2010-04) mod (Appendix 02L)
Mercury	<0.1 mg/kg	DIN EN 15763:2010 (2010-04) mod (Appendix 02L)

Abbreviations: CFU = colony forming unit; mod. = modified.

2.4 ANALYTICAL DATA

2.4.1 Compliance with Product Specifications

Analytical data for 5 independent batches of purified yeast cell wall representative of the commercial product are summarized in Table 2.3. Certificates of Analysis are provided in Appendices 03A to Q, 04A to E, and 05 A to E. The analytical batch data demonstrate that purified yeast cell wall can be manufactured in compliance with the product specifications and exhibits acceptable batch to batch variation.

Across the 5 batches tested, the beta-glucans content varied from 57 to 67% on an as-is basis, with a mean value of 61.6%. The CP, fat and crude ash contents were reported to vary from 3.3 to 3.6% (mean 3.5%), 11.2 to 15.2% (mean 13.0%), and 5.6 to 7.4% (mean 6.5%), respectively over the 5 batches. The moisture content did not exceed 3.1% for any of the batches tested. Although the batches analyzed are representative of the commercial material, continual monitoring of the CP content by Phileo indicates

that levels can also range up to 6 to 7%. Certificates of Analyses for batches of purified yeast in which the CP content is above 6% are provided in Appendix 03R.

No microorganisms were identified above detection limits in any of the 5 batches tested and the levels of heavy metals were well below the specification limits.

Table 2.3: Analytical Data for 5 batches of Purified Yeast Cell Wall

Parameter	Unit	Spec.	Analytical Data				
			Batch	Batch	Batch	Batch	Batch
Appearance		Light beige powder	(b) (4) (D) (4)				
Composition							
Mannans	%	≤5					
β-glucans	%	≥50					
Crude protein	%	≤8					
Fat	%	≤20					
Crude ash	%	≤10					
Moisture	%	≤6					
Microbiology							
Total plate count	CFU/g	<5 000					
Yeast	CFU/g	<100					
Molds	CFU/g						
Total coliforms	CFU/g	<100					
Escherichia coli	CFU/g	<10					
Salmonella	/25 g	Absent					
Heavy Metals							
Lead	mg/kg	<1					
Arsenic	mg/kg	<1					
Cadmium	mg/kg	<0.5					
Mercury	mg/kg	<0.1					

Abbreviations: CFU = colony forming unit; ND = not detected; spec. = specification.

2.4.2 Further Compositional Information on Purified Yeast Cell Wall

The overall composition of purified yeast cell wall is summarized in Table 2.4. The Certificates of Analysis for the glycogen analyses are provided in Appendices 03A to E. The analytical data presented in Table 2.3 and representative of the commercial product, indicate that purified yeast cell wall is primarily composed of beta-glucans (*ca.* 62%), along with minor amounts of CP (*ca.* 4%)[1], fat (*ca.* 13%), crude ash

[1] Note that additional CP analyses are provided in Appendix 03R. The mean value across the additional 4 batches of purified yeast is 6.5%. Taken together, the CP content across all batches presented in the GRAS notice is 4.8%,

(*ca.* 7%), moisture (*ca.* 2%) and glycogen (*ca.* 4%). Based on the available analytical results, around 91% of the purified yeast cell wall is accounted for and there will be some analytical variation expected based on the test methods used. Minor amounts of other carbohydrates present in yeast cell walls may also contribute to the overall composition but have not been analyzed by Phileo.

Table 2.4: Composition of Purified Yeast Cell Wall							
Parameter	**Unit**	**Analytical Data**					**Mean**
		Batch	**Batch**	**Batch**	**Batch**	**Batch**	
Mannans	%					(b) (4)	(b) (4)
β-glucans	%						
Crude protein	%						
Fat	%						
Crude ash	%						
Moisture	%						
Glycogen	%						
Total	**%**						

Abbreviations: ND = not detected.

2.4.3 Molecular Weight Distribution

Analysis of purified yeast cell wall as manufactured by Phileo indicates that the molecular weights of the individual components range from 500 to 4,000 kDa. A statement confirming the molecular weight distribution of the components of purified yeast cell well is provided in Appendix 03S.

2.4.4 Mineral Profile

The mineral profiles of 5 batches of purified yeast cell wall are provided in Table 2.5. The Certificates of Analysis are provided in Appendices 06A to E. The data indicate that the mineral content is consistent between batches of purified yeast cell wall. Calcium levels were reported to vary from 8,600 to 10,900 mg/kg, magnesium from 2,100 to 4,200 mg/kg and sodium from 9,600 to 15,700 mg/kg across the 5 batches tested. Potassium levels varied from 220 to 600 mg/kg, iron from 70 to 130 mg/kg, zinc from 600 to 1,800 mg/kg and chromium from 0.1 to 0.9 mg/kg. No selenium was identified above detection limits in any of the batches tested.

but for the purposes of this assessment, the mean of the 5 batches in Table 2.3 was considered appropriate to remain consistent with the other analyses reported.

Table 2.5: Mineral Profile of 5 Batches of Purified Yeast Cell Wall					
Parameter	Analytical Data (mg/kg)				
	Batch	Batch	Batch	Batch	Batch
Calcium	(b) (4)				
Chromium					
Iron					
Magnesium					
Potassium					
Selenium					
Sodium					
Zinc					

2.4.5 Other Inherent Impurities

As evidenced by the long and established history of safe use of *S. cerevisiae* and products derived thereof, in food for animals and humans, there are no inherent constituents of yeast cell walls that are anticipated to be present or to warrant further investigation. While bacterial membranes are widely recognized to contain endotoxins (lipopolysaccharides) and lipoproteins, with the potential to impart toxigenicity or pathogenicity, these inherent constituents are not associated with yeasts (e.g., Holst *et al.*, 1996; Nguyen *et al.*, 2020) as further discussed in Section 6.1.3.

2.5 STABILITY DATA

Phileo recommends storing purified yeast cell wall in cool and dry conditions in unopened packaging for up to 24 months. Purified yeast cell wall is packaged in 5 kg, three-layered bags (PE-OPA-PE) in order to minimize the potential for customers to reseal bags and to provide a barrier to moisture. By ensuring the moisture content remains below the specification limit of not more than 6%, the level of microbiological contamination will also be minimized.

A stability study has been conducted using 4 production batches of purified yeast cell wall stored in packaging representative of the commercial bags[2] under real-time conditions (25°C, 65% RH). The results of analysis of the batches at regular time points over a 24-month storage period are summarized in Table 2.6. The study report is provided in Appendix 07A (CONFIDENTIAL).

The stability study was started in August 2019 using batches manufactured in 2018 and 2019, and at the initial time point, all samples were demonstrated to be in conformance with the product specifications regardless of their manufacturing date. Furthermore, no significant (within analytical variation) difference in composition or microbiological quality of the batches was observed when comparing the

[2] PE bags were used which represents packaging used previously for commercial batches of purified yeast cell wall. The new packaging is also PE but comprises PE-OPA-PE layers which will provide a greater barrier to moisture than the previous PE bags. Thus, the packaging continues to be considered representative on the basis that PE continues to be the primary barrier material and that the new packaging material will be at least as effective as that used in the stability study.

analytical data obtained on manufacture and at the start of the stability study. Thus, the batches were all considered representative of the commercial material and suitable for inclusion in the stability study, with the slightly older samples in practice, providing evidence of greater than 24-months stability.

Purified yeast cell wall continued to conform to the product specifications for beta-glucans and crude protein content over the 24-month storage period. The analyzed beta-glucans contents of each of the 4 batches differed by no more than 6% between the start and end of the study[3]. Microbiological data were collected at study start and end (0 and 24 months) and all batches conformed with the product specifications. The dry matter content was observed to change by up to 6% and was below the specification limit of no less than 94% by weight at the end of the 24-month storage period in 3 of the 4 batches. Although the packaging used for the batches of purified yeast cell wall is representative of the commercial material, the volumes are much smaller. Phileo has conducted a study demonstrating that although the (b) (4) packaging material does provide a suitable barrier to moisture, the greater surface area (exchange surface) of the smaller batches used for the stability studies, results in dry matter contents over time which do not correlate with the commercial batches. A copy of the report is provided in Appendix 07C (CONFIDENTIAL). Evidence that the moisture content remains within the specified limit of not more than 6% over the shelf-life of purified yeast cell wall stored under typical warehouse conditions is provided by analysis of 3 retained batches analyzed at manufacture and after 2 to 4 years. The moisture content remained within the specification limit (4.7 to 6%) across the 3 batches. The Certificates of Analysis at manufacture and of the moisture content after storage for more than 24 months, are provided in Appendix 07D.

Taken together, the results of the stability study and additional data, support the proposed shelf-life of 24 months for purified yeast cell wall under the recommended conditions of storage.

[3] The analytical method was different to the one routinely used by Phileo currently – it has however, been extensively used by Phileo and an external laboratory in the past and the results shown to be comparable to those of the current method. Details of the method are provided in Appendix 07B.

Table 2.6: Results of a Stability Study on 4 Batches of Purified Yeast Cell Wall Stored under Real-Time Conditions							
Parameter	**Unit**	**Specification**	**T=0 Months**	**T=6 Months**	**T=12 Months**	**T=18 Months**	**T=24 Months[1]**
Batch (b) (4) **(Manufacturing Date: 03/25/2019**							(b) (4)
Dry matter	%	>94					
Crude protein	%	≤8					
Beta-glucans	%	≥50					
Total plate count	CFU/g	<5,000					
Yeast	CFU/g	<100					
Molds	CFU/g						
Total coliforms	CFU/g	<100					
Escherichia coli	CFU/g	<10					
Salmonella	/25 g	Absent					
Batch (b) (4) **(Manufacturing Date: 03/04/2019)**							
Dry matter	%	>94					
Crude protein	%	≤8					
Beta-glucans	%	≥50					
Total plate count	CFU/g	<5,000					
Yeast	CFU/g	<100					
Molds	CFU/g						
Total coliforms	CFU/g	<100					
Escherichia coli	CFU/g	<10					
Salmonella	/25 g	Absent					
Batch (b) (4) **(Manufacturing Date: 03/15/2018)**							
Dry matter	%	>94					
Crude protein	%	≤8					
Beta-glucans	%	≥50					
Total plate count	CFU/g	<5,000					
Yeast	CFU/g	<100					
Molds	CFU/g						
Total coliforms	CFU/g	<100					
Escherichia coli	CFU/g	<10					
Salmonella	/25 g	Absent					

Table 2.6: Results of a Stability Study on 4 Batches of Purified Yeast Cell Wall Stored under Real-Time Conditions							
Parameter	**Unit**	**Specification**	**T=0 Months**	**T=6 Months**	**T=12 Months**	**T=18 Months**	**T=24 Months[1]**
Batch (b) (4) **(Manufacturing Date: 03/30/2018)**							
Dry matter	%	>94	(b) (4)				
Crude protein	%	≤8					
Beta-glucan	%	≥50					
Total plate count	CFU/g	<5,000					
Yeast	CFU/g	<100					
Molds	CFU/g						
Total coliforms	CFU/g	<100					
Escherichia coli	CFU/g	<10					
Salmonella	/25 g	Absent					

Abbreviations: CFU = colony forming units;

[1]The quantitative results are not routinely reported by Phileo's internal laboratory, only the compliance with specification.

2.6 CONDITIONS OF INTENDED USE

Phileo's purified yeast cell wall is intended for use as a source of beta-glucans in food for all animal species. The ingredient will normally be incorporated as an ingredient in the complete feed of animals. The intended use levels of purified yeast cell wall and equivalent amount of beta-glucans assuming a minimum content of 50% by weight in the product, are summarized in Table 2.7 for the major species of animal. Exposure to purified yeast cell wall will vary from 0.125 to 1.2 g/kg complete feed, and from 0.05 to 10 g/head/day depending on the species and category of animal. The equivalent levels of beta-glucans exposure, based on the minimum specified content, will be between 63 and 600 mg beta-glucans/kg complete feed, or between 25 and 5,000 mg beta-glucans/head/day. Purified yeast cell wall is not intended for use as an ingredient in calf milk replacer for the first 2 days of life when the intestinal mucosal barrier is permeable.

The proposed use levels are based on current commercial feeding practices in the U.S. for another of Phileo's yeast cell wall derived products, Safmannan® composed of not less than 20% mannans and 20% beta-glucans. Considering that both beta-glucans and mannans are fermentable fibers which will be metabolized by comparable pathways in animals and provide similar nutritional value, information on Safmannan® has been used to estimate practical conditions of use of purified yeast cell wall rich in beta-glucans (Safglucan®) in feed. The current recommended inclusion levels of Safmannan® in feed are summarized in the product data sheet provided in Appendix 08. The use levels of Phileo's purified yeast cell wall will be further refined following commercial trials with customers in the U.S. but will not exceed the upper limits of the ranges given in Table 2.7 for major categories of animal.

Purified yeast cell wall will normally be incorporated as an ingredient in the complete feed of animals.

Table 2.7: Typical Use Levels of Purified Yeast Cell Wall in Animal Food

Animal Species	Use Levels
Poultry	0.125 to 0.15 g/kg complete feed – Equivalent to min. 62.5 to 75 mg beta-glucans/kg complete feed
Swine	0.5 g/kg complete feed – Equivalent to min. 250 mg beta-glucans/kg complete feed
Aquaculture	0.4 to 1.2 g/kg complete feed Equivalent to 200 to 600 mg beta-glucans/kg complete feed
Pets (cats and dogs)	0.25 to 1.0 g/kg complete feed – Equivalent to 125 to 500 mg beta-glucans/kg complete feed
Ruminants	10 g/head/day – larger ruminants (dairy and beef cattle, bison) – Equivalent to min. 5,000 mg beta-glucans/head/day 1 g/head/day – smaller ruminants (goats, sheep) – Equivalent to min. 500 mg beta-glucans/head/day 0.05 g/head/day – milk replacer (calves; min. 2 days of age) – Equivalent to min. 25 mg beta-glucans/head/day
Equine species	0.25 to 5 g/head/day – Equivalent to 125 to 2,500 mg beta-glucans/head/day

2.7 REGULATORY STATUS

2.7.1 Animal Feed Use in the U.S.

2.7.1.1 Yeast-Derived Products (Containing Beta-Glucans)

Various yeast-derived ingredients have a long and established history of use as ingredients in animal feed in the U.S. The yeast products currently defined in Chapter 6 of the AAFCO OP are summarized in Table 2.8 (Section 96 of Chapter 6; AAFCO, 2023). The ingredients are intended as nutrient sources in animal feed and there is no separation or removal of the yeast cell wall components during their manufacture. Thus, these ingredients will contain beta-glucans naturally as part of the cell wall.

Table 2.8: Related Yeast-Based Ingredients with a History of Use in Animal Feed in the U.S.

Reference	AAFCO Ingredient Definition
96.1	**Primary dried yeast** or dried yeast is the dried, non-fermentative yeast of the botanical classification *Saccharomyces* which has been separated from the medium in which propagated. It must consist of yeast cell walls with no fillers and contain not less than 40% crude protein.
96.2	**Active dry yeast** is yeast which has been dried in such a manner as to preserve a large portion of its fermenting power. It must contain no added cereal or filler and must contain not less than 15 billion live yeast cells per gram.
96.3	**Irradiated dried yeast, irradiated _____ dried yeast** is the dried, non-fermentative yeast which has been subject to ultraviolet rays in order to produce anti-rachitic potency
96.4	**Brewers dried yeast** is the dried, non-fermentative, non-extracted yeast of the botanical classification *Saccharomyces* resulting as a by-product from the brewing of beer or ale. It must contain not less than 35% crude protein. It must be labeled according to its crude protein content.
96.5	**Grain distillers dried yeast** is the dried, non-fermentative yeast of the botanical classification *Saccharomyces* resulting from the fermentation of grains and yeast, separated from the mash, either before or after distillation. It must contain not less than 40% crude protein.
96.8	**Yeast culture** is the dried product of yeast (*Saccharomyces* and/or *Kluyveromyces maxianus*) and the media on which it is grown, dried in such a manner as to preserve the fermenting activity of the yeast. The media must be stated on the label.
96.10	**Brewers liquid yeast** is the non-fermentative, non-extracted yeast of the botanical classification *Saccharomyces* resulting as a by-product of the brewing of beer and ale. It must contain not less than 35% crude protein on a dry weight basis. The guaranteed analysis shall include the maximum moisture.
96.11	**Yeast extract** is the concentrated solubles of mechanically ruptured cells of a selected strain of the yeast, *Saccharomyces cerevisiae*. It may be dried or concentrated. It must contain not less than 9% crude protein.
96.12	**Hydrolyzed yeast** is a concentrated, non-extracted, partially soluble, yeast digest. Solubilization is accomplished by enzymatic hydrolysis of whole Saccharomyces cerevisiae cells. Salts may be added as processing acids in accordance with good manufacturing practice. It must contain not less than 35% protein.

2.7.1.2 Beta-Glucans Products (Isolated Cell Wall Products)

Currently, there are no ingredients listed in the AAFCO OP, the Code of Federal Regulations (CFR) or GRAS Inventory of Animal Food which are composed of isolated yeast cell wall ingredients rich in beta-

glucans. Moreover, there are no listings for beta-glucans derived from other sources such as cereals and filamentous fungi.

2.7.2 Animal Feed Use in Canada

In Canada, yeast cell wall is listed in Schedule IV, Part II, Entry 8.76 of the Feed Regulations, 1983 for use as an ingredient in animal feed (CFIA, 2023). The definition of the ingredient is as follows:

*"**Yeast cell wall** is the product resulting from the extraction and purification of the structural components of the yeast cell wall from a fermentation conducted in accordance with good manufacturing practices. This fermentation is conducted for the production of beta-glucans, mannans and mannan oligo-saccharides using a non-pathogenic strain of the microorganism Saccharomyces cerevisiae, which does not contain a novel trait. It shall be labeled with the following statement: "this product is free of antimicrobial activity and is not a source of viable microbial cells" It shall also be labeled with guarantees for minimum beta-glucans and mannans and/or mannan oligosaccharides and maximum moisture."*

2.7.3 Animal Feed Use in the EU

Beta-glucans from the cell walls of *S. cerevisiae* and non-GM *Aspergillus niger* are listed in the Feed Materials Register in the EU under entries 001325-EN and 002922-EN, respectively (Feed Materials Register, 2023). Feed materials are not subject to pre-market approval in the EU but must be listed in the Feed Materials Catalogue published by the European Commission (EC) or the industry-maintained Feed Materials Register prior to use.

2.7.4 Human Food Use in the U.S.

A number of beta-glucans ingredients from fungal and cereal sources have been notified as GRAS to the U.S. FDA and are summarized in Table 2.9.

Table 2.9: Summary of Beta-Glucan Ingredients Notified as GRAS for Human Food Use			
Reference	**Source**	**Ingredient**	**Intended Use**
Fungal Beta-Glucans			
Biothera's notification of the GRAS status of yeast beta-glucans (GRN 000239; U.S. FDA, 2008)	*S. cerevisiae*	Min. 70% beta-glucans	Ingredient in a range of foods at levels of up to 200 mg beta-glucans/serving
Glucan Corporation's notification of the GRAS status of black yeast beta-glucans (GRN 000309, U.S. FDA, 2010)	Black yeast *Aureobasidium pulluns* ATCC 42023	Min. 40% beta-glucans	Ingredient in a range of foods at levels of up to 150 mg beta-glucans/serving
Super Beta Glucan's notification of the GRAS status of mushroom beta-glucans (GRN 000413, U.S. FDA, 2012)	*Ganoderma lucidum* mycelium	Min. 50% beta-glucans	Ingredient in a range of foods delivering 150 mg beta-glucans/serving
Cereal Beta-Glucans			
Cargill's notification of the GRAS status of barley beta-glucans (GRN 000207, U.S. FDA, 2006; and GRN 000344, U.S. FDA, 2011)	Barley	Min. 70% beta-glucans	Ingredient in a range of foods delivering between 0.75 to 3.0 g beta-glucans/serving
Garuda International's notification of the GRAS status of oat beta-glucans (GRN 000437; U.S. FDA, 2013)	Oat bran	Min. 55% beta-glucans	Ingredient in a range of foods at levels delivering between 0.75 to 3.0 g beta-glucans/serving
Tate & Lyle's notification of the GRAS status of oat beta-glucans (GRN 000544, U.S. FDA, 2015)	Oat bran	Min. 35% beta-glucans	Ingredient in a range of foods delivering between 0.75 to 3.0 g beta-glucans/serving

2.7.5 Human Food Use in the EU

In the EU, yeast beta-glucans (min. 75%) has gained novel food approval in accordance with Regulation (EU) No 2015/2283 (EC, 2015) for use as an ingredient in food supplement products (1.275 g/day for children over 12 years and adults; 0.675 g/day for children), in total diet replacement foods for weight control (1.275 g/day), in foods for special medical purposes (1.275 g/day), in fruit-, vegetable- and milk-based beverages (levels of 0.8 g/kg to 3.8 g/kg depending on the beverage), in breakfast cereals, cereal bars, cookies and crackers (levels of 6 g to 15.3 g/kg depending on the product), in soups and soup mixes (0.9 g/kg ready-to-eat), in chocolate and confectionary (4 g/kg), in protein bars and powders (19.1 g/kg) and in jellies and similar fruit spreads (11.3 g/kg) (EC, 2017).

Additionally, a positive opinion was recently issued by the European Food Safety Authority (EFSA) on the safety of dried whole *Euglena gracilis* containing at least 50% beta-glucans on a dry matter basis for use as a novel food ingredient in food supplement products, in total diet replacement foods for weight control and a range of conventional foods (EFSA, 2020a).

2.8. INFORMATION TO ESTABLISH UTILITY FOR THE TARGET ANIMAL

Purified yeast cell wall is intended for use as a source of beta-glucans in the diet of all animal species. The ingredient comprises at least 50% beta-glucans and analysis of 5 representative commercial batches indicate that the analyzed beta-glucans content is in the region of 62% (see Section 2.4). As further described below, beta-glucans are non-digestible polysaccharides that can act as fermentable fibers in the diet of animals. Under the conditions of intended use, purified yeast cell wall provides a supplementary source of beta-glucans functioning as fermentable fiber and will not replace any other fiber-containing nutrient sources in the diet. On this basis, there are no anticipated nutritional disadvantages associated with the intended use of purified yeast cell wall as a component of animal diets at levels ranging from 0.125 to 1.2 g/kg complete feed, and from 0.05 to 10 g/head/day depending on the species. Beyond confirming the nutritional value as a supplemental fermentable fiber source in the form of beta-glucans to support the above statements, the technical effect of purified yeast cell wall does not have any bearing on safety and no further evaluation of utility is warranted in accordance with 21 CFR §570.230(d) and likewise, the use levels intended for addition to feed do not require further justification.

2.8.1 Evidence of Beta-Glucans as a Fermentable Fiber Source

2.8.1.1 Structural Properties of Beta-Glucans

The structure of beta-glucans depends on the source, existing as D-glucose monomers linked by beta-1,3- and beta-1,6-glycoside bonds in yeast and mushrooms compared to beta-1,3-glycosidic bonds in bacteria and algae, and beta-1,4- and beta-1,3-glycosidic bonds in cereals. Beta-glucans from yeast, mushrooms and cereals such as barley and oats display branched structures in contrast to bacteria and algae in which linear structures are present. The chain length, linkages and degree of branching of beta-glucans varies between sources resulting in different spatial arrangements being adopted (Sletmoen & Stokke, 2008). These specific characteristics of beta-glucans are widely assumed to influence their physiological and biological response in animals including humans (e.g., Sletmoen & Stokke, 2008; Zhang *et al*., 2008; Jayachandran *et al*., 2018). Beta-glucans with 1,3-glycoside linkages for example, adopt a helical conformation which is thought to influence their biological properties (Sletmoen & Stokke, 2008). Furthermore, depending on their structure, isolated beta-glucans can be water soluble or insoluble with the extraction conditions largely responsible for the relative ratios of the soluble and insoluble fractions (Izydorczyk *et al*., 2000; Gajdošovà *et al*., 2007).

2.8.1.2 Functionality as a Fermentable Fiber

Animals are unable to digest carbohydrate polymers containing beta-glycosidic linkages (Lam & Cheung, 2013; Bach Knudsen, 2015; Wang *et al*., 2019) and therefore, absorption by the epithelium and significant systemic exposure to beta-glucans will not occur. The molecular size will also preclude absorption to any significant extent in the small intestine. Thus, the majority of the ingested beta-glucans will be transferred to the large intestine or rumen and subject to fermentation by resident microbiota (Lam & Cheung, 2013; Wang *et al*., 2019). The fermentation of beta-glucans to form short-chain fatty acids and other metabolic products which can generally support the digestive health of the animal is well-documented in the published literature (e.g., Li *et al*., 2006; Sayar *et al*., 2007; Cox *et al*.,

2010; Kim & White, 2010; Lam & Cheung, 2013; Ma *et al.*, 2015; Twari *et al.*, 2019). Four *in vitro* studies were identified in which the fermentation of beta-glucans from cereal or yeast sources by the intestinal digesta of pigs and dogs was investigated. The studies are summarized below and the data on cereal beta-glucans is considered pertinent to yeast beta-glucans on the basis that the beta-glycosidic bonds will not be digested from either source in the gastrointestinal (GI) tract of the animal.

2.8.1.3 *In Vitro* Fermentation Studies by Fecal Inoculum from Pigs

Jonathan et al. (2012)

In a study by Jonathan *et al.* (2012), the fermentation of oat beta-glucans along with 11 other dietary fibers by fecal inoculum from three multiparous Dutch Landrace pigs was evaluated. Gas production, short-chain fatty acids (SCFAs) and fiber degradation products were monitored during fermentation at 3.4, 5.1 and 15.3 hours for oat beta-glucans.

The primary findings of the study are summarized in Table 2.10. After 15.3 hours, cumulative gas production from oat beta-glucans was reported to be 393 mL gas/g organic matter during fermentation compared to 290 mL gas/g organic matter for cellulose, 344 mL gas/g organic matter for inulin and 424 mL gas/g organic matter for oligofructose. The concentration of SCFAs was observed to vary from 5.81 mmol/g organic matter for oat beta-glucans to 8.11, 4.52 and 6.46 mmol g/organic matter for cellulose, inulin and oligofructose, respectively. The acetate to propionate to butyrate ratio from the fermentation of oat beta-glucans was 59 to 22 to 19 vs. 50 to 44 to 6 for cellulose, 53 to 34 to 13 for inulin and 53:35:12 for oligofructose. SCFAs composition varied during fermentation and the proportion of butyrate was observed to increase over time. Lactate was also identified during fermentation and is an intermediate fermentation product of propionate or butyrate (not shown). Only 2% of the initial soluble sugar content of oat beta-glucans remained after 15.3 hours fermentation, with no detectable levels reported for inulin and oligofructose. Together these findings indicate that the fermentation of oat beta-glucans is comparable in pigs' digesta to that of the known fermentable fibers cellulose, inulin and oligofructose.

Table 2.10: Total Gas Production, Total SCFA, SCFAs Composition and Soluble Sugars After *In vitro* Fermentation of Dietary Fibers

Fiber	Gas Production (mL gas/g organic matter)	Total SCFAs (mmol/g organic matter)	Composition (Acetate: Propionate: Butyrate)	Soluble Sugars (% of initial total sugar)
Oat Beta-Glucans	393	5.81	59:22:19	2
Cellulose	290	8.11	50:44:6	NA
Inulin	344	4.52	53:34:13	0
Oligofructose	424	6.46	53:35:12	0

Abbreviations: NA = not analyzed because of fiber insolubility; SCFAs = short chain fatty acids.

Williams et al. (2011)

Another study by Williams *et al.* (2011) evaluated the fermentation kinetics and endpoints of a range of purified and semi-purified polysaccharides (soluble and insoluble) derived from cereals *in vitro* using pig fecal inoculum. The substrates used in the study included low, medium and high viscosity wheat

arabinoxylans and barley beta-glucans, as well as insoluble arabinoxylan, which were compared to monosaccharides (arabinose, xylose, and glucose) and maize and wheat starches. Cumulative gas production was measured with 17 gas readings taken over 48 hours. SCFAs and ammonia were determined in fermentation fluid after 48 hours, and the branched-chain ratio (BCR) of the polysaccharides calculated.

Both arabinoxylan and beta-glucans were reported to be rapidly fermented if soluble, while less soluble components were more slowly fermented. The cumulative gas production from low, medium and high viscosity beta-glucans were reported to be 405, 397 and 410 mL gas/g DM, respectively. There was little difference in cumulative gas production observed between the arabinoxylan and beta-glucan polymers, with values of 373, 402 and 416 mL gas/g DM reported for low, medium and high viscosity arabinoxylans, respectively. Cumulative gas production by simple sugars was also similar, with values of 401, 401 and 413 mL gas/g DM reported for arabinose, xylose and glucose, respectively. The rates of fermentation were comparable for simple sugars (arabinose, xylose and glucose), arabinoxylans and beta-glucans, indicating that depolymerization of the polysaccharides was not a limiting step. Fermentation endpoints were related to kinetics, with slow carbohydrate fermentation associated with an increase in protein fermentation. Overall it was concluded that beta-glucans and polymeric arabinoxylan (if soluble) are rapidly fermented at similar rates to monosaccharides.

Mikkelsen et al. (2011)

Mikkelsen *et al*. (2011) investigated the *in vitro* fermentation of a range of dietary fibers including mixed linkage beta-glucans (from barley) using pig fecal inoculum. The cumulative gas production was monitored for 48 hours with 17 readings taken. At the end of the fermentation process, the SCFAs concentrations were evaluated and BCR calculated as an indicator of relative protein fermentation.

The cumulative gas production was reported to be 420 mL gas/g DM for barley beta-glucans which was not different (P>0.05) to the values obtained for medium viscosity arabinoxylans and xyloglycans. The total SCFAs content after barley beta-glucan fermentation was 14.25 mmol/g DM and comprised of 65.7% acetate, 33.4% propionate and 0.9% butyrate. By comparison, similar (P>0.05) findings were reported for medium viscosity arabinoxylans with 14.33 mmol/g DM of SCFAs composed of 66.1% acetate, 32.8% propionate and 1.1% butyrate. Xyloglycan fermentation resulted in a slightly lower (P<0.05) SCFAs concentration of 13.21 mmol/g DM and comparable percentages of acetate, propionate and butyrate of 69.1, 29.7 and 1.2%, respectively. There were no differences (P<0.05) in ammonium concentrations between barley beta-glucans, medium viscosity arabinoxylans and xyloglycans (189.9, 169.6 and 167.6 mmol/L, respectively), or BCR content (0.090, 0.086 and 0.094%). Overall, barley beta-glucans displayed similar fermentability in the study to other soluble polysaccharides.

2.8.1.4 *In vitro* Simulation of Canine Gastrointestinal Tract

A study by Van den Abbeele *et al*. (2020) assessed the effect of a *S. cerevisiae*-based product consisting of 27.5% beta-glucans and 22.5% mannan-oligosaccharides (MOS), on modulation of the canine gut microbiota and fermentative metabolites using an *in vitro* simulation of the GI tract of dogs [Simulator of the Canine Intestinal Microbial Ecosystem (SCIME)]. The SCIME consisted of three different segments, with each segment consisting of a succession of three reactors simulating the different

regions of the GI tract, i.e., upper GI tract including subsequent simulation of stomach and small intestine, proximal colon (PC), and distal colon (DC), respectively. Upon inoculation of each PC and DC with the microbiota derived from fresh fecal matter obtained from a Beagle dog, a 2 week stabilization period was initiated to allow the microbial community to differentiate in the different reactors depending on the local environmental conditions, followed by a 2 week control period to determine the baseline microbial community composition and activity. Following the control period, the yeast-based test product was administered during each feeding cycle on top of the simulated nutritional medium (composed of dog food, mucin, cysteine, peptone and yeast extract; supplied to the model twice per day) to reach a daily dose of 0.5, 1.0, or 2.0 g/d over a 3-week treatment period. Additionally, the SCIME setup was modified by incorporating a mucosal environment. Given the regular flushing with nitrogen in the SCIME model to ensure anaerobiosis, gas production could not be monitored in the long-term setup. To evaluate total gas production, a single level of the yeast-based test product was supplied to a PC microbiota derived from the long-term SCIME during the control period and incubated with culture media and either 0.5, 1.0, and 2.0 g/d of yeast-based test product for 48 hours. SCFAs and branched-chain fatty acids (BCFAs) were extracted and analyzed. The microbiota profiling of each colon compartment was established both at the luminal and at the mucosal level and assessed by 16S-targeted Illumina sequencing.

Treatment with the yeast-based test product increased gas production at all of the time intervals, with the most pronounced effect observed during the first 6 hours of incubation. The total BCFAs concentration at 6 hours are summarized in Table 2.11. Overall, BCFAs production was low with a significantly increased production observed upon dosing with 1.0 and 2.0 g/day of the yeast-based product in both the PC and DC. BCFAs concentration in the PC and DC were 3.27 and 2.79 mM, and in the DC were 4.16 and 4.32 mM, at dosing of 1 and 2 g yeast-based test product/day, respectively.

Table 2.11: BCFAs Production in a Canine *In vitro* Digestibility Model using a Yeast-Based Test Product

Compartment	BCFAs Concentration (mM)					
	0.5 g/day		1 g/day		2 g/day	
	Control	Treatment	Control	Treatment	Control	Treatment
PC	1.48 ± 0.44	2.34[a] ± 1.10	2.08 ± 1.13	**3.27**[a] ± 0.42	2.00 ± 0.51	2.79[a] ± 0.44
DC	3.9 ± 0.14	3.94[a] ± 0.41	3.59 ± 0.37	**4.16**[a,b] ± 0.15	3.78 ± 0.16	**4.32**[b] ± 0.17

Abbreviations: BCFAs = branched-chain fatty acids; DC = distal colon; PC = proximal colon.
[1]Statistical significance relative to the control for any given treatment is indicated in **bold**, whereas significant difference between different treatments (0.5, 1 or 2 g/day) are indicated with a different letter (a,b; $P<0.05$).

The production of individual SCFAs (acetate, propionate and butyrate) were reported to increase in a dose-dependent manner along the entire length of the colon. Relative to the control, the levels of propionate were observed to increase ($P<0.05$) in the simulated PC and DC at each of the tested concentrations of the yeast-based product.

The changes in the fermentation pattern with yeast-based test article dosing were also linked to specific microbial alterations at the family level, such as the specific stimulation of the propionate-producing families Porphyromonadaceae and Prevotellaceae, upon product supplementation. Other consistent changes in community composition included the decrease in the Enterobacteriaceae and the

Fusobacteriaceae families. The study findings indicate that the beta-glucans and MOS components of the yeast-based product support microbial fermentation in dogs.

2.8.1.5 Summary of the *In Vitro* Fermentation Studies

The results of the fermentation trials using fecal inoculum from pigs indicate that cereal-derived beta-glucans serve as a fermentable fiber when consumed in the diet of animals. Similar findings were reported in a study in which a beta-glucans- and MOS-containing yeast-derived product was evaluated in an *in vitro* model mimicking the GI tract of dogs. Although there are some structural differences between beta-glucans from different sources, the fermentation behavior is expected to share many similarities based on the absence of any significant absorption, molecular size and ability to be fermented in the GI tract or rumen on the animal. Furthermore, the nutritional properties of yeast beta-glucans as fermentable fibers will be common to other species, including poultry, ruminants and aquaculture. Together, the available *in vitro* and *in situ* data support the function of purified yeast cell wall as a source of beta-glucans in animals. On this basis, as mentioned in Section 2.8.1.1, the technical effect of purified yeast cell wall will have no bearing on safety and does not warrant further evaluation.

PART 3. §570.235 – TARGET ANIMAL AND HUMAN EXPOSURES

3.1 ESTIMATED EXPOSURE BY ANIMALS

3.1.1 Estimated Exposure to Purified Yeast Cell Wall and the Beta-Glucans Component

The intended use and use levels of Phileo's purified yeast cell wall are described in Section 2.6 above. The exposure by animals to the purified yeast cell wall and the beta-glucans component were estimated based on representative body weight and feed intake data for major categories of animal as reported by EFSA in its guidance document on the safety of feed additives for target species (EFSA, 2017a). These intakes estimates are summarized in Table 3.1. Exposure to purified yeast cell wall was estimated to range from 9 to 14 mg/kg body weight/day for different categories of poultry, 17 to 25 mg/kg body weight/day for different categories of swine, 0.5 mg/kg body weight/day for calves, 15 to 25 mg/kg body weight/day for dairy and beef cattle, respectively, 17 mg/kg body weight/day for sheep and goats, 24 mg/kg body weight/day for salmon, 18 mg/kg body weight/day for medium-sized dogs and 21 mg/kg body weight/day for cats. Assuming the beta-glucans content is 50% of purified yeast cell wall, the equivalent intakes of this component are between 4.5 and 7 mg/kg body weight/day for different categories of poultry, 8.5 to 12.5 mg/kg body weight/day for different categories of swine, 0.25 mg/kg body weight/day for calves, 7.5 to 12.5 mg/kg body weight/day for dairy and beef cattle, respectively, 8 mg/kg body weight/day for sheep and goats, 12 mg/kg body weight/day for salmon, 9 mg/kg body weight/day for medium-sized dogs and 10.5 mg/kg body weight/day for cats.

Table 3.1: Estimated Intakes of Purified Yeast Cell Wall on a Body Weight Basis by Animals

Category	Body Weight (kg)	Feed Intake (kg feed/day)[1]	Maximum Use Level of Purified Yeast Cell Wall (g/kg feed)	Daily Intake of Purified Yeast Cell Wall (mg/kg body weight/day)	Daily Intake of Beta-Glucans (g/kg body weight/day)
Broilers (meat-type chickens)	2	0.180	0.15	14	7
Laying hens	2	0.120	0.15	9	4.5
Growing turkeys	3	0.2	0.15	10	5
Piglets	20	1	0.5	25	12.5
Grower/finisher pigs	60	2.5	0.5	21	10.5
Lactating sows	175	6	0.5	17	8.5
Veal calves (milk replacer)	100	1.89 (DM basis)	0.05 (g/head/day)	0.5	0.25
Dairy cattle	650	9	10 (g/head/day)	15	7.5
Beef cattle	400	3.6	10 (g/head/day)	25	12.5
Sheep/goat	60	23	1 (g/head/day)	17	8
Salmon	0.12	0.003	1.2	24	12
Dog (medium)	15	0.263	1	18	9
Cat	3	0.063	1	21	10.5

[1]Feed intake calculated from the default values reported by EFSA on a dry matter (DM) basis by assuming 12% moisture in feed for poultry and swine, and 5% moisture in kibbles for cats and dogs. The feed intake values were not used in the calculations for ruminants but the amounts reported assumed 55% moisture in the total mixed

ration of cattle and are on a DM basis for veal calves. Calculations (except ruminants): purified yeast cell wall (mg/kg complete feed in kg) x (default feed amount consumed per day in kg as-is)/body weight (kg). Calculations (ruminants): purified yeast cell wall (amount in mg/head)/body weight (kg). Calculations (calves): purified yeast cell wall (amount in mg/head)/body weight (kg). Calculations for beta-glucans exposure: purified yeast cell wall exposure x 0.5.

3.1.2 Estimated Exposure to Other Fermentable Fibers in the Diet of Animals

The yeast beta-glucans component of purified yeast cell wall is intended to provide a source of beta-glucans in the diet of animals. Further details on the structural properties of beta-glucans and their ability to function as fermentable fibers is evaluated in Section 2.8.

A number of other non-digestible polysaccharides such as fructooligosaccharides (FOS), inulin and xylooligosaccharides (XOS) have an established and well-documented history of use as sources of fermentable fibers in animal food (Patterson & Burkholder, 2003; Fahey *et al.*, 2004; Courtin *et al.*, 2008; de Lange *et al.*, 2010; Gaggìa *et al.*, 2010; Hajati & Rezaei, 2010; Ringø *et al.*, 2010; Patra, 2011; Heo *et al.*, 2012; De Maesschalck *et al.*, 2015; Uyeno *et al.*, 2015; Bednarczyk *et al.*, 2016; Gibson *et al.*, 2017; Singh *et al.*, 2017; Kritas, 2018). AAFCO ingredient definitions have been established for FOS and inulin as summarized in Table 3.2 for example, in recognition of their established role as fermentable fiber sources in the diet of animals in the U.S. Thus, purified yeast cell wall will provide a source of beta-glucans which presents an alternative fermentable fiber to these recognized counterparts.

Table 3.2: Examples of Other Polysaccharides as Fermentable Fibers in Animal Feed in the U.S.

Ingredient	AAFCO Ingredient Definition
Fructooligosaccharides (FOS)	**60.105** Carbohydrate product composed of short chain fructose units bound by β-2,1 linkages attached to a terminal glucose unit. The final product must contain a minimum of 80% fructooligosaccharide on a dry weight basis (AAFCO, 2023).
Inulin	**60.106** Polysaccharide product obtained from plant sources such as chicory (*Cichorium intybus*, L.), agave (Agave *azul tequilana*), and Jerusalem artichoke *(Hellianthus tuberosus*) by hot water extraction. It is intended as a source of soluble, fermentable fiber. It must contain not less than 90% inulin on a dry matter basis. It may contain products of partially hydrolyzed inulin (AAFCO, 2023).

3.1.3 Background Exposure to Beta-Glucans from the Diet vs. Purified Yeast Cell Wall

Animals will be exposed to beta-glucans in the diet from a range of sources including cereal products, soybean meal (SBM), distillers products and yeast-derived ingredients. The reported values for the beta-glucans content present naturally in cereals, SBM, distillers products and brewer's yeast are summarized in Table 3.3. The beta-glucans content of cereals is reported to vary between 0.5 to 7% in wheat, 2 to 9% in barley and 2 to 6% in oats (Izydorczyk *et al.*, 2000; Havrlentovà & Kraic, 2006; Gajdošovà *et al.*, 2007; Cui & Wang, 2009). The differences in beta-glucans content among cereal crops indicates that exposure to beta-glucans from the background diet will largely depend on the composition of the feed, with those fed barley-rich diets for example, likely to consume higher levels of the cell wall component than those fed wheat-based diets. Furthermore, beta-glucanase is present naturally in grains and on storage may act on beta-glucans and reduce the overall content (Gajdošovà *et*

al., 2007). Similarly, beta-glucanase can be added to feeds containing ingredients high in dietary fiber in order to increase digestibility which will also impact the background level of beta-glucans in the diet (AAFCO, 2023).

The beta-glucans levels in SBM used as a commercial feed ingredient are reported to be in the region of 0.3% (Ward, 2014). Animals will also consume beta-glucans from yeast-derived products directly or via their carry-over into distillers products. It can generally be assumed that *S. cerevisiae* contains around 30% cell wall on a dry basis of which around 37% is in the form of beta-glucans (Kessler & Nickerson, 1959; Fleet & Manners, 1977; Nguyen *et al*., 1998). This equates to a beta-glucans content in the region of 11% in dried yeast products commonly used in animal feed (see Table 3.4). Distillers products contain inactivated yeast as a carry-over from the fermentation process, with levels estimated to be in the region of 5% (Ingledew, 1999; Han & Liu, 2010) equivalent to 0.55% beta-glucans.

The reported values for the beta-glucans content of cereals, SBM, distillers products and brewer's yeast were used to estimate the likely exposure by animals as part of the normal diet and the results are provided in Table 3.2. The estimated dietary levels of beta-glucans in poultry feed containing 20% barley and 40% SBM was 13.2 g/kg complete feed. Complete swine feeds comprised of 5% wheat and 10% SBM were estimated to contain 2.8 g beta-glucans/kg. Similarly, the beta-glucans level was estimated to be 0.3 g/kg in complete feed for salmonids containing 10% SBM and 15 g/kg in dog food containing 25% barley.

Although the minimum content of beta-glucans in purified yeast cell wall is 50% by weight, analytical data on 5 representative commercial batches of product indicate the levels are around 62% in practice (see Section 2.4). Based on the analyzed beta-glucans content, when purified yeast cell wall is included in the diets of poultry, swine, aquaculture and pets at the maximum intended use levels of 0.15, 0.5, 1.2 and 1.0 g/kg complete feed, the equivalent intakes are 0.09, 0.3, 0.7 and 0.6 g beta-glucans/kg complete feed, respectively. Thus, background consumption of beta-glucans from the normal diet of poultry, swine and dog food has the potential to be higher (at least 2.5-fold higher) than from the intended use of purified yeast cell wall. The exception is aquaculture feeds which were estimated to contain approximately half the amount of beta-glucans potentially in the background diet compared to that provided by purified yeast cell wall under the conditions of intended use.

Likewise, a total mixed ration (TMR) for dairy cattle containing 15% SBM and 1% distillers products was estimated to contain around 0.6 g beta-glucans/kg DM compared to 0.3 g beta-glucans/kg DM from the intended use of purified cell wall. Thus, dairy cattle are potentially exposed to higher levels (2-fold) of beta-glucans from the background diet than from the intended use of purified yeast cell wall.

Overall, these data indicate that animals are potentially exposed to beta-glucans as a structural component of the endosperm wall or cell wall from various sources in the diet at levels often equivalent or higher than from the intended use of purified yeast cell wall. However, in these dietary sources, the beta-glucans form part of the polymeric structure of the endosperm or cell wall and is not available to animals in the same form. Purified yeast cell wall is intended as a supplemental beta-glucans source in the diet of animals and will only be added at low levels to the diet on the basis that there are various sources of fermentable fiber in the diet from other sources.

Table 3.3: Estimated Intakes of Beta-Glucans from the Background Diet				
Animal Category	**Feed Ingredient**	**Estimated Beta-Glucans Content (%)[1]**	**Potential Ingredient Contribution to the Diet (%)[2]**	**Estimated Dietary Beta-Glucans Levels (%)[5]**
Dietary Exposure (% in the Diet)				
Poultry	Barley	6	Max. 20	1.2
	SBM	0.3	Max. 40	0.12
	Distillers products[3]	0.6	Max. 15	0.09
	Brewer's yeast	11	Max. 0.2	0.02
Maximum estimated intakes of beta-glucans from the intended use of purified cell wall in poultry feeds – 0.09 g/kg complete feed vs. 13.2 g/kg complete feed containing 20% barley and 40% SBM				
Swine	Wheat	5	Max. 5	0.25
	Barley	6	Max. 5	0.3
	SBM	0.3	Max. 10	0.03
	Distillers products[3]	0.6	Max. 20	0.12
	Brewer's yeast	11	Max. 0.2	0.02
Maximum estimated intakes of beta-glucans from the intended use of purified yeast cell wall in swine feeds – 0.3 g/kg complete feed vs. 2.8 g/kg complete feed containing 5% wheat and 10% SBM				
Finfish	SBM	0.3	Max. 10	0.03
Maximum estimated intakes of beta-glucans from the intended use of purified yeast cell wall in salmonid feeds – 0.7 g/kg complete feed vs. 0.3 g/kg complete feed containing 10% SBM				
Dogs	Barley[4]	6	Max. 25	1.5
Maximum estimated intakes of beta-glucans from the intended use of purified yeast cell wall in dog food – 0.6 g/kg complete feed vs. 15 g/kg complete feed containing 25% barley				
Dairy cows	SBM	0.3	Max. 15 (DM)	0.05 (DM)
	Distillers products[3]	0.6	Max. 2 (DM)	0.01 (DM)
Maximum estimated intakes of beta-glucans from the intended use of purified yeast cell wall in cattle feeds – 6.2 g/head/day equivalent to 0.3 g/kg DM (assuming a TMR of 25 kg DM/cow/day) vs. 0.6 g/kg DM in a TMR containing 15% SBM and 1% distillers products (DM basis)				

Abbreviations: DM = dry matter; SBM = soybean meal; TMR = total mixed ration;

[1]Estimated beta-glucans content based on ranges reported in the literature and in particular the mean values reported by Havrlentovà & Kraic (2006) in barley and wheat;

[2]Estimated dietary levels of feed ingredients based on U.S. feeding practices (taken from NRC nutrient requirements for poultry, swine, dogs and dairy cattle (NRC, 1994; 2012; 2006 and 2001, respectively);

[3]The only contribution to the beta-glucans content of distillers grains was assumed to be the inactivated yeast component for the purpose of this assessment;

[4]Barley was assumed to represent 50% of the whole cereal content of a typical commercial dry dog food containing around 50% cereals;

[5]Calculation: % estimated beta-glucans content x (% ingredient contribution/100) = % estimated beta-glucans intake.

3.1.4 Estimated Exposure by Animals to Other Components of Purified Yeast Cell Wall

Analytical data on 5 representative batches of purified yeast cell wall (see Section 2.4) indicate that the ingredient is primarily composed of soluble beta-glucans (*ca.* 62%), along with minor amounts of CP (*ca.* 4%), fat (*ca.* 13%) and crude ash (*ca.* 7%) (see Section 2.4).

The mean results of the analytical data for 5 commercial batches of purified yeast cell wall were used to estimate the exposure by animals to the CP, fat and ash components under the intended conditions of use in animal food. The exposure estimates are summarized in Table 3.4 on a complete feed basis. Under the maximum conditions of intended use of purified yeast cell wall, the CP contribution to the diet of poultry, swine, aquaculture and dogs was estimated to range from 0.006 to 0.05 g/kg complete feed, and for ruminants and pseudo-ruminants to vary from 0.04 to 0.2 g/head/day. Likewise, the fat content was estimated to range from 0.02 to 0.2 g/kg complete feed for poultry, swine, aquaculture, cats and dogs, and from 0.13 to 1.3 g/head/day for ruminants and pseudo-ruminants. The ash content is calculated to vary from 0.01 to 0.08 g/kg complete feed, and 0.07 to 0.7 g/head/day for these same representative species. As mentioned in Section 2.6, calves will not be exposed to purified yeast cell wall for the first 2 days of their lives and only data on growing or reproducing animals is provided for ruminants.

Table 3.4: Estimated Exposure to the Components of Purified Yeast Cell Wall

Animal	Maximum Anticipated Use Level	Estimated Exposure (g/kg complete feed)			
		Beta-Glucans	Crude Protein	Fat	Ash
Poultry	0.15	0.09	0.006	0.02	0.01
Swine	0.5	0.3	0.02	0.07	0.04
Aquaculture	1.2	0.7	0.05	0.2	0.08
Cats and dogs	1.0	0.6	0.04	0.1	0.07
Animal	**Maximum Anticipated Use Level**	**Estimated Exposure (g/head/day)**			
		Beta-Glucans	**Crude Protein**	**Fat**	**Ash**
Large ruminants	10	6	0.4	1.3	0.7
Small ruminants	1	0.6	0.04	0.13	0.07
Equine species	5	3	0.2	0.7	0.4

The CP, fat and ash contents of some common feedstuffs are presented in Table 3.5. The CP content of purified yeast cell wall is around 4% on a DM basis compared to around 12% in barley, 9% in corn and 46% in SBM. Similarly, the fat content of purified yeast cell wall is in the region of 13% compared to 8% in SBM and 100% in soybean oil. The ash content of purified yeast cell wall is around 7% compared to 6% in SBM and 11% in molasses. Purified yeast cell wall will typically be included in the diet of animals at significantly lower levels of use (0.013 to 0.12%) compared to these common feedstuffs. As an example, SBM may be included in the diet of many food-producing animals at levels of 20%, delivering 9% CP, 4% fat and 1% ash. By comparison, 0.12% (or 1.2 g/kg complete feed) purified yeast cell wall in the diet of the same animals will provide 0.04 g of CP, 0.16 g fat and 0.08 g of ash. Overall, it may be concluded that purified yeast cell wall will not make a significant contribution to the CP, fat or ash contents of the complete feed under the intended conditions of use.

Table 3.5: Proximate Composition of Common Feedstuffs (NRC, 2001)				
Ingredient	**Composition (% DM)**			**Typical Dietary Level (%)[1]**
	Crude Protein	**Fat**	**Ash**	
Barley grain, rolled	12.4	2.2	2.9	5
Corn, cracked	9.4	4.2	1.5	40
Canola meal, mechanically extracted	37.8	5.4	7.4	10
Soybean meal	46.3	8.1	5.5	20
Soy oil	-	100	-	2
Molasses, beet sugar	8.5	0.2	11.4	2
Purified yeast cell wall	***3.5***	***13.0***	***6.5***	***0.013 to 0.12%***

Abbreviations: DM = dry matter;
[1]The levels of these ingredients in the diet of animal will vary between species and the values reported are intended to be indicative only of likely exposure to the proximate components.

3.2 ESTIMATED EXPOSURE BY HUMANS

Phileo's purified yeast cell wall is comprised of beta-glucans (*ca*. 62%), along with minor amounts of CP (*ca*. 4%), fat (*ca*. 13%), crude ash (*ca*. 7%), moisture (*ca*. 2%) and glycogen (*ca*. 4%). All of these components are found naturally in the diet of animals and humans. In particular, beta-glucans act as a fermentable fiber in animal food and will not be digested or absorbed to any significant extent by animals as outlined below.

3.2.1 Absorption, Distribution, Metabolism and Excretion (ADME) of Beta-Glucans Component

Animals are unable to digest carbohydrate polymers containing beta-glycosidic linkages (Bach Knudsen, 2015; Wang *et al*., 2019) and therefore, absorption by the epithelium and significant systemic exposure to beta-glucans will not occur. No studies were identified in the published literature in which the metabolism of yeast-derived beta-glucans was assessed in animals, but a number of studies evaluated the passage of plant beta-glucans through the GI tract. In ileal-cannulated pigs fed oat-derived beta-glucans (Bach Knudsen *et al*., 1993), no degradation of beta-glucans occurred during passage through the stomach and past the distal third of the small intestines, while some degradation began in the terminal ileum. The remaining beta-glucans were degraded in the cecum and proximal colon. However, as reported by Johansen *et al* (1993, 1997) and Holtekjølen *et al*. (2014) a substantial amount of depolymerization of the soluble fraction of beta-glucans derived from oats and barley occurs in the small intestine (50% reduction in the average molecular weight) before being fermented in the hindgut. This depolymerization may be due to hydrolytic enzymes excreted by microbiota in the upper digestive tract of pigs and/or retained endogenous hydrolase activities in the barley material.

While beta-glucans are not expected to be absorbed to any significant quantity by the animal, Sandvik *et al*. (2007) reported that in rats fed 5 to 6 mg yeast beta-glucans for 14 consecutive days, the total amount of beta-glucans in plasma was 30 ng. This level of beta-glucans in the plasma represents only a minute fraction of a single oral dose of beta-glucans and the biological relevance was considered by the authors to be uncertain. One hypothesis was that mucosal dendritic cells sample or interact with soluble beta-glucans via projections across the epithelium before being carried by the afferent

lymphatics to the mesenteric lymph nodes. Beta-glucans degradation within macrophages has been evaluated in a number of other studies reviewed by Samuelsen *et al*. (2014), and the results indicate that both soluble and insoluble beta-glucans have the potential to be taken up to a minor extent by animals.

3.2.2 ADME of Other Components

The other minor components of purified yeast cell wall such as CP, fat and micronutrients (minerals) will be metabolized by animals according to well-established pathways and are not expected to have any significant impact on the composition or quality of animal products.

3.3.3 Potential for Residues in Edible Tissues

On the basis that the components of purified yeast cell wall are macronutrients and micronutrients which will be metabolized via normal pathways in the animal, no residues of potential toxicological concern will deposit in animal products under the conditions of intended use of Phileo's purified yeast wall ingredient. In particular, there will be no exposure to beta-glucans from the consumption of purified yeast cell wall by animals.

PART 4. §570.240. SELF-LIMITING LEVELS OF USE

The use of purified yeast cell wall will be self-limiting on the basis that there are detrimental physiological effects associated with the intake of excessive levels of fermentable fiber.

PART 5. §570.245. EXPERIENCE BASED ON COMMON USE IN FOOD BEFORE 1958

Not applicable.

PART 6. §570.250. NARRATIVE

6.1 SAFETY FOR THE TARGET ANIMALS

6.1.1 Introduction

Phileo's purified yeast cell wall is the product resulting from the extraction and purification of the structural components of the yeast cell wall of *S. cerevisiae*. Compositional specifications have been established for purified yeast cell wall of not less than 50% beta-glucans. Analytical data on 5 representative batches of the commercial product indicate that purified yeast cell wall is primarily composed of soluble beta-glucans (*ca.* 62%), along with minor amounts of CP (*ca.* 4%), fat (*ca.* 13%), crude ash (*ca.* 7%), moisture (*ca.* 2%) and glycogen (*ca.* 4%) (see Section 2.4).

Purified yeast cell wall is intended for use as a source of beta-glucans in the diet of all animals. The beta-glycosidic bonds in beta-glucans from all sources, including yeast, are indigestible by animals and beta-glucans will therefore, act as a supplemental fermentable fiber for animals, similar to existing counterparts such as inulin and fructooligosaccharides (Lam & Cheung, 2013; Bach Knudsen, 2015; Wang *et al*., 2019).

Fungal beta-glucans are as highlighted above, primarily composed of beta-1,3- and 1,6-glycosidic bonds, in contrast to their cereal and legume counterparts which mainly contain unbranched beta-1,3 and 1,4-glycosidic bonds (Johansson *et al*., 2000). Beta-glucans from bacterial sources exhibit greater similarity to those from fungal than cereal and legume sources, containing mostly beta-1,3-glycosidic linkages (Gleeson, 2016). Although beta-glucans from all sources will act as fermentable fiber, in order that the source and manufacturing process are also taken into account, only data on fungal beta-glucans were evaluated in terms of safety for the target animal.

The safety of purified yeast cell wall for the target animal is assessed using a weight of evidence approach based on data generally available in the public domain on yeast-derived ingredients rich in beta-glucans. The individual components that comprise the safety evaluation are as follows: (a) the history of use of yeast-derived products in animal feed; (b) the generally recognized body of evidence to support the safety of the *S. cerevisiae* source; (c) studies in which yeast-derived ingredients rich in beta-glucans have been fed to animals; and (d) toxicological data on yeast-derived ingredients rich in beta-glucans.

As mentioned in Section 2.7.1.1, various yeast-derived ingredients have a long and established history of use in animal feed in the U.S. These ingredients are intended as nutritional ingredients (sources of nutrients such as vitamins, minerals and fermentable fibers) in animal feed and there is generally no separation or removal of the cell wall components. The relevance of these ingredients to the safety assessment of purified yeast cell wall is considered in Section 6.1.2.

There is a body of published data pertaining to the pathogenicity and toxigenicity potential of *S. cerevisiae* which supports the long and established history of safe use of yeast-derived ingredients in animal feed. A summary of the data supporting the safety of the *S. cerevisiae* source is provided in Section 6.1.3.

Numerous studies are available in the published literature in which yeast-derived ingredients rich in beta-glucans were fed to poultry, swine, aquaculture, ruminants, dogs and rabbits. These studies were generally designed to evaluate the utility of yeast-derived beta-glucans to serve as a source of fermentable fiber in the feed of animals, and only limited endpoints pertinent to safety, including performance, nutrient digestibility and immune response were evaluated. Detailed summaries of these studies are provided in Section 6.1.4. Together, the results of these studies provide corroborative evidence of the tolerability of yeast cell wall-based ingredients to animals.

Yeast-derived ingredients rich in beta-glucans have been the subject of safety evaluations for use in food in the EU and U.S. As a result, a number of toxicological studies are available on these related yeast products which provide corroborative evidence of the safety of purified yeast cell wall for animals. These studies are detailed in Section 6.1.5.

6.1.2 History of Safe Use of Yeast Products in Animal Feed

Various yeast-derived ingredients are defined in the AAFCO OP as summarized in Table 2.9. These ingredients include primary dried yeast, irradiated dried yeast, grain distillers yeast, yeast extract and hydrolyzed yeast. The cell wall is estimated to comprise up to 30% by weight on a DM basis of the yeast cell, of which 37% is estimated to be in the form of beta-glucans (Kessler & Nickerson, 1959; Fleet & Manners, 1977; Nguyen *et al*., 1998; Luo *et al*., 2015). This equates to a beta-glucans content of approximately 11% in dried yeast products commonly used in animal food. Generalizing that food for poultry, swine and aquaculture may contain in the region of 2 g/kg complete feed (0.2%) of dried yeast, the exposure by animals to cell wall components will be around 0.6 g/kg complete feed and to beta-glucans around 0.2 g/kg complete feed. By comparison, the maximum intended conditions of use of Phileo's purified yeast cell wall are in the range of 0.125 to 1.2 g/kg complete feed across different species, equivalent to a beta-glucans exposure (based on a typical 62% content) of 0.08 to 0.7 g/kg complete feed.

Likewise, ruminants may also be fed dried yeast in the diet, with levels typically in the range of 28 to 57 g/head/day for sheep and 85 to 113 g/head/day for beef cattle. At these inclusion levels, exposure to cell wall components is estimated to be 8 to 17 g/head/day by sheep and 26 to 34 g/head/day for beef cattle, and to beta-glucans 3 to 6 g/head/day for sheep and 9 to 12 g/head/day for beef cattle. By comparison, the recommended use levels of Phileo's purified yeast cell wall for sheep and beef cattle are lower, at 1 g/head/day and 10 g/head/day, respectively.

The long and established history of use of yeast products as ingredients in animal food in the U.S. therefore, provides supporting evidence for the safety of the components of purified yeast cell wall as components of the diet at the lower end of the intended conditions of use of Phileo's product. However, it is also noteworthy that in yeast products, the beta-glucans form part of the polymeric structure of the cell wall, whereas in Phileo's purified yeast cell wall, the beta-glucans are released and selectively isolated. Studies in the target animals (see Section 6.4) are required to further evaluate the tolerance of animals to purified yeast cell wall ingredients over the range of intended use levels.

6.1.3 Safety of the *S. cerevisiae* Source

6.1.3.1 Safety Evaluations by Scientific Bodies

In the EU, *S. cerevisiae* has Qualified Presumption of Safety (QPS) status for use as an ingredient in food and feed applications (EFSA, 2023). As part of the QPS assessment, the EFSA Panel on Biological Hazards (BIOHAZ) evaluated a range of *S. cerevisiae* in 2007 (EFSA, 2007) and concluded: "*the body of knowledge is considered sufficient (long history of use) with only 92 cases of pathogenicity involving S. cerevisiae reported in total (15 cases diagnosed before 1990); all patients had a least one condition facilitating the opportunistic development of S. cerevisiae….It is possible to propose some species of the genus [Saccharomyces] for QPS status with the following qualification: S. cerevisiae, subtype S. boulardii should certainly be contraindicated for patients of fragile health, as well as for patients with a central venous catheter in place….*". The BIOHAZ Panel has continued to monitor the safety of *S. cerevisiae* and update the QPS status on an annual basis (EFSA, 2008 to 2011a, 2012 to 2016, 2017b, 2018 to 2022 and 2020b). *S. cerevisiae* is currently considered to be suitable for QPS status provided that absence of resistance to antimycotics used for medical treatment of yeast infections is met for *S. cerevisiae* strains able to grow above 37°C and introduced as viable yeast into the food or feed chain.

6.1.3.2 Potential Pathogenicity of *S. cerevisiae*

The examples of *S. cerevisiae* infections in humans are rare and occur in those with compromised immune systems or predisposing factors. Enache-Angoulvant & Hennequin (2005) reviewed 92 cases of invasive *S. cerevisiae* infection, most frequently of the blood: 51.3% of these fungaemias comprised infection with the probiotic strain *S. cerevisiae* subtype *boulardii*, although in some patients who did not take the probiotic, this nosocomial infection was probably acquired by contamination of catheters. Predisposing factors included candidiasis, intravascular catheters, and antibiotic treatment, and co-morbidities were frequent, all patients having at least one condition predisposing to the opportunistic infection. Genetic analysis of some of these clinical strains has shown them to be very different to laboratory and industrial strains, in particular having acquired an ability to grow at higher temperatures (McCusker *et al*., 1994a; Murphy & Kavanagh, 1999), form pseudohyphae (chain of easily disrupted fungal cells), maintain a strong cell wall and detoxify the cell (McCusker *et al*. 1994b; Muller *et al*., 2011; Strope *et al*., 2015). The ability of *S. cerevisiae* to acquire pathogenicity is likely to be polygenic, and is suggested as a useful model for mechanisms of pathogenicity in fungal pathogens.

Investigations into the mechanism by which *S. cerevisiae* can cause infection have highlighted differences compared to related, virulent yeasts such as *Candida glabrata* and *Candida albicans* (Roetzer *et al*., 2011; Pérez-Torrado *et al*., 2012; Pérez-Torrado & Querol, 2016). Unlike these *Candida* species, *S. cerevisiae* has been shown to have low adhesion levels to human tissues, and to be likely only to perform opportunistic or passive crossings of the epithelial barrier when it is previously compromised (de Llanos *et al*., 2006 and 2011). Consistent with fungal pathogens generally however, enhanced resistance to oxidative stress has been shown to be characteristic of virulent *S. cerevisiae* strains (Llopis *et al*., 2012).

Any rare infection caused by *S. cerevisiae* can normally be treated using standard antifungal agents and isolates of this species are reported to typically be susceptible to antifungals. Only where *S. cerevisiae* is

able to grow at unusually high temperatures, exceeding 37°C, is the potential for antifungal resistance a consideration (Ghannoum & Rice, 1999; Anderson *et al*., 2003; Kanafani & Perfect, 2008; Miceli *et al*., 2011; White & Hoot, 2011).

Lipoproteins are membrane proteins widely accepted to be found in all bacteria where they play a variety of different roles including adhesion to host cells, modulation of inflammatory processes and translocation of virulence factors into host cells (Kovacs-Simon *et al*., 2011; Buddelmeijer, 2015; Nguyen *et al*., 2020). They contain an N-terminal lipid modified cysteine residue which by its hydrophilic nature, can anchor onto bacterial cell membranes. *Saccharomyces cerevisiae* is not characterized by the presence of lipoproteins, consistent with their not being generally associated with pathogenicity. Furthermore, the production process to purified yeast cell wall involves separation of fatty components from the polysaccharides-rich fraction. Thus, no substances such as lipoproteins (even if present) would carry over into the purified yeast cell wall.

In conclusion, *S. cerevisiae* is rarely implicated in infections despite the extensive use of the species by the food and feed industries. Taken together with the absence of any live yeast in the purified cell wall ingredient, pathogenicity is not expected to be a concern.

6.1.3.3 Potential Toxigenicity of *S. cerevisiae*

S. cerevisiae is not associated with the production of toxins and is generally regarded as non-toxigenic (U.S. EPA, 1997). There are reports of "killer toxin" production (e.g., proteins, glucoproteins) by some *S. cerevisiae* strains which are active against other yeasts but not against animals or humans (Reiter *et al*., 2005; Schmitt & Breinig, 2006).

Lipopolysaccharides or endotoxins, are cell-wall constituents of most gram-negative bacteria which provide innate immunity to the microorganism. At the same time, endotoxins are widely implicated in infection and trauma and associated with various pathophysiological reactions in animals and humans (Morrison & Ulvetich, 1978; Raetz, 1990; Holst *et al*., 1996; Raetz & Whitfield, 2002). Endotoxins are not associated with *Saccharomyces,* and *S. cerevisiae* cell wall and fermentation products have both been reported to reduce the concentrations of bacterial endotoxins in cattle (e.g., Razavi *et al*., 2019; Guo *et al*., 2022). Thus, endotoxins are not expected to be present or to pose a safety concern in purified yeast cell wall.

Taken together, toxigenicity is not considered a potential concern for the purified yeast cell wall ingredient.

6.1.4 Studies in Target Animals

Numerous studies were identified in the published literature in which yeast cell wall-based ingredients were fed to poultry, swine, aquaculture, ruminants, dogs and rabbits in order to evaluate the utility of beta-glucans as a source of fermentable fiber in the diet. As a consequence of the nutritional value of beta-glucans as a non-digestible carbohydrate source, the digestive health of the animal can be positively impacted leading to improvements in growth performance, nutrient digestibility and immune function. The studies identified were primarily designed to evaluate these indirect measures of utility and therefore, included only limited parameters relevant to safety such as body weight (BW), feed

intake or feed consumption (FI or FC), growth [average daily gain (ADG) and feed conversion ratio (FCR)], as well as general health. However, the studies can be used as part of a weight of evidence approach to support the safety of purified yeast cell wall as an ingredient in animal food at levels ranging from 0.125 to 1.2 g/kg complete feed, or 0.05 to 10 g/head/day, depending on the species. Notably, to avoid any penetration of the immature intestinal barrier, calves less than 2 days old will not be provided purified yeast cell wall.

The ability of fermentable fibers such as beta-glucans to positively influence the immune function of animals has led to a number of studies being performed under challenge conditions, i.e., studies in which poultry, swine or aquaculture are intentionally exposed to pathogenic bacteria or viruses in order to evaluate their susceptibility to infection when consuming diets containing supplemental sources of beta-glucans. Phileo's purified yeast cell wall ingredient is only intended for use in healthy animals as a source of beta-glucans and any secondary indirect effects on immune function or resistance to infection fall outside the scope of this GRAS determination. On this basis, studies investigating the resistance of animals to infection, or only considering factors related to immunomodulation, were not considered relevant to the safety evaluation and are not summarized in the sections below.

A range of yeast cell wall-based ingredients containing different beta-glucans content were used in the studies. A summary of the different test articles is provided in Section 6.1.4.1. The available studies in target animals are outlined in Sections 6.1.4.2 to 6.1.4.8 separated according to species/category (poultry, swine, aquaculture, ruminants, dogs and rabbits).

6.1.4.1 Test Articles used in Target Animal Studies

As mentioned briefly above, a range of yeast cell wall-based ingredients were used in the target animal studies with differing beta-glucans content. In a number of the studies, the test articles were purified hydrolyzed yeast products in which the beta-glucans content ranged from 50 to greater than 80%. Although the full details of the manufacturer are not available, it is anticipated that these ingredients were subject to similar processing to Phileo's purified yeast cell wall and only subject to physical separation techniques following alkaline treatment of the autolyzed yeast to release the beta-glucans component. Thus, for the purpose of this assessment, these ingredients are grouped together and referred to as "purified yeast cell wall-based ingredients".

In other studies, the test articles were hydrolyzed yeast products which were not purified to the same extent, resulting in beta-glucans contents of around 20 to 35% and significant mannans contents of 10 to 20%. By comparison, Phileo's purified yeast cell wall ingredient contains only a low level of mannans (≤5%). Considering that these ingredients are equivalent to intermediates in the manufacture of Phileo's purified yeast cell wall (i.e., crude hydrolyzed yeast extracts prior to further physical processing), they were also considered pertinent to the safety assessment. These ingredients are grouped together and referred to as "yeast cell wall-based ingredients".

In a number of the studies identified, the beta-glucans content of the test articles was not specified. However, in these studies the authors generally refer to the ingredients as "beta-glucans" rather than "yeast cell wall" and it was therefore, presumed that purification methods had been employed to selectively isolate the beta-glucans components. For the purpose of this assessment, these test articles

were assumed to fall within the above-defined category of "purified yeast cell wall-based ingredients" referring to products with a beta-glucans content of at least 50%.

The summaries of the target animal studies provided in Sections 6.1.4.2 to 6.1.4.8 all identify the nature of the test articles and the beta-glucans content (where reported). A comparison of each of the test articles used in the target animal studies is provided in Table 6.1.

As mentioned in Section 2.8.1, the structure of the beta-glucans component depends on the source, existing as D-glucose monomers linked by beta-1,3- and beta-1,6-glycosidic linkages in yeast and mushrooms compared to the beta-1,3- and beta-1,4-glycosidic bonds in cereals. Beta-glucans from yeast, mushrooms and cereals display branched structures in contrast to those from bacteria and algae in which linear structures exist. These specific characteristics of beta-glucans are widely reported to influence the physiological and biological response in animals (e.g., Zhang *et al.*, 2008, Sletmoen & Stokke, 2008; Jayachandran *et al.*, 2018). Consequently, only target animal studies using ingredients rich in fungal beta-glucans were considered pertinent to the safety assessment. The fungal source was normally *S. cerevisiae*, but studies using cell wall-derivatives from *Rhodoturula mucilaginosa* (Chen *et al.*, 2019), *Kluyveromyces fragilis* (Keimer *et al.*, 2018a,b), *Schizophyllum commune* (Chirapongsatonkul *et al.*, 2019) and *Saccharomyces uvarium* (Suphantharika *et al.*, 2003) were also included.

Table 6.1: Comparison of Yeast Cell Wall-Derived Ingredients used in Target Animal Studies

Beta-Glucans Content	References
Purified Yeast Cell Wall-Based Ingredients (Beta-Glucans, min. 50%)	
≥80%	**Poultry studies:** Ding *et al.*, 2019 Zhang *et al.*, 2008 Zhen *et al.*, 2020 Zhen *et al.*, 2021
	Swine studies: He *et al.*, 2022 (Biorigin) Li *et al.*, 2006 [Trade name: FUBON®] Szuba-Trznadel *et al.*, 2014 [Trade name: Betamune®] Wang *et al.*, 2008
	Aquaculture studies: Jiang *et al.*, 2019
	Ruminant studies: Ma *et al.*, 2015
	Dog studies: Rychlik *et al.*, 2013 [Biolex Beta HP]
50 to 70%	**Poultry studies:** Morales-Lopéz *et al.*, 2009 Rathgeber *et al.*, 2008
	Swine studies: Sweeney *et al.*, 2012
	Aquaculture studies: Aramli *et al.*, 2015 [MacroGard®] Bai *et al.*, 2010 Bagni *et al.*, 2005 [MacroGard®] Boonanuntanasarn *et al.*, 2016 [Trade name: Beta-S]

Table 6.1: Comparison of Yeast Cell Wall-Derived Ingredients used in Target Animal Studies	
Beta-Glucans Content	**References**
	Do Huu *et al.*, 2016 [MacroGard®] Domenico *et al.*, 2017 [MacroGard®] Ghaedi *et al.*, 2015 [MacroGard®] Ghaedi *et al.*, 2016 [MacroGard®] Kühlwein *et al.*, 2014 [MacroGard®] Munir *et al.*, 2016 [MacroGard®] Refstie *et al.*, 2010 [MacroGard®]
	Ruminant Studies: Eicher *et al.*, 2010 [BetaRight] Sowińska *et al.*, 2017 [Biolex® Beta-S] Zabek *et al.*, 2013 [Biolex® Beta-S] Zaleska *et al.*, 2015 [Biolex® Beta-S]
	Dogs studies: Stuyven *et al.*, 2010 [MacroGard®]
Beta-glucans content not reported	**Poultry studies:** An *et al.*, 2008 Cox *et al.*, 2010 Bar-Dagan *et al.*, 2023
	Swine studies: Ganner *et al.*, 2010 Hahn *et al.*, 2006 Kerkaert *et al.*, 2018 Wook Goh *et al.*, 2023a and b
	Aquaculture studies: López *et al.*, 2003 Sánchez-Martínez *et al.*, 2017 [Beta-G]
	Dogs and cat studies: Ferreira *et al.*, 2022 Fries-Craft *et al.*, 2023 González *et al.*, 2023
Yeast Cell Wall Ingredients (Beta-Glucans, 20 to 49%; Mannans 10 to 30%)	
10 to 49%	**Poultry studies:** Chae *et al.*, 2006 [Trade name: Glucagen] Cheraghi *et al.*, 2014 [Alphamune™] Keser *et al.*, 2012 Kyoung *et al.*, 2023 Morales-Lopéz *et al.*, 2010 Yalçin *et al.*, 2014 Zhang *et al.*, 2005
	Swine studies: Keimer *et al.*, 2018a
	Aquaculture studies: Abu-Elala *et al.*, 2018 [Immunowall®] Chen *et al.*, 2019 Hisano *et al.*, 2018 [Glucan-MOS®] Nazari et al., 2016 [Alphamune™] Selim & Reda, 2015 Suphantharika *et al.*, 2003

Table 6.1: Comparison of Yeast Cell Wall-Derived Ingredients used in Target Animal Studies	
Beta-Glucans Content	**References**
	Studies in Ruminants: Wojcik, 2010 [Biolex-MB40] Peng *et al*., 2020
	Study in Rabbits: Wu *et al*., 2011

6.1.4.2 Studies in Poultry using Yeast Cell Wall-Derived Ingredients

A number of studies were identified in broilers in which birds were fed diets containing beta-glucans rich yeast ingredients which are compositionally similar to Phileo's purified yeast cell wall. An overview of the key findings from each of the poultry studies is provided in Table 6.2. In the majority of the studies, newly hatched broilers were fed diets containing yeast cell wall-based ingredients for the entire production stage (34 to 49 days). In the studies in which the beta-glucans content of the yeast cell wall-based ingredients was at least 50%, broilers were fed diets containing levels of between 0.0025 to 0.2% (25 to 2,000 mg/kg complete feed) of the test article (Rathberger *et al*., 2008; Zhang *et al*., 2008; Morales-Lopéz *et al*., 2009; Ding *et al*., 2019;). In studies in which the beta-glucans content of the yeast cell wall-based ingredients were lower than 50%, or were not reported, the levels of incorporation in the diet was typically up to 0.1% (1,000 mg/kg complete feed), and in one study was 0.3% (3,000 mg/kg complete feed) (Zhang *et al*., 2005; Chae *et al*., 2006; An *et al*., 2008; Cox *et al*., 2010; Morales-Lopéz *et al*., 2010; Keser *et al*., 2012; Cheraghi *et al*., 2014; Bar-Dagan *et al*., 2023; Kyoung *et al*., 2023).

Treatment with yeast cell wall-based ingredients was observed to either have no impact, or to improve ($P \leq 0.05$) growth performance of broilers over the entire study period. A quadratic response in average daily gain was observed in a study by Zhang *et al*. (2008) in which male broilers were fed diets containing 0, 0.0025, 0.005, 0.0075, 0.01 or 0.0125% of a purified yeast cell wall ingredient containing 91.5% beta-glucans for 42 days. Although birds fed 0.005 or 0.0075% purified yeast cell wall ingredient displayed significantly higher average daily gains than the control birds ($P<0.05$), the remaining treatments (0.0025, 0.01 and 0.0125% purified yeast cell wall ingredient) were numerically but not significantly higher than the control animals. Likewise, FI was higher and feed/gain ratio was lower in birds fed 0.005 or 0.0075% purified yeast cell wall ingredient relative to controls. Under these study conditions, the authors concluded that maximal growth performance was obtained at a supplementation level of 0.005% (50 mg/kg complete feed) of purified yeast cell wall ingredient, which on a beta-glucans-basis is equivalent to around 0.0075% (75 mg/kg complete feed) of Phileo's purified yeast cell wall (*ca*. 62% beta-glucans content).

In two experiments conducted by Chae *et al*. (2006), nutrient digestibility was evaluated and increases in DM, calcium and phosphorus retention were reported at levels of 0.03 and 0.04% (300 or 400 mg/kg complete feed) of the yeast cell wall-derived ingredient containing a minimum of 50% beta-glucans in the feed of broilers. Morales-Lopéz *et al*. (2010) reported that supplementation of the diet of broilers with 0.05% (500 mg/kg complete feed) of a yeast cell wall-based ingredient containing 26% beta-glucans had no impact on crude fat or CP ileal digestibility coefficients, although intestinal viscosity was decreased relative to the control birds.

Blood parameters relating to immune function were either not affected or were positively influenced by the inclusion of yeast cell wall-based ingredients in the diet (Zhang *et al*., 2005 and 2008; Chae *et al*., 2006; An *et al*., 2008; Cox *et al*., 2010; Keser *et al*., 2012; Ding *et al*., 2018; Kyoung *et al*., 2023).

Bursa and spleen weights, and intestinal morphology were also evaluated in a number of studies (Rathgeber *et al*., 2008; Morales-Lopéz *et al*. 2009; Ding *et al*., 2019; Bar-Dagan *et al*., 2023; Kyoung *et al*., 2023) using yeast cell wall-based ingredients. In all studies, the yeast cell wall-based ingredients either positively impacted, or had no impact, on relative organ weights, villus height and crypt depth. There were no reports of yeast cell wall-based ingredients exhibiting a detrimental impact on immune response or the digestive tract under the conditions of the studies.

Taken together, under the conditions of these broiler studies, yeast cell wall-based ingredients were well-tolerated at levels of up to 2 g/kg complete feed (0.2%; 80% beta-glucans content) and 3 g/kg complete feed (0.3%; yeast cell wall components, beta-glucans content not known). The treatment levels in these studies were comparable to, and in some cases higher, than from the intended use of Phileo's purified yeast cell wall in poultry feed of between 0.125 to 0.15 g/kg complete feed (0.0125 to 0.015%). In the majority of these studies, the objective was to evaluate the ability of yeast cell wall-ingredients to elicit an immune response in animals relative to an established antibiotic. As previously mentioned, Phileo's purified yeast cell wall is intended only as a source of beta-glucans to help support digestive function in the animal, and the level required to achieve the desired effect in feed can differ to those reported in the published studies in broilers.

The feeding of yeast cell wall-based ingredients at levels higher than the intended use of Phileo's purified yeast cell wall provides a small margin of safety to be established. For example, 2 g/kg complete feed of purified yeast cell wall ingredient containing 80% beta-glucans (Ding *et al*., 2019) is the equivalent to 2.58 g of Phileo's purified yeast cell wall ingredient on a beta-glucans basis. Compared to the maximum intended use level of Phileo's purified yeast cell wall ingredient of up to 0.15 g/kg complete feed, the ingredient and beta-glucans inclusion levels are 13 and 17 times higher. Thus, the available studies in broilers provide supporting evidence for the safety of Phileo's purified yeast cell wall under the intended conditions of use. Moreover, these conclusions can be extrapolated to all categories and species of poultry on the basis of their physiological similarities.

Additionally, 3 studies were identified in which yeast cell wall-based ingredients were fed to laying hens (Yalçin *et al*., 2014; Zhen *et al*., 2020 and 2021). In the study by Yalçin *et al*. (2014), the yeast cell wall-based ingredient has no effect on performance or production parameters in laying hens when supplemented in the diet at levels of up to 4 g/kg complete feed. Similar findings were reported by Zhen *et al*. (2020 and 2021) at a supplementation level of 200 mg/kg complete feed of yeast cell wall-based ingredients with relatively high beta-glucans content (*ca*. 80%). Consistent with the findings in broiler studies, no adverse effects and some positive effects on immune function and intestinal health were reported. Together, these studies support the extrapolation of the available data in broilers to all categories and species of poultry.

Table 6.2: Summary of Studies in Poultry using Yeast Ingredients Rich in Beta-Glucans		
Reference and Objective	**Study Design**	**Key Findings Related to Safety and Utility**
Purified Yeast Cell Wall Ingredients (min. Beta-Glucans 50%, or Beta-Glucans indicated as Major Component but Content not Reported)		
Bar-Dagan *et al*., 2023 **Objective** Beta-glucans induce cellular immune training and changes in intestinal morphology in poultry	**Animals** 1-day old male and female Ross 308 broiler chicks (10 chicks/treatment) **Test Article** BG (purity not reported) **Treatment** Commercial feed diets containing 0, 250 or 1,000 mg/kg complete feed BG; 3 x 2 factorial arrangement (also using algae-derived BGs) **Duration** 5-weeks	**Intestinal Morphology** ↑'d villi length and numbers of goblet cells in ileum and jejunum in chicks fed diets supplemented with BGs vs. controls
Ding *et al*., 2019 **Objective** Effects of dietary yeast beta-1,3-1,6-glucan on growth performance, intestinal morphology and chosen immunity parameters changes in Haidong chicks	**Animals** 1-day old Haidong chicks (30 chicks/replicate; 5 replicates/treatment) **Test Article** BG (80%) Taylor rhizomorph (as an antibiotic) **Treatment** Corn/SBM-based diets containing 0.0% (-ve control; C), 0.02% Taylor rhizomorph (+ve; T), 0.05% (G1), 0.1% (G2) or 0.2% BG (G3) **Duration** 42 days **Conditions** High altitude (hypoxic conditions)	**Performance Parameters** ↑'d BW, BWG and FI in animals fed diets supplemented with 0.05, 0.1 or 0.2% BG (P<0.05) vs. controls **Blood Parameters** NSD in RBC count, and IgA and IgM levels among treatment groups ↑'d WBC counts and lymphocytes levels in BG-fed birds vs. T and C and ↑'d IgG in G3 vs. T, C, G1 (P<0.05) **Intestinal Morphology** Villus height, crypt depth and goblet cell density were positively impacted by BG dietary supplementation
Keser *et al*., 2012 **Objective** Effects of chitosan oligosaccharide (COS) and/or β-glucan supplementation to diets containing organic zinc (Or.Zn) on	**Animals** 1-day old Ross x Ross male broiler chicks (15 chicks/replicate; 6 replicates/treatment) **Test Article** BG (purity and source not stated) Or.Zn COS	**Performance** NSD in BW, ADG, ADFI and FCR among treatment groups **Blood Parameters (d42)** NSD in serum total cholesterol, HDL-cholesterol, VLDL-cholesterol, urea, insulin and glucose levels among treatment groups ↓'d GOT levels in control vs. other treatments (P<0.05)

Table 6.2: Summary of Studies in Poultry using Yeast Ingredients Rich in Beta-Glucans		
Reference and Objective	**Study Design**	**Key Findings Related to Safety and Utility**
performance and some blood indices in broilers	**Treatment** Corn/SBM-based diets containing 0, 1% Or.Zn, 0.025% COS, 0.05% BG, 1% Or.Zn + 0.025% COS or 1% Or.Zn + 0.05% BG **Duration** 42 days	
Morales-Lopéz *et al.*, 2009 **Objective** Use of yeast cell walls; beta-1,3/1,6-glucans and mannoproteins	**Experiment 1** **Animals** 1-day old Ross 308 male broiler chicks (23 chicks/replicate; 6 replicates/treatment) **Test Article** BG (55%) MP (55% mannans) YCW (29% BG; 19% mannans) [Test articles supplied by Lesaffre] **Treatment** Wheat-based diets containing 0% (-ve control), 0.001% Avilamycin (antibiotic; +ve control), 0.05% YCW, 0.0095% MP, 0.0145% BG, or 0.0095% MP + 0.0145% BG **Duration** 42 days	**Experiment 1** **Performance** NSD in average BW, DFI among treatment groups at d42; at d21, FCR was impaired in birds fed YCW or MP + BG vs. control, AVI or BG **Organ Weights** ↑'d relative weight of thymus in animals fed MP + BG ($P \leq 0.05$)
	Experiment 2 **Animals** 1-day old Ross 308 male broiler chicks (40 chicks/replicate; 5 replicates/treatment) **Test Article** BG (55%) MP (55% mannans) YCW (25% BG; 21% mannans) **Treatment** Wheat based diets containing 0 (control), 0.05% YCW, 0.019% MP or 0.0227% BG **Duration** 42 days	**Experiment 2** **Performance** NSD in average BW, DFI and FCR among treatment groups at d42 **Intestinal Morphology** ↑'d villus height in birds fed YCW, MP or BG vs. control (*P* **Organ Weights** ↓'d relative % liver weight in birds fed YCW vs. control (but NSD vs. BG-fed birds) ($P \leq 0.01$)

Table 6.2: Summary of Studies in Poultry using Yeast Ingredients Rich in Beta-Glucans		
Reference and Objective	**Study Design**	**Key Findings Related to Safety and Utility**
Rathgeber *et al.*, 2008 **Objective** Growth performance and spleen and bursa weight of broilers fed yeast beta-glucans	**<u>Experiment 1</u>** **Animals** 1-day old Ross x Ross male and female broiler chicks [38 chicks/replicate; 8 replicates/treatment (4 replicates of males + 4 replicates of females)] **Test Article** BG (min. 70%) Virginiamycin (antibiotic) **Treatment** Corn/wheat/SBM-based diets containing 0% BG or Virginiamycin (-ve control),0.025% Virginiamycin (+ve control) or 0.004% BG (starter), 0.002% BG (grower and finisher stages) **Duration** 38 days (starter d0-14; grower d14-24; finisher d24-38)	**<u>Experiment 1</u>** **Performance (d38)** ↑'d BW in BG-fed birds vs. control birds ($P \leq 0.05$); FCR was poorer in control birds vs. BG-fed birds (numerically over d0-38 but significantly poorer in starter phase, $P \leq 0.05$) **Spleen and Bursa Weights (d14, d38)** NSD in spleen weights among treatment groups but bursa weights did not decline with age in control birds as they did for BG-fed groups
	<u>Experiment 2</u> **Animals** 1-day old Ross x Ross male and female broiler chicks [38 chicks/replicate; 8 replicates/treatment (4 replicates of males + 4 replicates of females)] **Test Article** BG (min. 70%) Virginiamycin **Treatment** Corn/SBM-based diets containing 0% BG or Virginiamycin (-ve control), 0.025% Virginiamycin (+ve control) or 0.004% BG (starter)/0.002% (grower and finisher stages) **Duration**	**<u>Experiment 2</u>** **Performance (d38)** NSD in BW or FCR among treatment groups **Spleen and Bursa Weights (d14, d38)** NSD in spleen or bursa weights among treatment groups

Table 6.2: Summary of Studies in Poultry using Yeast Ingredients Rich in Beta-Glucans		
Reference and Objective	**Study Design**	**Key Findings Related to Safety and Utility**
	38 days (starter d0-14; grower d14-24; finisher d24-38)	
	<u>Experiment 3</u> **Animals** 1-day old Ross x Ross male and female broiler chicks [38 chicks/replicate; 4 replicates/treatment (2 replicates of males + 2 replicates of females)] **Test Article** BG (min. 70%) Virginiamycin **Treatment** Corn/SBM-based diets containing 0% BG or Virginiamycin (-ve control), 0.025% Virginiamycin (+ve control) or 0.004% BG (starter)/0.002% (grower and finisher stages) **Duration** 38 days (starter d0-14; grower d14-24; finisher d24-38)	**<u>Experiment 3</u>** **Performance (d38)** NSD in BW or FCR among treatment groups **Spleen and Bursa Weights (d14, d38)** NSD in spleen and bursa weights among treatment groups

Table 6.2: Summary of Studies in Poultry using Yeast Ingredients Rich in Beta-Glucans		
Reference and Objective	**Study Design**	**Key Findings Related to Safety and Utility**
Zhang *et al.*, 2008 **Objective** Modulating effect of beta-1,3/1,6-glucan supplementation in the diet on performance and immunological responses of broiler chickens	**Animals** 1-day old Arbor Acres male broiler chicks (8 chicks/replicate; 5 replicates/treatment) **Test Article** BG (91.5%) **Treatment** Corn/SBM-based diets containing 0, 0.0025, 0.005, 0.0075, 0.01 or 0.0125% BG **Duration** 42 days (6 weeks)	**Performance** Quadratic response in ADG in BG-fed animals; ↑'d ADG, FI and improved feed/weight gain ratio in birds fed diets supplemented with 0.005 or 0.0075% BG ($P \leq 0.05$) vs. controls; NSD in ADG, FI or feed/weight gain ratio between birds fed 0.01 or 0.0125% BG vs. controls **Immunological Parameters** ↑'d plasma globulin concentrations in all diets supplemented with BG ($P<0.01$) vs. control ↑'d serum IgG levels in all diets supplemented with BG (except for the diet containing 0.0125% BG on day 42) ($P<0.05$) vs. control ↑'d intestinal IgA in all diets supplemented **Lymphoid-Organ Indices** ↑'d lymphoid-organ index analysis in thymus, bursa and spleen indices over weeks 0 to 3 and 4 to 6 ($P<0.01$) of BG-fed birds vs. controls except for bursa index in birds fed 0.0125%
Zhen *et al.*, 2020 **Objective** Dietary yeast beta-glucans supplementation improves eggshell color and fertile egg hatchability as well as enhances immune function in breeder laying hens	**Animals** 43-wk old Hyline Brown breeder hens (4 hens/cage; 8 cages/treatment) **Test Article** BGs (77.8%) **Treatment** Corn/SBM-based diets containing 0, 50, 100 or 200 mg/kg complete feed BGs **Duration** 8-weeks	**Performance and Production** NSD in performance but ↑'d hatchability ($P<0.05$) and ↓'d mortality in hens fed diets supplemented with BGs vs. controls **Egg Properties** Improved egg shell color in hens fed diets supplemented with BGs vs. controls **Immune Parameters** ↑'s serum cytokines, total IgA and antibody titers against BSA, AIV and NDV vaccine; and ↑'d lymphocyte proliferation response to LPS, peripheral blood CD3+ T cells and phytohemagglutin skin response in hens fed diets supplemented with BGs vs. controls

Table 6.2: Summary of Studies in Poultry using Yeast Ingredients Rich in Beta-Glucans		
Reference and Objective	**Study Design**	**Key Findings Related to Safety and Utility**
Zhen *et al*., 2021 **Objective** Yeast beta-glucan altered intestinal microbiome and metabolome in older hens	**Animals** 43-wk old Hyline Brown breeder hens (2 hens/cage; 8 cages/treatment) **Test Article** BGs (77.8%) **Treatment** Corn/SBM-based diets containing 0, 50, 100 or 200 mg/kg complete feed BGs **Duration** 9-weeks	**Intestinal Parameters** Hens fed diets containing BGs displayed altered gut microbiomes and metabolite profiles, with downregulation of intestinal mucosa immune response
Yeast Cell Wall Ingredients (Beta-Glucans Contents 10 to 49%)		
An *et al*., 2008 **Objective** Growth performance and antibody response of broiler chicks fed yeast derived beta-glucan and single-strain probiotics	**Animals** 1-day old Ross male broiler chicks (30 chicks/replicate; 3 replicates/treatment) **Test Article** BG (purity not reported) *Bacillus amyloliquefaciens* KU801 (BA) **Treatment** Corn/SBM-based diets containing 0 (control; no BG or BA), 0.025% BG, 0.05% BG, 0.1% BG, 0.05% BA, 0.1% BA or 0.2% BA **Duration** 35 days	**Performance (BG-Fed Birds vs. Controls)** ↑'d BWG in animals fed diets supplemented with 0.025 or 0.1% BG ($P<0.05$) vs. control NSD in FI or feed/gain ratio between diets containing BG vs. control **Blood Parameters** NSD in GOT, GPT, total cholesterol or HDL cholesterol levels among treatment groups **Carcass Characteristics** NSD in relative weight of liver, abdominal fat, breast muscle and spleen among treatment groups **Antibody Production** ↑'d titers against NDV and IBV in all treated groups vs. control except for group fed 0.025% BG **Cecal Microbial Population** NSD in total microbes, coliforms or lactic acid bacteria in BG-fed birds vs. controls
Chae *et al*., 2006 **Objective** Effects of supplementation of beta-glucans on the growth	**Experiment 1 (Feeding System x Treatment; 2 x 3 Factorial Study)** **Animals**	**Experiment 1** **Performance (d0-34)** ↑'d BWG and FI in open floor housed broilers vs. caged broilers ($P=0.0001$) but NSD in BWG, FI or feed/gain ratio with BG dietary levels ($P=0.0228$)

Table 6.2: Summary of Studies in Poultry using Yeast Ingredients Rich in Beta-Glucans		
Reference and Objective	**Study Design**	**Key Findings Related to Safety and Utility**
performance and immunity in broilers	3-day old Ross 308 broiler chicks (8 chicks/replicate; 3 replicates/treatment) housed in cages or open pens **Test Article** BG (Glucagen; min. 40%) **Treatment** Corn/SBM-based diets containing 0.0% (control), 0.02 or 0.04% BG **Duration** 34 days	**Nutrient Digestibility (Caged Broilers)** ↑'d retention of DM birds fed 0.02 or 0.04% BG vs. controls (*P*=0.0373); NSD in GE, CP, EE, crude ash, or Ca and P retention with BG dietary levels **Immunological Parameters (d42)** ↑'d B-lymphocyte population, and levels of CD8 and TCR 1 cells in birds fed 0.04% BG vs. controls; NSD in MHC-Class II, CD4 and TCR 2 among treatment groups
	<u>Experiment 2</u> **Animals** 3-day old Ross 308 broiler chicks (8 chicks/replicate; 3 replicates/treatment) **Test Article** BG (min. 40%) Flavomycin **Treatment** Corn/SBM-based diets containing 0% BG or Flavomycin (-ve control; T1), 0.03% BG (T2), 0.1% Flavomycin (T3) or 0.03% BG and 0.1% Flavomycin (+ve control; T4) **Duration** 34 days	**<u>Experiment 2</u>** **Performance (d0-34)** Trend to ↑'d FI in birds fed 0.03% BG vs. –ve control (*P*<0.01) but NSD in BWG or feed gain ratio among treatment groups **Nutrient Digestibility (BG-Fed Birds vs. Controls)** ↑'d DM, Ca and P retention birds fed 0.03% BG vs. –ve control; NSD in GE, CP, EE or crude ash
Cheraghi *et al.*, 2014 **Objective** Effects of dietary levels of yeast extracted β-glucans and α-mannans (Alphamune™) on performance of broiler chicken raised in normal and thermal-stressed conditions	**<u>Experiment 1</u>** **Animals** 1-day old Ross 308 broiler chicks (40 chicks/replicate; 5 replicates/treatment) **Test Article** BG + mannans (Alphamune™; 26% BG and 15% mannans) **Treatment**	**Performance (d1-49)** ↑'d BWG in birds fed 0.075% BG + mannans vs. controls (linear response *P*=0.0179); ↑'d FI during d1-21 but ↓'d FI (linear response *P*=0.0481) accompanied by improved FCR (linear response *P*=0.0237) in birds fed BG + mannans over entire period (d1-49) ↑'d PEI in birds fed 0.075% BG vs. controls (*P*<0.05) ↓'d mortality in BG + mannans-fed birds vs. controls **Carcass Characteristics (d49)**

Table 6.2: Summary of Studies in Poultry using Yeast Ingredients Rich in Beta-Glucans		
Reference and Objective	**Study Design**	**Key Findings Related to Safety and Utility**
	Corn/SBM-based diets containing 0 (control), 0.05, 0.075, 0.1 or 0.125% BG + mannans **Duration** 49 days	↓'d % abdominal fat in birds fed 0.125% BG + mannans vs. controls; NSD in carcass yield among treatment groups) **Litter Characteristics and Flock Uniformity** NSD in litter pH, moisture content, temperature or % caking among treatment groups NSD in flock uniformity (skewness and coefficient of variation) among treatment groups
	Experiment 2 Studies in cold- or heat-stressed broiler chicks to evaluate effects on conventional economic parameters (not considered relevant to the safety assessment)	The authors concluded there was a positive effect of BG + mannans on the production performance of chickens fed diets containing BG + mannans under fluctuating conditions
Cox *et al*., 2010 **Objective** Performance and immune responses to dietary β-glucan in broiler chicks	**Animals** 1-day old Ross male broiler chicks (30 chicks/treatment) **Test Article** BG (purity not reported) **Treatment** Corn/SBM-based diets containing 0, 0.02 or 0.1% BG **Duration** 14 days	**Performance (d0-14)** NSD in BW or BWG among treatment groups **Bursa and Spleen Weights; Gene Expression (d7 and d14)** NSD in bursa or spleen weights among treatments; **Peripheral Blood Cell Profiling (d14)** NSD in heterophil:lymphocyte ratios among treatment groups Some differences in cytokine-chemokine balance observed in BG-fed birds vs. controls but no robust immune response (likely due to absence of challenge)
Kyoung *et al*., 2023 **Objective** Dietary yeast cell wall enhanced health of broiler chicks by modulating intestinal integrity, immune responses, and microbiota	**Animals** 1-day old Ross 308 broiler chicks (40 birds/replicate; 10 replicates/treatment) **Test Article** Yeast Cell Wall (YCW; 28.2% BGs) **Treatment** Corn/SBM-based diets containing 0 or 0.05% YCW **Duration** 5-weeks	**Performance** Improved FCR in broilers fed YCW vs. controls ($P<0.05$) **Intestinal Morphology** ↑'d villus height to crypt depth ratio ($P<0.05$) in duodenum, jejunum and ileum, and tendency to higher numbers of goblet cells vs. controls ($P<0.05$) **Serum Immune Parameters** ↑'d scrum TGF-β1, ileal gene expression of claudin family in broilers fed YCW vs. controls ($P<0.05$) ↓'d TNF-α IL-1β, and IL-6 in broilers fed YCW vs. controls ($P<0.05$ or 0.1)

Table 6.2: Summary of Studies in Poultry using Yeast Ingredients Rich in Beta-Glucans		
Reference and Objective	**Study Design**	**Key Findings Related to Safety and Utility**
		Microbiota ↑'d relative abundance of *Lactobacillus*, *Prevotella* and *Enterococcus* vs. controls ↓'d relative abundance of *Clostridium* vs. controls
Zhang *et al.*, 2005 **Objective** Effects of yeast (*S. cerevisiae*) cell components on growth performance, meat quality, and ileal mucosa development of broiler chicks	**Animals** 1-day old male Ross broiler chicks (10 chicks/replicate; 6 replicates/treatment) **Test Article** Yeast Cell Wall (YCW; purity not given) Whole Yeast (WY; *S. cerevisiae*) *S. cerevisiae* extract (YE) **Treatment** Corn/SBM-based diets containing 0, 0.5% WY, 0.3% YE or 0.3% YCW **Duration** 35 days (5 weeks)	**Performance** ↑'d BWG in birds fed 0.3% YCW from weeks 0 to 3 vs. controls ($P \leq 0.05$) but NSD in BWG in over weeks 0 to 5 **Shear Force of Meat** NSD in shear force of breast meats among treatment groups; numerical decrease in shear force of raw drumstick and boiled breast and drumstick from birds fed 0.3% WY, YE or YCW vs. controls **TBARS Value of Meat and Skin** ↓'d TBARS of breast meat at d10 of incubation in meat from birds fed 0.3% YW, YE or YCW vs. controls ($P \leq 0.05$) ↓'d TBARS of skin at d10 of incubation in meat from birds fed 0.3% YCW and YE vs. control ($P \leq 0.05$) **Ileal Mucosal Development (d21)** ↑'d villus height and villus/crypt depth ratio in birds fed 0.3% YW and YCW vs. controls ($P \leq 0.05$); NSD in crypt depth among treatment groups
Morales-Lopéz *et al.*, 2010 **Objective** Effects of different yeast cell wall supplements added to maize- or wheat-based diets for broiler chickens	**<u>Experiment 1 (Wheat-Based Diets)</u>** **Animals** 1-day old Ross 308 male broiler chicks (20 chicks/replicate; 6 replicates/treatment) **Test Article** Brewery-YCW (22.86% BG; 17.24% mannans) Yeast extract-YCW (26.03% BG; 21.6% mannans) [Test articles supplied by Lesaffre] **Treatment** Wheat-based diets containing 0 (-ve control; T1), 0.001% Avilamycin (+ve control; T2), 0.05% Yeast	**<u>Experiment 1</u>** **Performance (d0-42)** ↑'d ABW and DFI in T3 vs. T1 ($P<0.05$) but NSD in FCR among treatment groups **Ileal Parameters** NSD in crude fat or CP ileal digestibility coefficients among treatment groups ↓'d intestinal viscosity content in T2 and T3 vs. T1 NSD in ileal bacterial counts among treatment groups

Table 6.2: Summary of Studies in Poultry using Yeast Ingredients Rich in Beta-Glucans		
Reference and Objective	**Study Design**	**Key Findings Related to Safety and Utility**
	extract-YCW (0.0133% BG; T3) or 0.05% Brewery-YCW (0.0117% BG; T4) **Duration** 42 days	
	Experiment 2 (Maize-Based Diets) **Animals** 1-day old Ross 308 male broiler chicks (22 chicks/replicate; 6 replicates/treatment) **Test Article** Brewery-YCW (22.86% BG; 17.24% mannans) Yeast extract-YCW (26.03% BG; 21.6% mannans) **Treatment** Maize-based diets containing 0 (-ve control; T1), 0.001% Avilamycin (+ve control; T2), 0.05% Yeast extract-YCW (0.0133% BG; T3) or 0.05% Brewery-YCW (0.0117% BG; T4) **Duration** 39 days	**Experiment 2** **Performance (d0-39)** NSD in ABW, DFI and FCR among treatment groups (although FCR was improved over d0-14 in T3 and T4 vs. T1 ↓'d mortality in T4 vs. T3 ($P<0.05$) **Ileal Parameters** NSD in crude fat or CP ileal digestibility coefficients among treatment groups ↓'s intestinal viscosity content in T2 and T3 vs. T1 NSD in ileal bacterial counts among treatment groups
	Experiment 3 (Diet x Treatment; 2 x 2 Factorial Study) **Animals** 1-day old Ross 308 male broiler chicks (23 chicks/replicate; 5 replicates/treatment) **Test Article** Yeast extract-YCW (26.03% BG; 21.6% mannans) **Treatment** Maize or wheat-based diets containing 0 (control) or 0.05% Yeast extract-YCW (0.0133% BG) (2x2 factorial experiment; treatment x diet) **Duration** 43 days	**Experiment 3** **Performance (d0-43)** NSD in average ABW and DFI among treatment groups Improved FCR in birds fed yeast extract-YCW vs. control ($P<0.05$) ↑'d DFI and FCR in birds fed wheat diets vs. maize diets NSD in mortality among treatment groups **Intestinal Morphology** ↑'d villus height, mucus thickness, no. goblet cells in birds fed yeast extract-YCW vs. control ($P<0.0001$) NSD in intestinal viscosity content among treatment groups

Table 6.2: Summary of Studies in Poultry using Yeast Ingredients Rich in Beta-Glucans		
Reference and Objective	**Study Design**	**Key Findings Related to Safety and Utility**
Yalçin *et al*., 2014 **Objective** Effects of dietary yeast cell wall on performance, egg quality and humoral immune response in laying hens	**Animals** 39-wk old Hyline Brown laying hens (9 hens/replicate; 5 replicates/treatment) **Test Article** Yeast Cell Wall (YCW; BGs content not reported) **Treatment** Corn/SBM-based diet containing 0,1,2,3 or 4 g/kg complete feed YCW **Duration** 26-weeks	**Performance and Production** NSD on body weight, FI, FCR, egg production or egg weight among treatments **Egg Properties** ↓'d egg yolk cholesterol (P<0.05) of hens fed diets containing 1 or 2 g/kg complete feed of YCW vs. controls **Immune Parameters** ↓'d serum cholesterol and TGs levels with YCW supplementation of the diet (P<0.01) and ↑ in antibody titer to SRBC

Abbreviations: ↑'d, increased; ↓'d, decreased; ABW = average body weight; ADFI = average daily feed intake; ADG = average daily gain; AVI = avilamycin; BA = *Bacillus amyloliquiefaciens*; BG = beta-glucans; BW = body weight; BWG = body weight gain; COS = chitin oligosaccharides; CP = crude protein; d = day; DFI = daily feed intake; DM = dry matter; EE = ether extract; FCR = feed conversion ratio; FI = feed intake; GE = gross energy; GOT = glutamic oxaloacetic transaminase; GPT = glutamic pyruvic transaminase; HDL = high density lipoprotein; IBV = infectious bronchitis virus; IgA = immunoglobulin A; IgG = immunoglobulin G; IgM = immunoglobulin M; MHC = major histocompatibility complex; MP = mannoprotein complex; NDV = Newcastle disease virus; NSD = no significant difference; Or.Zn = organic zinc; RBC = red blood cells; SBM = soybean meal; TBARS = 2-thiobarbituric acid reactive substances; TCR = T-cell receptor; VLDL = very low density lipoprotein; WBC = white blood cells; WY = whole yeast; YCW = yeast cell wall; YE = yeast extract;
Note: All test article concentrations in the feed are presented on a percentage basis to allow comparison between studies.

6.1.4.3 Studies in Swine using Yeast Cell Wall-Derived Ingredients

A number of studies were identified in which weaned piglets were fed diets containing yeast cell wall-derived ingredients that are compositionally similar to Phileo's purified yeast cell wall. An overview of the key findings from each of the swine studies is provided in Table 6.3. A number of the studies were relatively short in duration (e.g., 14 days) but there are studies in which weaned piglets were fed the experimental diets for between 28 and 41 days (or 6 weeks). Of these studies, weaned piglets were generally fed diets containing yeast cell wall-based ingredients (beta-glucans content >50% or not reported) at levels of between 0.02 and 0.04% (Hahn *et al*., 2006; Li *et al*., 2006; Szuba-Trzandel *et al*., 2014; Wang *et al*., 2008; Wook Goh *et al*., 2023b), although in one study, animals were supplied with between 1 and 5% of a yeast cell wall-based ingredient containing 12% beta-glucans (Keimer *et al*., 2018a).

Treatment with yeast cell wall-based ingredients was observed to either have no impact, or to improve ($P \leq 0.05$) growth performance of weaned piglets over the entire study period in a number of the studies (Hahn *et al*., 2006; Sweeney *et al*., 2012; Szuba-Trznadel *et al*., 2014; Kerkaert *et al*., 2018; Wook Goh *et al*., 2023a and b). In these studies, weaned piglets were fed diets containing 0.01 to 3% purified yeast cell wall ingredient, and sows 0.01 to 0.02% purified yeast cell wall ingredient. Conversely, Li *et al*., (2006) reported a quadratic response to average daily gain in piglets fed diets containing increasing levels of purified yeast cell wall ingredient containing 86.1% beta-glucans, with an optimal inclusion level determined to be 0.005% (50 mg/kg complete feed). Likewise, piglets fed increasing levels of dietary purified yeast cell wall ingredient containing 91.5% beta-glucans were reported by Wang *et al*. (2008) to numerically follow a quadratic response in average daily gain and average daily feed intake but with no significant differences among treatment groups or between the pigs fed the highest levels of dietary purified yeast cell wall ingredient (0.02% or 200 mg/kg complete feed) and the controls. The authors of these 2 studies (Li *et al*., 2006 and Wang *et al*., 2008) considered that there may be differences in the optimum level of purified yeast cell wall in the diet depending on the purity, conformation, degree of branching and extraction methods. As previously mentioned, Phileo has extrapolated data on Safmannan® in pigs to derive the proposed use levels of purified yeast cell wall for swine and no adverse effects on performance are anticipated.

In 3 of the studies identified, nutrient digestibility parameters were evaluated with a significant ($P \leq 0.05$) increase in DM, gross energy, CP, ethyl ester, and calcium and phosphorus digestibilities reported at a level of 0.04% purified yeast cell wall-derived ingredient in the diet (Hahn *et al*., 2006), and an increase in ileal CP and ethyl ester digestibility observed in a study using a yeast cell wall-derived ingredient with lower beta-glucans content (12%) (Keimer *et al*., 2018a). Conversely, there was no significant difference ($P > 0.05$) in DM, organic matter, ash or gross energy observed in a study by Sweeney *et al*. (2012) in which piglets were fed diets containing 0.025% of a purified yeast cell wall ingredient. Parameters relating to immune function were analyzed in the blood of piglets in 5 of the studies with no adverse findings reported (Hahn *et al*., 2006; Wang *et al*., 2008; Ganner *et al*., 2010; Sweeney *et al*., 2012; Szuba-Trznadel *et al*., 2014).

A study by Wook Goh *et al*. (2023a) evaluated the effect of yeast cell wall-based ingredient supplementation at levels of 0.1 and 0.2% in the diet on sows and their litters. No adverse effects were

observed and supplementation of the diet with 0.1% yeast cell wall-based ingredient was associated with improved growth performance of piglets by leading to an increased feed intake by sows.

Additionally, a study was conducted by He *et al*. (2022) in which finisher pigs were fed 0.2% yeast cell wall-based ingredient and the effect on meat quality was assessed. The pH and water-holding capacity of fresh meat was improved in finisher pigs fed the yeast cell wall-based ingredient in the diet.

Taken together, under the conditions of these weaned piglet studies, yeast cell wall-based ingredients were well-tolerated at levels ranging 0.4 g/kg complete feed (0.04%; beta-glucans content not reported) which is in the same range albeit slightly lower than the intended use of Phileo's purified yeast cell wall of up to 0.5 g/kg complete feed (0.05%). In some of the studies, lower levels of yeast cell wall-derived ingredients in the diet were considered optimal. Additionally, a study in sows and another in finisher pigs indicates that 200 mg/kg complete feed (0.2%) of beta-glucans in the diet is not associated with any adverse effects on reproductive performance or meat quality, respectively. It is anticipated that the optimum level of inclusion may differ between specific products and therefore, Phileo will work alongside the U.S. swine industry to identify the appropriate conditions of use, where 0.5 g/kg complete feed will be the maximum fed. Overall, these data provide only corroborative evidence of the tolerability of pigs to Phileo's purified yeast cell wall but combined with available data in other animal species, including toxicology studies in rats, it is reasonable to conclude that levels of up to 0.05% (0.5 g/kg complete feed) will not be associated with adverse effects. Moreover, these conclusions can be extrapolated to all categories of swine on the basis of their physiological similarities.

Table 6.3: Summary of Studies in Swine using Yeast Cell Wall-Derived Ingredients		
Reference and Objective	**Study Design**	**Key Findings Related to Safety and Utility**
Purified Yeast Cell Wall Ingredients (min. Beta-Glucans 50%, or Beta-Glucans indicated as Major Component but Content not Reported)		
Ganner *et al.*, 2010 **Objective** *Ex vivo* effect of yeast beta-glucan on lymphocyte viability and plasma IL-18 in weaning piglets	**Animals** 4-week-old, mixed-sex weaning piglets (no further details given) (2 piglet/replicate; 3 replicates/treatment) **Test Article** BG (purity not reported) **Treatment** Corn/SBM-based diets containing 0 (control), 0.01, 0.025 or 0.05% BG **Duration** 21 days	**Immunological Parameters** ↑'d lymphocyte viability after 21 days in pigs fed diets supplemented with 0.025 and 0.05% BG vs. controls
Hahn *et al.*, 2006 **Objective** Effects of supplementation of β-glucans on growth performance, nutrient digestibility, and immunity in weanling pigs	**Experiment 1** **Animals** Duroc × Landrace × Yorkshire castrated male weanling pigs (14 pigs/replicate; 3 replicates/treatment) **Test Article** BG (Glucagen; purity not reported) **Treatment** Corn/SBM-based diets containing 0 (control), 0.01, 0.02, 0.03 or 0.04% BG **Duration** 35 days (5 weeks; Phase I diets weeks 1-2; Phase II diets weeks 3-5)	**Experiment 1** **Performance (d0-35)** ↑'d ADG in BG-fed pigs vs. control (linear response P=0.068); NSD in ADFI or gain/feed ratio among treatment groups **Nutrient Digestibility (d32-35)** ↑'d digestibility of DM, GE, CP, EE, Ca and P in BG-fed pigs vs. control (linear response, P=0.001 to 0.004); quadratic response in digestibility of Ca with BG supplementation (P=0.055; all BG-fed pigs displayed % Ca digestibility numerically above those of the controls)
	Experiment 2 **Animals** Duroc × Landrace × Yorkshire castrated male weanling pigs (12 pigs/replicate; 3 replicates/treatment) **Test Article** BG (Glucagen; purity not reported) Antibiotics (Apramycin + Carbadox in Phase I, Chlorotetracycline + Carbadox in Phase II) **Treatment**	**Experiment 2** **Performance (d0-35)** NSD in ADG, ADFI and gain/feed ratio between BG-fed pigs (T2) vs. controls (P>0.05) **Nutrient Digestibility (d32-35)** ↑'d digestibility of DM and GE in BG-fed pigs (T2) vs. controls (P<0.05); numerical increase in digestibility of CP, EE, Ca and P in BG-fed pigs vs. controls **Antibody Titers**

Table 6.3: Summary of Studies in Swine using Yeast Cell Wall-Derived Ingredients		
Reference and Objective	**Study Design**	**Key Findings Related to Safety and Utility**
	Corn/SBM-based diets containing 0 (control; T1), 0.02% BG (T2), antibiotics (T3) or 0.02% BG + antibiotics (T4) **Duration** 35 days (immune response monitored until day 56)	↑'d antibody titers to *Pasteurella multocida* at 15 days post-vaccination in BG-fed and antibiotic-fed pigs (T2-T4) vs. controls **Immunological Parameters** Trend to ↑'d MHC class-II lymphocytes content in BG-fed pigs vs. other treatment groups ($P<0.10$) at week 4 ↑'d CD4 cell content ($P<0.05$) and a trend for an increase in CD8 cell content ($P<0.10$) in BG-fed pigs vs. controls at week 8
He *et al*., 2022 **Objective** Effects of dietary beta-glucan supplementation on meat quality, antioxidant capacity and gut microbiota of finishing pigs	**Animals** Duroc x Landrace x Yorkshire finishing pigs (70.47±0.04 kg) (18 pigs/treatment) **Test Article** BGs (>90%; Biorigin) **Treatment** Corn/SBM-based diets supplemented with 0, 50, 100, 200 or 400 mg/kg BGs **Duration** 40-days	**Meat Quality** ↑'d pH (45 mins; linear and quadratic $P<0.01$), a* (45 mins) (linear $P<0.05$), and reduced cooking loss (linear $P<0.05$) and drip loss (quadratic $P<0.05$) in meat of pigs fed diets supplemented with BGs vs. controls [200 mg/kg complete feed of BGs was considered optimal] ↑'d catalase, SOD, and total antioxidant capacity in skeletal muscle of pigs fed 200 mg BGs in the diet vs. controls
Kerkaert *et al*., 2018 [Conference Abstract] **Objective** Immunological response of pigs by lymphocyte proliferation by the supplementation of β-Glucans	**Animals** 21-day-old, weaned piglets (no further details given) (4 pigs/replicate; 6 replicates/treatment) **Test Article** BG (purity not reported) Antibiotics (identity not reported) **Treatment** Standard nursery Phase II based diet including 0 (-ve control) antibiotics (+ve control), 1 or 3% BG **Duration** 14 days	**Performance** NSD in ADG, ADFI and feed/gain ratio in BG-fed pigs vs. controls **Immunological Parameters** NSD in lymphocyte proliferation in response to Concanavalin A (ConA) + Phytohaemagglutinin (PHA-P) in BG-fed pigs vs. controls

Table 6.3: Summary of Studies in Swine using Yeast Cell Wall-Derived Ingredients		
Reference and Objective	**Study Design**	**Key Findings Related to Safety and Utility**
Li *et al*., 2006 **Objective** Effects of beta-glucan extracted from *Saccharomyces cerevisiae* on growth performance, and immunological and somatotropic responses of pigs challenged with *Escherichia coli* lipopolysaccharide	**Experiment 1** **Animals** 28 day old, Landrace × Large White, crossbred weaned pigs [4 pigs (2 males and 2 females) /replicate; 5 replicates/treatment] **Test Article** BG (86.1%) **Treatment** Corn/SBM-based diets containing 0 (control), 0.0025, 0.005, 0.01, or 0.02% BG **Duration** 35 days **Experiments 2 and 3** Studies conducted under challenge condition and therefore, not considered relevant to the safety determination	**Performance** Quadratic response in ADG with BG dietary level (P=0.08) with greatest numerical ADG observed in pigs fed 0.005% BG during d14-28 (P=0.041) and d0-28 (P=0.031; 438, 488, 503, 425 or 412 g/day with increasing BG dietary level) No linear or quadratic responses in ADFI and gain/feed with dietary BG levels Note: Optimal concentration of BG to improve growth was recognized by authors to be lower than previously reported – differences postulated to arise from purity, molecular weight, conformation and extraction methods **Experiments 2 and 3** Some effects were noted on immune parameters which were considered potentially beneficial and not detrimental
Sweeney *et al*., 2012 **Objective** Effect of purified b-glucans derived from *Laminaria digitata*, *Laminaria hyperborea* and *Saccharomyces cerevisiae* on piglet performance, selected bacterial populations, volatile fatty acids and pro-inflammatory cytokines in the gastrointestinal tract of pigs	Animals 49-day-old, weaned (at 26 days) (8 pigs/ treatment) Test Article BG (65%) from *S. cerevisiae* Laminarin (99%) extracted from *L. digitata* Laminarin (99%) extracted from *L. hyperborea* **Treatment** Wheat/SBM-based diets containing 0 (control T1), 0.025% Laminarin from *L. digitata* (T2), 0.025% Laminarin from *L. hyperborean* (T3) or 0.025% BG (T4) **Duration** 14 days	**Performance** NSD in ADG, ADFI and FCR in 0.025% BG-fed pigs vs. controls (P<0.05) **Nutrient Digestibility** NSD in digestibility of DM, OM, ash, N or gross energy in 0.025% BG-fed pigs vs. controls **Immunological Parameters** ↓'d expression of pro- and anti-inflammatory cytokines (IL-1a, IL-10, TNF-α and IL-17A) was down-regulated in the colon in 0.025% BG-fed pigs vs. controls (P<0.05)

Table 6.3: Summary of Studies in Swine using Yeast Cell Wall-Derived Ingredients		
Reference and Objective	**Study Design**	**Key Findings Related to Safety and Utility**
Szuba-Trznadel *et al*., 2014 **Objective** The influence of (1,3)-(1,6)-β-D-glucan on the production results of sows and their offspring	**Animals** 49-day-old, Polish Large White × Polish Landrace and their progeny (total 411) (10 sows/ treatment) **Test Article** BG (Betamune®; min. 80%) **Treatment** Wheat/barley based diets containing 0 (Group I), 0.01 (Group II), 0.02 (Group III) or 0.03% BG (Group IV) **Duration** 45 days	**Performance** NSD in BW and BW loss during lactation on supplementation of sow diets with 0.01, 0.02 or 0.03% BG vs. control ↑'d final BW and improved FCR in piglets from sows fed diets supplemented with 0.02 and 0.03% BG vs. control **Immunological Response** NSD in immune response parameters in sows or piglets after 45 days vs. control ↑'d γ-globulin in milk whey from sows fed diets containing 0.01 and 0.02% BG (P<0.05) vs. control
Wang *et al*., 2008 **Objective** Effect of dietary β-1,3/1,6-glucan supplementation on growth performance, immune response and plasma prostaglandin E2, growth hormone and ghrelin in weanling piglets	**Animals** 28-day old, Landrace × Large White, crossbred weanling piglets (6 pigs/replicate; 6 replicates/treatment) **Test Article** BG (91.5%) **Treatment** Corn/SBM-based diets containing 0 (control), 0.0025, 0.005, 0.01, or 0.02% BG **Duration** 28 days	**Performance (d1-14; d14-28; d0-28)** NSD in ADG, ADFI, gain/feed ratio, diarrhea ratio and mortality ratio observed among treatment groups over d0-14 Quadratic response for gain/feed ratio (P=0.026) and quadratic trend for ADG (P=0.089) with dietary BG level over d14-28 No linear or quadratic responses to ADG or ADFI over d0-28 (P=0.627, 0.790, 0.267 and 0.535) although ADG and ADFI were numerically higher in pigs fed 0.005% BG and (401, 414, 458, 423, 395 g/day ADG and 660, 611, 652, 629 and 660 g/day ADFI with increasing dietary BG-level); gain/feed ratio displayed quadratic response with BG dietary level over d0-28 **Immunological Parameters** NSD in lymphocyte proliferation and serum IgG levels among treatment groups

Table 6.3: Summary of Studies in Swine using Yeast Cell Wall-Derived Ingredients		
Reference and Objective	**Study Design**	**Key Findings Related to Safety and Utility**
		Note: the authors noted that the optimal level of BG supplementation for improvement in ADG was lower than reported in other studies; it was postulated that the purity, molecular weight, confirmation and degree of branching may contribute to the differences A numerical increase in diarrhea rate with increasing BG dietary level (1.82. 1.97, 1.83, 2.63 and 3.07%) was also reported which the authors attributed to BG potentially being repartitioned towards the immune system and not growth
Wook Goh *et al.*, 2023a **Objective** Effects of beta-glucan with vitamin E supplementation on physiological response, litter performance, blood profiles, immune response, and milk composition of lactating sows	**Animals** Multiparous F1 sows (Yorkshire x Landrace) (BW av. 233 kg and av. parity of 4.00) and litters (1 sow/replicate; 10 replicates/treatment) **Test Article** BG (not reported) **Treatment** Corn/SBM-based diets containing 0 (control), 0.1 or 0.2% BGs and 110 IU vitamin E/kg complete feed **Duration** Post-partum and 21-days of lactation	**Performance** ↑'d ADFI in sows fed diets containing BGs vs. controls (P<0.01) with 0.1% BGs inclusion appearing to be optimal **Reproductive Performance** Trend to increased piglet weights at weaning, and in litter weight and litter weight gain at 21st day of lactation from sows fed diets containing 0.1% BGs vs. controls **Immune Response** ↓'d tumor necrosis factor-α in lactating sows fed diets containing 0.1% BGs and their piglets
Wook Goh *et al.*, 2023b **Objective** Effects of beta-glucan with vitamin E supplementation on the growth performance, blood profiles, immune response, fecal microbiota, fecal score and nutrient digestibility in weaning pigs	**Animals** Weaned piglets (BW av. 7.6 kg) (10 pigs/pen; 4 pens/treatment) **Test Article** BG (not reported) **Treatment** Corn/SBM-based diets containing 0 (control), 0.1 or 0.2% BGs and 0.02% vitamin E/kg complete feed **Duration** 6 weeks	**Performance** ↑'d BW, ADG and ADFI in piglets fed diets containing BGs vs. controls ↓'d fecal score in piglets fed diets containing BGs vs. controls **Nutrient Digestibility** NSD among treatments **Immune Response** NSD among treatments

<table>
<tr><th colspan="3">Table 6.3: Summary of Studies in Swine using Yeast Cell Wall-Derived Ingredients</th></tr>
<tr><th>Reference and Objective</th><th>Study Design</th><th>Key Findings Related to Safety and Utility</th></tr>
<tr><td colspan="3">Yeast Cell Wall Ingredients (Beta-Glucans Content 10 to 49%)</td></tr>
<tr><td rowspan="2">Keimer et al., 2018a

Objective
Influence of differently processed yeast (Kluyveromyces fragilis) on feed intake and gut physiology in weaned pigs</td><td><u>Experiment 1</u>
Animals
25-day-old, Danbred × Piètrain, post-weaning, male and female piglets (25 pigs/replicate; 4/5 replicates/treatment)
<table>
<tr><td></td><td>0</td><td>HY1</td><td>HY3</td><td>HY5</td><td>NHY3</td></tr>
<tr><td>Replicates</td><td>4</td><td>4</td><td>5</td><td>4</td><td>5</td></tr>
</table>
Test Article
Hydrolyzed yeast (HY; 12% BG and 7.9% mannans)
Non-hydrolyzed yeast (NHY; 11.6% BG and 9.5% mannans) from K. fragilis
Treatment
Wheat/SBM-based diets containing 0 (control), 1%, 3%, 5% HY or 3% NHY
Duration
41 days</td><td><u>Experiment 1</u>
Performance
NSD in ADG, ADFI and gain/feed among treatment groups
Nutrient Digestibility
↑'d apparent Ileal digestibility of CP (P=0.058) and EE (P=0.080) in 1% HY-fed pigs vs. controls</td></tr>
<tr><td><u>Experiment 2</u>
Animals
25-day-old, Danbred × Piètrain, post-weaning, male and female piglets (2 pigs/replicate; 8 replicates/treatment)
Test Article
Hydrolyzed yeast (HY) (12% BG and 7.9% mannans)
Non-hydrolyzed yeast (NHY) (11.6% BG and 9.5% mannans) from K. fragilis
Treatment
Wheat/SBM-based diets containing 0 (control), 1 % NHY or 1 % HY
Duration
14 days</td><td><u>Experiment 2</u>
Performance
↑'d ADFI in 1% HY-fed pigs vs. controls (P=0.076)
NSD in ADG, ADFI and gain/feed among treatment groups</td></tr>
</table>

Abbreviations: ↑'d = increased; ↓'d = decreased; ADFI = average daily feed intake; ADG = average daily gain; BG = beta-glucans; BW = body weight; ConA = concanavalin A; CP = crude protein; d= day; DM = dry matter; EE = ether extract; FCR = feed conversion ratio; GE = gross energy; HY = hydrolyzed yeast; IgG =

immunoglobulin G; IL-1a = interleukin 1a; IL-10 = interleukin 10; IL-17A = interleukin 17 a; NHY = non-hydrolyzed yeast; MHC = major histocompatibility complex; NSD = no significant difference; OM = organic matter; PHA-P = phytohaemagglutinin; SBM = soybean meal; TNF-α = tumor necrosis factor alpha;
Note: All test article concentrations in the feed are presented on a percentage basis to allow comparison between studies.

6.1.4.4 Studies in Aquaculture

A number of studies were identified in aquaculture in which fin fish (salmonids and non-salmonids) and shrimp were fed diets containing yeast cell wall-derived ingredients that are compositionally similar to Phileo's purified yeast cell wall. An overview of the key findings from each of the aquaculture studies is provided in Tables 6.4 (salmonids), 6.5 (non-salmonids) and 6.6 (shellfish).

Studies in Salmonids

Four studies were identified in which salmonid fish were fed diets containing yeast cell wall-derived ingredients which are compositionally similar to Phileo's purified yeast cell wall. The studies were conducted over a period of time that allows changes in growth performance and tolerability to be evaluated (60 to 90 days). In three of the studies, fish were fed diets containing a purified yeast cell wall ingredient containing 55% beta-glucans at levels of up 0.1 to 0.2% (Ghaedi *et al*., 2015, 2016; Refstie *et al*., 2010), though Nazari *et al*. (2016) used a product containing 24% beta-glucans in combination with 15% MOS at treatment levels up to 0.4%. Treatment with yeast cell wall-based ingredients was observed to either have no impact, or to improve ($P \leq 0.05$) growth performance.

Blood biochemistry and hematological analyses were conducted in Rainbow trout broodstock by Ghaedi *et al*. (2015) and indicated that yeast cell wall-derived ingredients may impact white blood cell, total protein and albumin levels but these slight changes were considered by the study authors to be consistent with the ingredient exerting a positive effect on immune response in the fish. In the same study, there was no effect of the yeast cell wall-derived ingredient on blood parameters in fry produced from the brood fish.

Parameters related to immune function, fry performance, nutrient digestibility and lice infestation (as a measure of gastrointestinal health) were included in different studies (Ghaedi *et al*., 2015, Refstie *et al*., 2010). There were no reports of adverse findings in any of these parameters, with yeast cell wall-derived ingredients generally having no impact on nutrient digestibility. The yeast cell wall-derived ingredient at a dietary level of 0.1% appeared to decrease lice infestation in Atlantic salmon.

Overall, under the experimental conditions, purified yeast cell wall ingredients containing 55% beta-glucans were well-tolerated by Rainbow trout and Atlantic salmon, at levels ranging from 1 to 2 g/kg complete feed (0.1 to 0.2%). These use levels are comparable, and slightly higher than, those proposed by Phileo for purified yeast cell wall of up to 1.2 g/kg complete feed (0.12%) for aquaculture. It is recognized, that a relatively limited number of safety-related parameters were included in the individual studies but blood parameters, nutrient digestibility and growth performance were evaluated. Thus, the results of the studies in salmonids provide supporting evidence for the safety of Phileo's yeast cell wall ingredient under the intended conditions of use.

<table>
<tr><th colspan="3">Table 6.4: Summary of Studies in Salmonids using Yeast Ingredients Rich in Beta-Glucans</th></tr>
<tr><th>Reference and Objective</th><th>Study Design</th><th>Key Findings Related to Safety and Utility</th></tr>
<tr><td colspan="3">Purified Yeast Cell Wall Ingredients (min. Beta-Glucans 50%, or Beta-Glucans indicated as Major Component but Content not Reported)</td></tr>
<tr>
<td>Ghaedi et al., 2015

Objective
Effects of dietary beta-glucan on maternal immunity and fry quality of rainbow trout (Oncorhynchus mykiss)</td>
<td><u>Trial 1 (Rainbow Trout Broodstock)</u>
Animals
Female rainbow trout with an average initial weight of 4 kg (5 fish/replicate; 3 replicates/treatment)
Test Article
BG (MacroGard®; min. 55%)
Treatment
0 (control), 0.1% or 0.2% BG
Duration
3 months prior to spawning

<u>Trial 2 (Fry)</u>
Animals
Rainbow trout fry from brood fish (mean initial BW 184 mg; 300 fish/replicate; 3 replicates/treatment)
Test Article
BG (MacroGard®; min. 55%)
Treatment
<table>
<tr><th></th><th>BG in broodstock diet</th><th>BG in fry diet</th></tr>
<tr><td>L1</td><td>0%</td><td>0%</td></tr>
<tr><td>L2</td><td>0%</td><td>0.10%</td></tr>
<tr><td>L3</td><td>0%</td><td>0.20%</td></tr>
<tr><td>L4</td><td>0.10%</td><td>0%</td></tr>
<tr><td>L5</td><td>0.10%</td><td>0.10%</td></tr>
<tr><td>L6</td><td>0.20%</td><td>0%</td></tr>
<tr><td>L7</td><td>0.20%</td><td>0.20%</td></tr>
</table>
Duration
2 months
Challenge Trial
A short trial was also conducted under challenge conditions but the results are not considered relevant</td>
<td><u>Trial 1</u>
Fry Survival
NSD in survival rates (from fertilization to swim-up stages) among treatments
Hematological and Biochemical Analyses
↑'d WBC counts in females fed 0.2% BG diet vs. 0.1% BG diet or control diet (P<0.05)
↑'d total protein, globulin and albumin contents in BG-fed fish vs. controls (P<0.05)
NSD among biochemical parameters of oocytes obtained from different treatments (P>0.05); the changes were attributed by the authors to the positive impact of BG on immune function of brood fish
Immunological Parameters
↑ in ACH_{50}, lysozyme activity, total Ig and IgM values with BG dietary levels reaching significance at 0.2% BG in the diet (ACH50 and lysozyme) or 0.1% BG (total Ig and IgM) (P<0.05)
↑'d ACH_{50} in oocytes of 0.2% BG-fed fish vs. controls (P<0.05) but NSD in other immune parameters

<u>Trial 2</u>
Fry Performance
NSD in mortality among treatments
↑'d WG and SGR in BG-fed fish of L2, L3 L5 and L7 groups vs. controls (P<0.05) but NSD in FCR among treatments
Hematological and Biochemical Analyses
NSD among treatments
Immunological Parameters
↑'d ACH_{50}, lysozyme, total Ig and IgM values in fry fed 0.2% BG (L3) vs. controls and comparable to those in 0.2% BG-fed fry originating from 0.2% BG-fed brood fish</td>
</tr>
</table>

<table>
<tr><th colspan="3">Table 6.4: Summary of Studies in Salmonids using Yeast Ingredients Rich in Beta-Glucans</th></tr>
<tr><th>Reference and Objective</th><th>Study Design</th><th>Key Findings Related to Safety and Utility</th></tr>
<tr><td>Ghaedi et al., 2016

Objective
Proteomic analysis of muscle tissue from rainbow trout (Oncorhynchus mykiss) fed dietary beta-glucan</td><td>Animals
Rainbow trout larvae (No. of fish/replicate not reported; 3 replicates/treatment)
Test Article
BG (MacroGard®; min. 55%)
Treatment
0 (control; low BG-content basal diet), 0.1% or 0.2% BG
Duration
2 months</td><td>Performance
↑'d BWG and FE in fish fed 0.2% BG vs. controls
Structural Protein Expression
Some significant changes in expression of structural proteins consistent increased growth observed on dietary supplementation with BG</td></tr>
<tr><td>Refstie et al., 2010

Objective
Effects of dietary yeast cell wall β-glucans and MOS on performance, gut health, and salmon lice resistance in Atlantic salmon (Salmo salar) fed sunflower and soybean meal</td><td>Animals
Atlantic salmon (Salmo salar) (mean initial BW 0.68 g; 150 fish/replicate; 3 replicates/treatment)
Test Article
BG (MacroGard®; min. 55%)
Mannan (MOS; PatoGard™; purity not given)
Treatment
Low temperature FM diets with either SBM (S) or SBM + SFM (SS) substituted into the feed

<table><tr><th>Ingredient (% in diet)</th><th>FM Control</th><th>FM+S Basic</th><th>FM+SS Basic</th></tr><tr><td>FM</td><td>52.5</td><td>24.2</td><td>30.0</td></tr><tr><td>SBM</td><td>-</td><td>32.0</td><td>13.5</td></tr><tr><td>SFM</td><td>-</td><td>-</td><td>13.5</td></tr><tr><td>Wheat gluten</td><td>-</td><td>1.0</td><td>-</td></tr><tr><td>Wheat</td><td>18.8</td><td>10.05</td><td>11.65</td></tr><tr><td>Fish oil</td><td>28.6</td><td>30.5</td><td>29.1</td></tr><tr><td>Lysine</td><td>-</td><td>0.1</td><td>0.1</td></tr><tr><td>Methionine</td><td>-</td><td>0.15</td><td>0.15</td></tr><tr><td>MCP</td><td>0.1</td><td>2.0</td><td>2.0</td></tr></table></td><td>Performance
NSD among treatments
Diarrhea, Nutrient Digestibility and Retention
SBM-induced enteritis in fish fed diets containing S or SS was not alleviated by dietary supplementation with BG
NSD in nutrient digestibility or N retention on addition of BG to diet
Lice Infestation
Moderate infestation was observed which was not impacted by dietary BG supplementation
In fish fed FM + SS diet, 0.1% BG supplementation significantly reduced lice infestation (28%)</td></tr>
</table>

Table 6.4: Summary of Studies in Salmonids using Yeast Ingredients Rich in Beta-Glucans		
Reference and Objective	**Study Design**	**Key Findings Related to Safety and Utility**
	Following extrusion diets FM+S and FM+SS were supplemented with BG or MOS FM (control), FM+S, FM+S & 0.05% BG, FM+S & 0.1% BG, FM+S & 0.1% MOS, FM+S & 0.2% MOS, FM+SS, FM+SS & 0.1% BG or FM+SS & 0.2% MOS **Duration** 70 days	
Yeast Cell Wall Ingredients (Beta-Glucans, 20 to 49%; Mannans 10 to 30%)		
Nazari *et al.*, 2016 **Objective** Effect of different Alphamune levels in artificial diet on growth parameters, digestibility and enzyme activity of rainbow trout, *Oncorhynchus mykiss*	**Animals** Rainbow trout (initial BW 40.01±0.11g; 25 fish/replicate; 3 replicates/treatment) **Test Article** Alphamune (24% BG, 15% MOS) **Treatment** 0 (control), 0.05, 0.1, 0.2 or 0.04% BG **Duration** 8 weeks	**Growth Performance** ↑'d BWG in BG-fed fish vs. controls (P<0.01) **Nutrient Digestibility and Enzyme Activities (Amylase, Lipase)** NSD among treatment groups (P>0.05)

Abbreviations: ↑'d = increased; ↓'d = decreased; ACH_{50} = alternative complement activity; av. = average; BG = beta-glucans; BW = body weight; BWG = body weight gain; DGC = daily growth coefficient; FCR = feed conversion ratio; FE = feed efficiency; FM = fish meal; Ig = immunoglobulin; IgM = immunoglobulin M; MCP = mono calcium phosphate; MOS = mannan-oligosaccharides; N = nitrogen; NSD = no significant difference; SBM = soybean meal; SFM = sunflower meal; SGR = specific growth rate; WBC = white blood cells; WG = weight gain;
Note: All test article concentrations in the feed are presented on a percentage basis to allow comparison between studies.

Studies in Non-Salmonids

Twelve studies were identified in which non-salmonids were fed purified yeast cell wall-derived ingredients over a period of 30 days or longer. The studies were primarily designed to evaluate the effect of yeast cell wall-derived ingredients on immune response fish. However, a number of the studies were conducted for 56 days or longer allowing growth performance and tolerability to be evaluated. In the majority of the studies, fish were fed diets containing a purified yeast cell wall ingredient containing 55% beta-glucans at levels of up 0.1 to 0.4% (Bagni *et al*., 2005; Do Huu *et al*., 2016; Munir *et al*., 2016; Chen *et al*., 2019), except in one study in which 2% was incorporated into the feed (Külwein *et al*., 2014). Treatment with yeast cell wall-derived ingredients was observed to either have no impact, or to improve (P<0.05), growth performance. In a 30-day feeding trial in Pacu, dietary inclusion of 0.2% of a yeast cell wall-based ingredient containing 30% beta-glucans content was reported to result in optimum performance (Hisano *et al*., 2018).

Although the primary endpoints measured in the study related to immune response, these included selected hematological parameters relevant to safety. Yeast cell wall-derived ingredients were observed to either have no impact on hematological parameters, or to display changes consistent with improved immune status (Külwein *et al*., 2014; Do Huu *et al*., 2016; Domenico *et al*., 2017; Sanchez-Martinez *et al*., 2017; Hisano *et al*., 2018). All of the studies included specific endpoints relevant to immune status of fish and no adverse findings were reported. In 3 of the studies, intestinal morphology was evaluated and yeast cell wall-derived ingredients reported to positively impact villus height and immune parameters (Külwein *et al*., 2014; Selim & Reda, 2015; Hisano *et al*., 2018).

In one study, nutrient digestibility was evaluated with a significant increase in relative protein digestibility (P<0.05) reported in fish fed diets containing 0.2% purified yeast cell wall (55% beta-glucans) relative to the control group. Additionally, Selim & Reda (2015) observed that the protein and fat content of the whole body of Nile Tilapia was increased following dietary administration of 0.3% of a yeast cell wall ingredient containing 30% beta-glucans.

Taken together, under the experimental conditions, purified yeast cell wall-derived ingredients containing 55% beta-glucans were well-tolerated by a range of non-salmonid fish at levels ranging from 1 to 4 g/kg complete feed (0.1 to 0.4%). These use levels are comparable, and slightly higher than, those proposed by Phileo for purified yeast cell wall of between 0.4 and 1.2 g/kg complete feed (0.04 to 0.12%) for aquaculture. Thus, the results of the studies in non-salmonids provide supporting evidence for the safety of Phileo's yeast cell wall ingredient under the intended conditions of use.

Table 6.5: Summary of Studies in Non-Salmonids Fish using Yeast Ingredients Rich in Beta-Glucans		
Reference and Objective	**Study Design**	**Key Findings Related to Safety and Utility**
Purified Yeast Cell Wall Ingredients (min. Beta-Glucans 50%, or Beta-Glucans indicated as Major Component but Content not Reported)		
Aramli *et al*., 2015 **Objective** Effects of dietary β-glucan on the growth and innate immune response of juvenile Persian sturgeon, *Acipenser persicus*	**Animals** Juveniles of Persian sturgeon, *A. persicus,* with an initial BW 20.1 ± 0.8 g (15 fish/replicate; 3 replicates/treatment) **Test Article** BG (MacroGard®; min. 55%) **Treatment** FM and SBM based diets containing 0 (control), 0.1, 0.2 or 0.3% BG **Duration** 42 days	**Growth Performance** ↑'d FW SGR and FCR (P<0.05) in fish fed diets supplemented with 0.1, 0.2 or 0.3% BG vs. control **Immunological Parameters** ↑'d WBC count and lymphocytes (%) in fish fed diets containing 0.2% and 0.3% BG (P<0.05) vs. control ↑'d lysozyme activity and alternative complement activity in fish fed diets supplemented with 0.2% and 0.3% BG (P<0.05) vs. control
Bagni *et al*., 2005 **Objective** Short- and long-term effects of a dietary yeast beta-glucans and alginic acid preparation on immune response in sea bass (*Dicentrarchus labrax*)	**Short-term Sampling** **Animals** Sea bass (*D. labrax*) with an initial BW of 80 ± 12 g (150 fish/replicate; 2 replicates/treatment)**Test Article** BG (MacroGard®; min. 55%)**Treatment** Diets based on normal fish feed formulation (Trouwit, Hendrix) and containing 0 (control), 0.5% alginic acid or 0.1% BG **Duration** 60 days. Fish were fed the experimental diets for 15 days and then the commercial diet for 45 days	**Short-term Sampling** **Growth Performance** NSD in growth parameters (FW, SGR, FCR) between fish fed diets supplemented with 0.1% BG vs. control **Immunological Parameters** NSD at the end of study in serum complement activity, serum lysozyme activity, and HSP expression in the gill and liver in fish fed diets supplemented with 0.1% BG vs. controls
	Long-term Sampling **Animals** Sea bass (*D. labrax*) adults with an initial BW of 80 ± 12 g) 150 fish/replicate; 2 replicates/treatment **Test Article** BG (MacroGard®; min. 55%) **Treatment** Diets based on normal fish feed formulation (Trouwit, Hendrix) and containing 0 (control), 0.5% alginic acid or 0.1% BG **Duration**	**Long-term Sampling** **Growth Performance** NSD in growth parameters between fish fed diets containing 0.1% BG vs. controls **Immunological Parameters** NSD at the end of study in innate (serum complement activity, serum lysozyme activity and total proteins) and specific immune parameters (leukocyte subpopulation) in fish fed diets supplemented with 0.1% BG vs. control

Table 6.5: Summary of Studies in Non-Salmonids Fish using Yeast Ingredients Rich in Beta-Glucans		
Reference and Objective	**Study Design**	**Key Findings Related to Safety and Utility**
	ca. 245 days. Fish were fed the experimental diets for 15 days and then the commercial diet for 45 days; 4 cycles were completed	
Do Huu *et al.*, 2016 **Objective** Dietary b-glucan improved growth performance, *Vibrio* counts, hematological parameters and stress resistance of pompano fish, *Trachinotus ovatus* Linnaeus, 1758	**Animals** Pompano fish, (*T. ovatus)* initial BW 6.45 g ± 0.06 (24 fish/replicate; 6 replicates/treatment) **Test Article** BG (MacroGard®; min. 55%) **Treatment** FM and wheat based diets containing 0 (control) 0.05, 0.10, 0.2 or 0.4% BG **Duration** 56 days	**Growth Performance** ↑'d DGC at 56 days, in fish fed diets containing 0.1% BG ($P \leq 0.002$) vs. control NSD in DGC at 56 days, in fish fed diets containing 0.05, 0.2 and 0.4% BG vs. control **Hematological Parameters** ↑'d WBC count at 56 days, in fish fed diets containing 0.05, 0.1 or 0.2% BG ($P \leq 0.01$) vs. control **Immunological Parameters** ↑'d leukocytes, lymphocyte and monocyte contents at 56 days, in fish fed diets containing 0.10, 0.2 or 0.4% BG ($P \leq 0.05$) vs. control NSD in neutrophil, eosinophil and basophil counts in fish fed diets containing BG vs. control
Domenico *et al.*, 2017 **Objective** Immuno-modulatory effects of dietary β-glucan in silver catfish (*Rhamdia quelen*)	**Animals** Silver catfish (*R. quelen*) juveniles with an initial BW of 70-90 g (15-17 fish/replicate; 2 replicates/treatment)**Test Article** BG (MacroGard®; min. 55%) **Treatment** Pelleted feed containing 0 (control) 0.01% or 0.1% BG **Duration** 28 days **Challenge Conditions** The effect of treatment under challenge conditions was also evaluated but the findings are not considered relevant	**Growth Performance** The study was conducted to investigate the mechanisms by which BG can influence innate immune response and no growth performance parameters were included or reported **Hematological Parameters** NSD among treatments and all values fell within normal ranges for the species **Immunological Blood Parameters and Antibody Production** NSD in serum myeloperoxidase activity or capacity of serum to agglutinate among treatments NSD on ability of fish to produce antibodies among treatments
Jiang *et al.*, 2019 **Objective**	**Animals** Turbot (*S. maximus*) female with an initial BW of 3-4 kg (40 fish/treatment) **Test Article**	**Growth Performance** The study was conducted to investigate the effect of BG on immune function and no growth performance parameters were included or reported

Table 6.5: Summary of Studies in Non-Salmonids Fish using Yeast Ingredients Rich in Beta-Glucans		
Reference and Objective	**Study Design**	**Key Findings Related to Safety and Utility**
Administration of yeast glucan on immunity of offspring in Turbot *Scophthalmus maximus*: A trans-generational immune-enhancing effect	BG (80%) **Treatment** Sandlaunce (*Ammodytes personatus*) based diet containing 0 (control) or 0.2-0.3% BG Each turbot was fed with yeast glucan at a rate of approximately 2% body weight per day **Duration** 80 days	**Immunological Parameters (Egg and Serum)** ↑'d component 3, factor B and lysozyme levels in serum and egg cytosol of BG-fed fish (P<0.05) ↑'d lysozyme activity in serum and egg cytosol of BG-fed fish (P<0.05)
Kühlwein *et al*., 2014 **Objective** Effects of dietary beta-1,3/1,6-D-glucans supplementation on growth performance, intestinal morphology and hemato-immunological profile of mirror carp (*Cyprinus carpio L.*)	**Animals** Mirror carp (*C. carpio L.*) with an initial BW of approximately 7 g (25 fish/replicate; 3 replicates/treatment) **Test Article** BG (MacroGard®; min. 55%) **Treatment** Fish protein and wheat starch meal-based diets containing 0 (control), 0.1, 1 or 2% (w/w) BG Carp were carefully acclimatized for 6 weeks before they received the basal diet at 2% BW per day **Duration** 56 days	**Growth Performance** ↑'d BWG, SGR and FCR in fish fed 1 or 2% BG in the diet vs. controls (P<0.05) **Hematological Parameters** NSD in WBC counts among treatment groups ↑'d hematocrit levels in 2% BG-fed fish vs. controls (P<0.05) ↑'d blood monocyte fraction in fish fed 1 or 2% BG in the diet **Intestinal Histomorphology** ↑'d infiltration of leucocytes into the epithelial layer of the anterior intestine of fish fed 1 or 2% BG in the diet vs. controls; no effects on the posterior intestine NSD in intestinal absorptive surface area of goblet cells in either intestinal region
Munir *et al*., 2016 **Objective** Dietary prebiotics and probiotics influence growth performance, nutrient digestibility and the expression of immune regulatory genes in snakehead (*Channa striata*) fingerlings	**Animals** Snakehead (*C. striata*) fingerlings with an initial BW of 22.40 g ± 0.06 (400 fish/replicate; 3 replicates/treatment) **Test Article** BG (MacroGard®; min. 55%) Galacto-oligosaccharide (GOS) Mannan-oligosaccharides (MOS) Live yeast (*S. cerevisiae*) *Lactobacillus acidophilus*, powder **Treatment (16 weeks feeding time)**	**Growth Performance** ↑'d SGR in 0.2% BG-fed fish vs. controls across both phases (P<0.05) Improvement in FCR (at 8 and 16 weeks) and PER (at 8 weeks) in BG-fed fish in phase 1 vs. controls (P<0.05) **Nutrient Digestibility** ↑'d relative protein digestibility in 0.2% BG-fed fish vs. controls (P<0.05) **Expression of Immune Regulatory Genes** ↑'d expression of immune regulatory genes (TGFβ1 and NFκB) in fish fed supplemented diets (P<0.05) vs. control

Table 6.5: Summary of Studies in Non-Salmonids Fish using Yeast Ingredients Rich in Beta-Glucans		
Reference and Objective	**Study Design**	**Key Findings Related to Safety and Utility**
	Danish FM and Korean corn starch based diets containing 0 (control) 0.2% BG, 1% GOS, 0.5% MOS, 1 % live yeast or 0.01% *Lactobacillus acidophilus* **Duration** 24 weeks over 2 phases Phase 1: the experimental diet was administered for 16 weeks (growth evaluated at weeks 8 and 16) Phase 2: fish from all treatments were fed the control diet for a further 8 weeks to evaluate the efficacy of the prebiotic and probiotic intake	
Sánchez-Martínez *et al.*, 2017 **Objective** Effect of β-glucan dietary levels on immune response and hematology of channel catfish *Ictalurus punctatus* juveniles	**Animals** Channel catfish (*Ictalurus punctatus*) juveniles with an initial BW of 35.04 ± 6.95 g (15 fish/replicate; 3 replicates/treatment) **Test Article** BG (Beta G®; purity not reported) **Treatment** Commercial pelleted catfish food (32% protein; Purina®) was ground to powder and mixed with 0 (control) 0.05, 0.1 or 0.5% BG The diets were homogenized and pelletized for the experiment **Duration** 35 days	**Mortality** No fish mortalities were reported for the duration of the study **Hematological Parameters (Sampled Weekly)** NSD in hematology parameters (no diet or diet x week interactions identified) except for decrease in Ht level and RBC count over time in BG-fed fish (P<0.05; week interaction) **Immunological Parameters** ↑'d leukocyte count, in fish fed 0.05% BG diets in weeks 2, 3 and 4 vs. controls (P<0.05) No consistent effect of BG on blood NbT, spleen NbT, NbT HK or IgM levels
Abu-Elala *et al.*, 2018 **Objective** Efficacy of dietary yeast cell wall supplementation on the nutrition and immune response of Nile tilapia	**Animals** Nile tilapia (*Oreochromis niloticus*) with an initial BW 50.7 ± 0.8 g; 3 replicates/treatment) **Test Article** YCW (Immunowall®; 30% BG; 18% mannans) **Treatment** FM and SBM based diets containing 0 (control), 0.1% YCW or 0.2% YCW **Duration**	**Growth Performance** ↑'d final BWG, FI, SGR, PER and improved FCR in YCW-fed fish vs. controls (P<0.05) **Immunological Parameters (Including Gene Expression)** ↑'d WBC counts, total protein concentration and globulin concentration in YCW fed-fish vs. controls (P<0.05) ↑'d phagocytic activity, lysozyme activity, catalase activity, glutathione-reductase activity and immune-related genes expression in fish fed 0.2% YCW in diet vs. controls (P<0.05)

Table 6.5: Summary of Studies in Non-Salmonids Fish using Yeast Ingredients Rich in Beta-Glucans		
Reference and Objective	**Study Design**	**Key Findings Related to Safety and Utility**
	60 days	
Yeast Cell Wall Ingredients (10 to 49% Beta-Glucans Content)		
Chen *et al*., 2019 **Objective** Effects of dietary hydrolyzed yeast (*Rhodotorula mucilaginosa*) on growth performance, immune response, antioxidant capacity and histomorphology of juvenile Nile tilapia (*Oreochromis niloticus*)	**Animals** Nile tilapia (*O. niloticus*) with an initial BW of 19.1 ± 0.01 g (30 fish/replicate; 4 replicates/treatment) **Test Article** HY (24.3% BG; 14.2% mannan) **Treatment** FM/SBM-based diets containing 0 (control), 0.125, 0.25, 0.5 or 1% HY **Duration** 56 days	**Growth Performance** ↑'d SGR and improved FCR in 1% HY-fed fish vs. controls (P<0.05) ↓'d HSI in 0.25% HY-fed fish vs. controls (P<0.05) **Immunological Parameters** ↑'d lysozyme activity in the serum of HY-fed fish vs. controls (P<0.05) ↑'d complement 3 content in in the serum of 0.5 or 0.1% HY-fed fist vs. controls (P<0.05) ↑'d total antioxidant capacity and superoxide dismutase activity in the serum of HY-fed fish vs. controls (P<0.05) ↓'d serum myeloperoxidase activity in HY-fed fish vs. controls (P<0.05)
Hisano *et al*., 2018 **Objective** Dietary β-glucans and manno-oligosaccharides improve growth performance and intestinal morphology of juvenile pacu *Piaractus mesopotamicus*	**Animals** Pacu (*P. mesopotamicus*) juveniles with an initial BW of 30.93 ± 0.46 g (8 fish/replicate; 4 replicates/treatment) **Test Article** YCW (Glucan-MOS®; 30% BG; 12% mannans) **Treatment** Corn/SBM-based diets containing 0 (control) 0.1, 0.2. 0.4 or 0.8% YCW **Duration** 30 days	**Growth Performance** Quadratic response on WG, FCR and PER on YCW dietary supplementation; 0.2% YCW-fed fish displayed the optimum growth performance **Hematological Parameters** NSD among treatment groups **Intestinal Morphology** BG positively impacted villus height and perimeter
Selim & Reda, 2015 [Abstract only] **Objective** β-Glucans and mannan oligosaccharides enhance growth and immunity in Nile Tilapia	**Animals** Nile Tilapia (*Oreochromis niloticus*) fingerlings with an initial weight of 8.7 ± 0.4 g (120 fish/treatment) **Test Article** YCW (30% BG; 10% Mannans) **Treatment** Basal diet (further details not provided) containing 0 (control; G1) 0.15 (G2) or 0.3% YCW (G3)	**<u>Subgroup A (60 days)</u>** **Growth Performance** ↑'d WG and feed utilization in fish fed 0.3% YCW (G3) vs. controls ↑'d WG and improved FCR in fish fed 0.15% YCW (G2) vs. controls **Intestinal Histomorphology**

Table 6.5: Summary of Studies in Non-Salmonids Fish using Yeast Ingredients Rich in Beta-Glucans		
Reference and Objective	**Study Design**	**Key Findings Related to Safety and Utility**
	Duration 60 days Subgroup A or 30 day subgroup B	Villus height, number of goblet cells and number of intraepithelial lymphocytes increased with YCW dietary levels **Body Composition** ↑'d whole body protein and fat content in fish fed 0.3% YCW (G3) vs. controls **Subgroup B (30 days)** **Immunological Parameters** ↑'d serum killing percentage and phagocytic activity in fish fed 0.3% YCW (G3) vs. controls ↑'d serum lysozyme activity was significantly higher in YCW-fed fish vs. control (G1)

Abbreviations: ↑'d = increased; ↓'d = decreased; BG = beta-glucans; BWG = body weight gain; DGC = daily growth coefficient; FCR = feed conversion ratio; FI = feed intake; FW = final weight; GOS = galacto-oligosaccharides; HK = head kidney; HSI = hepatopancreas somatic index; HSP = heat shock proteins; Ht = hematocrit; HY = hydrolyzed yeast; IgM = immunoglobulin M; MOS = mannan-oligosaccharides; NbT= nitroblue tetrazolium; NFκB = nuclear factor kappa-light-chain-enhancer of activated B cells; NSD = no significant difference; PER = protein efficiency ratio; RBC = red blood cells; SBM = soybean meal; SGR = specific growth rate; TGFβ1 = transforming growth factor beta 1; WBC = white blood cells; WG = weight gain; YCW = yeast cell wall;
Note: All test article concentrations in the feed are presented on a percentage basis to allow comparison between studies.

Studies in Shellfish

Four studies were identified in shellfish of which one study was only short-term (3-days) and was not considered relevant to the safety evaluation (Suphantharika *et al*., 2003). Of the other 3 studies, 2 were around 40 days in duration (Bai *et al*., 2010; López *et al*., 2003) and the other 90 days (Boonanuntanasarn *et al*., 2016). In the 90-day study, white shrimp fed diets containing 0.05% purified yeast cell wall (70% beta-glucans) displayed improved growth ($P<0.05$) relative to the control group although feed efficiency was unaffected ($P>0.05$) by treatment. Survival rates of shellfish were also unaffected by treatment. Selected hemolymph parameters were also evaluated with no reported differences among treatment groups. Evaluation of the intestinal morphology and microbiota of the shrimp indicated that villi height was increased on supplementation of the diet with purified yeast cell wall and there were some minor variations, not considered adverse, in microbial populations.

Consistent with the findings of the 90-day feeding study, dietary treatment with 0.2% purified yeast cell wall (70% beta-glucans or beta-glucans not reported) for around 40 days, was associated with improvements in performance ($P<0.05$). In these studies, blood parameters relating to immune function were also measured and no adverse findings were reported.

Taken together, under the experimental conditions, a purified yeast cell wall ingredient containing 70% beta-glucans was well-tolerated at levels of 0.5 and 2 g/kg complete feed (0.05 and 0.2%) by shrimp. These use levels are comparable, and slightly higher than, those proposed by Phileo for purified yeast cell wall of between 0.4 and 1.2 g/kg complete feed (0.04 to 0.12%) for aquaculture. Thus, the available data in shrimp provide supporting evidence for the safety of Phileo's purified yeast cell wall under the conditions of intended use. Considering that shellfish are physiologically similar and that shrimp are likely to consume a relatively high amount of food on a body weight basis, the findings of these studies can be extrapolated to other shellfish species.

Table 6.6: Summary of Studies in Shellfish using Yeast Ingredients Rich in Beta-Glucans		
Reference and Objective	**Study Design**	**Key Findings Related to Safety and Utility**
Purified Yeast Cell Wall Ingredients (min. Beta-Glucans 50%, or Beta-Glucans indicated as Major Component but Content not Reported)		
Bai *et al*., 2010 **Objective** Effects of discontinuous administration of β-glucan and glycyrrhizin on the growth and immunity of white shrimp *Litopenaeus vannamei*	**Animals** White shrimp (*Litopenaeus vannamei)* (mean initial BW of 4.70 ± 0.20 g; 50 shrimp/replicate; 10 replicates/treatment) **Test Article** BG (Fubon Yeast BG; 70%) Glycyrrhizin extracted from glycyrrhiza root (GZN) **Treatment** FM/shrimp head-based diets containing 0 (control;), or 0.2% BG continuously (T1); 0.06% GZN continuously (T2); 0.2% BG for 7 days, then 7 days control (T3); 0.2% BG for 2 days, then 5 days control (T4) 0.2% BG for 7 days, then 7 days 0.06% GZN (T5) [The objective of the dietary treatments was to evaluate diets containing BG or GZN when fed continuously or discontinuously to shrimp] **Duration** 42 days	**Growth Performance** ↑'d SGR in shrimp fed 0.2% BG in the diet vs. controls (P<0.05) **Immunological Parameters (THC, PO, SOD)** NSD among treatment groups
Boonanuntanasarn *et al*., 2016 **Objective** Effects of dietary supplementation with β-glucan and synbiotics on growth, hemolymph chemistry, and intestinal microbiota and morphology in the Pacific white shrimp	**Animals** White shrimp (*Litopenaeus vannamei)* (mean initial BW 0.15 ± 0.03 g; 20 shrimp/replicate; 5 replicates/treatment) **Test Article** BG (BETA-S; 70%) Micro-encapsulated probiotics from *Bacillus subtilis* and *Pediococcus acidilactici*. *B. subtilis* and *P. acidilactici* added to diet at a concentration of 5 x 10^7 CFU/kg of diet **Treatment** FM/SBM-based diets containing 0 (control) 0.05% BG, 0.05% BG + *B. subtilis* or 0.05% BG + *P. acidilactici* **Duration** 90 days	**Growth Performance and Survival** ↑'d FW, BWG and body length in shrimp fed 0.05% BG in the diet vs. controls (P<0.05) NSD in survival or feed efficiency among treatments **Hemolymph Chemistry** NSD between 0.05% BG-fed fish vs. controls **Intestinal Microbiota and Morphology (d45 and d90)** Some minor variations in microbial populations were observed among treatment groups but were not considered adverse by the study authors Increased villi height in shrimp fed BG-containing diets vs. controls **Shrimp Meat Composition** ↑'d moisture and protein content in shrimp meat from shrimp fed BG-containing diets vs. controls

Table 6.6: Summary of Studies in Shellfish using Yeast Ingredients Rich in Beta-Glucans		
Reference and Objective	**Study Design**	**Key Findings Related to Safety and Utility**
López *et al*., 2003 **Objective** Physiological, nutritional, and immunological role of dietary h 1-3 glucan and ascorbic acid 2-monophosphate in *Litopenaeus vannamei* juveniles	**Animals** Whiteleg shrimp (*L. vannamei)* juveniles with an initial wet weight of 2.01 ± 0.02 g (10 shrimp/replicate; 5 replicates/treatment) **Test Article** BG (Stanguard; BG content not reported) Vitamin C **Treatment** FM/SBM-based diets containing 0.2% BG (T1), 0.02% vitamin C (T2), 0% BG + 0% vitamin C (control; T3), 7 days 0.2% BG + 7 days 0% BG + 0% vitamin C (T4) or 7 days 0.15% vitamin C + 7 days 0% BG + 0% vitamin C (T5) **Duration** 40 days	**Growth Performance** ↑'d DGC in shrimp fed diets containing BG (T1 + T4) (*P*<0.05) vs. control (*P*<0.05) **Immunological Parameters (PO, THC, Granular Cells)** NSD in BG-fed fish (T1 and T4) vs. controls [Changes in blood metabolites were also evaluated after exposure to salinity shock but were not considered relevant to the safety evaluation]
Suphantharika *et al*., 2003 **Objective** Preparation of spent brewer's yeast β-glucans with a potential application as an immunostimulant for black tiger shrimp, *Penaeus monodon*	**Animals** Black tiger shrimp (*Penaeus monodon)* adult (mean initial BW 22-27 g; 30 shrimp/replicate; 3 replicates/treatment) **Test Article** Alkaline extracted Brewer's yeast (BYG; 50.5% BG) Commercial bakers' yeast BG (YGT; 71.2% BG) **Treatment** FM/SBM-based diets containing 0 (control) 0.2% BYG or 0.2% YGT Shrimp were fed the above diets at 10% body weight/day three times a day for 3 days **Duration** 3 days	**Immunological Parameters (PO)** ↑'d PO activity in shrimp hemolymph fed BYG or YGT-containing diets vs. controls (*P*≤0.05) at day 3 [*In vitro* experiments were also conducted using shrimp hemolymph but were not considered relevant to the safety evaluation]

Abbreviations: ↑'d = increased; ↓'d = decreased; BG = beta-glucans; BW = body weight; BWG = body weight gain; BYG = alkaline extracted brewer's yeast; CFU = colony forming unit; DGC = daily growth coefficient; FM = fish meal; FW = final weight; GZN = glycyrrhizin; NSD = no significant difference; PO = phenoloxidase; SBM = soybean meal; SGR = specific growth rate; SOD = superoxide dismutase; THC = total hemocyte count; YGT = commercial bakers' yeast beta-glucans;
Note: All test article concentrations in the feed are presented on a percentage basis to allow comparison between studies.

6.1.4.5 Studies in Ruminants

A number of studies were identified in ruminants in which animals were fed diets containing beta-glucan rich yeast ingredients which are compositionally similar to Phileo's purified yeast cell wall. An overview of the key findings from each of the ruminant studies is provided in Tables 6.7 (cattle), 6.8 (calves) and 6.9 (sheep and lambs).

Studies in Cattle

Two studies were identified in cattle in which the effect of purified yeast cell wall-derived ingredients on performance and nutrient digestibility were evaluated. Cherdthong *et al*. (2018) fed cattle 0, 1.6, 3.1 or 4.1 g/head/day of purified yeast cell wall (90% beta-glucans content) for 21 days in a 4 x 4 Latin square design study. Purified yeast cell wall supplementation was observed to increase feed intake linearly. Nutrient digestibility was generally unaffected by treatment except for an increase in the CP digestibility coefficient in cattle fed diets containing 4.7 g/head/day. Similarly, supplementation of the diet of cattle with 20 g of yeast cell wall (30 to 35% beta-glucans)/head/day for 70 days was not associated with any changes in performance or nutrient digestibility (P<0.05) (Peng *et al*., 2020). On a beta-glucans basis, 20 g of yeast cell wall is equivalent to around 12 to 14 g/head/day of Phileo's purified yeast cell wall assuming a 50% beta-glucans content. Therefore, although the parameters evaluated in the study are limited to performance and nutrient digestibility, and do not include blood parameters, the findings provide supporting evidence for the tolerability of Phileo's purified yeast cell wall ingredient to cattle at levels of up to 10 g/head/day.

Although only two studies were identified in cattle, yeast and yeast-derived products have a long and established history of use in ruminant feed (Shurson, 2018). The background exposure by ruminants to beta-glucans as a component of the cell wall of yeast-derived products provides further corroborative evidence of safety. Additionally, there are a number of studies in calves and sheep which, based on their physiological similarities, provide further support of the safety of purified yeast cell wall under the conditions of intended use in cattle.

Table 6.7: Summary of Studies in Cattle using Yeast Ingredients Rich in Beta-Glucans		
Reference and Objective	**Study Design**	**Key Findings Related to Safety and Utility**
Purified Yeast Cell Wall Ingredients (min. Beta-Glucans 50%, or Beta-Glucans indicated as Major Component but Content not Reported)		
Cherdthong *et al*., 2018 **Objective** Effect of beta-glucan supplementation on feed intake, digestibility of nutrients and ruminal fermentation in Thai native beef cattle	**Animals** 4 Thai native beef cattle were randomly assigned according to a 4 × 4 Latin square design **Test Article** BG (FUBON®; min. 90%) **Treatment** Diets based on casava chips and rice bran containing 0, 1.6, 3.1 or 4.7 g/head/day BG **Duration** 4 periods of 21 days (after 14-days adaption and before 7-day collection period)	**Performance** ↑'d FI with dietary BG content (P=0.02) **Nutrient Digestibility** ↑'d digestibility of CP on dietary supplementation with 4.7 g BG/head/day (P=0.03) NSD in estimated energy intake, digestibility of DM, OM, NDF or ADF with treatment **Ruminal pH and Ammonia-N (0 and 4 h Post-Feeding)** NSD with BG-supplementation level **Blood Urea-N (0 and 4 h Post-Feeding)** NSD with BG-supplementation level
Yeast Cell Wall Ingredients (10 to 49% Beta-Glucans Content)		
Peng *et al*., 2020 **Objective** Effects of yeast and yeast cell wall polysaccharides supplementation on beef cattle growth performance, rumen microbial populations and lipopolysaccharides production	**Animals** Qinchuan cattle (1 cow/replicate; 10 replicates/treatment) **Test Article** YCW (30-35% BG; 28-32% mannans) Live yeast (2×10^{10} live cell g^{-1}) **Treatment** Corn grain and rapeseed meal-based diets containing 0 (control), 2 g live yeast/head/day or 20 g YCW, head/day **Duration** 70 days	**Performance** NSD in DMI ADG and FCR between animals fed diets containing YCW vs. control **Nutrient Digestibility** NSD in Apparent nutrient digestibility (DM, OM, CP, NDF, ADF, EE, Ca and P) between animals fed diets containing YCW vs. control **Immunological Parameters** NSD in plasma inflammatory parameters (acute phase protein, histamine and lipopolysaccharides) in animals fed a diet YCW vs. control

Abbreviations: ↑'d = increased; ↓'d = decreased; ADF = acid detergent fiber; ADG = average daily gain; BG = beta-glucans; CP = crude protein; DM = dry matter; DMI = dry matter intake; EE = ether extract; FCR = feed conversion ratio; FI = feed intake; NDF = neutral detergent fiber; NSD = no significant difference; OM = organic matter; YCW = yeast cell wall.

Studies in Calves

Two studies were identified in which calves were fed milk replacer containing purified yeast cell wall (70 or 83% beta-glucans content) for a period of 28 or 56 days (Eicher *et al*., 2010; Ma *et al*., 2015). The results were relatively inconsistent, with calves in the study by Eicher *et al*. (2010) displaying reduced intake and a depression in blood parameters related to immune function when purified yeast cell wall (70% beta-glucans content) was included in milk replacer at a level of 0.0034%. The authors postulated that any beneficial effects of supplementing the diet of calves with purified yeast cell wall may be delayed and exhibited after 28 days. Although DM intake was reduced, body weight was not significantly impacted by treatment.

By comparison, calves fed a combination of milk replacer and starter feed containing up to 0.02% purified yeast cell wall (83% beta-glucans content) exhibited improved growth, and no significant changes in hematological and immunological blood parameters (Ma *et al*., 2015). In the same study, nutrient digestibility was improved in calves fed 0.02% purified yeast cell wall containing diets.

Assuming the body weight of the calves in the studies was around 55 kg and DM intake was 1.5 kg/day (Eicher *et al*., 2010), animals were estimated to be exposed to 0.051 g or 0.3 g/head/day in the 2 studies. Phileo's purified yeast cell wall ingredient is proposed for use in milk replacer at levels of up to 0.05 g/head/day and only once the calf is at least 2 days of age. Intestinal permeability of calves is highest during the first 24 to 36 hours after birth in order to facilitate the transfer of immunoglobulins from maternal colostrum across the small intestinal epithelium (Bush & Stanley, 1980; Araujo *et al*., 2015). As the first protective barrier to exogenous pathogens, integrity of the intestinal epithelium ensures appropriate nutrient absorption and avoids translocation of pathogens. By only administering purified yeast cell wall to calves via milk replacer from 48 hours (2 days) after birth, the potential for absorption from intestinal permeability will be minimized.

Although the available data in calves provides only limited direct evidence of the safety of purified yeast cell wall under the conditions of intended use, it is pertinent that bovine milk, similar to other mammalian milk is rich in oligosaccharides, particularly for the first few days after calving (Tao *et al*., 2009). These oligosaccharides while structurally different to beta-glucans are composed of monosaccharides linked by glycosidic bonds and are established to play a critical role in the development of the gut in immature animals. Thus, calves over the age of 2 days are anticipated to tolerate relatively high levels of fermentable fibers, including beta-glucans as components of the diet.

Table 6.8: Summary of Studies in Calves using Yeast Ingredients Rich in Beta-Glucans		
Reference and Objective	**Study Design**	**Key Findings Related to Safety and Utility**
Purified Yeast Cell Wall Ingredients (min. Beta-Glucans 50%, or Beta-Glucans indicated as Major Component but Content not Reported)		
Eicher *et al.*, 2010 **Objective** Yeast cell-wall products containing beta-glucan plus ascorbic acid affect neonatal *Bos taurus* calf leukocytes and growth after a transport stressor	**Animals** 6 day old, calves (20 bulls, 19 heifers) (13 calves/treatment) **Test Article** BG (BetaRight; 70%) YCW (1.8% BG) **Treatment** MR containing 0% (control), 2.5% YCW + 0.0056% L-ascorbic acid or 0.0034% BG + 0.0056% L-ascorbic acid **Duration** 28 days	**Performance** ↓'d calf starter intake and DMI in animals fed diets supplemented with 0.0034% BG or 2.5% YCW ($P \leq 0.05$) vs. control **Hematological and Immunological Parameters** Hematocrit level tends to be greater in YCW vs. BG-fed calves ($P=0.09$) ↓'d WBC count at 28 days for 0.0034% BG-fed calves vs. controls ($P=0.04$) Fewer PBMC in 0.0034% BG-fed calves vs. controls ↓'d Phagocytosis for 0.0034% BG-fed calves vs. controls **Fecal Microbial Populations** Slight variations in fecal microbial populations and no increase in *E. coli* shedding with 0.0034% BG-fed calves
Ma *et al.*, 2015 **Objective** Effects of dietary yeast beta-glucans on nutrient digestibility and serum profiles in pre-ruminant Holstein calves	**Animals** Neonatal Holstein male calves (39.6±4.2 kg; 7 calves/treatment) **Test Article** BG (82.75%) **Treatment** Calves received a basal diet consisting of a MR and a starter feed containing 0 (control), 0.0025, 0.005, 0.0075, 0.01 or 0.02% BG on a DMI basis **Duration** 56 days	**Growth Performance** ↑'d ADG in calves fed 0.0075% BG in the diet a diet supplemented with 0.0075% BG DMI ($P<0.05$) vs. controls NSD in FI among treatments **Nutrient Digestibility (d14-20; d42-48)** ↑'d apparent digestibility of DM, CP, EE, and P ($P<0.05$) in calves fed 0.0075% BG diets vs controls ($P<0.05$) over both collection periods **Hematological and Immunological Parameters** NSD in serum total protein, albumin, serum urea-N and glucose levels among treatments ↓'d serum TGs and TC in BG-fed calves vs. controls ($P<0.05$)

Table 6.8: Summary of Studies in Calves using Yeast Ingredients Rich in Beta-Glucans		
		↑'d serum ALP activity in BG-fed calves vs. controls (P<0.05) Quadratic response in serum IgG and IgM concentrations, and lysozyme activity with increased BG-supplementation; optimum immune response was observed at 0.0075% BG supplementation **Intestinal Morphology** Lowest crypt depth and higher villous height/crypt depth was detected in 0.0075% BG-fed calves (P<0.05); NSD in mucosal thickness among treatments

Abbreviations: ↑'d = increased; ↓'d = decreased; ADG = average daily gain; ALP = alkaline phosphatase; BG = beta-glucans; CP = crude protein; DM = dry matter; DMI = dry matter intake; EE = ether extract; FI = feed intake; MR = milk replacement; NSD = no significant difference; PBMC = peripheral blood mononuclear cells; TC = total cholesterol; TGs = triglycerides; WBC = white blood cells; YCW = yeast cell wall.

Studies in Ewes and Lambs

Two studies were identified in which lactating ewes were fed diets supplemented with purified yeast cell wall containing 70% beta-glucans at a level of 1.8 g/head/day for the 70-day lactation period (Zabek *et al*., 2013), or 0.9 g/head/day for the 3-week tupping period and then separately during the 70-day lactation period (Zaleska *et al*., 2015). In both studies, milk production was increased in lactating ewes fed diets supplemented with purified yeast cell wall. Reproductive performance was comparable to, or improved, in ewes relative to the control group (Zaleska *et al*., 2015). Additionally, in the study by Zabek *et al*. (2013), no effect of treatment was observed on the growth performance of lambs but improved muscle development was reported.

In an additional two studies, lambs were fed diets supplemented at a level of 0.05 or 1.5 kg/head/day, increasing by 0.05 kg every 10 days, of a concentrate containing 3 g of purified yeast cell wall (70% beta-glucans content; Sowińska *et al*., 2017) or yeast cell wall (25 to 30% beta-glucans content; Wojcik *et al*., 2010) for 100 or 60 days, respectively. These levels of supplementation were equivalent to an initial yeast cell wall ingredient exposure of 0.15 or 4.5 g/head/day by lambs. There were no reports of adverse findings on performance, hematological and immunological parameters or meat quality reported in the studies.

Overall, the results of the studies in lactating ewes indicate that levels of up to 1.8 g/head/day of purified yeast cell wall-derived ingredients were not associated with any adverse effects. Levels of up to 4.5 g/head/day of purified yeast cell wall-derived ingredients were also well-tolerated by lambs. These levels of supplementation are higher than the proposed maximum use level of Phileo's purified yeast cell wall ingredient of up to 1 g/head/day in small ruminants.

Combined with the data summarized above in beef cattle and calves, the findings in sheep indicate that ruminants, including pre-ruminants can tolerate purified yeast cell wall-derived ingredients at levels anticipated to be relevant to the intended use of Phileo's ingredient. Based on their physiological similarities, it is reasonable to extrapolate these findings to all ruminants and pre-ruminants.

Table 6.9: Summary of Studies in Sheep and Lambs using Yeast Ingredients Rich in Beta-Glucans		
Reference and Objective	**Study Design**	**Pertinent Findings Related to Safety and Utility**
Purified Yeast Cell Wall Ingredients (min. Beta-Glucans 50%, or Beta-Glucans indicated as Major Component but Content not Reported)		
Lactating Ewes (and Lambs)		
Zabek *et al.*, 2013 **Objective** Effect of β-1,3/1,6-D-glucan in diet on productivity and humoral and cellular defense mechanisms in sheep	**Animals** Polish Longwool Kamieniecka variety of suckling ewes (13 ewes/treatment) and their lambs (21 lambs/treatment) **Test Article** BG (Biolex® Beta-S; 70%) **Treatment** Ewes received diets based on silage from pre-dried grasses and papilionaceous plants plus 3 g/kg (equating to around 0.6 kg/head/day) of a concentrate delivering around 1.8 g BG/head/day **Duration** 70 day lactation period	**Suckling (Lactating) Ewes** **Milk Parameters (d28, d70)** ↑'d milk performance in ewes (13.5 to 14%) fed BG vs. controls Lower somatic cell count in milk from ewes fed BG vs. controls ↑'d % of fat (15-30%) and protein (11%) composition of milk from ewes fed BG vs. controls **Immunological Parameters (d28, d70)** Ewes administered BG displayed markers of non-specific humoral immunity vs. controls (P<0.01) **Lambs** **Lamb Performance (d28, 70)** NSD in BWG between treatments, although numeric ↑ in growth rate in lambs from ewes fed BG vs. controls **Meat Quality at Days 28 and 70)** Lambs from BG fed ewes displayed better muscle development vs. controls
Zaleska *et al.*, 2015 **Objective** Impact of *S. cerevisiae* supplementation on reproductive performance, milk yield in ewes and offspring growth	**Experiment 1** **Animals** 3-5-year-old Polish Longwool ewes and lambs (breeding herd) (40 ewes/treatment; 190 lambs total) **Test Article** BG (Biolex® Beta-S; 70%) Dried brewer's yeast (DBY; Inter Yeast®) **Treatment (Tupping Period)** Pasture-based diets plus a concentrate (0.3 kg/animal/day, containing 0.9 g BG/animal/day) **Treatment (Lactation and Lamb-rearing period)**	**Experiment 1** **Reproductive Performance** ↑'d prolificacy and reproductive performance in sheep administered BG vs. controls NSD in fertility or lamb rearing (reared/live-born lambs) among treatments **Milk Performance (d28, d70)** ↑'d milk yield of ewes fed BG vs. control (P≤0.05) ↑'s DM and fat content in milk of ewes fed BG vs. controls (P≤0.01) **Lamb Performance** ↑'d final BW, BWG and growth in lambs from ewes fed BG in diet vs. controls (P<0.05) over whole rearing period

Table 6.9: Summary of Studies in Sheep and Lambs using Yeast Ingredients Rich in Beta-Glucans		
Reference and Objective	**Study Design**	**Pertinent Findings Related to Safety and Utility**
	Meadow hay and Lucerne silage-based diets plus a concentrate (0.7 kg/animal/day, containing 3.1 g BG/animal/day) **Treatment (Lambs)** Concentrate starting at 0.05 kg/head/day at day 11 and increasing by 0.05 kg every 10 days from day 11 to 30 **Duration** 3-week tupping period for ewes; 70-day lactation; and rearing period for lambs	
	<u>Experiment 2</u> Only dried brewer's yeast was evaluated	
Lambs		
Sowińska *et al*., 2017 **Objective** The effect of dietary supplementation with β-1,3/1,6-D-glucan on stress parameters and meat quality in lambs	**Animals** 2-day old Kamieniecka male lambs, from 3-year-old ewes (20 lambs/treatment) **Test Article** BG (Biolex® Beta-S; 70%) **Treatment** Lambs received diets based on hay silage of grass and legumes plus CJ concentrate at levels increasing every 10 days from 0.05 kg/animal/day (starting at 0.05 kg/animal/day at day 10) CJ containing 3 g BG/kg [Starting BG supplementation at day 11 was 0.15 g/animal/day] **Duration** 100 days; 12 h fasting followed by transportation to slaughterhouse	**Performance** ↑'d ABW in lambs fed a diet supplemented with BG (P<0.05) vs. controls **Growth Performance** ↑'d BW after rearing, pre-slaughter starvation and transport in BG-fed lambs vs. controls (P≤0.05) **Hematological Parameters** NSD in RBC count, WBC count, HGB level and HCT level in BG-fed lambs vs. controls (P≤0.05) ↓'d cortisol concentrations in BG-fed lambs vs. controls (P≤0.05) ↑'d glucose level in BG-fed lambs v. controls (P≤0.05) **Meat Quality** NSD in color parameters in meat between treatments ↓ pH, expressed juice area, shear force and color parameter

Table 6.9: Summary of Studies in Sheep and Lambs using Yeast Ingredients Rich in Beta-Glucans		
Reference and Objective	**Study Design**	**Pertinent Findings Related to Safety and Utility**
Yeast Cell Wall Ingredients (10 to 49% Beta-Glucans Content)		
Lambs		
Wojcik, 2010 **Objective** Effect of brewer's yeast (*S. cerevisiae*) extract on selected parameters of humoral and cellular immunity in lambs	**Animals** 30-day old Kamieniec suckling lambs (16 lambs/treatment) **Test Article** Extract from brewer's yeast cell wall (YB; Biolex-MB40; 25-30% BG; 10-15% mannans) **Treatment** Lambs received diets based on hay silage of grass and legumes plus CJ concentrate at levels increasing every 10 days from 0.05 kg/animal/day (starting at 0.15 kg/animal/day at day 10) CJ containing 3 g BG/kg [Starting BG supplementation was 4.5 g/animal/day) **Duration** 60 days	**Immunological Parameters (d0, d15, d30 and d60)** ↑'d respiratory burst activity, potential killing activity, lysozyme activity, ceruloplasmin activity, γ-globulin content and lymphocyte proliferation after mitogen activation in animals fed diets 0.3% YB vs. controls ($P \leq 0.05$)

Abbreviations: ↑'d = increased; ↓'d = decreased; ABW = average body weight; BG = beta-glucans; BW = body weight; BWG = body weight gain; CJ = commercial feed mixture for lambs; d = day; DBY = dried brewer's yeast; DM = dry matter; HCT = hematocrit; HGB = hemoglobin; NSD = no significant difference; RBC = red blood cells; WBC = white blood cells; YB = extract from brewer's yeast.

6.1.4.6 Studies in Dogs

Four studies were identified in dogs in which animals were fed yeast ingredients rich in beta-glucans that are compositionally similar to Phileo's purified yeast cell wall. An overview of the key findings from each of the studies in dogs is provided in Table 6.10. In 3 of the studies were specifically designed to evaluate the ability of beta-glucans to stimulate the immune system and are therefore limited in their relevance to the safety assessment on the basis that Phileo uses purified yeast cell wall as a source of beta-glucans only (i.e., nutritional functionality as a fermentable fiber).

In the first study, healthy dogs were fed diets supplemented with 0.012 or 0.023% beta-glucans (test article not fully described), for 24 days (Fries-Craft *et al*., 2023). Dietary treatment with beta-glucans was reported to have no adverse effects on routine blood parameters with the only observed effect, a slight increase in mean corpuscular hemoglobin concentrations (MCHC) levels relative to control animals which was not considered by the study authors to be adverse.

In another study, dogs with inflammatory bowel disease (IBD) were fed diets supplemented with 7 mg purified yeast cell wall (85% beta-glucans content)/kg body weight/day for 42 days (Rychlik *et al*., 2013). Dietary treatment with purified yeast cell wall was reported to positively impact indicators of IBD in dogs with no adverse findings on performance of general health noted by the authors.

Additionally, a study by Stuyven *et al*. (2010) evaluated the effect of supplementation of the diet of Beagle dogs with 225 mg purified yeast cell wall (55% beta-glucans content)/animal/day for 28 days on measures of immune function in the blood. Some changes in immune parameters were reported in dogs fed purified yeast cell wall-containing supplements relative those fed the placebo, but these disappeared within one week of ending the treatment.

In the fourth study, Ferreira *et al*. (2022), the effect of dietary supplementation with 0.1% beta-glucans (no further details provided) was evaluated in dogs classified as "healthy and normal" or "obese with insulin resistance". Beta-glucans appeared to be well-tolerated by the dogs over a 90-day feeding period with no reports of adverse effects and a reduction in plasma glycemic values in obese dogs after beta-glucan supplementation.

Assuming that a medium-sized dog weighs 15 kg and consumes 250 g food/day, supplementation of 7 mg/kg body weight/day of purified yeast cell wall is equivalent to an intake level of 0.420 g purified yeast cell wall/kg complete food. Likewise, 225 mg purified yeast cell wall/day equates to a dietary level of 900 mg/kg complete food. In the other studies identified, dogs were fed diets containing 0.1, 0.12 or 0.23% (1.0, 1.2 or 2.3 g/kg complete food). These estimated levels of exposure by dogs to purified yeast cell wall from the studies summarized in Table 6.9 are similar to those proposed for Phileo's ingredient of 0.25 to 1.0 g/kg complete feed. Thus, although limited in scope, these studies indicate that Phileo's purified yeast cell wall will be well-tolerated by dogs under the conditions of intended use.

Table 6.10: Summary of Studies in Dogs using Yeast Ingredients Rich in Beta-Glucans		
Reference and Objective	**Study Design**	**Key Findings Related to Safety and Utility**
Purified Yeast Cell Wall Ingredients (min. Beta-Glucans 50% or Not Reported)		
Ferreira *et al.*, 2022 **Objective** Metabolic variables of obese dogs with insulin resistance supplemented with yeast beta-glucan	**Animals** Different breeds of dogs from different households Obese group: 7 dogs (males and females) aged 4 to 10 years; classified as "obese" with BCS of 8-9 Lean group: 7 dogs (males and females) aged 1 to 4 years; classified as "normal" with BCS of 5 Supplemented obese group: dogs in obese group after 90-days of BG dietary supplementation **Test Article** BG (not reported) **Treatment** NRC and AAFCO-compliant diets were supplemented with 0 (lean group) or 0.1% (obese group and supplemented obese group) BG in the diet **Duration** 90-days (obese group, lean group) (15-day adaptation period)	**Performance** No changes in BW of dogs were observed during over the experimental period **Metabolic Variables** ↓'d plasma basal glycemic values (P<0.05) and serum cholesterol and TG levels in obese dogs (P=0.05) vs. supplemented obese dogs ↓'d TNF-α in supplemented obese dogs (P<0.05) vs. obese dogs ↑'d GLP-1 in supplemented obese group (P=0.02) vs. obese group and lean group
Fries-Craft *et al.*, 2023 **Objective** Dietary yeast beta-1,3/1,6-glucan supplemented to adult Labrador Retrievers alters peripheral blood immune cell responses to vaccination challenge without affecting protective immunity	**Animals** Adult Labrador dogs (males and females) (8 dogs/treatment) **Test Article** BGs (not reported) **Treatment** AAFCO-compliant diets were supplemented with 0, 0.12 or 0.23% BGs **Duration** 24 days (prior to administration of a vaccine); 24-hour digestibility study (at Day 24) [Only the 24-day feeding period prior to challenge reported herein]	**Adverse Effects** No adverse effects were reported on supplementation of the diet of dogs with BGs **Blood Parameters** Routine blood parameters were within normal ranges for all dogs except for hematocrit and mean corpuscular hemoglobin concentrations (MCHC); MCHC was higher in dogs fed diets supplemented with BGs **Immune Response** NSD in antibody serum titers or serum cytokines amongst treatments

Table 6.10: Summary of Studies in Dogs using Yeast Ingredients Rich in Beta-Glucans		
Reference and Objective	**Study Design**	**Key Findings Related to Safety and Utility**
Rychlik *et al.*, 2013 **Objective** The effectiveness of natural and synthetic immunomodulators in the treatment of inflammatory bowel disease in dogs	**Animals** 2-6 year old dogs (BW between 5 and 30 kg) of both sexes and various breeds displaying symptoms of IBD including chronic diarrhea specific of the small intestine and vomiting of varied intensity and frequency All dogs were classed as having mild to moderate IBD which could be treated by dietary intervention Group I (7 dogs/4M+3F; 2-6 years old) Group II (7 dogs/2M+5F; 2-4 years old) Group III (7 dogs/4M+3F; 3-6 years old) Group IV (7 dogs/4M+3F; 3-6 years old) **Test Article** BG (Biolex-Beta HP; min. 85%) β-hydroxy-β-methyl butyrate (HMB) **Treatment** Dry food (Bestfeed, Poland) supplemented with BG: Group I – 7 mg/kg BW BG (~122.5 mg/dog/day) Group II – 2 mg/kg BW HMB (~35 mg/dog/day) Group III – 2 mg/kg BW levamisole (~35 mg/dog/day) every five days Group IV – control **Duration** 42 days [dogs received treatment for 42 days but were monitored for 6 months after the study]	**Effect on IBD Symptoms** ↓'d CIBDAI to <1 in BG-fed dogs vs. controls (P=0.01) No recurrence of IBD was observed in BG-fed dogs after 6 months **Immunological Parameters (d0 vs. d42 – Before/After Modulation)** ↓'d (91% drop) in IL-6, ↑'d IL-10 in BG-fed dogs and ↑'d respiratory burst activity, potential killing activity, lymphocyte proliferation after mitogen activation and lipopolysaccharides levels in BG-fed dogs
Stuyven *et al.*, 2010 **Objective** Evaluate the effect of oral administration of β-1,3/1,6-glucan in dogs changes on total and antigen-specific IgA and IgM	**<u>Experiment 1</u>** **Animals** 3-4 year old female Beagle dogs (9 dogs/control group and 10 dogs/treatment group) **Test Article** BG tablets (Per table – 150 mg BG) (MacroGard®; min. 55%) Placebo tablet **Treatment**	**Immunological Parameters** ↓'d serum IgA content after d21 in BG-administered dogs vs. controls (P=0.015) rising after cessation of BG administration ↑'d serum IgM content after day 21 (P=0.048) and day 28 (P=0.045) in BG-administered dogs vs. controls NSD in serum total IgM

Table 6.10: Summary of Studies in Dogs using Yeast Ingredients Rich in Beta-Glucans		
Reference and Objective	**Study Design**	**Key Findings Related to Safety and Utility**
	Dogs received orally either 1.5 placebo tablets (control) or 1.5 BG tablets (150 mg BG, ~225 mg BG/dog/day) **Duration** 28 days	[Similar effects were also observed in saliva and tears from dogs]
	<u>Experiment 2</u> Vaccinated dogs received orally either 1.5 placebo tablets (control) or 1.5 BG tablets (150 mg BG, ~225 mg BG/dog/day) **Duration** 28 days	**Immunological Parameters** ↓'d serum IgA content after d21 in BG-administered dogs vs. controls (P=0.015) rising after cessation of BG administration ↑'d serum IgM content after day 21 (P=0.048) and day 28 (P=0.045) in BG-administered dogs vs. controls NSD in serum total IgM

Abbreviations: ↑'d = increased; ↓'d = decreased; BG = beta-glucans; BW = body weight; CIBDAI = canine inflammatory bowel disease activity index; d = day; HMB = β-hydroxy-β-methyl butyrate; IBD = inflammatory bowel disease; IgA = Immunoglobulin A; IgM = immunoglobulin M; IL-6 = interleukin 6; IL-10 = interleukin 10; MCHC = mean corpuscular hemoglobin concentrations; NSD = no significant difference.

6.1.1.7 Studies in Cats

One study was identified in which cats were fed diets containing 0 (control), 1.0 or 3.0 g/kg complete food of yeast cell wall (beta-glucans content not reported) for 27 days. The yeast cell wall ingredient was provided as top-dressing to commercial kibble diets. Fecal samples were taken at days 16 to 22 and 22 to 27 for digestibility and fecal analyses. There was no effect of treatment on feed intake by the cats. Supplementation of yeast cell wall in the diet resulted in an increase in CP, organic matter and gross energy apparent digestibility coefficients. Fecal pH and fermentation products were not influenced by dietary supplementation with yeast cell wall but greater diversity of species in the microbiome was reported. Phileo's purified yeast cell wall is intended for inclusion in cat food at levels of up to 1.0 g/kg complete food. Thus, the findings of the study support that purified yeast cell wall is well-tolerated by cats at amounts comparable or higher (3x), than the intended use level.

Table 6.11: Summary of a Study in Cats using a Yeast Ingredient Rich in Beta-Glucans		
Reference and Objective	**Study Design**	**Pertinent Results**
Yeast Cell Wall Ingredient (Beta-Glucans Content Not Reported)		
González *et al*., 2023 **Objective** Yeast cell wall compounds on the formation of fermentation products and fecal microbiota in cats: an *in vivo* and *in vitro* approach	**Animals** Adult mongrel cats (9 males and 9 females; ~5 years of age) (6 cats/group) **Test Article** Yeast Cell Wall (YCW; BG content not reported) **Treatment** Commercial kibble containing 0, 0.15 or 0.30% YCW commercial blend (in abstract reported as 46.2 and 92.4 mg/kg YCW) **Duration** 27-days (14-day adaptation period); digestibility and fecal scores evaluated on Days 16-22 and 22-27 [Note: additional in vitro studies were performed to assess microbiota – no findings were considered adverse]	**Performance** No effect of YCW dietary supplementation on FI **Nutrient Digestibility** ↑'d apparent digestibility coefficients of CP, OM and GE as well as ME, and higher fecal score in cats fed 0.3% YCW in the diet vs. controls No effect of YCW dietary supplementation on fecal pH or fermentation products **Microbiota** Shannon's alpha diversity index was higher in cats fed 0.3% YCW in the diet vs. controls

Abbreviations: ↑'d = increased; ↓'d = decreased; BG = beta-glucans; CP = crude protein; FI = feed intake; GE = gross energy; ME = metabolizable energy; OM = organic matter; YCW = yeast cell wall.

6.1.4.8 Studies in Rabbits

A study was identified in which rabbits were fed dietary beta-glucans in order to evaluate the effects on reproductive performance and immunity (Wu *et al*., 2011). An outline of the study is provided in Table 6.12. Pregnant rabbits were fed diets containing yeast cell wall (16% beta-glucans content) at levels of 0, 0.064 or 0.128% from day 14 of gestation to day 28 of lactation (Wu *et al*., 2011). Dietary supplementation with yeast cell wall was observed to reduce FI relative to the control group during the first 14 days but had no adverse effects on reproductive performance or body weight of does. There were some reported changes in immune function on dietary supplementation with yeast cell wall but overall, these were not considered adverse. The study is limited in design but the results indicate that yeast cell wall-based ingredients are tolerated by rabbits. No optimum use level of Phileo's purified yeast cell wall ingredient has been determined in rabbits, but it is anticipated that the amount would fall within the range 0.06 to 0.13%. These data therefore, provide supporting evidence of safety of Phileo's purified yeast cell wall for rabbits.

Table 6.12: Summary of a Study in Rabbits using a Yeast Ingredient Rich in Beta-Glucans		
Reference and Objective	**Study Design**	**Pertinent Results**
Yeast Cell Wall Ingredient (10 to 49% Beta-Glucans Content)		
Wu *et al*., 2011 **Objective** Effect of dietary supplementation of β-1,3–1,6-glucan on reproductive performance and immunity of New Zealand White does and their pups	**Animals** Pregnant multiparous New Zealand White does (10 rabbits/treatment) **Test Article** YCW (16% BG) **Treatment** Yellow corn and SBM based diets containing 0 (control), 0.064% or 0.128% YCW **Duration** Day 14 of gestation to day 28 of lactation (~42 days)	**Performance** ↓'d in FI during gestation period (d14-28) in 0.128% YCW-fed vs. controls (P<0.05); NSD in FI between treatments during the lactation period (d0-28) ↑ in BW changes in YCW-fed does vs. controls during gestation period (d14-28) but NSD during lactation (d0-28) Lower swelling response at 24 and 48 h after injection of phytohemoagglutinin-P in 0.064% BG-fed rabbits [evaluation of hypersensitivity] **Reproductive Performance** NSD in reproductive performance among treatments **Immunological Parameters (Does)** ↓'d serum IgG concentration in YCW-fed does at gestation d28 vs. controls (P<0.05) ↑'d serum IgM and IgG concentrations at lactation d28 in YCW-fed rabbits vs. controls (P<0.05) NSD in lymphocyte subsets of does between treatments ↓'d %CD4+ and CD8+ during lactation period in YCW-fed does vs. controls (P<0.05) **Immunological Parameters (Weanling Pups)** ↑'d subsets of CD4+ lymphocytes % in weanling pups of YCW-fed does vs. controls (P<0.05)

Abbreviations: ↑'d = increased; ↓'d = decreased; BG = beta-glucans; BW = body weight; FI = feed intake; IgG = immunoglobulin G; IgM = immunoglobulin M; NSD = no significant difference; SBM = soybean meal; YCW = yeast cell wall.

6.1.4.9 Overall Conclusions from the Available Target Animal Studies

Taken in their totality, the results of the available studies in poultry, swine, aquaculture, ruminants, dogs and rabbits indicate that yeast cell wall-based ingredients were well-tolerated by animals under conditions of use comparable to those proposed for Phileo's purified yeast cell wall.

The available data cover major classes of food-producing animals, i.e., chickens, pigs, finfish, shellfish, cows and sheep. Based on the similarity in physiology, these findings can also be extrapolated to support major and minor species within the categories of poultry, swine, aquaculture and ruminants. Although equine species and cats are non-physiologically similar to other animals, on a body weight basis, exposure to purified yeast cell wall is not anticipated to differ significantly. Additionally, studies are available in dogs and cats which although not fully meeting regulatory requirements, indicate that yeast cell wall-based ingredients are well-tolerated at levels relevant to the intended use of Phileo's purified yeast cell wall. As detailed in Part 3 of the GRAS Notice, cats weighing 3 kg and consuming 0.063 kg food/day containing 1 g/kg of purified yeast cell wall will be exposed to approximately 21 mg/kg body weight/day. Similarly, a horse of 500 kg and consuming 5 g/head/day of purified yeast cell wall will be exposed to around 10 mg /kg body weight/day. By comparison, broilers are anticipated to be exposed to 14 mg/kg body weight/day, piglets 25 mg/kg body weight/day and beef cattle 12.5 mg/kg body weight/day. These exposure estimates in major categories of food-producing animals are similar or slightly higher than that anticipated by cats and horses. Furthermore, considering purified yeast cell wall is not absorbed to any significant extent by animals and will act as a source of beta-glucans which are fermented in the lower intestine, it is reasonable to assume that tolerability will be similar among species. Thus, the available data in poultry, swine, ruminants, dogs, cats and rabbits may reasonably be extrapolated to support use by all animal species.

It is recognized that the ability of certain fibers to alter the rate of passage through the digestive tract of animals may, in turn, affect nutrient absorption (Fahey *et al*., 2004). Nutrient digestibility was only evaluated to a limited extent in the available studies in target animals but no reductions in the phosphorus or calcium digestibilities were reported. Phileo's purified yeast cell wall is intended for use at relatively low inclusion levels in the diet of animals and no significant impact on nutrient digestibility is anticipated.

6.1.5 Toxicological Information

As mentioned previously, chain length, linkages and degree of branching can vary in beta-glucans from different sources (see Section 2.8.1). Fungal beta-glucans, including yeast beta-glucans, are primarily composed of a backbone chain of 1,3-linked β-glucopyranosyl units with randomly dispersed β-D-glucopyranosyl units attached by 1,6-linkages. On the basis of their structural similarities, toxicological information on beta-glucan rich ingredients from fungal sources was considered pertinent to this safety assessment. These studies have been previously evaluated by EFSA and the U.S. FDA as part of novel foods and GRAS determinations for beta-glucans rich ingredients for use in human food as summarized in Table 6.13. Acute oral toxicity, subchronic and chronic toxicity, and mutagenicity and genotoxicity data were identified in the published literature including these authoritative body evaluations using test articles containing >75% yeast beta-glucans, 50 to 60% mushroom beta-glucans and a fungal chitin-glucan product, and are summarized in turn below. Although the test article has a higher level of beta-

glucans than typically found in Phileo's purified yeast cell wall (i.e., >75% vs. approximately 62% based on analytical data), on the basis that the source and primary component are equivalent, the data were considered relevant to this GRAS determination.

Table 6.13: Summary of Scientific Evaluations of Fungal-Derived Ingredients Rich in Beta-glucans for Use in Human Food

Reference	Source	Ingredient	Intended Use	Pertinent Safety Information
Fungal Beta-Glucans				
Biothera's request for novel foods approval of yeast beta-glucans (EFSA, 2011b)	*S. cerevisiae*	Min. 70% beta-glucans	Food supplements at levels of up to 375 mg/day Foods for particular nutritional uses at levels of up to 600 mg/day (excluding infant formula and follow-on formulae)	Although, sub-chronic data were available, data from these studies were considered to lead to an overly conservative (i.e., too low) NOAEL. Thus, EFSA considered that safety could be established on the basis of the significant history of use, the established safety of beta-glucans from other sources and the absence of any concerns from available toxicological information
Biothera's notification of the GRAS status of yeast beta-glucans (GRN 000239; U.S. FDA, 2008)			Ingredient in a range of foods at levels of up to 200 mg/serving	Safety determination based on the compositional similarity of the substance to baker's yeast glycan, as well as published and unpublished rodent and human studies
Glucan Corporation's notification of the GRAS status of black yeast beta-glucans (GRN 000309, U.S. FDA, 2010)	Black yeast *Aureobasidium pulluns* ATCC 42023	Min. 40% beta-glucans	Ingredient in a range of foods at levels of up to 150 mg beta-glucans/serving	Safety determination based on the compositional similarity to yeast beta-glucans, as well as published and unpublished rodent and human studies
Super Beta Glucan's notification of the GRAS status of mushroom beta-glucans (GRN 000413, U.S. FDA, 2012)	*Ganoderma lucidum* mycelium	Min. 50% beta-glucans	Ingredient in a range of foods delivering 150 mg beta-glucans/serving	Safety determination based on the history of consumption of the source organism, its established absence of pathogenicity or toxigenicity, and published rodent studies; available data on yeast-derived ingredients rich in beta-glucans was also considered relevant on the basis of the compositional similarities of yeast and mushroom beta-glucans.

Abbreviation: EFSA = European Food Safety authority; NOAEL = No-Observed-Adverse-Effect-Level.

6.1.5.1 Acute Oral Toxicity Studies

In an acute oral toxicity study in F344 rats consistent with OECD 420 testing guidelines, the LD_{50} value for a yeast-derived beta-glucans ingredient ("WGP-3-6"; >75% beta-glucans) was reported to be greater than 2,000 mg/kg body weight, the highest dose tested (Babíček *et al*., 2007). The test article was reported to be well-tolerated over the 14-day observation period and there were no indications of adverse clinical effects or significant differences in body weight gain or gross observations at necropsy.

In ICR mice (5/sex/group), the oral LD_{50} value for a beta-glucan ingredient derived from *Agrobacterium* sp. ZX09 ("Salecan"; 77.13% beta-glucans) was greater than 3,000 mg/kg body weight, the highest dose tested (Xiu *et al*., 2011). No deaths or clinical signs of toxicity occurred at this dose during the 14-day observation period. In addition, there was no impact on the body weight of mice compared to controls.

6.1.5.2 Subchronic/Chronic Toxicity

Babíček *et al*. (2007) conducted a 90-day subchronic toxicity study in F344 rats (10/sex/group) that were administered yeast-derived beta-glucans ingredient (WGP 3-6; >75% beta-glucans) by gavage at doses of 0, 2, 33.3, or 100 mg/kg body weight/day. This study was consistent with OECD testing guideline 408 and all standard toxicological endpoints were monitored. There was no mortality or clinical signs of toxicity in any of the groups, and no statistically significant differences with regards to body weight or feed consumption were reported. There were also no test article-related impacts on clinical pathology, functional/behavioral, microscopic, or gross observations indicative of toxicity compared to the control group. Sporadic changes were noted in some biochemical and hematological parameters; however, these were not considered to be of toxicological significance, as these were within normal references ranges and/or not dose dependent. The investigators determined a No-Observed-Adverse-Effect-Level (NOAEL) of 100 mg/kg body weight/day, the highest dose tested.

In another 90-day study, Chen *et al*. (2011) evaluated the safety of an ingredient rich in mushroom beta-glucans extracted from *Ganoderma lucidum* (50-60% beta-glucans) in Sprague-Dawley rats (12/sex/group) administered the test article by gavage at doses of 0 (control), 500 (low-dose), 1,000 (mid-dose), or 2,000 (high-dose) mg/kg body weight/day. The study was conducted consistent with OECD Testing Guideline 408 and Good Laboratory Practices (GLP). Throughout the study, the animals were observed for clinical signs of toxicity and mortality/morbidity, detailed clinical observations, body weight and feed consumption, and ophthalmologic examinations (start and end of study). Hematology, serum chemistry, and urinalysis measurements were performed for surviving animals after 13 weeks of treatment. At termination, necropsy was performed and tissue weights for all major organs/tissues were recorded.

There were no treatment-related mortalities or clinical signs of toxicity in any of the animals. Body weight, feed consumption, and urinalysis parameters of the treated groups were comparable to the control group animals throughout the treatment period. There were no treatment-related, biologically significant adverse effects on hematology or serum chemistry values; however, some statistically significant differences were noted. In female rats in the high-dose group, a statistically significant increase in mean corpuscular and mean corpuscular hemoglobin levels was noted compared to controls. Similarly, a significant increase in hematocrit levels was noted in the low dose treated group. In low-

and high-dose male rats and mid- and high-dose female rats, a significant increase in serum sodium level was observed compared to the control group. These statistically significant changes were considered by investigators to be incidental changes/variations and not adverse effects as the effects were not observed in both sexes, were not associated with correlating changes in other hematology parameters indicative of toxicity, were of small magnitude, were within normal reference values for rats, and/or were not noted in a dose-related manner.

No toxicologically significant changes in organ weights were observed when compared to the control group. Although statistically lower testes weight were reported in low-dose level males, and significantly lower heart weights reported in the high-dose group, these were not considered to be test article-related as the changes were of small magnitude, and/or were not noted in a dose-related manner (Chen *et al*., 2011). There were no treatment-related macroscopic or histopathological findings in any of the groups. All changes noted were considered to be spontaneous and/or incidental in nature, without dose-dependence, and without adverse impact on the organ or tissue. Based on a lack of treatment-related adverse effects in any of the treatment groups, the NOAEL derived by the authors was 2,000 mg/kg body weight/day, the highest dose tested.

In a GLP-compliant 90-day subchronic oral toxicity study conducted according to OECD testing guideline 408, Jonker *et al*. (2010) administered *Aspergillus niger*-derived chitin-glucan (94% chitin-glucan), to groups of male and female Wistar rats (20/sex/group) [Crl:WI(WU)] in diet at concentrations of 0 (control), 1 (low-dose), 5 (mid-dose), or 10% (high-dose). These levels were equivalent to 0, 632, 3,217, and 6,589 mg/kg body weight/day, respectively, for males and 0, 684, 3,437, and 7,002 mg/kg body weight/day, respectively, for females.

All animals were observed for mortality and clinical signs of toxicity with body weight, food and water intakes measured throughout the study. Opthalmoscopic observations were recorded for all rats prior to the administration period and in control and high-treated rats at the end of the study period. During week 13, neurobehavioral investigations were conducted using functional observation battery tests on 10 animals/sex/group. Hematology and clinical chemistry analyses were conducted on 10 animals/sex/group on days 8, 45 (males) or 44 (females), and 91 at termination of the administration period. Urinalysis was conducted on 10 animals/sex/group at week 13. The weights of the adrenals, brain, epididymides, heart, cecum, kidneys, liver, ovaries, spleen, testes, thymus, thyroid with parathyroids, and uterus were measured.

No compound-related mortality or adverse effects were reported in relation to general health, neurobehavior, growth, food or water intake, ophthalmoscopy, hematology and clinical chemistry values, urinalysis, organ weights, or pathological findings. Food intake was significantly increased in high-dose animals compared to controls, but without any effects on body weight. This was attributed by authors to the lower energy content of the high-dose diets, which were high in indigestible fiber.

Enlargement of full and empty cecum in mid- and high-dose males, as well as high-dose females was reported; however, this was attributed to the established response in rats to exposures to high levels of indigestible fiber in the diet and not a toxicological concern (Jonker *et al*., 2010). Numerous statistically significant, sporadic differences were reported in some parameters related to drinking water intake, hematology, clinical chemistry, and urinalysis; however, these differences were not considered to be

treatment-related or of toxicological relevance as the effects were: observed only in one sex, observed at interim evaluations only, but not at the end of the study, were without a treatment level-dependent relationship, were within normal reference values for rats, and/or were not associated with any pathological effects or correlated parameters. Based on the results of the study, the authors concluded that the NOAEL was 10% in the diet, which was equivalent to an overall estimated daily intake of 6.6 and 7.0 g/kg body weight in male and female rats, respectively, the highest level tested.

In a subchronic study in ICR mice (10/sex/group), Xiu *et al.* (2011) administered diets containing 0, 1.0, 2.5 and 5.0% of an *Agrobacterium sp*. ZX09-derived beta-glucans ingredient ("Salecan"; 77.13% beta-glucans) for 13 weeks. Body weights, individual food and water consumption were recorded weekly, while standard hematology and blood chemistry evaluations were conducted at the start and at the end of the study. At study termination, spleen, heart, liver, lung, cholecyst, pancreas, stomach, colon, kidney and urinary bladder, were observed macroscopically and compared with those in the control group. Histopathological examinations were conducted on the liver, kidney, spleen and heart from all animals. There was no mortality or clinical signs of toxicity in any of the groups of animals fed beta-glucans. Feed conversion efficiency (calculated weekly), as well as body weight, water intake and organ weights were statistically comparable between groups throughout the study compared to the control groups. Beta-glucan-fed groups had increased fecal bulk and moisture content vs. the control groups; however, fecal excretion on a dry basis was statistically comparable between groups. Histopathology did not reveal any treatment-related effects or abnormalities in any of the organs examined. As well, hematology and clinical chemistry parameters, including liver enzyme levels, were not statistically different compared to control groups and remained within normal reference ranges for mice. A statistically significant decrease in inorganic phosphorus in high-dose (5.0%) males compared with control animals was found but did not correlate with concurrent changes in other clinical parameters indicative of an adverse effect and remained within normal reference ranges. Fasting glucose levels were statistically significantly decreased in mid- and high-dose females (similar trend in male mice but without reaching statistically significance) compared to the control groups. Beta-glucans are known for the ability to reduce blood glucose levels in human studies and have been investigated for diabetic glucose control (Andrade *et al*., 2015); which is not considered to be an adverse or toxicological effect. As such, based on a lack of adverse effects related to the administration of *Agrobacterium sp*. ZX09-derived beta-glucan, the authors concluded that the NOAEL was 14,478 mg/kg body weight/day, the highest level tested (Xiu *et al.*, 2011).

In a 52-week chronic toxicity study in rats, Feletti *et al*. (1992) administered a *Candida albicans*-derived ingredient rich in beta-glucans (99.4% beta-glucans) to Sprague Dawley rats (20/sex/group) at levels of 0, 50, 100, or 200 mg/kg body weight/day by gavage. No mortality or clinical signs of toxicity were reported in any of the dosed animals. There were no statistically significant differences in body weight, feed, or water intake compared to the control group. Organ weights, hematology, blood chemistry, and urinalysis parameters were not impacted by the administration of yeast beta-glucans in any group and were within normal references ranges for rats. In the highest-dosed group (200 mg/kg group), soft stools/ diarrhea and cecal enlargement with variable hyperplasia of the colon mucosa were observed. These symptoms have been established to be typical of dietary exposure to high levels of indigestible fiber in the diet and not considered toxicologically significant or not relevant to humans (WHO, 1987).

Although the investigators estimated the no-observed-effect-level (NOEL) to be 100 mg/kg/day, a NOAEL of 200 mg/kg/day, the highest dose tested, is more appropriate. This is consistent with the conclusions of the EFSA panel in its safety review of yeast beta-glucans as a novel food (EFSA, 2011b).

6.1.5.3 Genotoxicity Studies

Chen *et al*. (2011) conducted a battery of mutagenicity/genotoxicity tests on a *Ganoderma lucidum*-derived ingredient rich in beta-glucans (50-60% beta-glucans), including an Ames assay, *in vitro* chromosome aberration test, and an *in vivo* micronucleus test in mice. These studies are summarized in turn below.

Ames Assay

In a bacterial reverse mutation assay, the mutagenicity of a mushroom-derived ingredient rich in beta-glucans was evaluated in *Salmonella typhimurium* strains TA98, TA 100, TA102, TA1535, and TA1537 using the plate incorporation method at concentrations of 0.313, 0.625, 1.25, 2.5, and 5 mg/plate in the presence or absence of S9 metabolic activation. Mitomycin C and benzo(a)pyrene were used as positive control substances for assays without or with metabolic activation. There was no significant increase in the number of revertant colonies at any concentration with or without S9, compared to negative control, which were within the range of historic control data. The results of this assay support that mushroom-derived beta-glucans were not genotoxic.

In Vitro Chromosome Aberrations

In an OECD-compliant *in vitro* chromosome aberration assay conducted in Chinese hamster ovary cells (CHO-K1), Chen *et al*. (2011) assessed a mushroom-derived ingredient rich in beta-glucans (50 to 60%) at concentrations of 0.313, 0.625, 1.25, 2.5, and 5 mg/mL with and without exogenous metabolic activation. The positive controls included mitomycin C (3- and 18-hour incubation) and benzo(a)pyrene (3-hour incubation).

Based on the cytotoxicity assay, concentrations of 1.25, 2.5, and 5 mg/mL of the mushroom-derived ingredient rich in beta-glucans were evaluated with and without exogenous S9 in the short-term incubation (3 hours) and without S9 in the long term incubation (18 hours). There were no statistically significant increases in the incidence of structural *chromosomal aberrations* in beta-glucan treated cells at any concentration *vs*. negative control. Positive control samples elicited the required response to support the validity of the test. This study supports that mushroom-derived beta-glucans were not genotoxic in the *in vitro* chromosome aberration assay.

In vivo Micronucleus Assay

A mushroom-derived ingredient rich in beta-glucans (50-60%) was assessed for genotoxicity in an OECD testing guideline-compliant peripheral blood micronucleus study in which ICR mice (5/sex/group) were administered the substance by gavage at dose levels of 0, 1,250, 2,500, and 5,000 mg/kg body weight (Chen *et al*., 2011). Intraperitoneally administered cyclophosphamide was used as the positive control. Peripheral blood samples from the tail vein were collected at 24, 48, and 72 hours after dosing.

There was no mortality of clinical signs of toxicity in any of the groups of animals. There was no statistically significant decrease in the polychromatic erythrocyte (PCE) percentage in any of the dosed groups compared to the negative control group, whereas the positive control response was as expected, supporting the validity of the test. There was also no statistically significant difference in micronucleus frequency (per 1,000 PCE) between the three treatment groups and the negative control group. The results of this study support that the mushroom-derived ingredient rich in beta-glucans did not inhibit erythropoiesis and were not genotoxic in the micronucleus assay.

6.1.5.4 Critical Evaluation of the Toxicological Information

A purified yeast cell wall ingredient containing >75% beta-glucans was not associated with any adverse effects when administered as a single oral dose of 2,000 mg/kg body weight/day. Thus, purified yeast cell wall may be considered to exhibit relatively low toxicity consistent with minimal absorption from the GI tract.

No toxicologically significant treatment-related changes were identified in one published and one unpublished 90-day repeated-dose oral toxicity studies in rats using purified yeast cell wall-based ingredients (>75% beta-glucans), as well as published studies conducted using mushroom-derived beta-glucans rich ingredients (50 to 60%) and chitin-glucan (94%), respectively. In two of the studies, cecal enlargement and greater fecal bulk were reported in the higher treatment level groups. These findings may be considered a physiological response to the feeding of relatively high levels of fiber in the diet (WHO, 1987) and are not considered relevant to the safety assessment of Phileo's purified yeast cell wall under the intended conditions of use of 0.125 to 1.2 g/kg complete feed or 0.05 to 10 g/head/day depending on the species and category of animal.

The NOAEL determined for each of the studies conducted according to OECD guidelines ranged from 100 mg/kg body weight/day for purified yeast cell wall (>75% beta-glucans) to 2,000 mg/kg body weight/day for a mushroom-derived beta-glucans rich ingredient (50-60% beta-glucans). The NOAEL assigned in all of the studies was the highest level tested and largely reflected the amount of fiber than could be feasibility included in the diet without observing changes due to nutritional imbalances or tolerability.

The exposure by animals to Phileo's purified yeast cell wall was estimated on a body weight basis in Part 3 of the GRAS Notice (Section 3.1.1). The intakes of purified yeast cell wall by representative major categories of monogastric target animals were estimated to be 14 mg/kg body weight/day for broilers, 25 mg/kg body weight/day by piglets, 18 mg/kg body weight/day by medium-sized dogs and 21 mg/kg body weight/day by cats. In comparison to the NOAEL established from the 90-day study on purified yeast cell wall ingredient (>75% beta-glucans) of 100 mg/kg body weight/day, equivalent to 75 mg beta-glucans/kg body weight/day a margin of safety of between 4 and 7 can be estimated on an ingredient basis for the different categories of animal. The equivalent intakes of beta-glucans was estimated to be 7 mg/kg body weight/day for broilers, 12.5 mg/kg body weight/day for piglets, 9 mg/kg body weight/day for medium-sized dogs and 10.5 mg/kg body weight/day for cats, allowing a margin of safety of between 12 to 21 to be calculated. Likewise, relative to the NOAEL established from the 90-day study on a mushroom-derived ingredient rich in beta-glucans (50-60% beta-glucans) of 2,000 mg/kg body weight/day, equivalent to around 1,100 mg beta-glucans/kg body weight/day, a margin of safety of

between 190 and 285 can be estimated on an ingredient and beta-glucans basis (i.e., both Phileo's ingredient and the mushroom-derived ingredient display similar beta-glucans contents).

The findings of the *in vitro* and *in vivo* battery of mutagenicity/genotoxicity studies using mushroom-derived ingredient rich in beta-glucans (50-60%) were negative. Thus, lifetime exposure of cats, dogs and other pets to fungal-derived beta-glucans is not expected to pose any genotoxicity concerns.

Overall, the battery of toxicology studies summarized above on related test articles to Phileo's yeast cell wall provide corroborative evidence of safety under the conditions of intended use in animal food.

6.2 HUMAN FOOD SAFETY EVALUATION

Phileo's purified yeast cell wall is comprised of beta-glucans (*ca*. 62%), along with minor amounts of CP (*ca*. 4%), fat (*ca*. 13%), crude ash (*ca*. 7%), moisture (*ca*. 2%) and glycogen (*ca*. 4%). All of these components are found naturally in the diet of animals and humans. In particular, beta-glucans act as a fermentable fiber in animal food and will not be digested or absorbed to any significant extent by animals. The other minor components will be metabolized by animals according to well-established pathways and are not expected to have any significant impact on the composition or quality of animal products. Thus, no residues will deposit in animal products under the conditions of intended use of Phileo's purified yeast wall ingredient which may pose a safety concern to humans consuming animal products.

6.3 SUMMARY AND BASIS FOR THE GRAS CONCLUSION

Phileo intends to market purified yeast cell wall under the trade name Safglucan® for use as a source of beta-glucans in feed for all categories and species of animal in the U.S. The ingredient will primarily be formulated into the complete feed of animals.

Purified yeast cell wall is obtained by extraction and purification of the structural components of the cell wall of *S. cerevisiae*. The manufacturing process to purified yeast cell wall consists of three stages: (a) the fermentation process to yield a cream yeast (*S. cerevisiae*); (b) autolysis of the cream yeast; and (c) extraction and purification of the beta-glucan-rich component of the yeast cell wall. The commercial manufacturing process is conducted in accordance with cGMP and a HACCP plan is in place.

Appropriate feed-grade specifications have been established for purified yeast cell wall which set a minimum beta-glucans content (50%) and include criteria for controlling the levels of heavy metals and microorganisms. The results of analysis of 5 representative batches of purified yeast cell wall confirm conformance with the proposed specifications and acceptable batch to batch variability. Purified yeast cell wall is demonstrated analytically to be composed of beta-glucans (*ca*. 62%), along with minor amounts of CP (*ca*. 4%), fat (*ca*. 13%), crude ash (*ca*. 7%), moisture (*ca*. 2%) and glycogen (*ca*. 4%).

A shelf-life of 24 months is proposed for purified yeast cell wall when stored unopened in the original packaging under cool and dry conditions. The findings of a stability study conducted using 4 representative batches of purified yeast cell wall under real-time conditions indicated that the ingredient continues to conform to the product specification after 24-months storage. The primary effect of prolonged storage appears to be increased moisture content, and Phileo has designed its

packaging to minimize exposure to humidity or water. Moreover, retained batches of purified yeast stored under warehouse conditions were demonstrated to have low moisture contents (i.e., within product specifications) after storage of at least 24 months.

Purified yeast cell wall is intended for use as a source of beta-glucans in the food of all animals at levels ranging from 0.125 to 0.15 g/kg complete feed for poultry, up to 0.5 g/kg complete feed for swine, 0.4 to 1.2 g/kg complete feed for aquaculture, 0.25 to 1.0 g/kg complete feed for cats and dogs, up to 0.05 g/head/day for calves, up to 1 g/head/day for small ruminants, up to 10 g/head/day for large ruminants and 0.25 to 5 g/head/day for equine species. Calves less than 2 days of age will not be provided purified yeast cell wall on the basis that their intestinal barrier may not yet be fully formed. A number of other non-digestible polysaccharides have a long and established history of use as source of fermentable fiber in the diet of animals and purified yeast cell can be considered an alternative to these existing counterparts. The amount of purified yeast cell wall intended for addition to feed is based on previous experience marketing a yeast-derived mannans ingredient and the utility of the beta-glucans component as a fermentable fiber under the conditions of intended use, has no bearing on safety.

Animals will be exposed to beta-glucans in the diet naturally from a range of sources including cereal products, SBM, distillers products and yeast-derived products. Except for fish, background exposure to beta-glucans by poultry, swine, dogs and dairy cows from the normal diet was estimated to be comparable or higher than that from the intended use of purified yeast cell wall. However, in these normal dietary sources, the beta-glucans component forms part of the polymeric structure of the endosperm and cell wall whereas in purified yeast cell wall, it has been released and isolated.

Fungal beta-glucans, including yeast beta-glucans are primarily composed of a backbone chain of 1,3-linked β-glucopyranosyl units with randomly dispersed side chains of β-D-glucopyranosyl units attached by 1,6-linkages. Yeast beta-glucans generally exhibit moderate branching with the exact degree of branching likely to depend on growth conditions (Klis *et al*., 2002; Lam & Cheung, 2013; Zhu *et al*., 2016). Nutritionally, beta-glucans from different sources will serve as fermentable fibers. However, in order to take into account the source and effect of the manufacturing process, only fungal beta-glucans were considered pertinent to the evaluation of the safety of purified yeast cell wall for the target species.

Animals are unable to digest carbohydrate polymers containing beta-glycosidic linkages and therefore, no significant systemic exposure by animals to beta-glucans will occur (Bach Knudsen, 2015; Wang *et al*., 2019). The majority of ingested beta-glucans will be transferred to the large intestine or rumen and subject to fermentation by resident microbiota. Published *in vitro* fermentation studies designed to mimic the GI tract of pigs and dogs, and using beta-glucans from cereal and yeast sources, respectively indicate that the fermentation of beta-glucans is comparable to other known fermentable fibers such as inulin and fructooligosaccharides.

The safety of purified yeast cell wall for the target animal is assessed using a weight of evidence approach based on data generally available in the public domain on yeast-derived ingredients rich in beta-glucans. The individual components comprising the safety evaluation are as follows: (a) the history of use of yeast-derived products in animal feed; (b) the generally recognized body of evidence to support the safety of the *S. cerevisiae* source; (c) studies in which yeast-derived ingredients rich in beta-

glucans have been fed to animals; and (d) toxicological data on yeast-derived ingredients rich in beta-glucans.

Various yeast-derived ingredients are defined in the AAFCO OP including primary dried yeast, yeast extract and hydrolyzed yeast. The yeast cell wall is a component of these products and it was estimated that animal food may contain in the region of 2 g/kg complete feed of dried yeast of which 0.6 g/kg complete feed was the yeast cell wall components. By comparison, the maximum intended conditions of use of purified yeast cell wall are in the range of 0.125 to 1.2 g/kg complete feed across different species, equating to 0.08 to 0.7 g beta-glucans/kg complete feed. Likewise, dried yeast can also supplement the diet of ruminants with exposure by sheep and beef cattle for example, estimated to be 28 to 57 g/head/day and 85 to 113 g/head/day, equating to 8 to 17 g/head/day and 26 to 34 g/head/day of yeast cell wall components, respectively. The long and established history of use of yeast products as ingredients in animal food in the U.S. therefore, provides supporting evidence for the safety of purified yeast cell wall under the conditions of intended use.

S. cerevisiae has QPS status for use as an ingredient for food and feed applications in the EU (EFSA, 2023). The QPS status was established on the basis of the long history of use of yeast products in food and feed, together with the absence of any pathogenicity or toxigenicity concerns. *S. cerevisiae* is rarely implicated in infections despite the extensive use of the species by the food and feed industries, and generally occur in individuals with compromised immune systems of predisposing factors (Enache-Angoulvant & Hennequin, 2005).

Numerous studies were identified in the published literature in which fungal cell wall-based ingredients were fed to poultry, swine, aquaculture, ruminants, dogs, cats and rabbits in order to evaluate the utility of beta-glucans as a source of fermentable fiber in the diet. As a consequence of the nutritional value of beta-glucans as a non-digestible carbohydrate source, the digestive health of animals can be positively impacted leading to improvements in growth performance, nutrient digestibility and immune response. The studies identified in the literature were primarily designed to evaluate these indirect measures of utility and therefore, only included limited parameters pertinent to the safety evaluation. The fungal-derived test articles used in the target animal studies contained a range of different beta-glucans contents but were considered compositionally similar to Phileo's purified yeast cell wall. Taken in totality, the findings of the available studies in target animals indicate that inclusion of yeast cell wall-based ingredients in the diet at levels comparable to those of the intended use of Phileo's purified yeast cell wall were not associated with any adverse effects on growth, nutrient digestibility or measures of immune function in the blood. Together these studies therefore, provide evidence of the ability of yeast cell wall ingredients to be well-tolerated by animals under the conditions of intended use of Phileo's ingredient. Considering the range of species for which data are available, it is reasonable to extrapolate these findings to support use in all species and categories of animal.

Additionally, a battery of toxicology studies were identified in the published and unpublished literature on fungal-derived ingredients rich in beta-glucans which further support the safety of purified yeast cell wall for use in food for all animals.

No toxicologically significant treatment-related changes were identified in one published and one unpublished 90-day repeated-dose oral toxicity studies in rats using purified yeast cell wall-based

ingredients (>75% beta-glucans), as well as published studies conducted using mushroom-derived beta-glucans rich ingredients (50 to 60%) and chitin-glucan (94%), respectively. In two of the studies, cecal enlargement and greater fecal bulk were reported in the higher treatment level groups. These findings may be considered a physiological response to the feeding of relatively high levels of fiber in the diet (WHO, 1987) and are not considered relevant to the safety assessment of Phileo's purified yeast cell wall under the intended conditions of use of 0.125 to 1.2 g/kg complete feed or 0.05 to 10 g/head/day depending on the species and category of animal.

The NOAEL determined for each of the studies conducted according to OECD guidelines ranged from 100 mg/kg body weight/day for purified yeast cell wall (>75% beta-glucans) to 2,000 mg/kg body weight/day for a mushroom-derived beta-glucans rich ingredient (50-60% beta-glucans). The NOAEL allocated for all studies was the highest levels tested and largely reflected the amount of fiber that could be feasibly included in the diet without observing changes due to nutritional imbalances or tolerability.

The intakes of purified yeast cell wall by representative major categories of monogastric target animals were estimated to be 14 mg/kg body weight/day for broilers, 25 mg/kg body weight/day by piglets, 18 mg/kg body weight/day by medium-sized dogs and 21 mg/kg body weight/day by cats. In comparison to the NOAEL established from the 90-day study on purified yeast cell wall ingredient (>75% beta-glucans) of 100 mg/kg body weight/day, equivalent to 75 mg beta-glucans/kg body weight/day a margin of safety of between 4 and 7 can be estimated on an ingredient basis for the different categories of animal. The equivalent intakes of beta-glucans was estimated to be 7 mg/kg body weight/day for broilers, 12.5 mg/kg body weight/day for piglets, 9 mg/kg body weight/day for medium-sized dogs and 10.5 mg/kg body weight/day for cats, allowing a margin of safety of between 12 to 21 to be calculated. Likewise, relative to the NOAEL established from the 90-day study on a mushroom-derived ingredient rich in beta-glucans (50-60% beta-glucans) of 2,000 mg/kg body weight/day, equivalent to around 1,100 mg beta-glucans/kg body weight/day, a margin of safety of between 190 and 285 can be estimated on an ingredient and beta-glucans basis (i.e., both Phileo's ingredient and the mushroom-derived ingredient display similar beta-glucans contents). Thus, the toxicology data can be extrapolated to support the use of purified yeast cell wall for all animal species.

The findings of the *in vitro* and *in vivo* battery of mutagenicity/genotoxicity studies using mushroom-derived ingredient rich in beta-glucans (50-60%) were negative. Thus, lifetime exposure of cats, dogs and other pets to fungal-derived beta-glucans is not expected to pose any genotoxicity concerns.

Phileo's purified yeast cell wall is primarily composed of beta-glucans (min. 50% and typically in the region of 62%) which is minimally absorbed from the GI tract. Thus, no deposition of residues in the tissues of food-producing animals will occur and no food safety concerns are anticipated for humans consuming animal products.

Following critical evaluation of the data and information summarized above, it can be concluded that purified yeast cell wall produced by Phileo using suitable food-grade materials in accordance with cGMP and meeting appropriate feed-grade specifications, is safe and suitable for use as a source of beta-glucans in the food of all species and categories of animals at levels ranging from 0.125 to 1.2 g/kg complete feed across different species or from 0.05 to 10 g/head/day. It is further concluded that purified yeast cell wall is GRAS for the intended use in feed based on scientific procedures.

PART 7. §570.255. LIST OF SUPPORTING DATA AND INFORMATION

7.1 LIST OF APPENDICES

APPENDICES 01, 01A TO 01M, 02A AND 02B, AND 07A AND 07C (HIGHLIGHTED IN GREY) ARE CONFIDENTIAL AND CONTAIN PROPRIETARY MANUFACTURING OR BUSINESS INFORMATION THAT SHOULD NOT BE DISCLOSED

Appendix 01	Manufacturing Process (CONFIDENTIAL)
Appendix 01A	Certificate of Deposition (Strain) (CONFIDENTIAL)
Appendix 01B	Raw Materials Specifications – Sugar Source (CONFIDENTIAL)
Appendix 01C	Raw Materials Specifications – Acid (CONFIDENTIAL)
Appendix 01D	Raw Materials Specifications – Base (CONFIDENTIAL)
Appendix 01E	Raw Materials Specifications – Processing Aid (CONFIDENTIAL)
Appendix 01F	Raw Materials Specifications – Salt (CONFIDENTIAL)
Appendix 01G	Raw Materials Specifications – Vitamin (CONFIDENTIAL)
Appendix 01H	Raw Materials Specifications – Vitamin (CONFIDENTIAL)
Appendix 01I	Raw Materials Specifications – Acid (CONFIDENTIAL)
Appendix 01J	Raw Materials Specifications – Water (CONFIDENTIAL)
Appendix 01K	Raw Materials Specifications – Processing Aid (CONFIDENTIAL)
Appendix 01L	Raw Materials Specifications – Base (CONFIDENTIAL)
Appendix 01M	Raw Materials Specifications – Base (CONFIDENTIAL)
Appendix 01N	Production Site Certificate – GMP
Appendix 01O	Production Site Certificate - ISO
Appendix 02A	Mannans Method (CONFIDENTIAL)
Appendix 02B	Beta-Glucans Method (CONFIDENTIAL)
Appendix 02C	Kjeldahl Method
Appendix 02D	Gravimetric Fat Method
Appendix 02E	Gravimetric Ash Method
Appendix 02F	Dehydration Method
Appendix 02G	APC Method (NF EN ISO 4833-1)
Appendix 02H	Yeast & Mold Method (ISO 6611 2004)
Appendix 02I	Coliforms Method (NF V08-050 Method [FR])
Appendix 02J	*E. coli* Method (NF ISO 16649-2)
Appendix 02K	*Salmonella* Method (NF EN ISO 6579-1)
Appendix 02L	Heavy Metals Method [DIN EN 15763 2010 (2010-04)]
Appendix 03A	Certificate of Analysis Batch (b) (4) (Mannans, Beta-Glucans, Glycogen)
Appendix 03B	Certificate of Analysis Batch (Mannans, Beta-Glucans, Glycogen)
Appendix 03C	Certificate of Analysis Batch (Mannans, Beta-Glucans, Glycogen)
Appendix 03D	Certificate of Analysis Batch (Mannans, Beta-Glucans, Glycogen)
Appendix 03E	Certificate of Analysis Batch (Mannans, Beta-Glucans, Glycogen)
Appendix 03F	Certificate of Analysis Batch (Protein, Fat & Moisture Content)
Appendix 03G	Certificate of Analysis Batch (Protein, Fat & Moisture Content)
Appendix 03H	Certificate of Analysis Batch (Protein, Fat & Moisture Content)
Appendix 03I	Certificate of Analysis Batch (Protein & Fat Content)
Appendix 03J	Certificate of Analysis Batch (Protein & Fat Content)

Appendix 03K	Certificate of Analysis Batch	(b) (4) (Moisture Content)
Appendix 03L	Certificate of Analysis Batch	(Moisture Content)
Appendix 03M	Certificate of Analysis Batch	(Ash Content)
Appendix 03N	Certificate of Analysis Batch	(Ash Content)
Appendix 03O	Certificate of Analysis Batch	(Ash Content)
Appendix 03P	Certificate of Analysis Batch	(Ash Content)
Appendix 03Q	Certificate of Analysis Batch	(Ash Content)
Appendix 03R	Additional Batch Analysis (Protein)	
Appendix 03S	Molecular Weight Distribution Report	
Appendix 04A	Certificate of Analysis Batch	(b) (4) (Microbiology)
Appendix 04B	Certificate of Analysis Batch	(Microbiology)
Appendix 04C	Certificate of Analysis Batch	(Microbiology)
Appendix 04D	Certificate of Analysis Batch	(Microbiology)
Appendix 04E	Certificate of Analysis Batch	(Microbiology)
Appendix 05A	Certificate of Analysis Batch	(Heavy Metals)
Appendix 05B	Certificate of Analysis Batch	(Heavy Metals)
Appendix 05C	Certificate of Analysis Batch	(Heavy Metals)
Appendix 05D	Certificate of Analysis Batch	(Heavy Metals)
Appendix 05E	Certificate of Analysis Batch	(Heavy Metals)
Appendix 06A	Certificate of Analysis Batch	(Mineral Profile)
Appendix 06B	Certificate of Analysis Batch	(Mineral Profile)
Appendix 06C	Certificate of Analysis Batch	(Mineral Profile)
Appendix 06D	Certificate of Analysis Batch	(Mineral Profile)
Appendix 06E	Certificate of Analysis Batch	(Mineral Profile)
Appendix 07A	Shelf-Life Stability Report (CONFIDENTIAL)	
Appendix 07B	Mannans & Beta-Glucans Method (Stability Study)	
Appendix 07C	Humidity Exchange Surface Study (CONFIDENTIAL)	
Appendix 07D	Commercial Retain Sample (Moisture)	
Appendix 08	Safmannan® Product Data Sheet	

7.2 LIST OF ABBREVIATIONS

↑'d	Increased
↓'d	Decreased
AAFCO	Association of American Feed Control Officials
ABW	Average Body Weight
ACH_{50}	Alternative Complement Activity
ADF	Acid Detergent Fiber
ADFI	Average Daily Feed Intake
ADG	Average Daily Gain
ADME	Absorption, Distribution, Metabolism and Excretion
ALP	Alkaline Phosphatase
BCFAs	Branched-Chain Fatty Acids
BCR	Branched-Chain Ratio
BG	Beta-Glucans
BIOHAZ	EFSA Panel on Biological Hazards
BW	Body Weight
BWG	Body Weight Gain
BYG	Alkaline Extracted Brewer's Yeast
CFIA	Canadian Food Inspection Agency
CFR	Code of Federal Regulations
CFU	Colony Forming Unit
cGMP	current Good Manufacturing Practices
CIBDAI	Canine Inflammatory Bowel Disease Activity Index
CJ	Commercial Feed Mixture for Lambs
ConA	Concanavalin A
COS	Chitin Oligosaccharides
CP	Crude Protein
DBY	Dried Brewer's Yeast
DC	Distal Colon
DFI	Daily Feed Intake
DGC	Daily Growth Coefficient
DM	Dry Matter
DMI	Dry Matter Intake
EC	European Commission
EE	Ether Extract
EFSA	European Food Safety Authority
EU	European Union
FC	Feed Consumption
FCR	Feed Conversion Ratio
FDA	Food and Drug Administration
FE	Feed Efficiency
FFDCA	Federal Food, Drug and Cosmetic Act
FI	Feed Intake
FM	Fish Meal
FOS	Fructooligosaccharides

FW	Final Weight
GE	Gross Energy
GI	Gastrointestinal
GLP	Good Laboratory Practices
GOS	Galacto-Oligosaccharides
GOT	Glutamic Oxaloacetic Transaminase
GPT	Glutamic Pyruvic Transaminase
GRAS	Generally Recognized As Safe
GZN	Glycyrrhizin
HACCP	Hazard Analysis Critical Control Point
HCT	Hematocrit
HDL	High Density Lipoprotein
HGB	Hemoglobin
HMB	β-hydroxy-β-methyl butyrate
HSI	Hepatopancreas Somatic Index
HSP	Heat Shock Proteins
HY	Hydrolyzed Yeast
IBD	Inflammatory Bowel Disease
IBV	Infectious Bronchitis Virus
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL-1a	Interleukin 1a
IL6	Interleukin 6
IL-10	Interleukin 10
IL-17A	Interleukin 17 A
IS	Spleen Somatic Index
MCHC	Mean Corpuscular Hemoglobin Concentrations
mod.	Modified
MHC	Major Histocompatibility Complex
MOS	Mannan-Oligosaccharides
MP	Mannoprotein Complex
MR	Milk Replacement
NA	Not Analyzed
NbT	Nitroblue Tetrazolium
ND	Not Detected
NDF	Neutral Detergent Fiber
NDV	Newcastle Disease Virus
NFκB	Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells
NHY	Non-Hydrolyzed Yeast
NOAEL	No-Observed-Adverse-Effect-Level
NOEL	No-Observed-Effect-Level
NSD	No Significant Difference
OECD	Organization for Economic Cooperation and Development
OM	Organic Matter
OP	Official Publication

Or.Zn	Organic Zinc
PA	*Pediococcus acidilactici*
PBMC	Peripheral Blood Mononuclear Cells
PC	Proximal Colon
PCE	Polychromatic Erythrocytes
PER	Protein Efficiency Ratio
PHA-P	Phytohaemagglutinin
PO	Phenoloxidase
QPS	Qualified Presumption of Safety
RBC	Red Blood Cells
SBM	Soybean Meal
SCFAs	Short-Chain Fatty Acids
SCIME	Simulator of the Canine Intestinal Microbial Ecosystem
SFM	Sunflower Meal
SGR	Specific Growth Rate
SOD	Superoxide Dismutase
Spec.	Specification
TC	Total Cholesterol
TCR	T-Cell Receptor
TGFβ1	Transforming Growth Factor beta 1
TGs	Triglycerides
THC	Total Hemocyte Count
TMR	Total Mixed Ration
TNF-α	Tumor Necrosis Factor Alpha
U.S.	United States
VLDL	Very Low Density Lipoprotein
WBC	White Blood Cells
WG	Weight Gain
XOS	Xylooligosaccharides
YB	Extract From Brewer's Yeast
YBG	Yeast Beta-Glucans
YCW	Yeast Cell Wall
YGT	Commercial Bakers' Beta-Glucans

Note: Every abbreviation in the text is worded completely the first time and the abbreviation given in (). From then onwards, only the abbreviation is given in the text.

7.3 REFERENCES

AAFCO, 2023. Chapter Six – Feed Terms and Ingredient Definitions, in: *Association of American Feed Control Officials (AAFCO) Official Publication (OP)*, pp.347-546.

Definition	Ingredient Name
60.105	Fructooligosaccharides
60.106	Inulin
96.	Yeast

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Confidential Manufacturing Information: Purified Yeast Cell Wall

2.2 Manufacturing Process to Purified Yeast Cell Wall

(b) (4)

2.2.1 List of Raw Materials Used in the Manufacture of the Cream Yeast

Purified yeast cell wall will be manufactured from non-genetically modified *S. cerevisiae* strains that form part of Lesaffre's industrial strain collection. Currently, a strain deposited in the Centre National de Collection Microorganismes ("CNCM") under the designation I-5268 is used for the production of purified yeast cell wall and a copy of the Certificate of Deposition is provided in Appendix 01A (CONFIDENTIAL).

The raw materials used in the fermentation of *S. cerevisiae* as the source of the cell wall extract are listed in Table 2.1. All raw materials used in the fermentation process are considered safe and suitable for use in the production of animal feed. Specifications for each of the raw materials are provided in Appendices 01B to 01J (CONFIDENTIAL).

Table 2.1: Raw Materials and Processing Aids Used in the Manufacture of *S. cerevisiae* (Source of Beta-Glucans)

Material	Function	Regulatory Status	Quality
(b) (4)			

Table 2.1: Raw Materials and Processing Aids Used in the Manufacture of *S. cerevisiae* (Source of Beta-Glucans)			
Material	**Function**	**Regulatory Status**	**Quality**
(b) (4)			

2.2.2 Raw Materials Used in the Manufacture of Purified Yeast Cell Wall from Cream Yeast

The raw materials used in the extraction and purification of the cell wall components of *S. cerevisiae* to yield the beta-glucans rich ingredient are listed in Table 2.2. All raw materials are considered safe and suitable for the production of purified yeast cell wall as an ingredient for animal feed. Specifications for the raw materials and processing aids are provided in Appendices 01C, 01E, 01I to 01M (CONFIDENTIAL).

Table 2.2: List of Raw Materials Used in the Manufacture of Purified Yeast Cell Wall

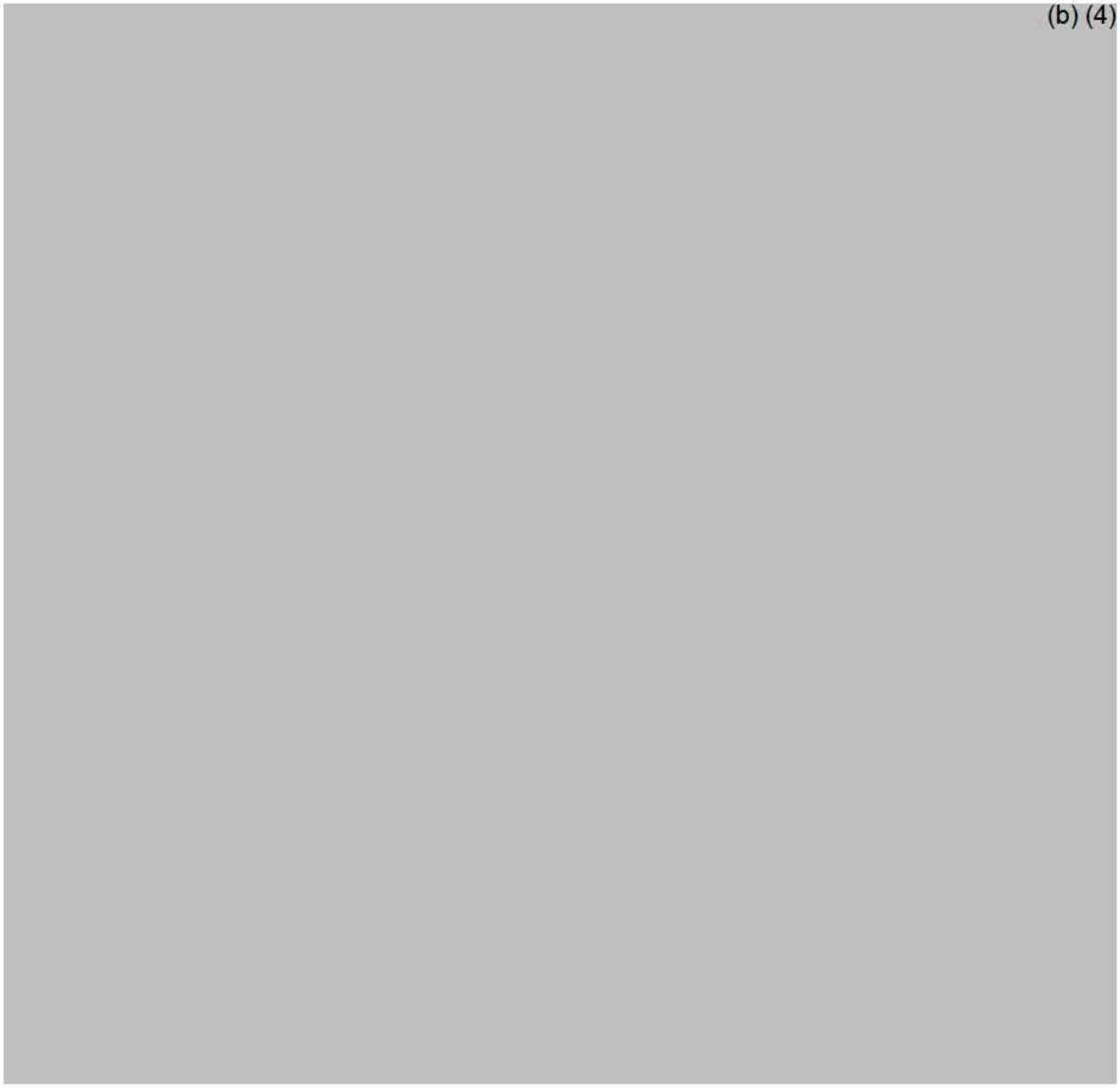
Material	Function	Regulatory Status	Quality
(b) (4)			

Table 2.2: List of Raw Materials Used in the Manufacture of Purified Yeast Cell Wall			
Material	**Function**	**Regulatory Status**	**Quality**
(b) (4)			

2.2.3 Manufacture of *S. cerevisiae* (Cream Yeast)

An overview of the manufacturing process to *S. cerevisiae* is provided in Figure 2.1 and each step is described below. The processes and controls employed are typical of practices followed by the yeast manufacturing industry (U.S. EPA, 1995; Bekatorou *et al.*, 2006; Gélinas, 2014 and 2016).

Figure 2.1: Overview of the Manufacturing Process to *S. cerevisiae* (Cream Yeast)



Isolation and Storage of the Strain

(b) (4)

Laboratory-Scale Propagation

(b) (4)

(b) (4)

Pure Culture Fermentation

(b) (4)

Seed Fermentation

(b) (4)

Yeast Separation

(b) (4)

Commercial (Main) Fermentation

(b) (4)

(b) (4)

Yeast Separation

(b) (4)

2.2.4 Manufacture of Purified Yeast Cell Wall

An overview of the manufacturing process to purified yeast cell wall is provided in Figure 2.2 and each step is described below. The principles of the extraction and purification process follow those reported in the published literature and critically reviewed by Zhu *et al*. (2016).

Figure 2.2: Overview of the Manufacturing Process to Purified Yeast Cell Wall

(b) (4)

Autolysis

(b) (4)

Separation

(b) (4)

Hydrolysis

(b) (4)

Separation

(b) (4)

Filtration and Drying

(b) (4)

in Table 2.3.

(b) (4)

Sieving and Packing

(b) (4)

2.2.5 Production Controls

(b) (4)

Table 2.4: Production Controls for *S. cerevisiae* Fermentation Process	
Isolation and storage of the strain	(b) (4)
Propagation of the strain in the laboratory	
Industrial propagation	

[1]Bacteriology controls: total plate count and coliforms;
[2]Physiochemical controls: dry matter and nitrogen contents.

2.2.6 References

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Definition	Ingredient Name
30.1	Enzymes/Source organisms acceptable for use in animal feed [Papain]
57.19	Phosphoric Acid, _%
57.102	Potassium Chloride
57.124	Potassium Hydroxide
63.7	Cane Molasses
87.138	Riboflavin
96.	Yeast

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21 CFR Section	**Name**
582.1073	Phosphoric acid
582.1095	Sulfuric acid
582.1139	Ammonium hydroxide
582.1631	Potassium hydroxide
582.1763	Sodium hydroxide
582.5622	Potassium chloride
582.5695	Riboflavin
582.5875	Thiamine hydrochloride

Zhu, F., Du, B. and Xu, B., 2016. A critical review on production and industrial applications of beta-glucans. *Food Hydrocolloids*, *52*, pp.275-288.

TRAITÉ DE BUDAPEST SUR LA RECONNAISSANCE
INTERNATIONALE DU DÉPÔT DES MICRO-ORGANISMES
AUX FINS DE LA PROCÉDURE EN MATIÈRE DE BREVETS

FORMULE INTERNATIONALE

DESTINATAIRE

LESAFFRE ET COMPAGNIE
41 RUE ETIENNE MARCEL
75001 PARIS

NOM ET ADRESSE
DU DÉPOSANT

RÉCÉPISSÉ EN CAS DE DÉPÔT INITIAL
délivré en vertu de la règle 7.1 par
l'AUTORITÉ DE DÉPÔT INTERNATIONALE
identifiée au bas de cette page

(b) (4)

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

RECIPIENT

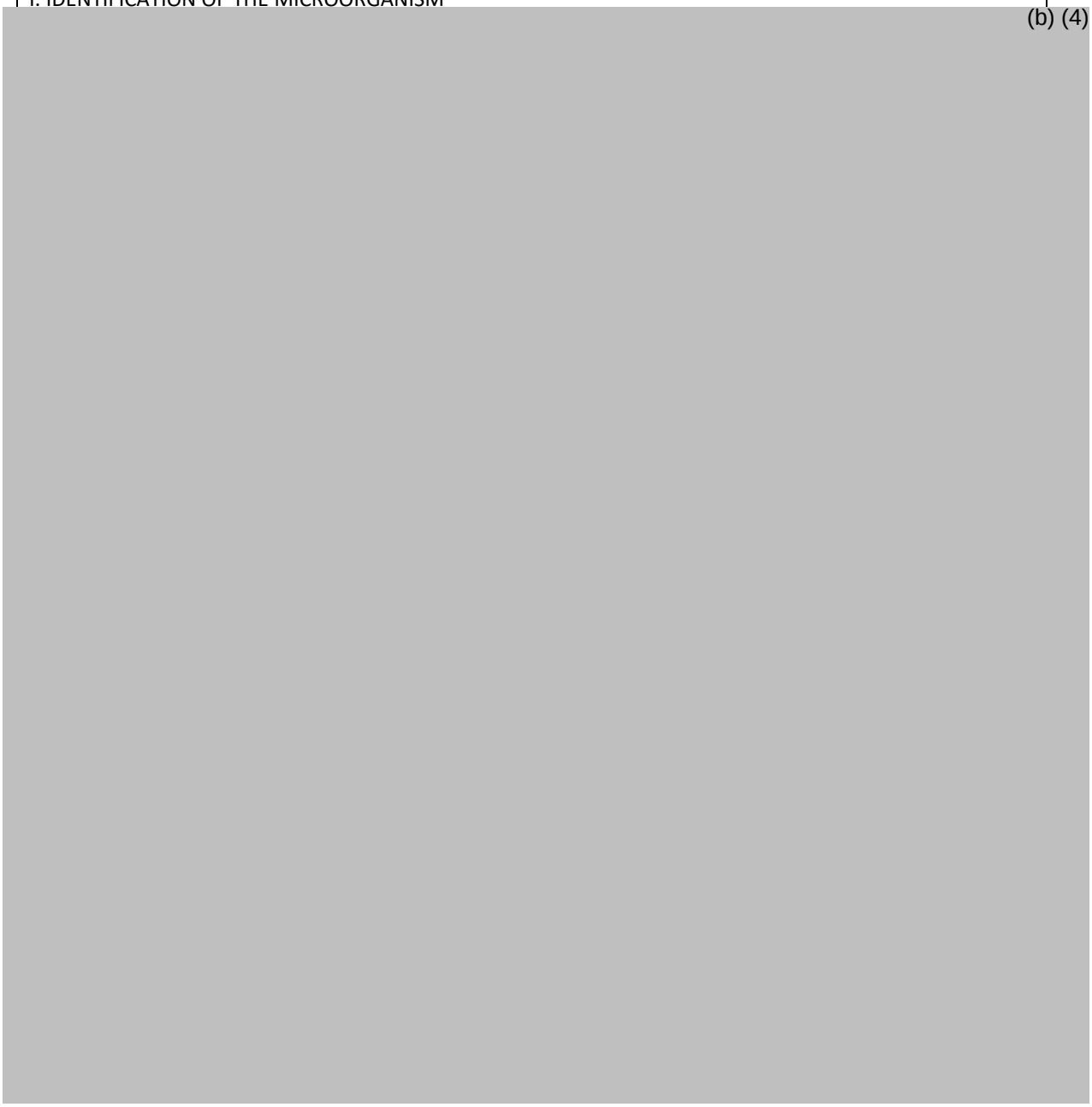
LESAFFRE ET COMPAGNIE
41 RUE ETIENNE MARCEL
75001 PARIS

NAME AND ADDRESS
OF THE DEPOSITOR

RECEIPT IN THE CASE OF INITIAL DEPOSIT
issued pursuant to rule 7.1 by
THE INTERNATIONAL DEPOSITARY AUTHORITY
indicated at the foot of this page

I. IDENTIFICATION OF THE MICROORGANISM

(b) (4)



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ICS 67.180.10

Classification No.: X34

Filing No.: (b) (4)

Light Industry Standard of the People's Republic of China

(b) (4)

Cane molasses

Issued on 2005-03-19 **Implemented on 2005-09-01**

Issued by National Development and Reform Commission of the People's Republic of China

Foreword

This standard was proposed by the (b) (4)

This standard is under the jurisdiction of Subcommittee on Sugar Production of National Technical Committee 64 on Food Industry of Standardization Administration of China.

This standard was drafted by the (b) (4)
(b) (4)

The main drafters of this standard are as follows: (b)(6)
(b)(6)

This standard was first issued.

(b) (4)

Cane molasses

1 Scope

This standard specifies the requirements, test methods, inspection rules, transportation and storage of cane molasses.

This standard applies to cane molasses which is the final molasses separated from sugar paste and used as raw materials for the production of alcohol, yeast, monosodium glutamate and other products.

2 Normative References

The following normative documents contain provisions which, through reference in this text, constitute provisions of this standard. For dated references, subsequent amendments (excluding the corrections), or revisions, of any of these publications do not apply to this standard. However, parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies.

GB/T 4789.1 Microbiological examination of food hygiene-General guidelines

GB/T 4789.2 Microbiological examination of food hygiene-Aerobic plate count

GB/T 5009.13 Determination of copper in foods

3 Requirements

3.1 Sensory requirements

(b) (4)

See Table 1 for quality requirements.

Table 1 Quality requirements

Items		Indicators
Total sugar (sucrose content+reducing sugar content)/%	≥	(b) (4)
Purity (total sugar/refractive Brix)/%	≥	
Acidity	≤	
Total ash (sulfated ash)/%	≤	
Copper (as Cu)/(mg/kg)	≤	
Aerobic bacterial count/(cfu/g)	≤	
Note: When the total reducing sugar and purity are lower than the above indicator values, and the acidity, total ash and aerobic bacterial count are higher than the above indicator values, if the buyer and the seller still need to trade, a detailed contract can be drawn up and the price will be based on quality.		

4 Test Methods

4.1 Total sugar

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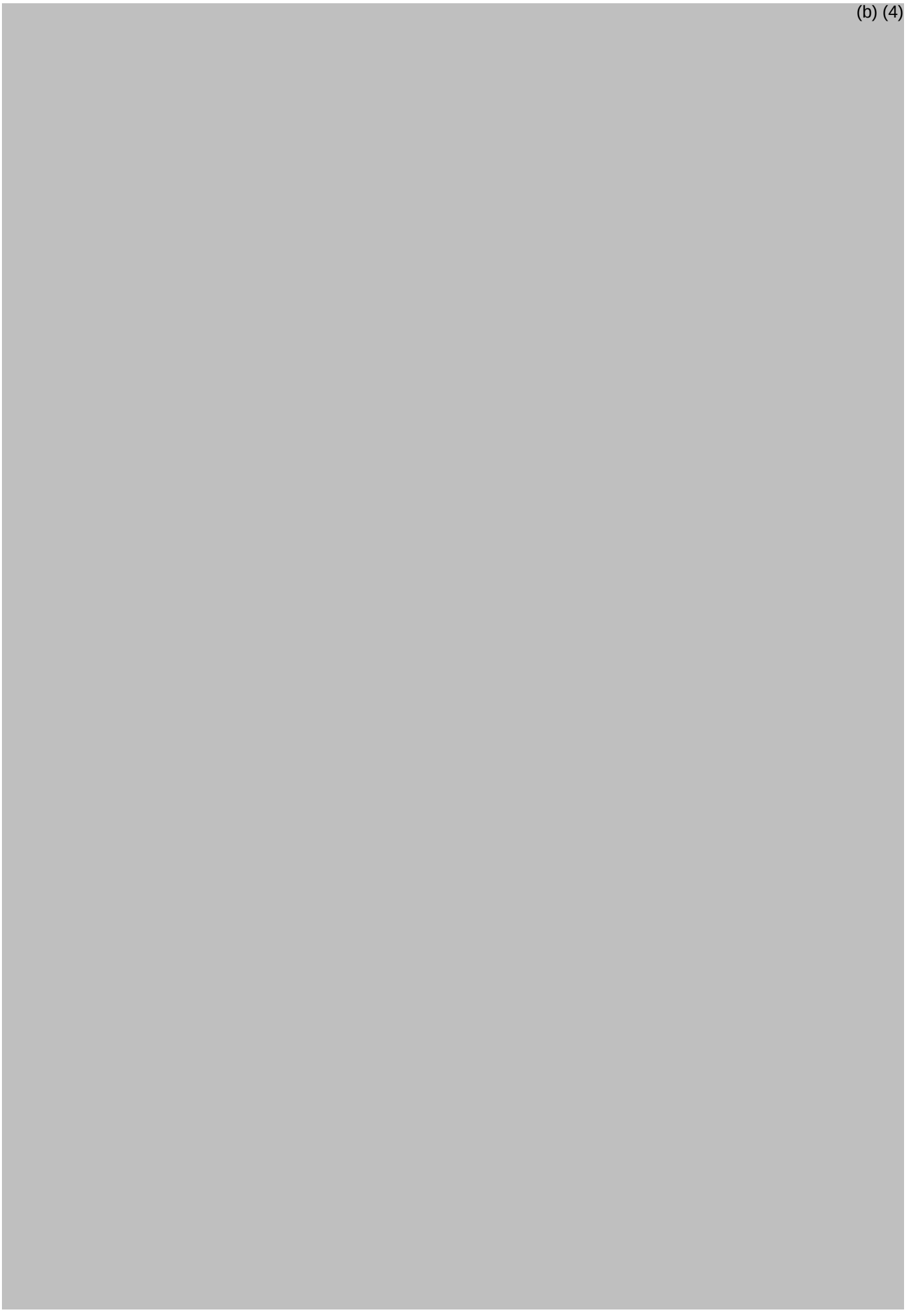
(b) (4)



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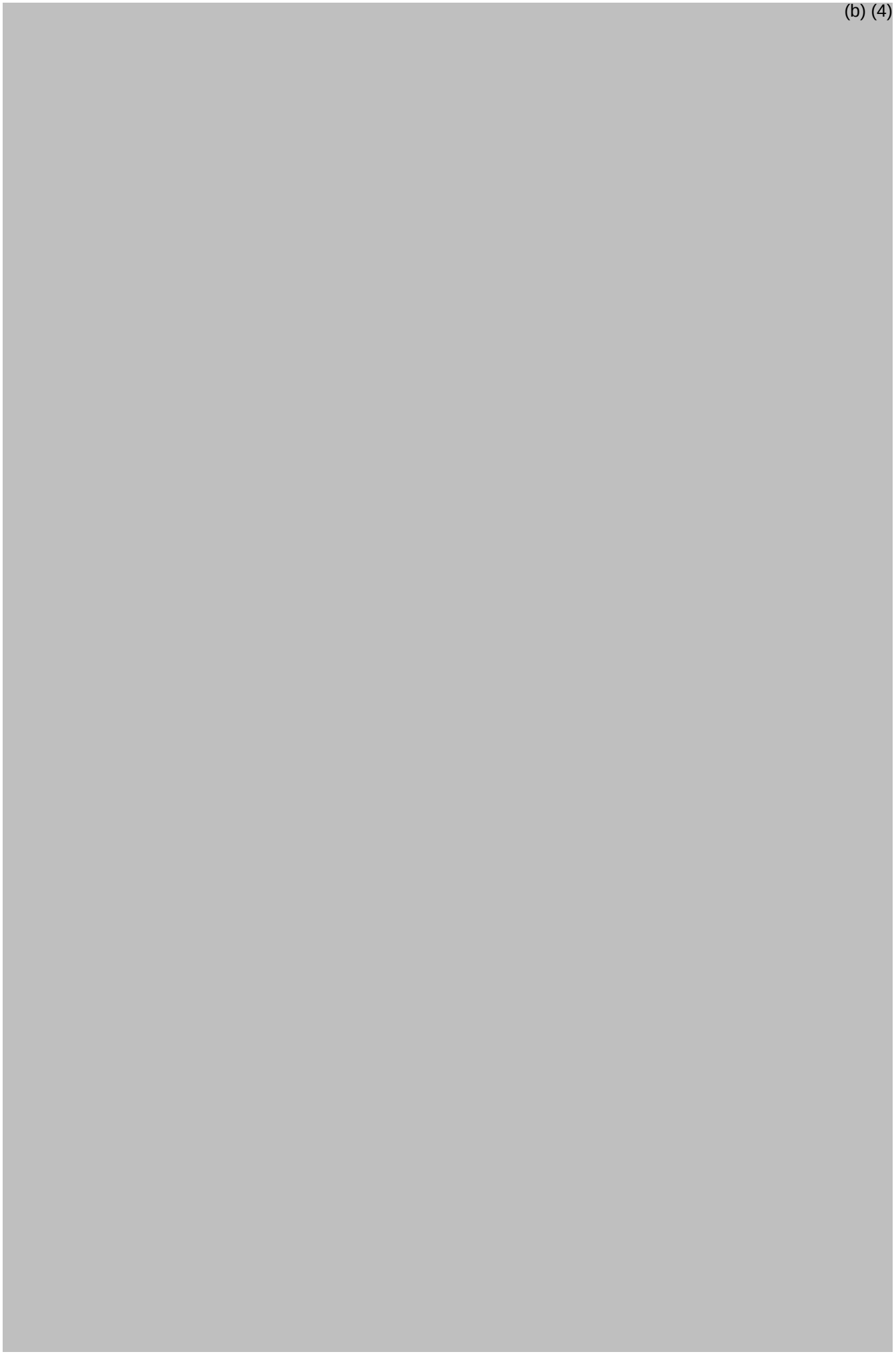

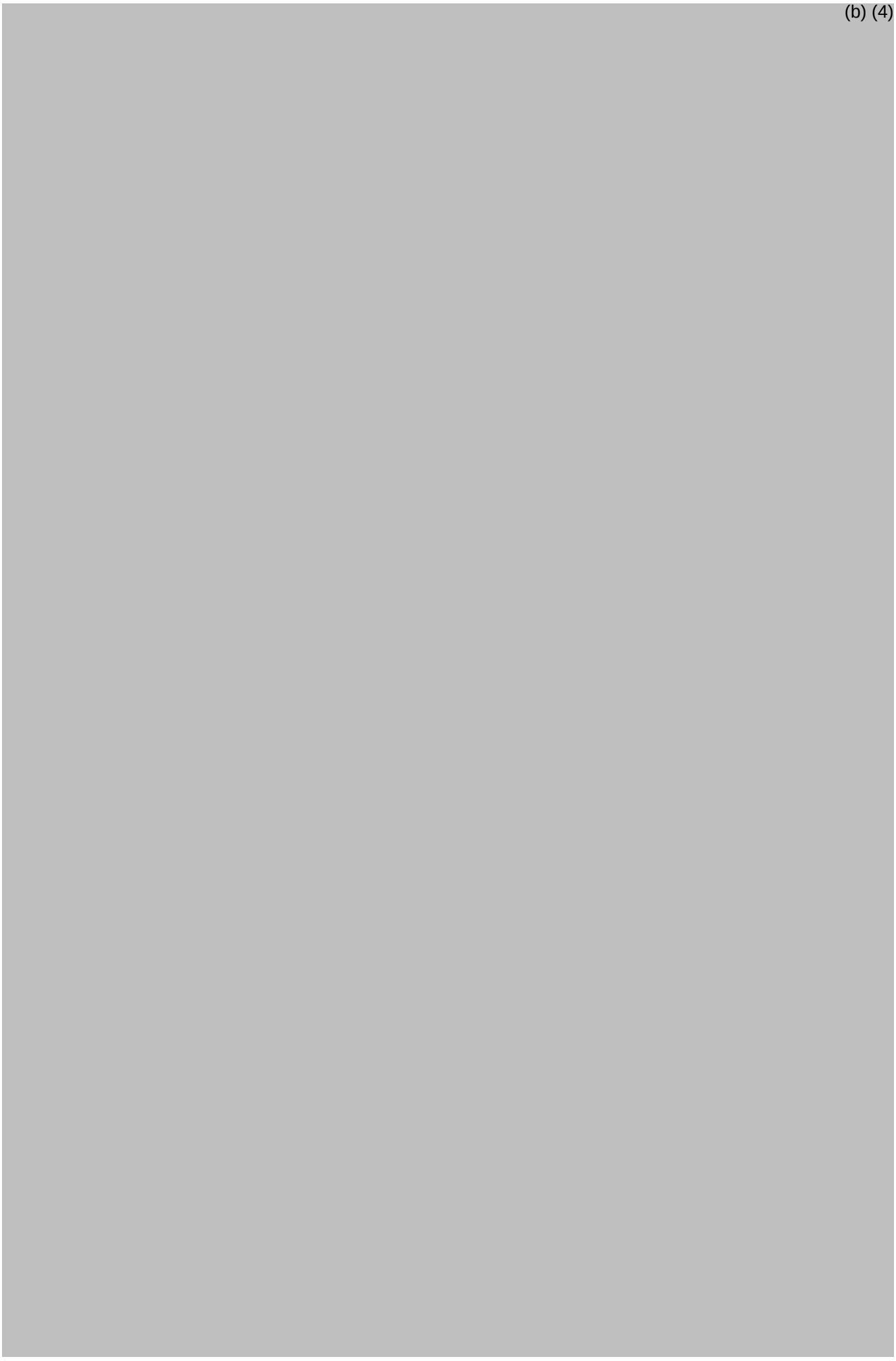
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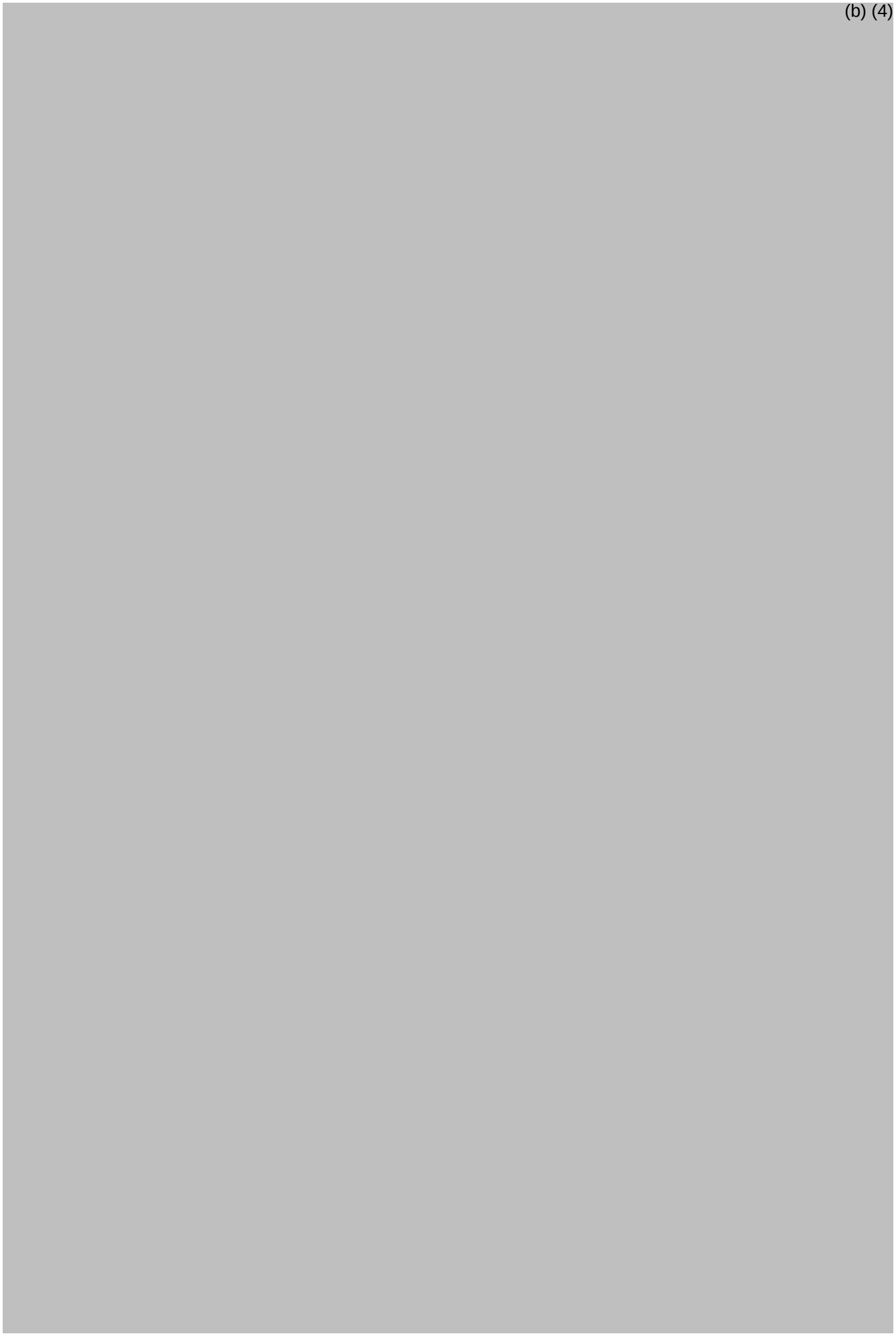

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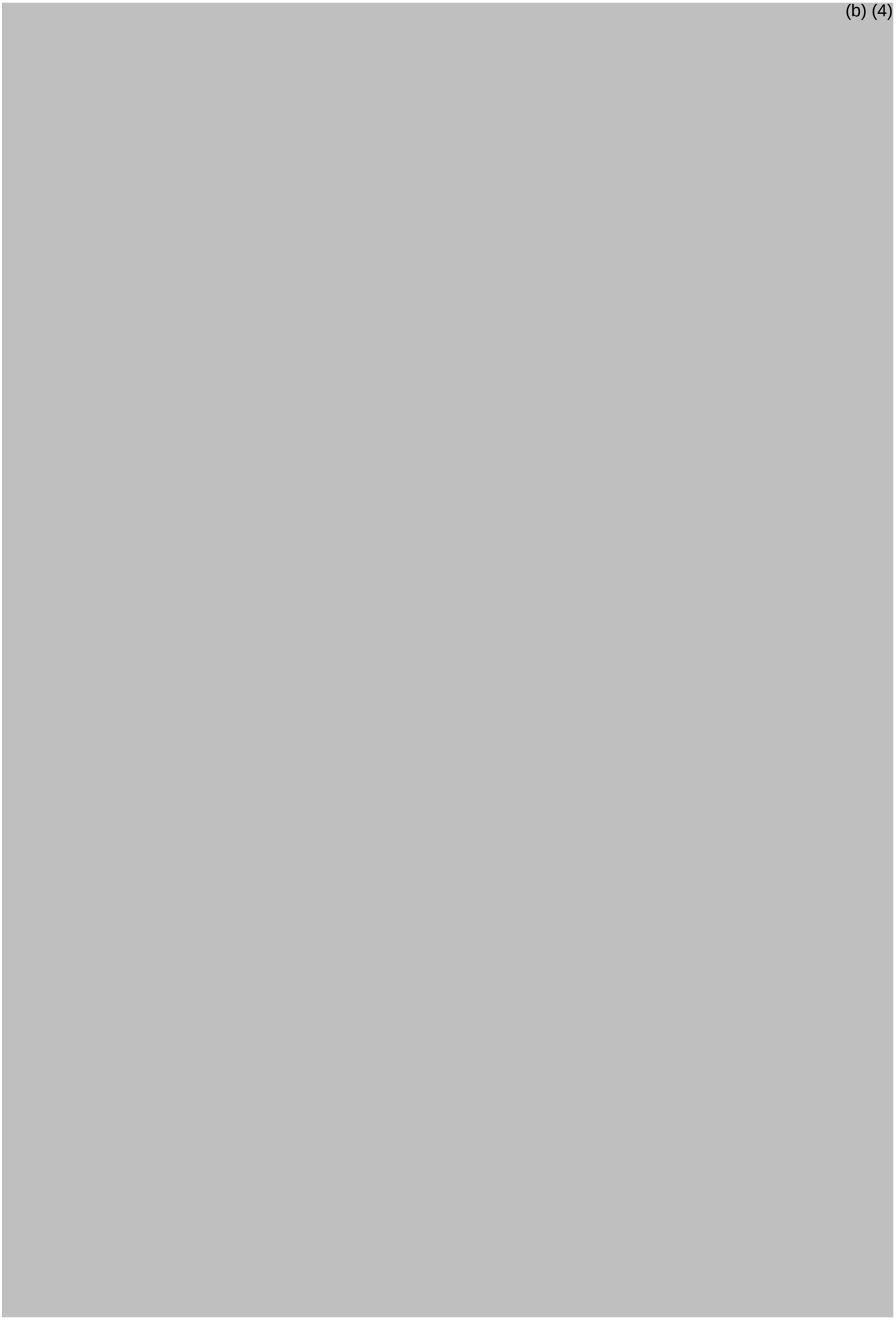
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National Food Safety Standard Food Additive Phosphoric Acid

食品安全国家标准 食品添加剂 磷酸

Competent authority: National Health and Family Planning Commission of the People's Republic of China (NHFPC)

(b) (4)

Date of Publication: Sep. 22, 2015
Date of Implementation: Mar. 22, 2016

(b) (4)

Disclaimer

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National Standard of the People's Republic of China

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National Food Safety Standard

Food Additive Phosphoric Acid

食品安全国家标准

食品添加剂 磷酸

Date of Publication: Sep. 22, 2015 Date of Implementation: Mar. 22, 2016

Issued by:

(b) (4)

Preface

This standard replaces (b) (4) "Food additive Phosphoric acid".

Compared with (b) (4), the main changes of this standard are as follows:

——the standard name has been modified to "National food safety standard – Food additive Phosphoric acid".

(b) (4)

(b) (4)

National Food Safety Standard
Food Additive Phosphoric Acid

1. Scope

This standard is applicable to the food additive phosphoric acid produced by thermal process.

2. Molecular Formula and Relative Molecular Mass

2.1 Molecular formula

H_3PO_4

2.2 Relative molecular mass

97.99 (According to atomic weights of the elements 2007)

3. Technical Requirements

3.1 Sensory requirements

It shall comply with the provisions in Table 1.

Table 1 Sensory requirements

Items	Requirements	Test methods
Color	(b) (4)	
State		

3.2 Physicochemical indicators

(b) (4)

It shall comply with the provisions in Table 2.

Table 2 Physicochemical indicators

Items	Indicators	Test methods
Phosphoric acid (H_3PO_4) content, *w*/%	(b) (4)	A.4 in Annex A
Fluoride (as F)/(mg/kg) ≤		A.5 in Annex A
Readily oxidized substances (as H_3PO_3), *w*/% ≤		A.6 in Annex A
Arsenic (As)/(mg/kg) ≤		A.7 in Annex A
Heavy metals (as Pb)/(mg/kg) ≤		A.8 in Annex A

(b) (4)

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Annex A
Test methods

A.1 Safety Instructions

As some reagents used in the test methods are toxic, corrosive and inflammable, the operator should be quite careful. If splashed on the skin, flush immediately with water. Seek medical attention immediately in severe cases. When using inflammable substances, heating with open fire is strictly prohibited.

A.2 General Provisions

Unless otherwise specified, the reagents and water used in this standard all refer to the analytically pure reagents and the Grade 3 water stipulated in GB/T 6682. The standard solutions, standard solutions for impurity, preparations and products used in the test, unless otherwise specified, shall be prepared according to the provisions of GB/T 601, GB/T 602 and GB/T 603. The solutions used in the test all refer to aqueous solution when there is no indication on the solvents used for preparation.

A.3 Identification Test

A.3.1 Reagents and materials

(b) (4)

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(b) (4)

A.4 Determination of Phosphoric Acid (H_3PO_4) Content

A.4.1 Gravimetric method (arbitration method)

A.4.1.1 Method summary

(b) (4)

A.4.1.2 Reagents and materials

A.4.1.2.1 Hydrochloric acid

(b) (4)

A.4.1.3 Apparatus and equipment

(b) (4)

(b) (4)

(b) (4)

A.4.1.4 Analysis procedure

A.4.1.4.1 Preparation of test solution

(b) (4)

A.4.1.4.2 Preparation of blank test solution

(b) (4)

A.4.1.4.3 Determination

(b) (4)

(b) (4)

(b) (4)

A.4.1.5 Result calculation

(b) (4)

A.4.2 Volumetric method

A.4.2.1 Method summary

(b) (4)

A.4.2.2 Reagents and materials

(b) (4)

(b) (4)

A.4.2.3 Analysis procedure

(b) (4)

A.4.2.4 Result calculation

(b) (4)

A.5 Determination of Fluoride (as F)

A.5.1 Method summary

(b) (4)

(b) (4)

(b) (4)

A.5.2 Reagents and materials

(b) (4)

(b) (4)

(b) (4)

A.5.4.2 Preparation of test solution

(b) (4)

A.5.4.3 Determination

(b) (4)

(b) (4)

A.5.5 Result calculation

(b) (4)

A.6 Determination of Readily Oxidized Substances (as H_3PO_3)

A.6.1 Method summary

(b) (4)

A.6.2 Reagents and materials

(b) (4)

(b) (4)

(b) (4)

A.6.2.5 Ferroin indicator solution

A.6.3 Analysis procedure

(b) (4)

(b) (4)

(b) (4)

A.7 Determination of Arsenic (As)

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A.7.1.4.2 Detemination

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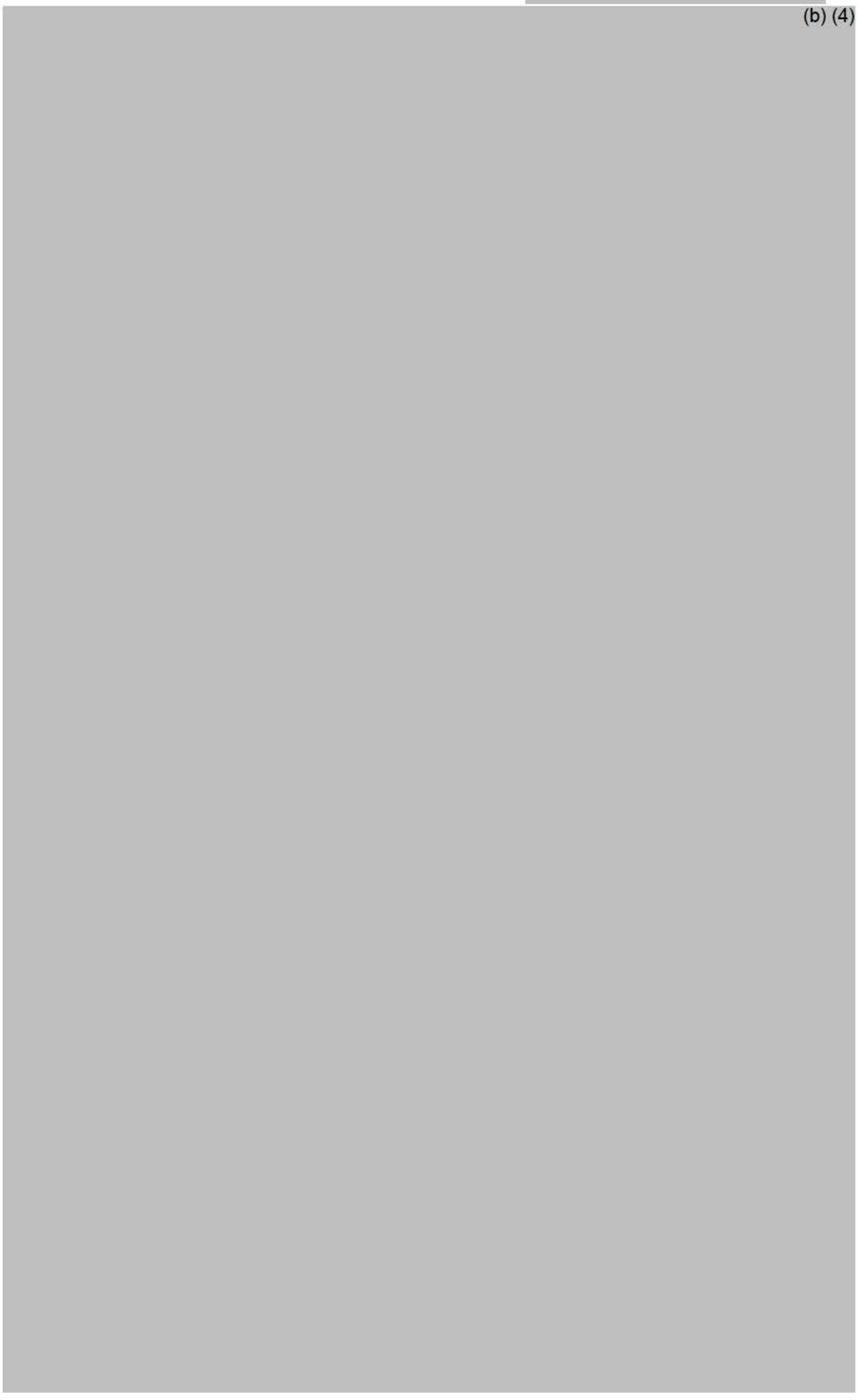
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A.8.2 Reagents and materials

(b) (4)

A.8.3 Analysis procedure

A.8.3.1 Preparation of test solution

(b) (4)

A.8.3.2 Determination

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A.8.4 Result calculation

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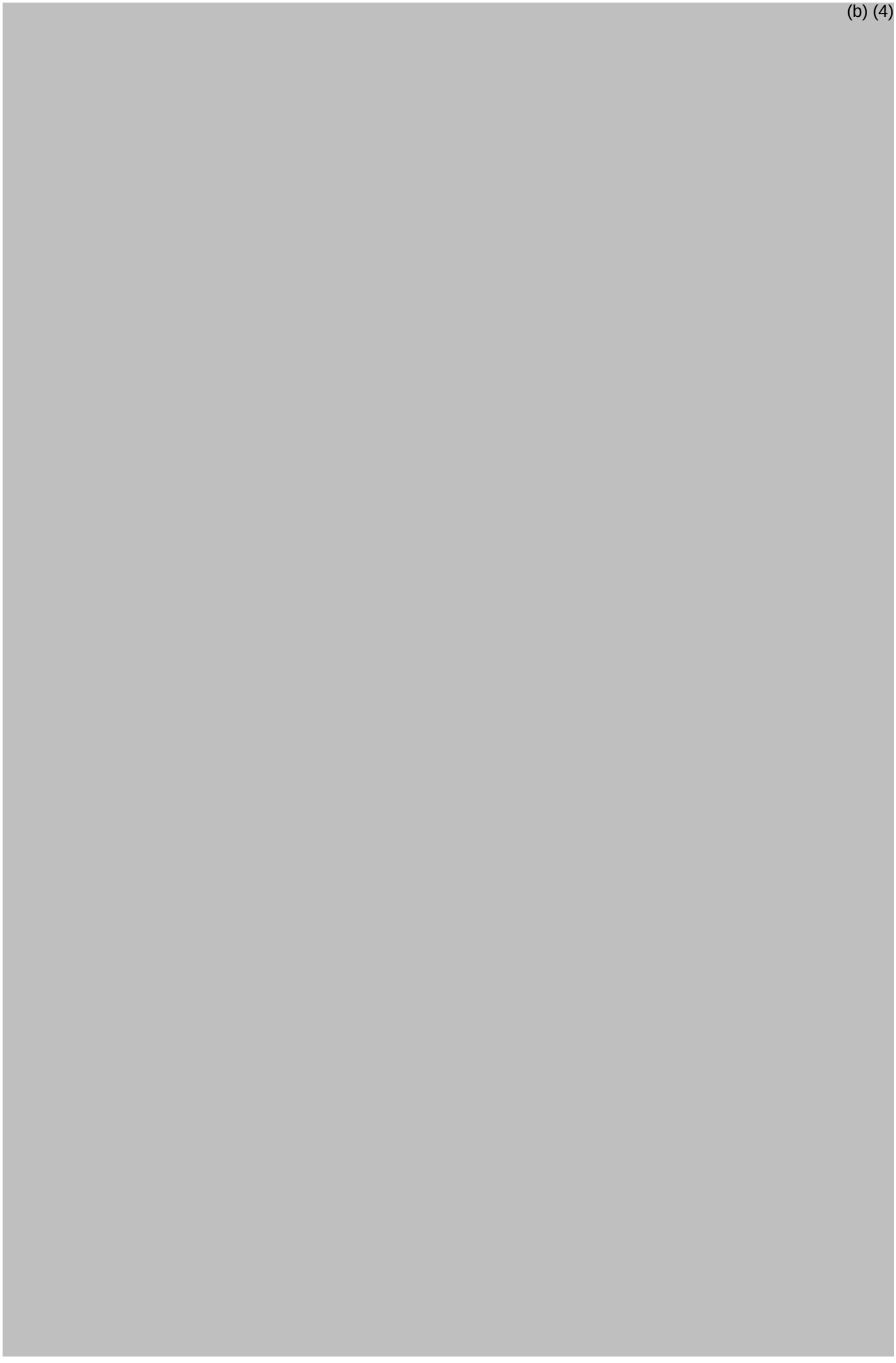

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National Standard of the People's Republic of China

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National food safety standard
Food additive Ammonia water

Issued on 2012-12-25 **Implemented on 2013-01-25**

Issued by Ministry of Health of the People's Republic of China

(b) (4)

National food safety standard
Food additive Ammonia water

1 Scope

This standard applies to the food additive ammonia water produced with liquid ammonia as raw material.

2 Chemical Name, Molecular Formula and Relative Molecular Mass

2.1 Chemical name

Ammonium hydroxide

2.2 Molecular formula

$NH_3 \cdot H_2O$

2.3 Relative molecular mass

33.05 (according to the international relative atomic mass in 2007)

3 Technical Requirements

3.1 Sensory requirements: it shall comply with the provisions of Table 1.

Table 1 Sensory requirements

Items	Requirements	Test methods
Color	(b) (4)	Take an appropriate amount of test sample and place in a 50-mL beaker, and observe the color and state under natural light.
State		

3.2 Physicochemical indicators: it shall comply with the provisions of Table 2.

Table 2 Physicochemical indicators

Items		Indicators	Test methods
Ammonia (NH_3) content, *w*/%		(b) (4)	A.4 in Annex A
Lead (Pb)/(mg/kg)	≤		A.5 in Annex A
Residue on evaporation, *w*/%	≤		A.6 in Annex A
Readily oxidizable substance			A.7 in Annex A

(b) (4)

Annex A

Test methods

A.1 Caution

Some reagents used in the test methods of this standard are toxic or corrosive, so appropriate safety and protective measures shall be taken during operation thereof.

A.2 General Provisions

Unless otherwise specified, the reagents and water used in this standard all refer to the analytically pure reagents and Grade 3 water specified in (b) (4) The standard volumetric solutions, standard solutions for impurity, preparations and products used in the test, without other requirements noted, shall be prepared according to the provisions of (b) (4) The solutions used in the test, without indication on the solvents used for preparation, shall refer to aqueous solution.

A.3 Identification Test

A.3.1 Reagents and materials

(b) (4)

A.3.2 Identification methods

(b) (4)

A.4 Determination of Ammonia (NH_3) Content

A.4.1 Method summary

(b) (4)

(b) (4)

(b) (4)

A.5 Determination of Lead

A.5.1 Reagents and materials

(b) (4)

Atomic absorption spectrophotometer: equipped with lead hollow cathode lamp.

A.5.3 Analysis procedures

A.5.3.1 Preparation of test solution

(b) (4)

A.5.3.2 Plotting of working curve

(b) (4)

A.5.3.3 Determination

(b) (4)

A.5.4 Result calculation

(b) (4)

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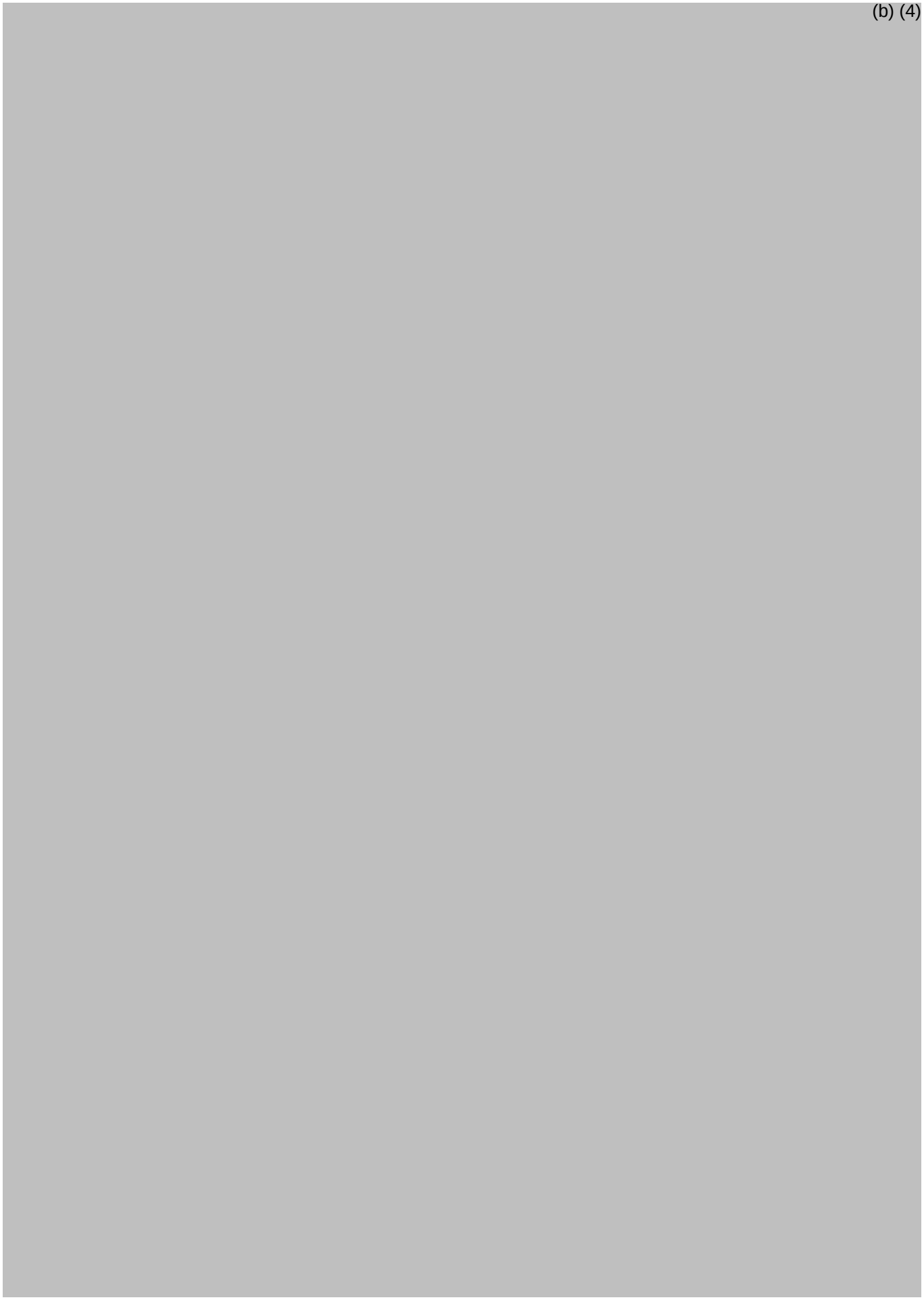
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National Food Safety Standard
Food Additive Potassium Chloride

食品安全国家标准
食品添加剂 氯化钾

Ministry of Health China (MOH)
(b) (4)

Release Date: 2010/12/21
Implementation Date: 2011/02/21

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National Standard of the People's Republic of China

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National Food Safety Standard

Food Additive Potassium Chloride

食品安全国家标准

食品添加剂 氯化钾

Date of Publication: Dec. 21, 2010 Date of Implementation: Feb. 21, 2011

Issued by:

Ministry of Health of the People's Republic of China

(b) (4)

Preface

Annex A of this standard is normative annex.

National Food Safety Standard
Food Additive Potassium Chloride

1. Scope

This standard applies to the food additive potassium chloride which is refined by the potassium chloride produced by rock salt carnallite or sea salt taking magnesium chloride and potassium chloride as main components.

2. Normative References

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies to this standard. For undated references, the latest edition of the referenced document (including any amendments) applies to this standard.

3. Molecular Formula and Relative Molecular Mass

3.1 Molecular formula

KCl

3.2 Relative molecular mass

74.55 (according to the international relative atomic mass in 2007)

4. Technical Requirements

4.1 Sensory requirements

Sensory requirements shall comply with provisions of Table 1.

Table 1 Sensory requirements

Items	Requirements	Test methods
Color	(b) (4)	Take appropriate amount of test sample, place in a 50mL beaker, and observe its color and structural state under natural light.
Structural state		

4.2 Physicochemical indicators

Physicochemical indicators shall comply with provisions of Table 2.

Table 2 Physicochemical indicators

Items	(b) (4)	Test methods
Potassium chloride (on dry basis), *w*/% ≥		A.4 in Annex A
Loss on drying, *w*/% ≤		A.5 in Annex A
pH value		A.6 in Annex A
Iodide and bromide		A.7 in Annex A
Sodium (Na), *w*/% ≤		A.8 in Annex A
Heavy metal (as Pb)/(mg/kg) ≤		A.9 in Annex A
Arsenic (as As)/(mg/kg) ≤		A.10 in Annex A

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Annex A
(Normative annex)
Test methods

A.1 Caution

(b) (4)

A.2 General Provisions

(b) (4)

A.3 Identification Test

A.3.1 Identification of potassium ion

(b) (4)

A.3.2 Identification of chloride ion

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A.4 Determination of Potassium Chloride

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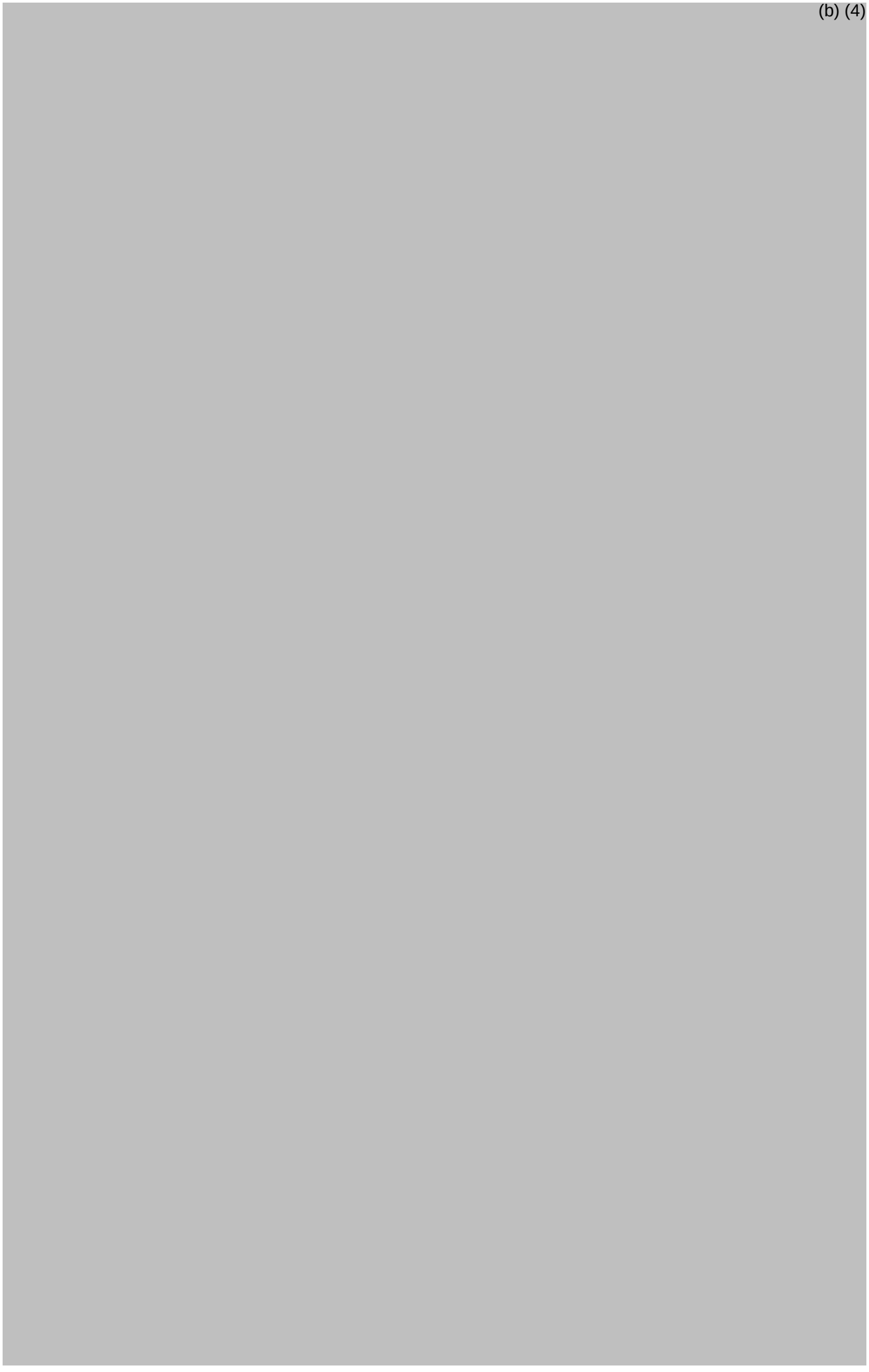

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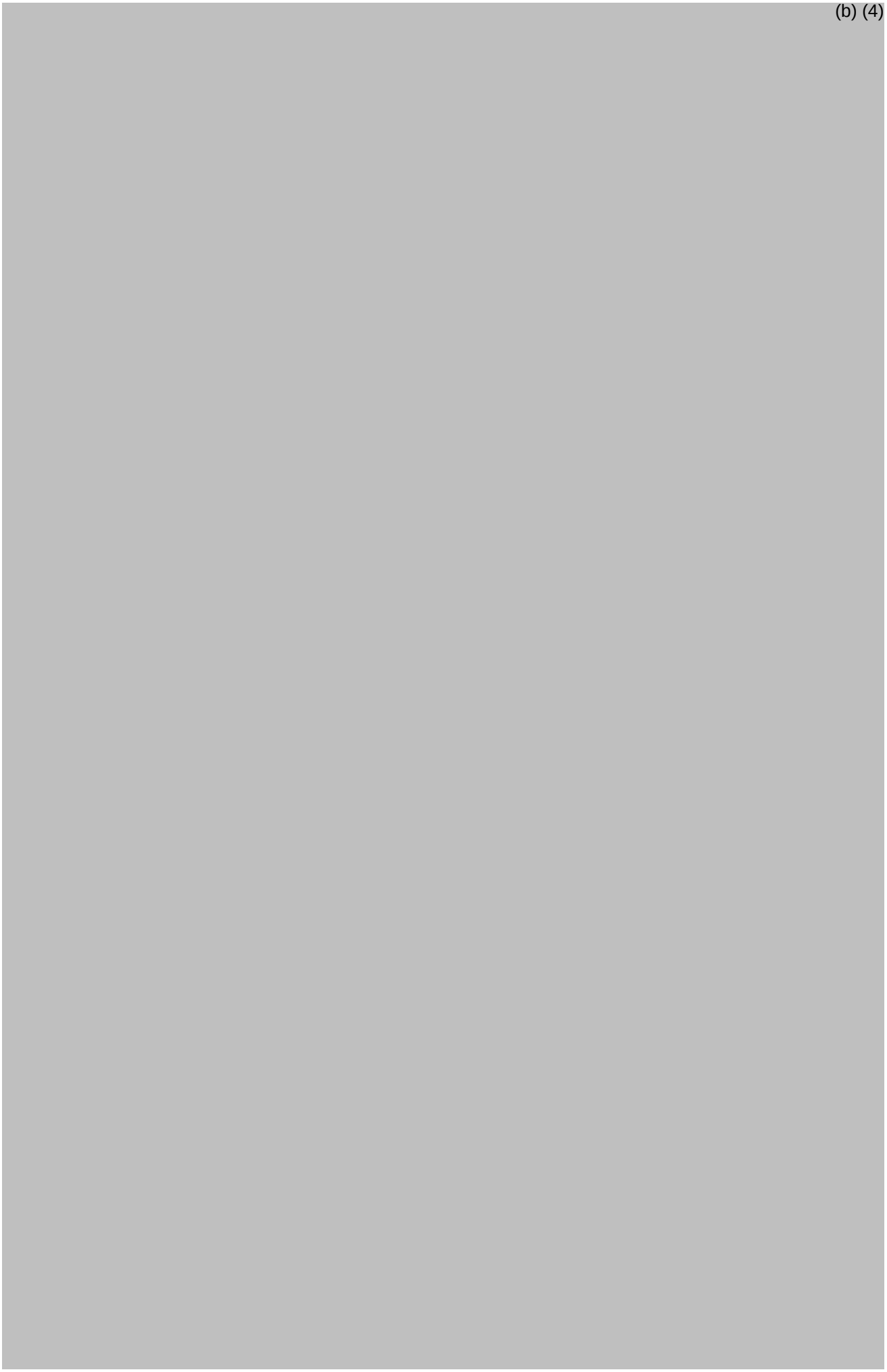

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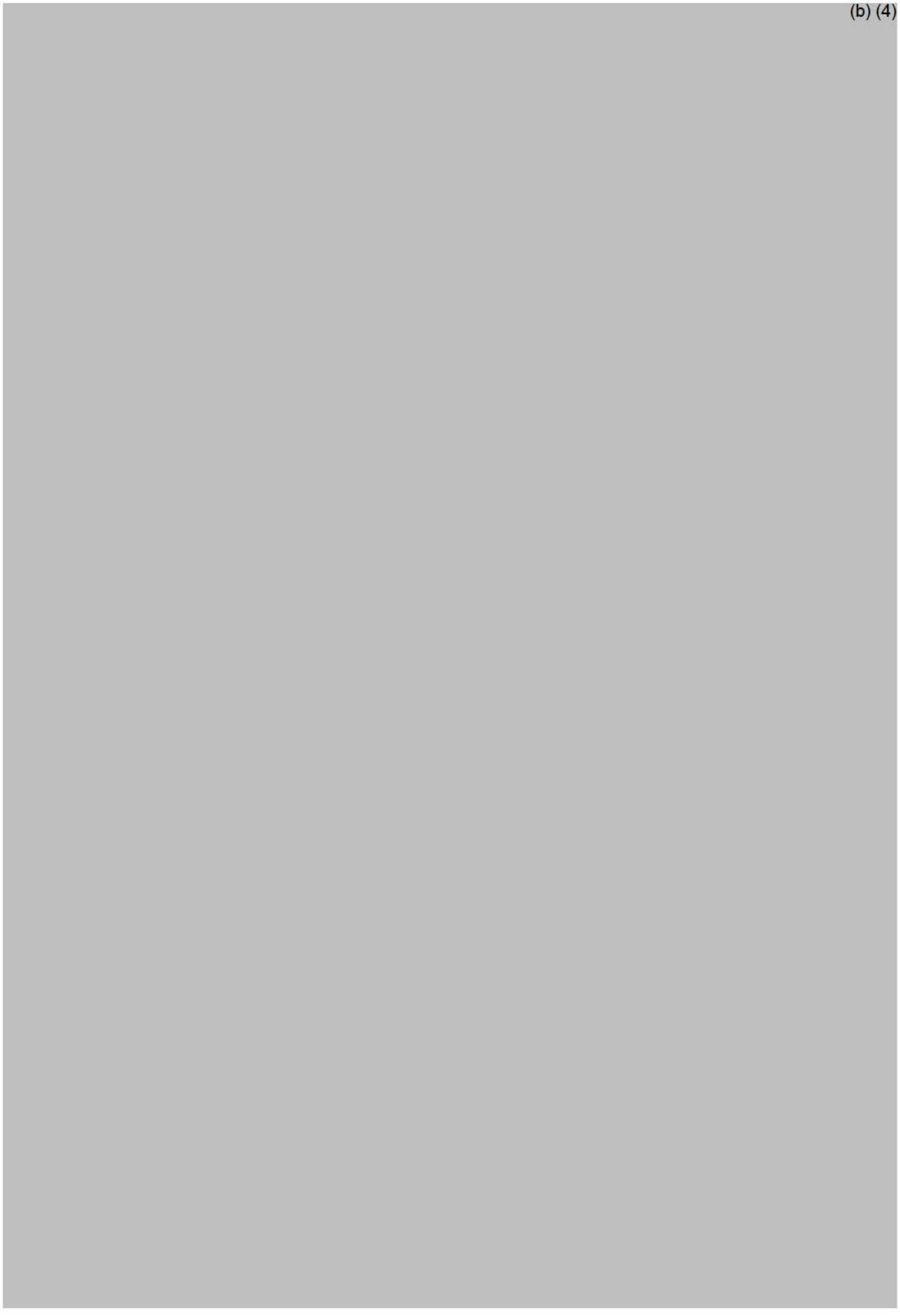
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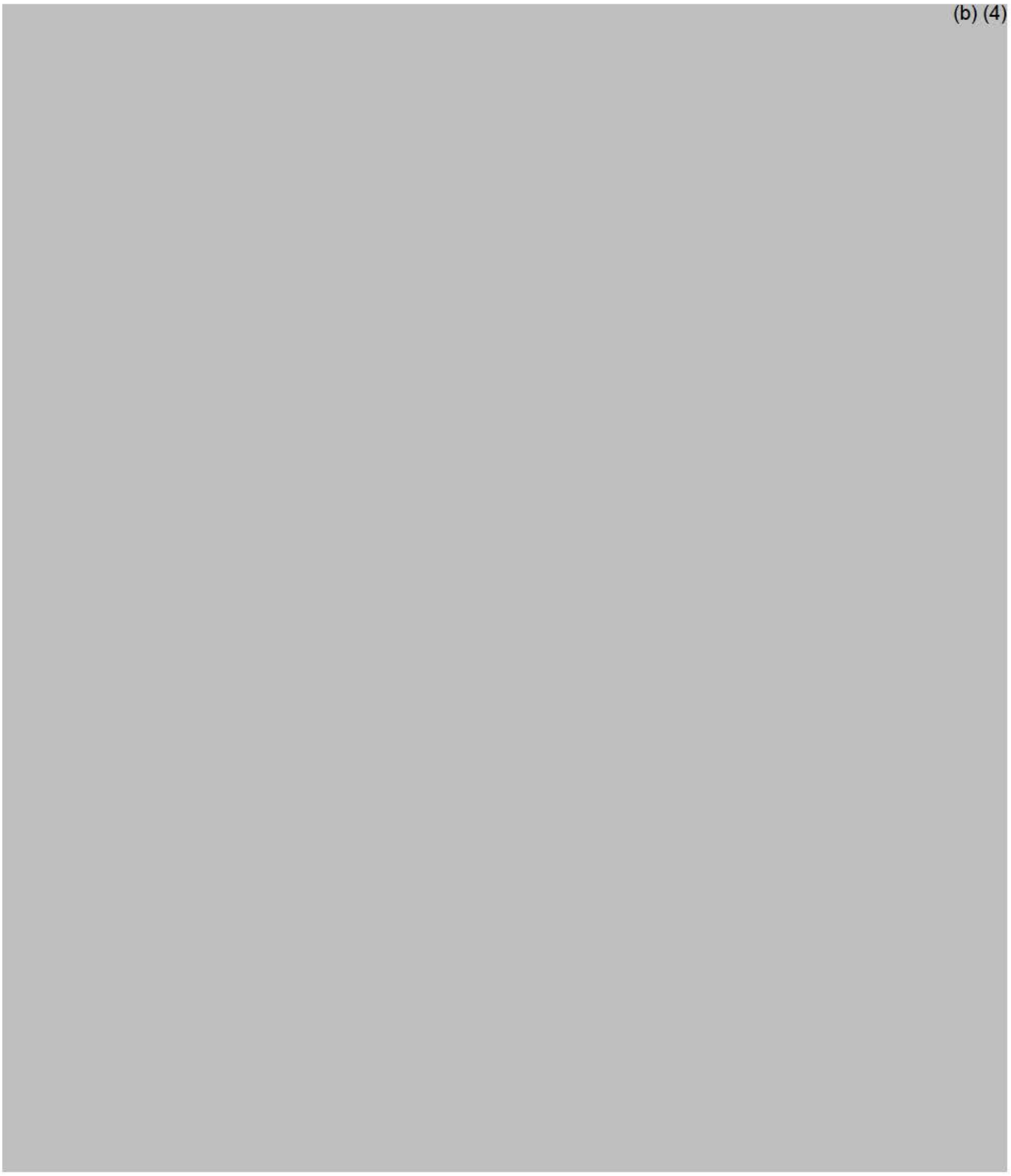
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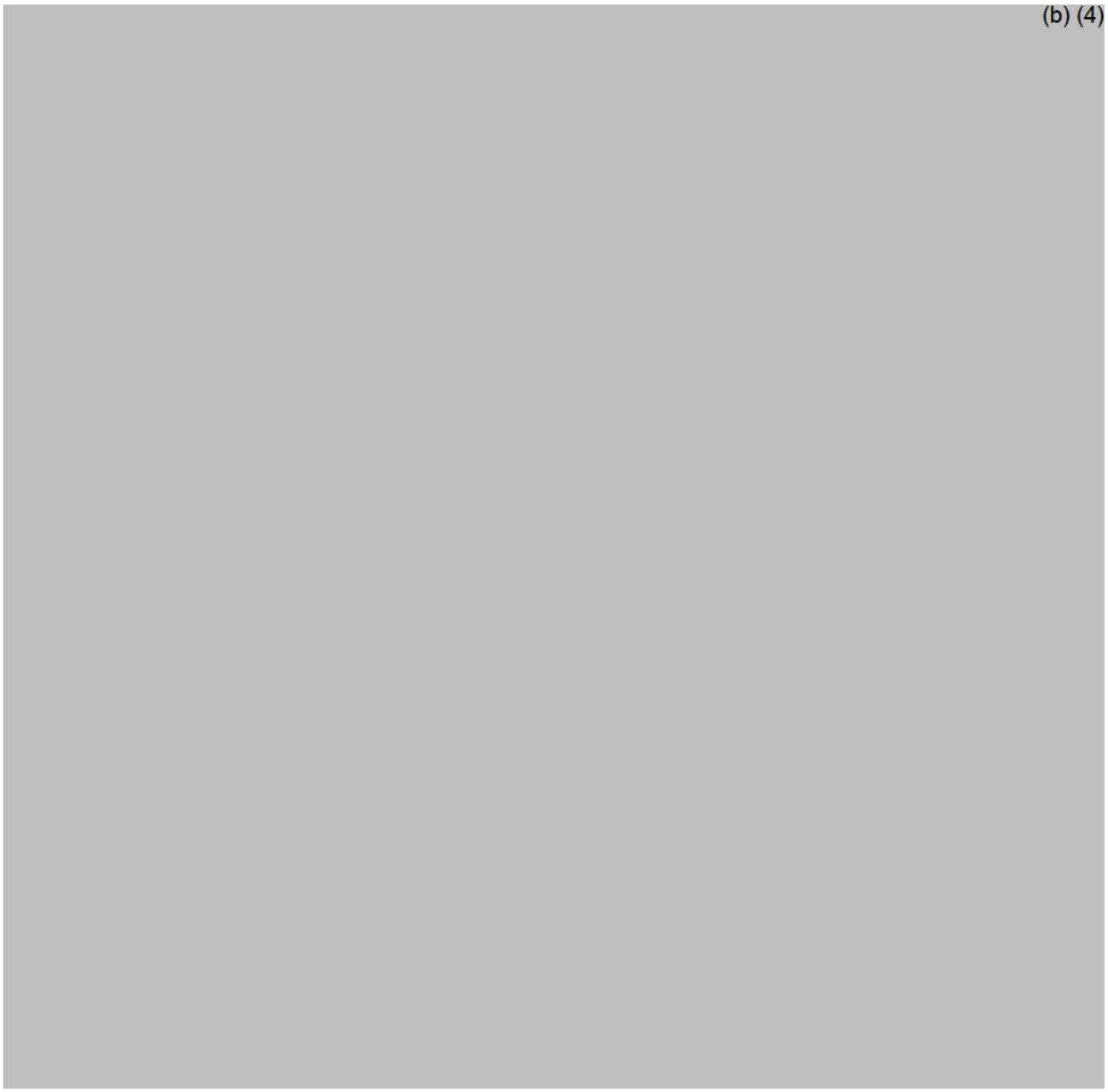
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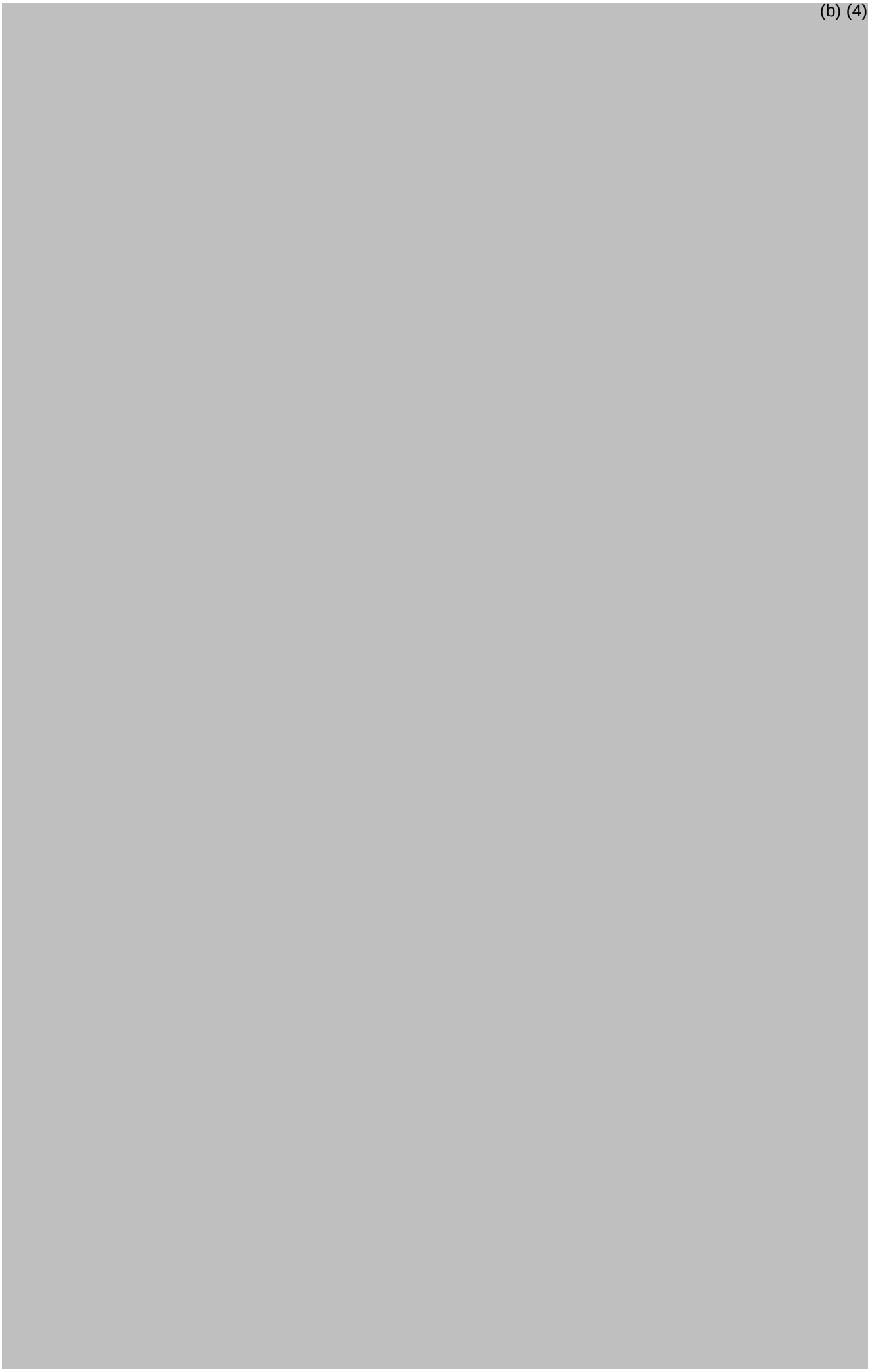

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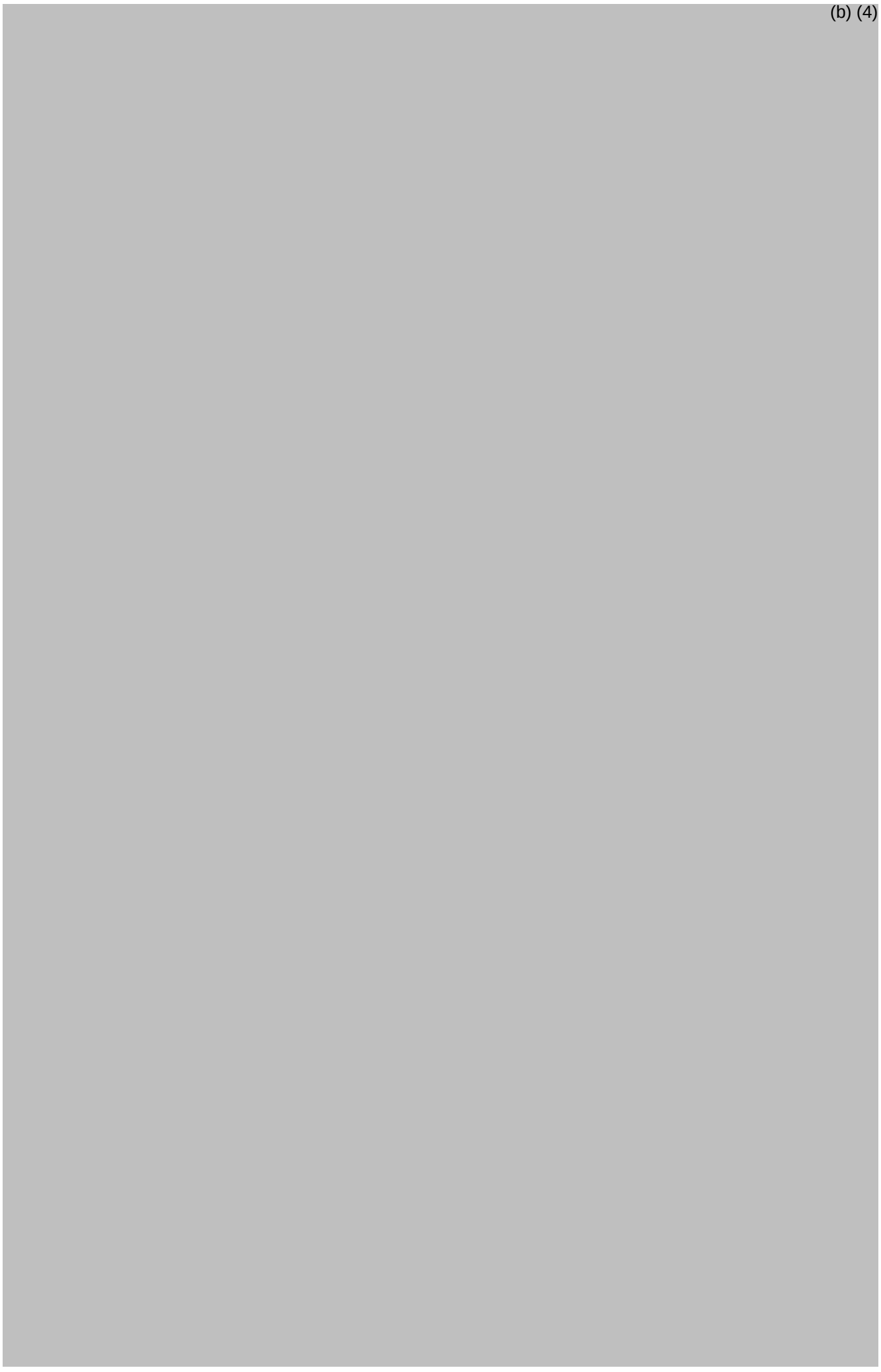
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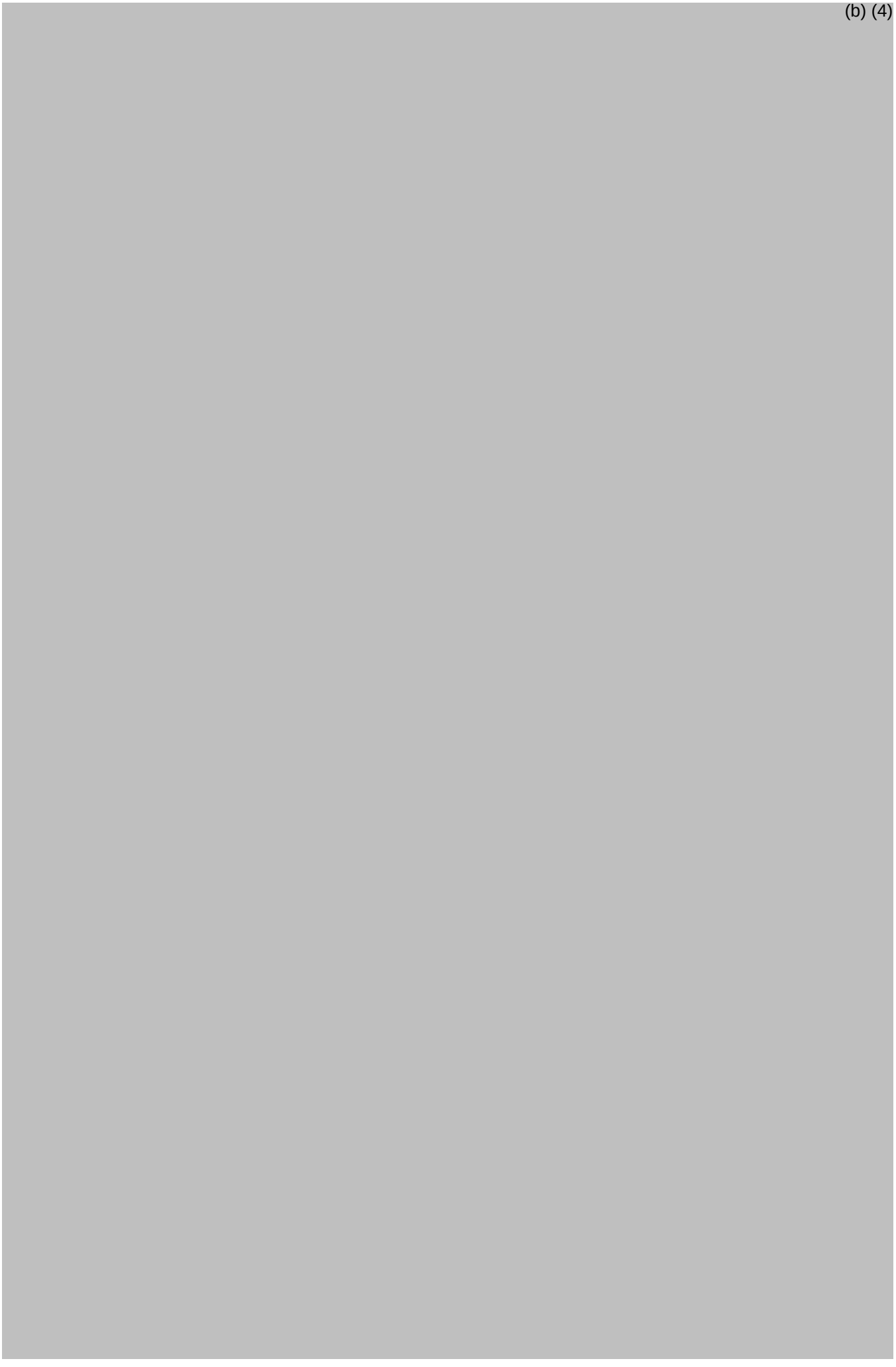

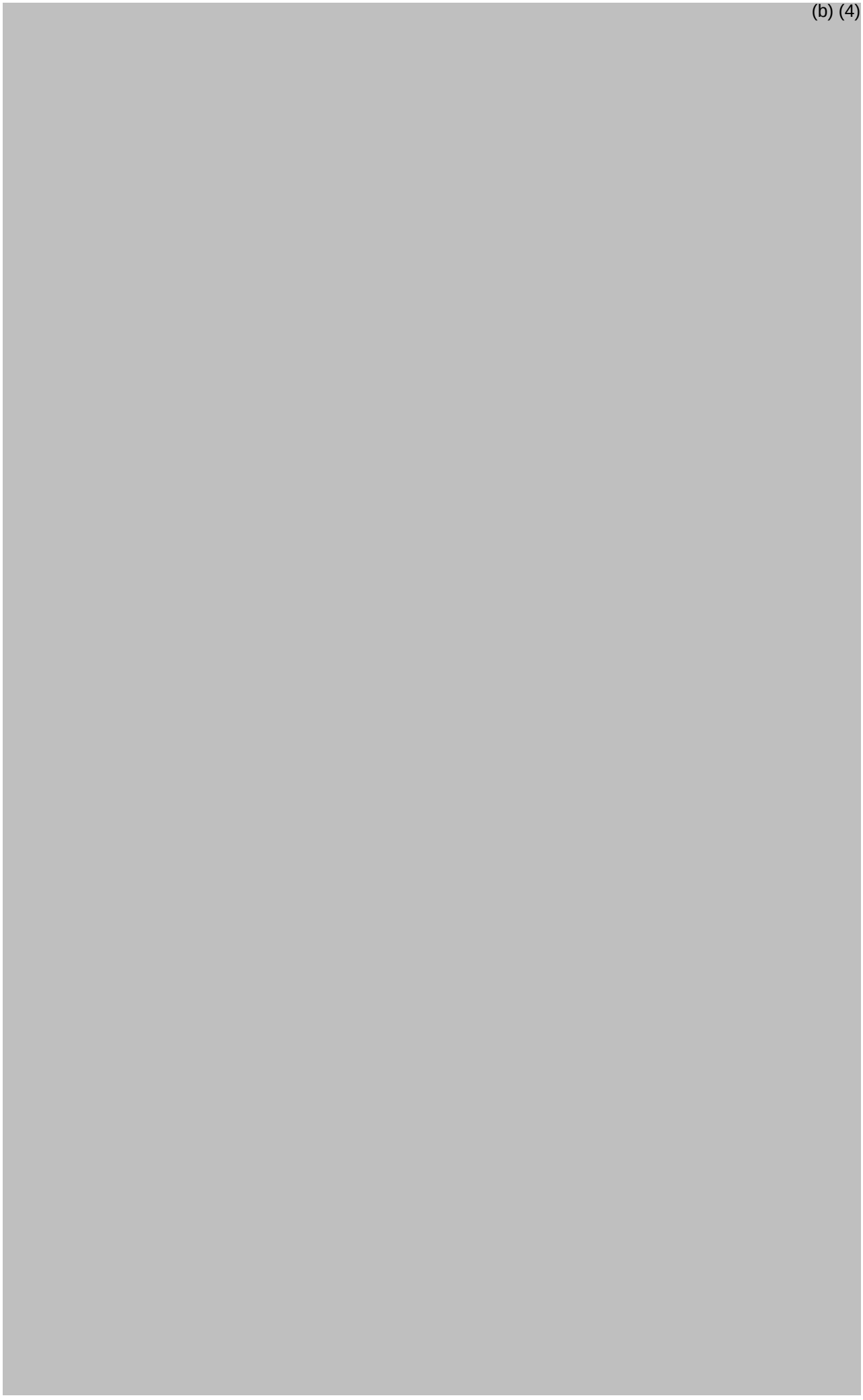
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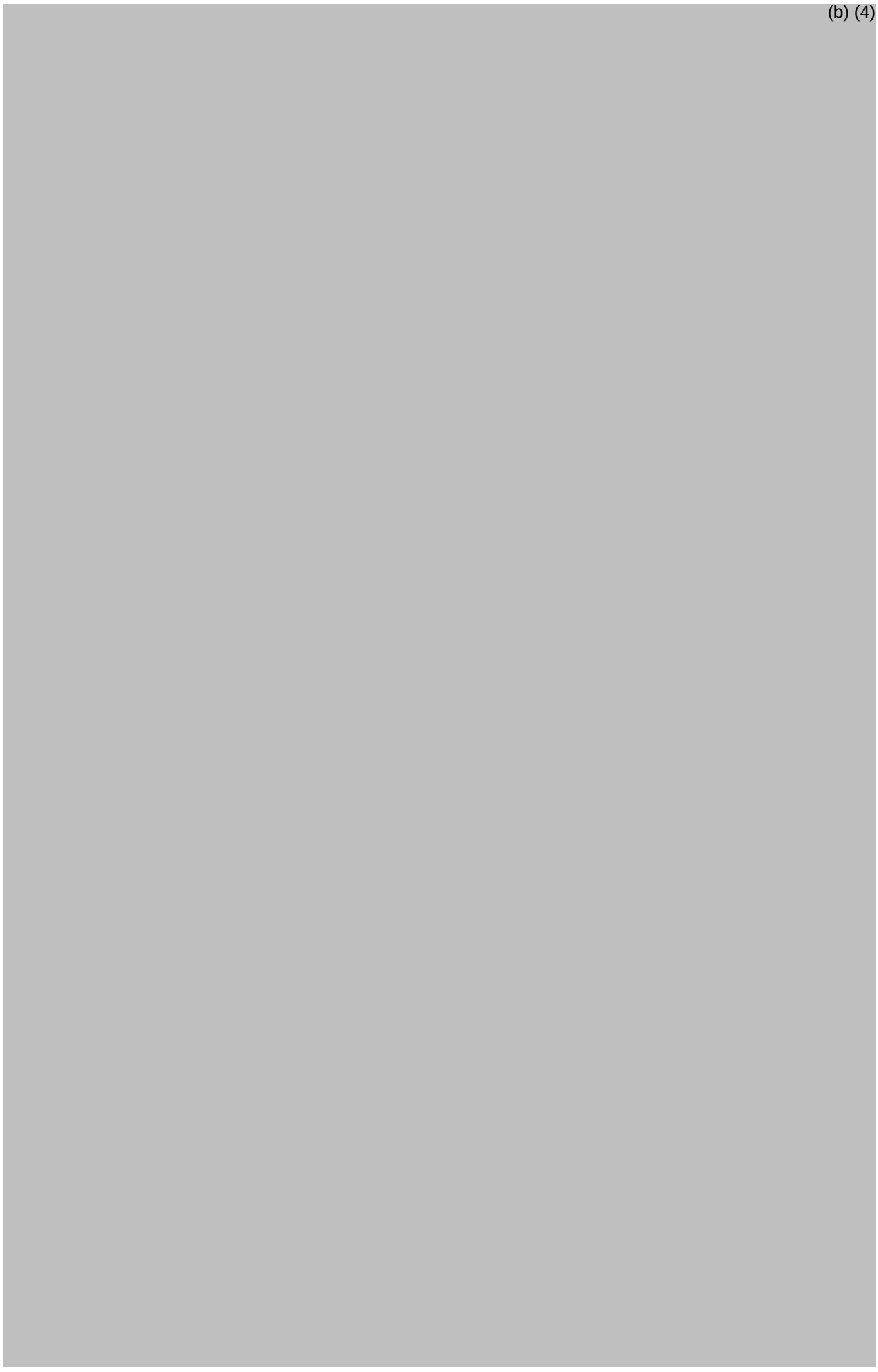

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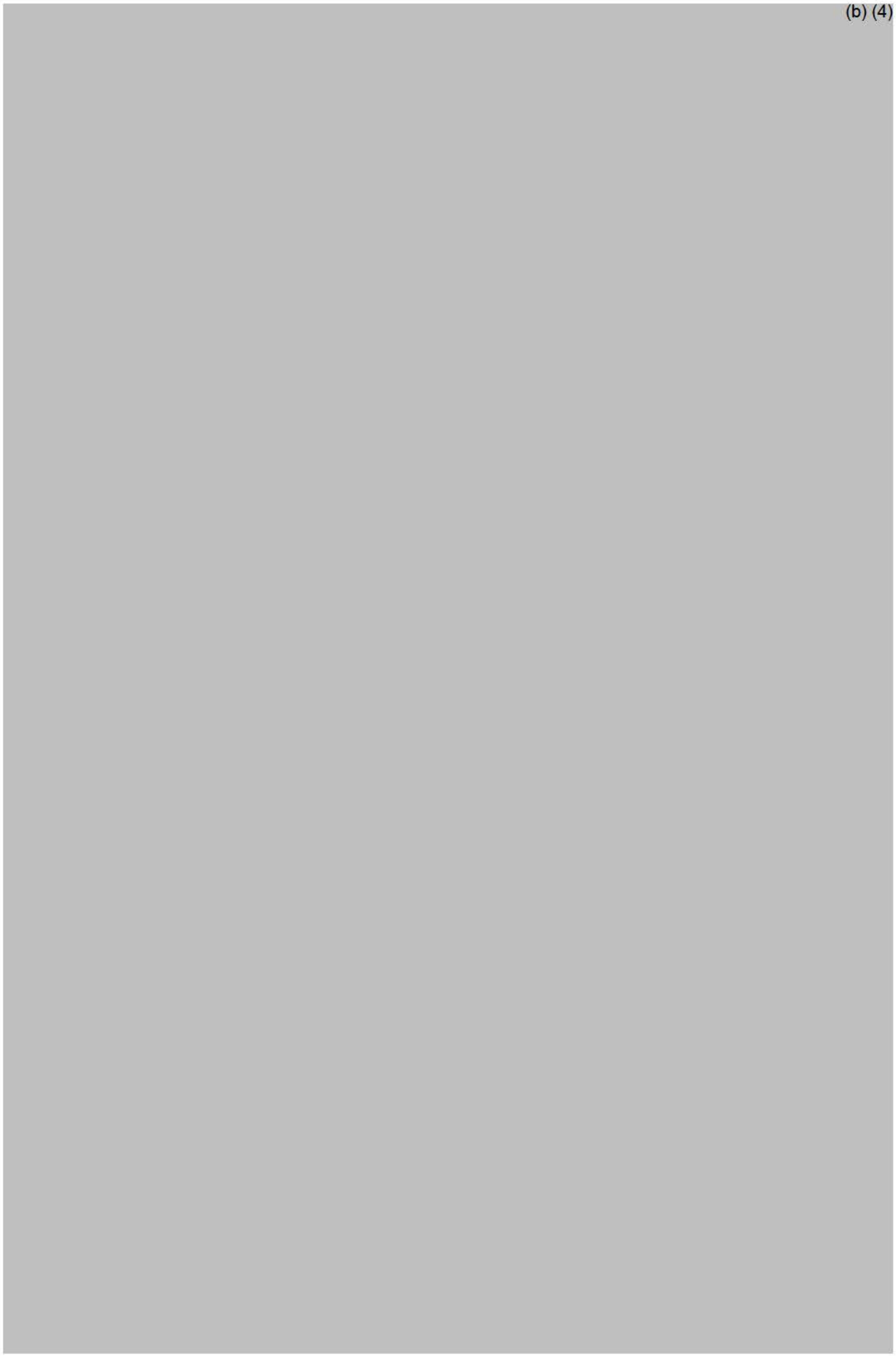


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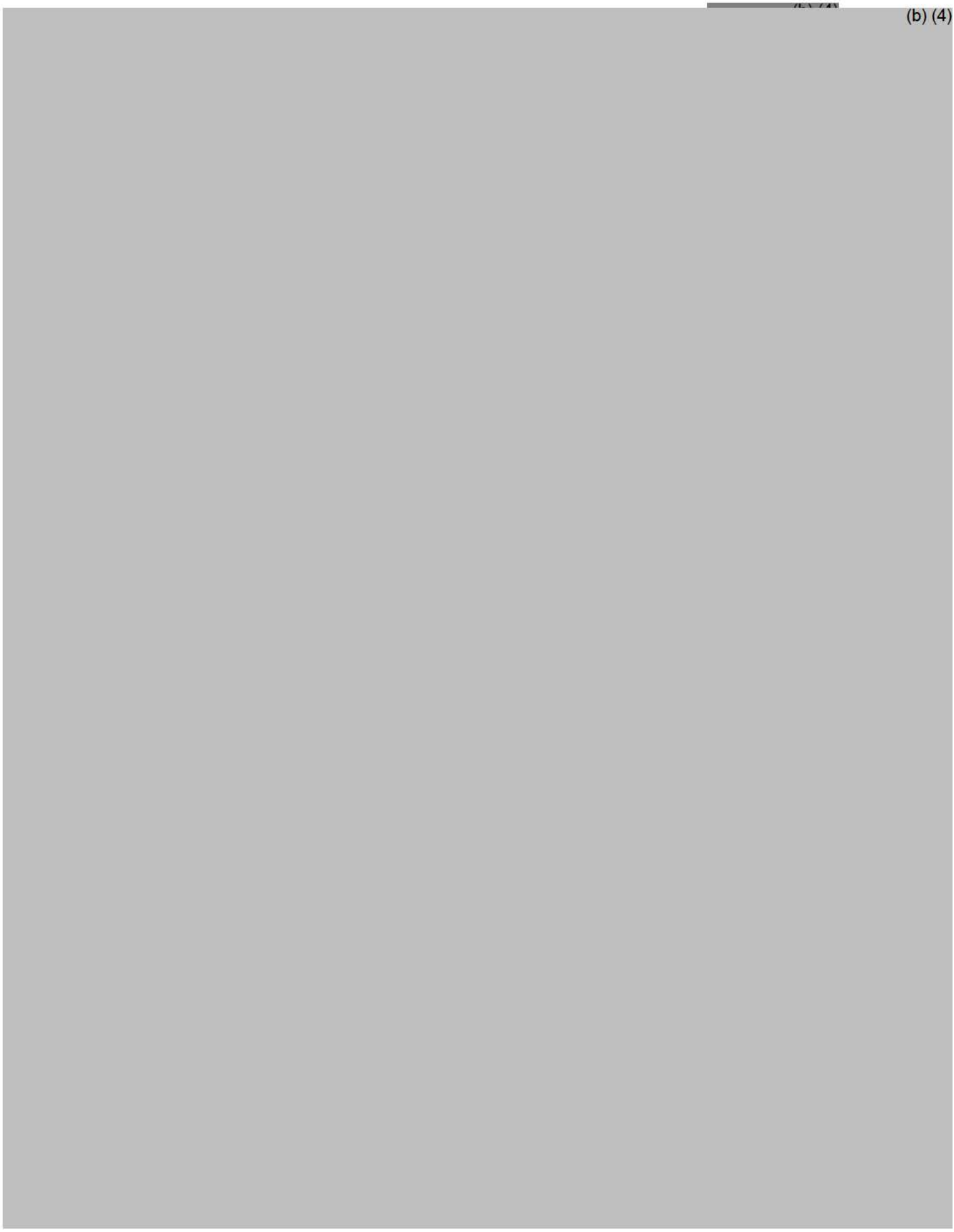
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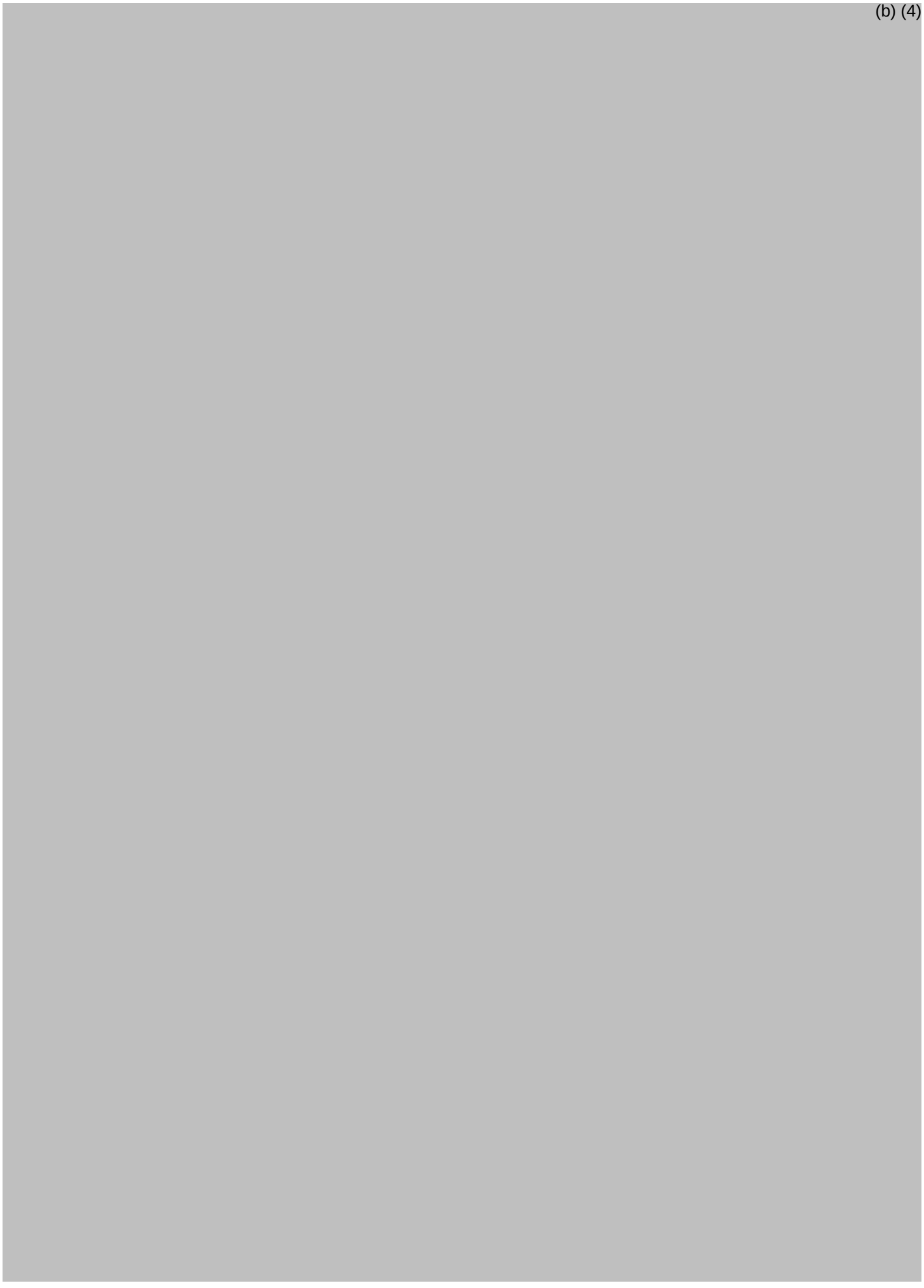
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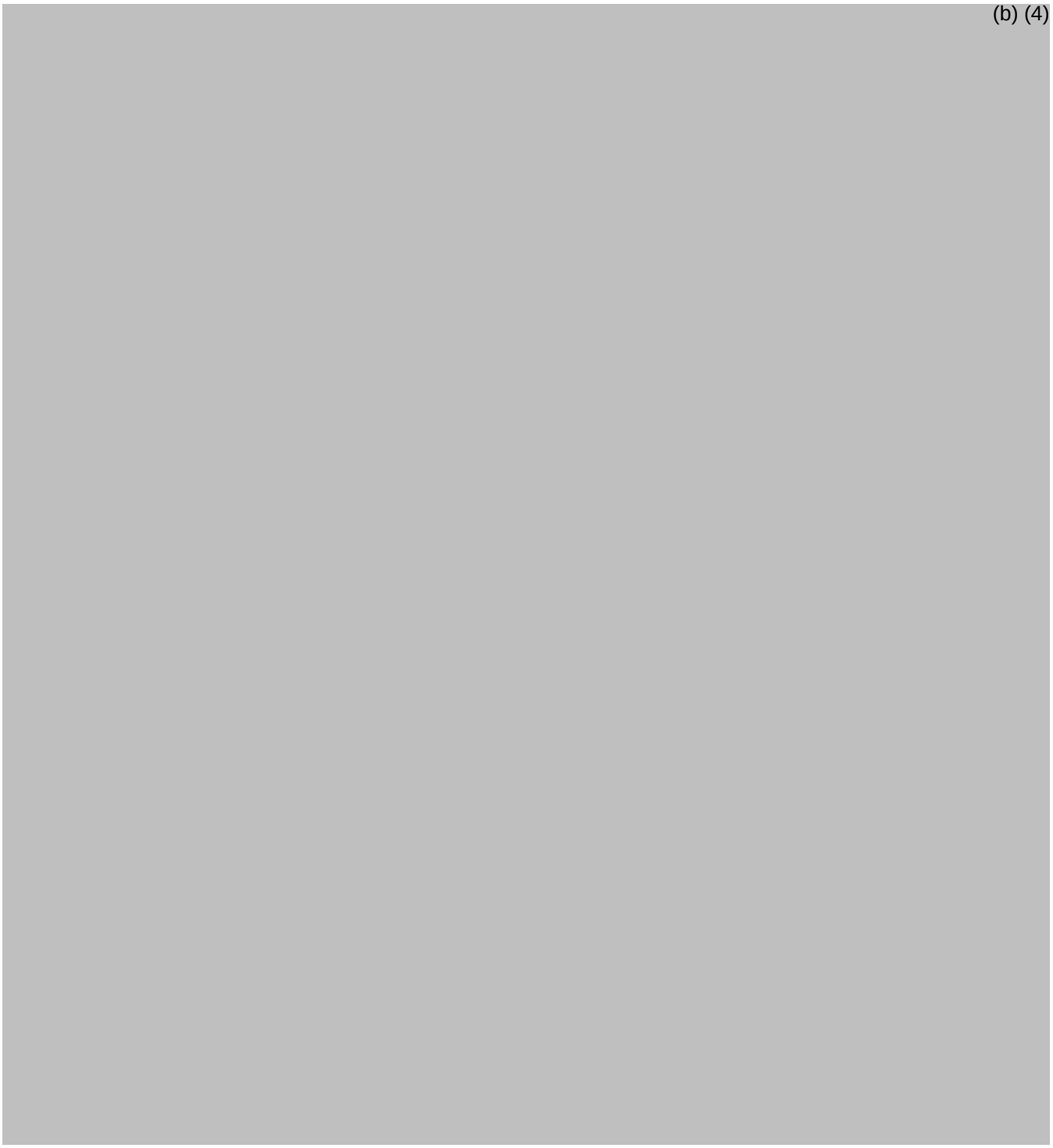
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SPECIFICATION of Sulfuric acid

Products Name: Sulfuric acid

Molecular Formula

H_2SO_4

Specification:

- Standard: GB2
- Appearance: (b) (4)
- Sulfuric acid content: (b) (4)
- Pb (b) (4)
- As
- Se

Packing
Special trunk transport.

Quality guarantee: one year from production date.

.

GB 5749-2006

Standards for Drinking Water Quality

生活饮用水卫生标准

(b) (4)

Disclaimer

(b) (4)

(b) (4)

Standards for Drinking Water Quality

生活饮用水卫生标准

Contents

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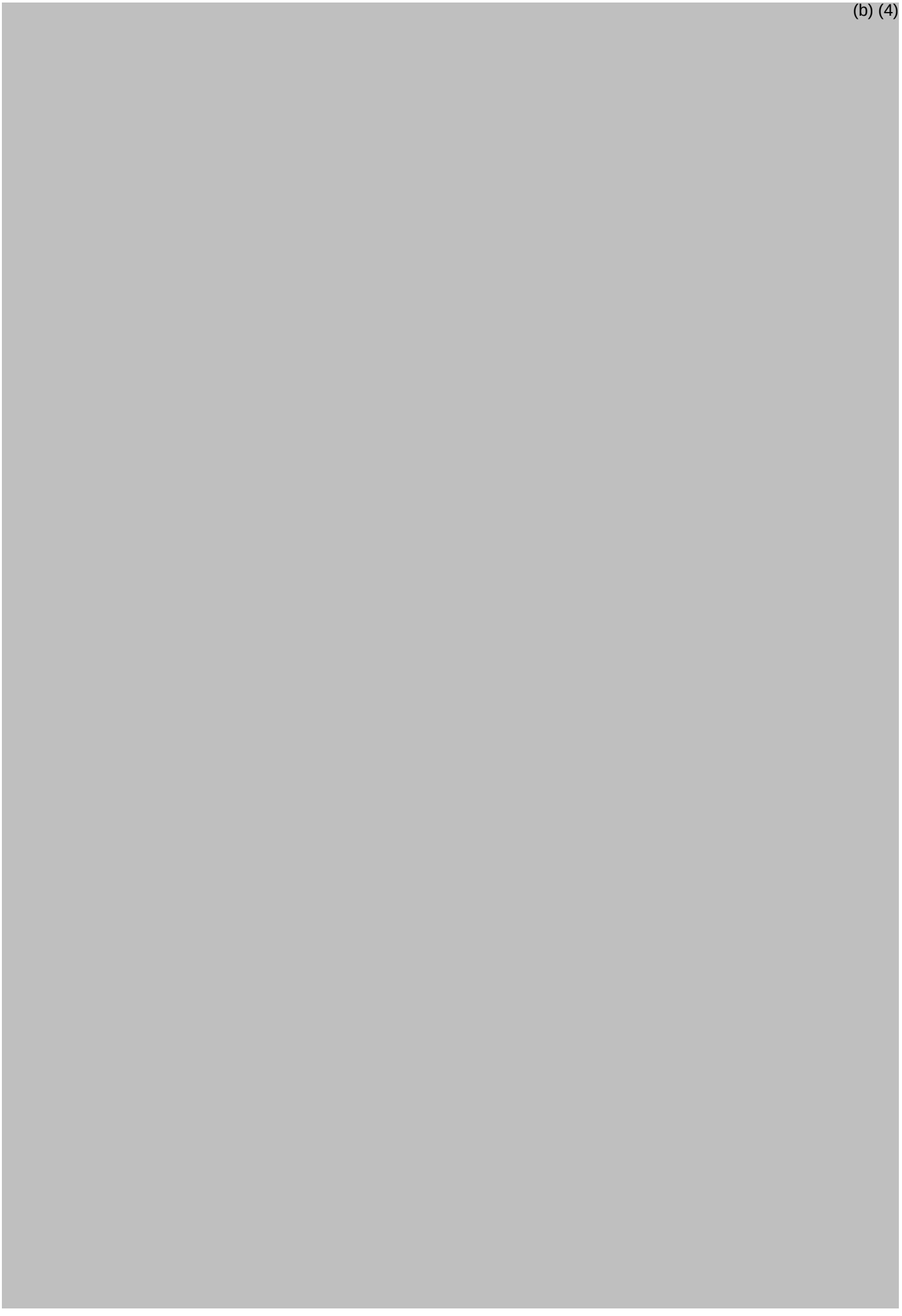
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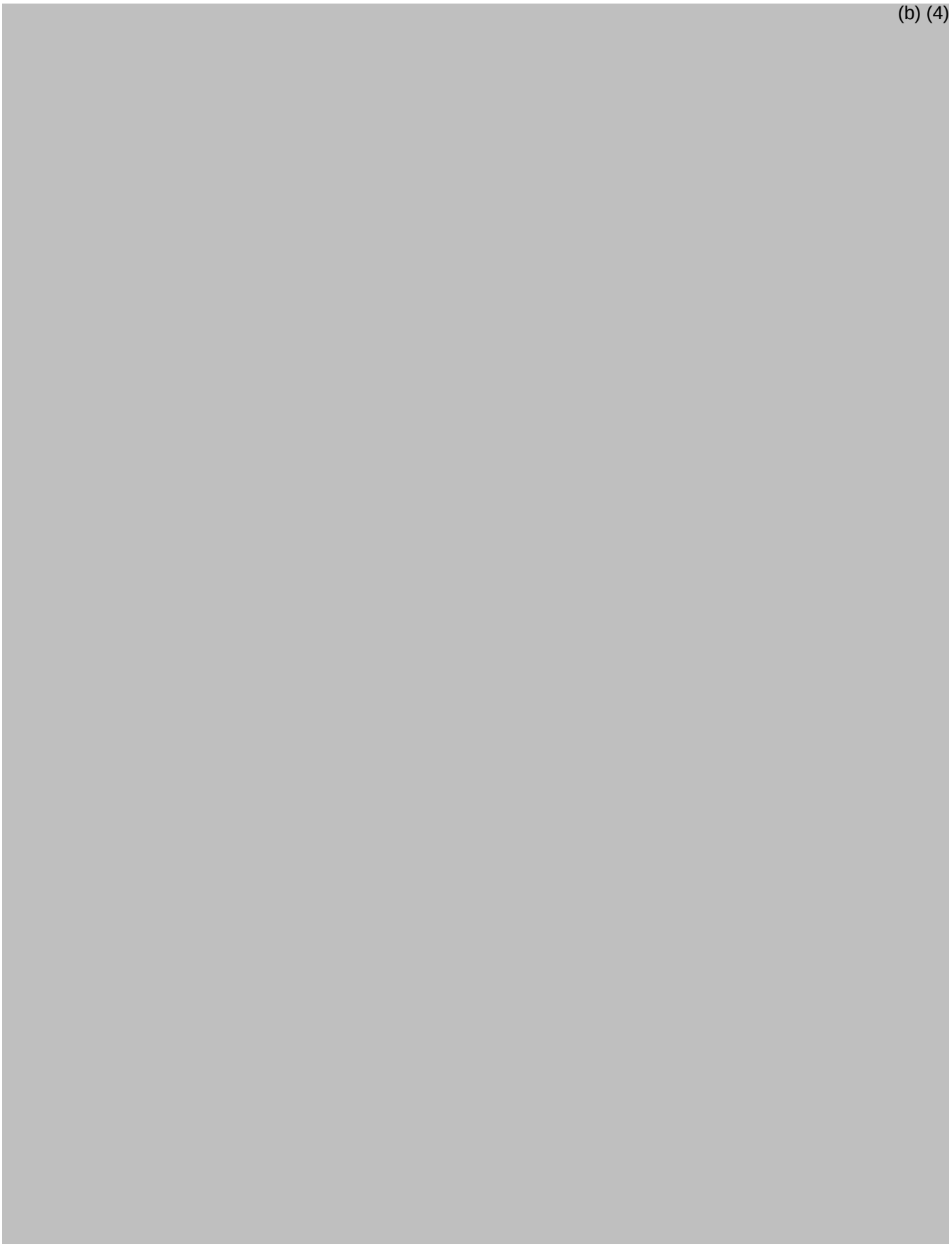
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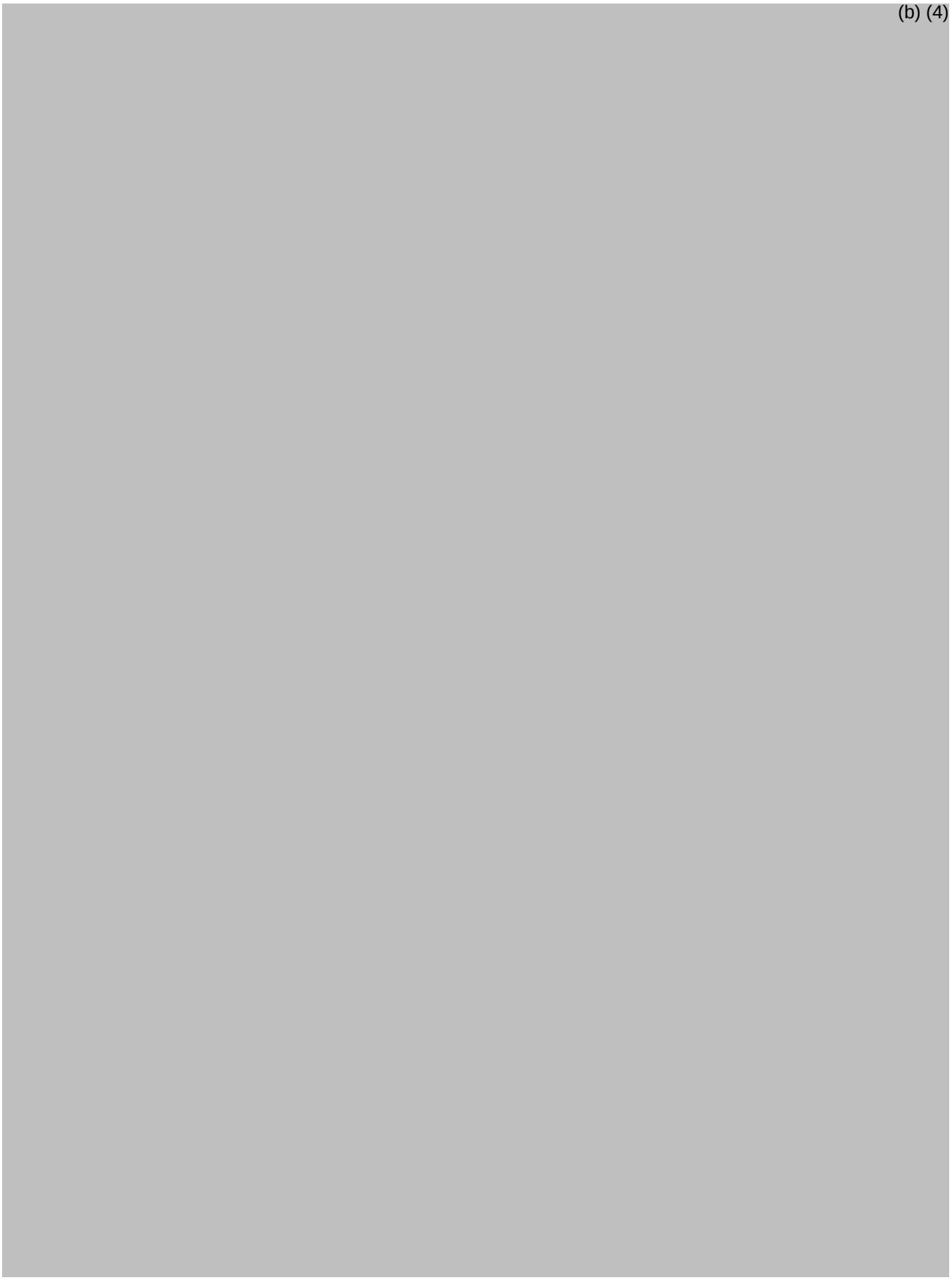
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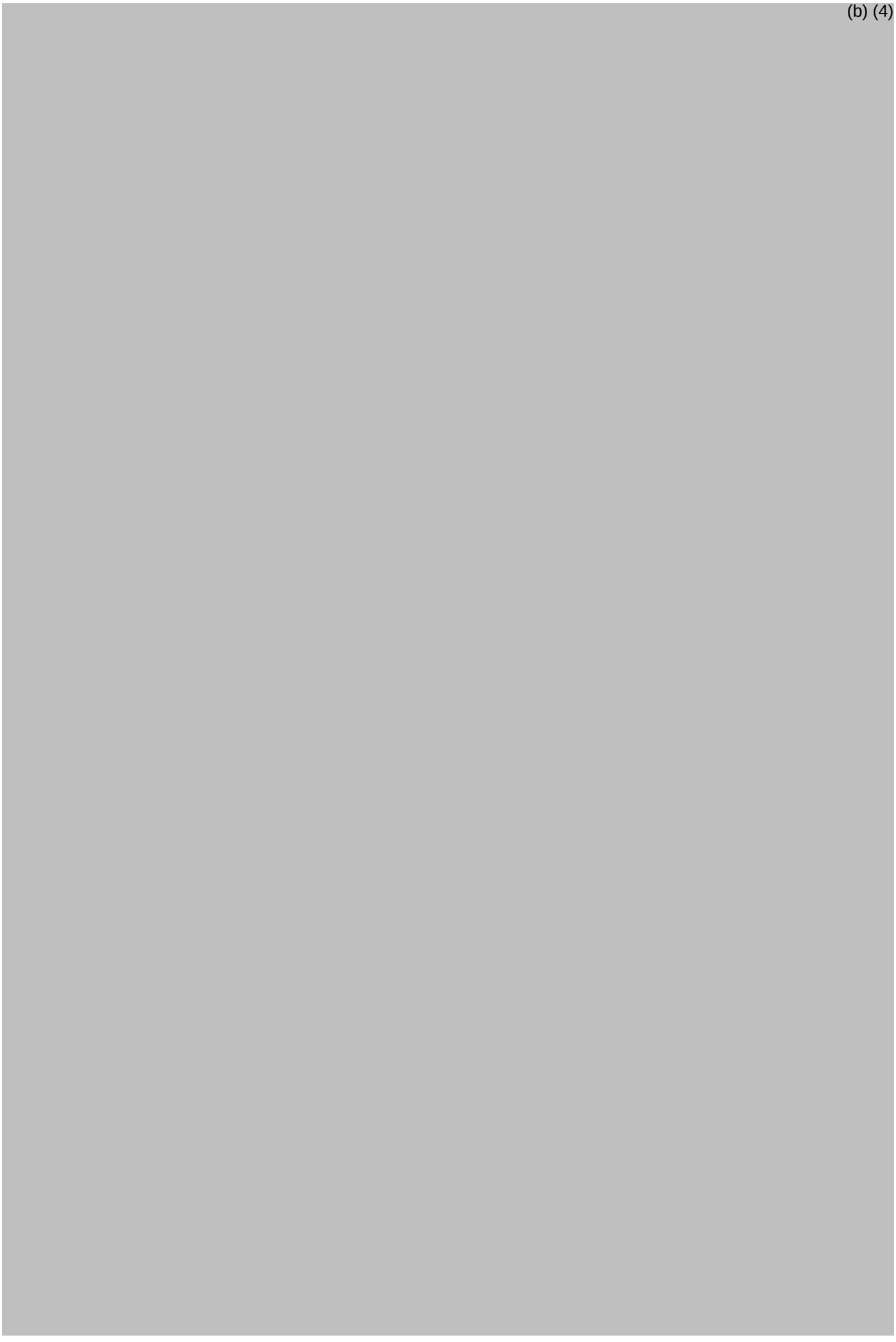

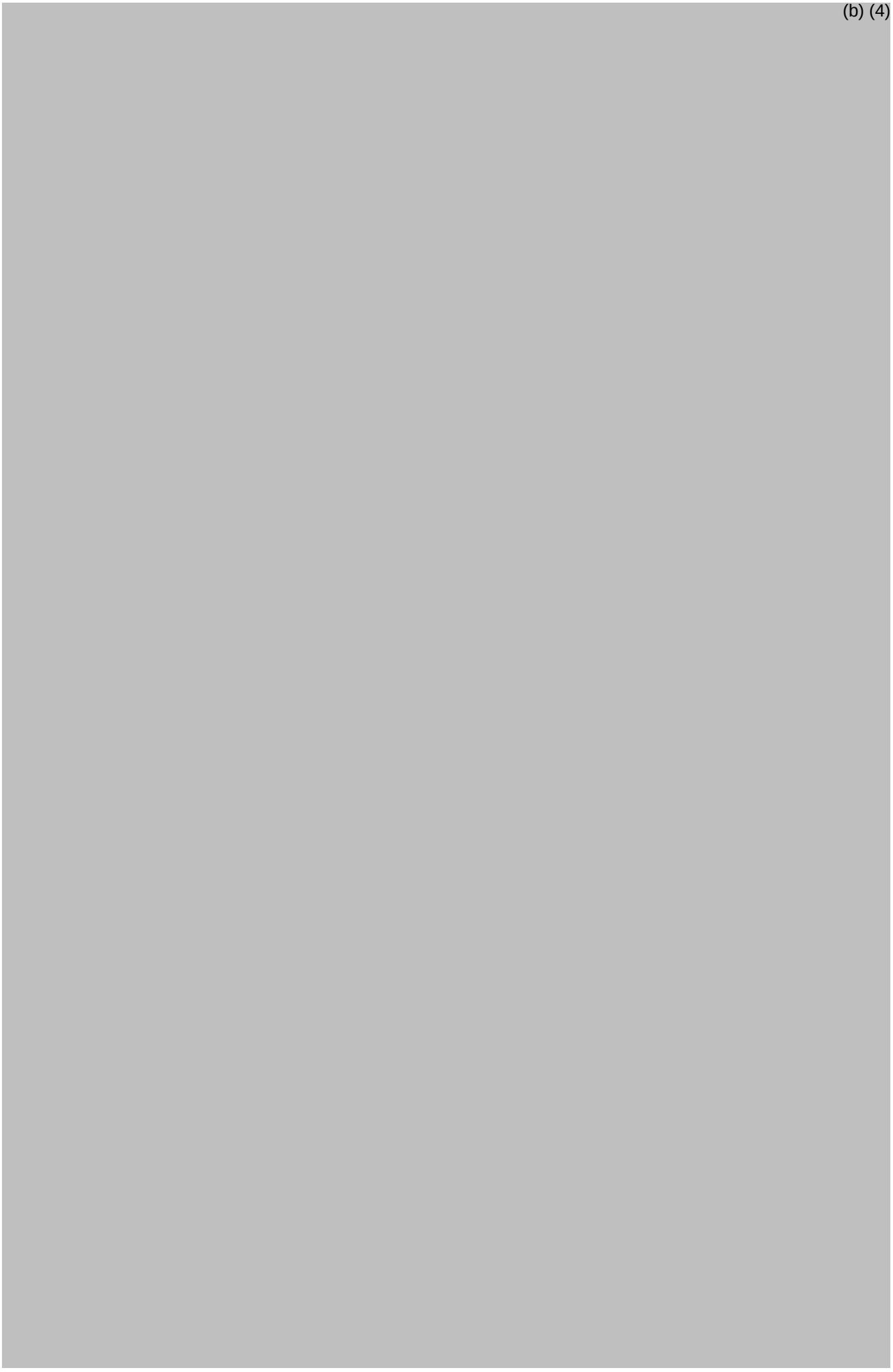
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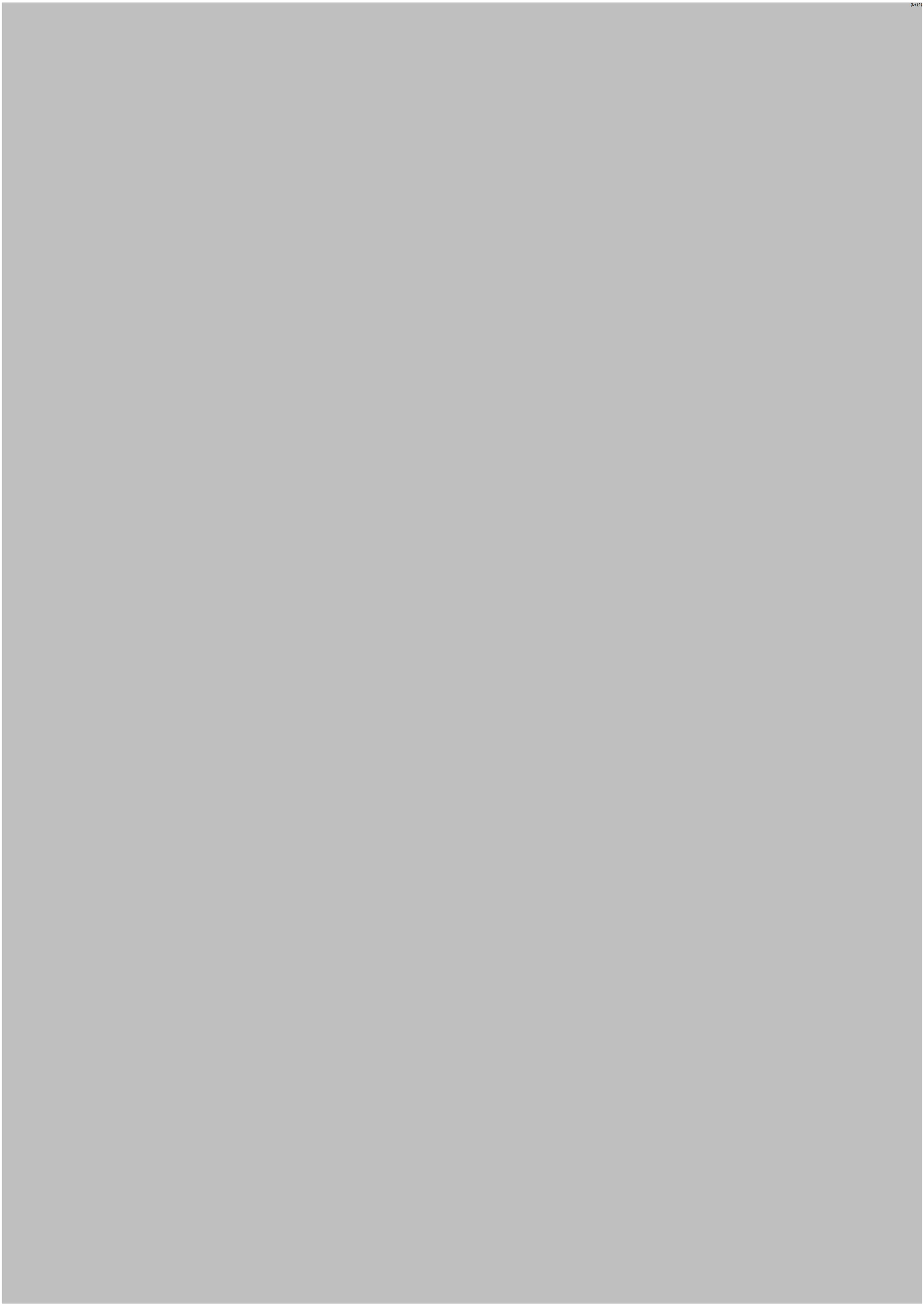

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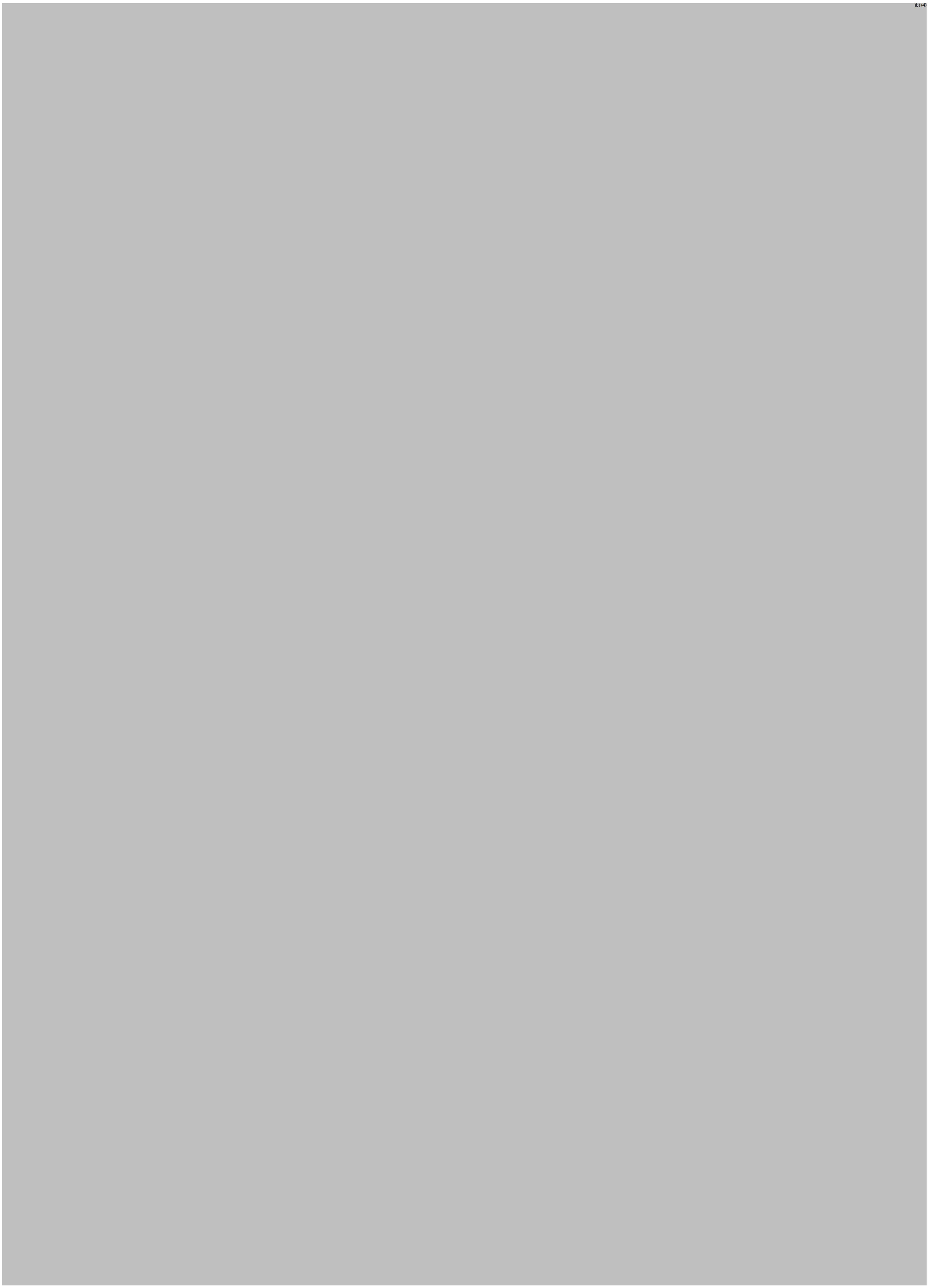

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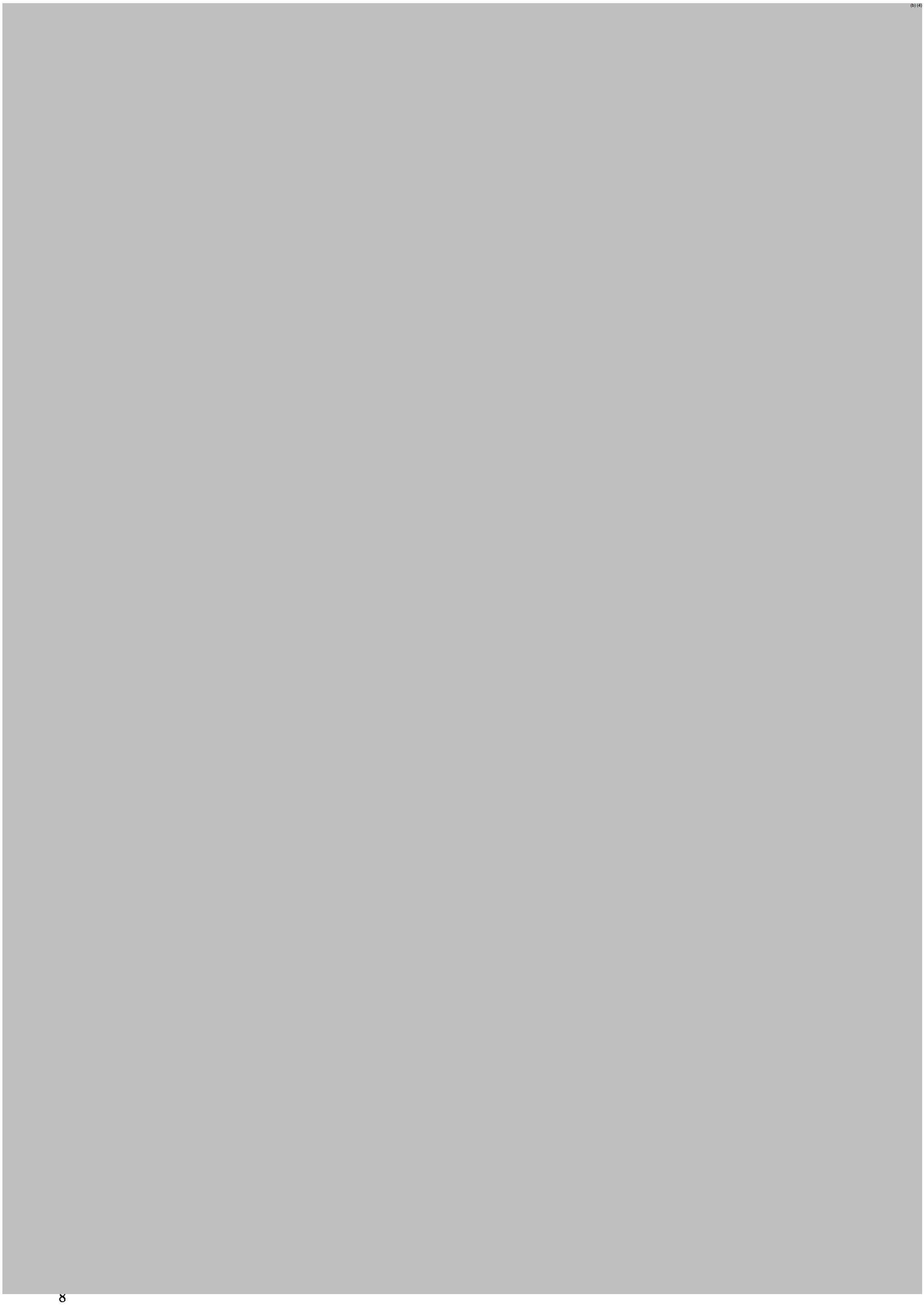

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Papain derived from papaya and meeting AAFCO ingredient definition 30.1 for enzymes and their sources is used as the protease. Copies of two manufacturer specifications are enclosed, both of which comply with GB 1886.174 National Standard on Enzyme Preparations for the Food Industry

[redacted]

SPECIFICATION ENZYME PRODUCT

Product Name:	PAPAIN	Part Plant:	PAPAYA
Origin:	CHINA	Manufacturer:	[redacted]
ITEMS OF ANALYSIS		**SPECIFICATIONS**	**Method**
Physical data			
Appearance		(b) (4)	Conform
Odor			Conform
Activity of enzyme			Q/NPB 01-2009
Loss on drying			GB1886.174-2016
Loss on ignition			GB1886.174-2016
Heavy metals (as Pb)			GB1886.174-2016
Heavy metals (as As)			GB1886.174-2016
Microbiology			
Total Plate Count		(b) (4)	GB1886.174-2016
Coliforms			GB1886.174-2016
Salmonella			GB1886.174-2016
Yeast and mould			GB1886.174-2016
Storage		STORED HUMIDITY PROTECTED (b) (4)) AT TEMPERATURE BELOW (b)	
Shelf Life		1 Year when Properly Stored	
Packaging		On request ((b) (4))	

All results to be reported on the COA with agreed specification.

TECHNICAL DATA SHEET

[redacted]

[redacted]

DESCRIPTION

(b) (4)

PHYSICOCHEMICAL CHARACTERISTICS

Appearance Color Odor Density pH	(b) (4)

CHEMICAL SPECIFICATION LIMITS

Heavy metals	Arsenic (As) Cadmium (Cd) Led (Pb) Mercury (Hg)	(b) (4)
Pesticides	(b) (4) (detection limit)	
Aflatoxins	Aflatoxin-B1 Aflatoxin-B2 Aflatoxin-G1 Aflatoxin-G2	(b) (4)

[redacted]

[redacted]

MICROBIOLOGICAL SPECIFICATION LIMITS

Total Plate Count	(b) (4)
Yeasts & Moulds	
Coliforms	
Escherichia coli	
Salmonella	
Staphylococcus aureus	

Methods and internal results are mentioned on the 'Certificate of Analysis'.

ENZYMATIC ACTIVITY

Assay

(b) (4)

Specification limits

Target	(b) (4)
LSL	
USL	

[redacted]

[redacted]

[redacted]

REACTION CONDITIONS

(b) (4)

[redacted]

STORAGE CONDITIONS

Store product in a cool and dry place (max. (b) (4) out of direct light exposure and in original non opened packaging.

SHELF LIFE

In storage conditions, at least 12 months. Re-evaluation after declared shelf life is feasible.

[redacted]

[redacted]

PACKAGING

[redacted] (b) (4) [redacted].

CERTIFICATIONS

[redacted] is a non-GMO product.

[redacted] is produced compliant to our ISO 9001-2008 management system. This is certified by *AIB-Vinçotte*.

[redacted] is Kosher. This is certified by *Orthodox Union*.

[redacted] is Halal. This is certified by *Halal Food Council of Europe*.

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A.2.4 Reagents and materials

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A.3 Determination of Glucoamylase (Amyloglucosidase) Activity (From Aspergillus niger and Variant)

A.3.1 Glucoamylase (amyloglucosidase)

With starch as the substrate and under certain conditions, the amyloglucosidase can hydrolyze α-1, 4, α-1, 6 and α-1, 3 glucosidic bonds from the non-reducing end of starch with the release of glucose.

A.3.2 Glucoamylase (amyloglucosidase) activity

One unit of enzyme activity is marked as U/mL or U/g, and defined as "1mL of liquid enzyme or 1g of enzyme powder hydrolyzes soluble starch to get 1mg of glucose in 1h at 40℃ and pH 4.6".

A.3.3 Reagents and materials

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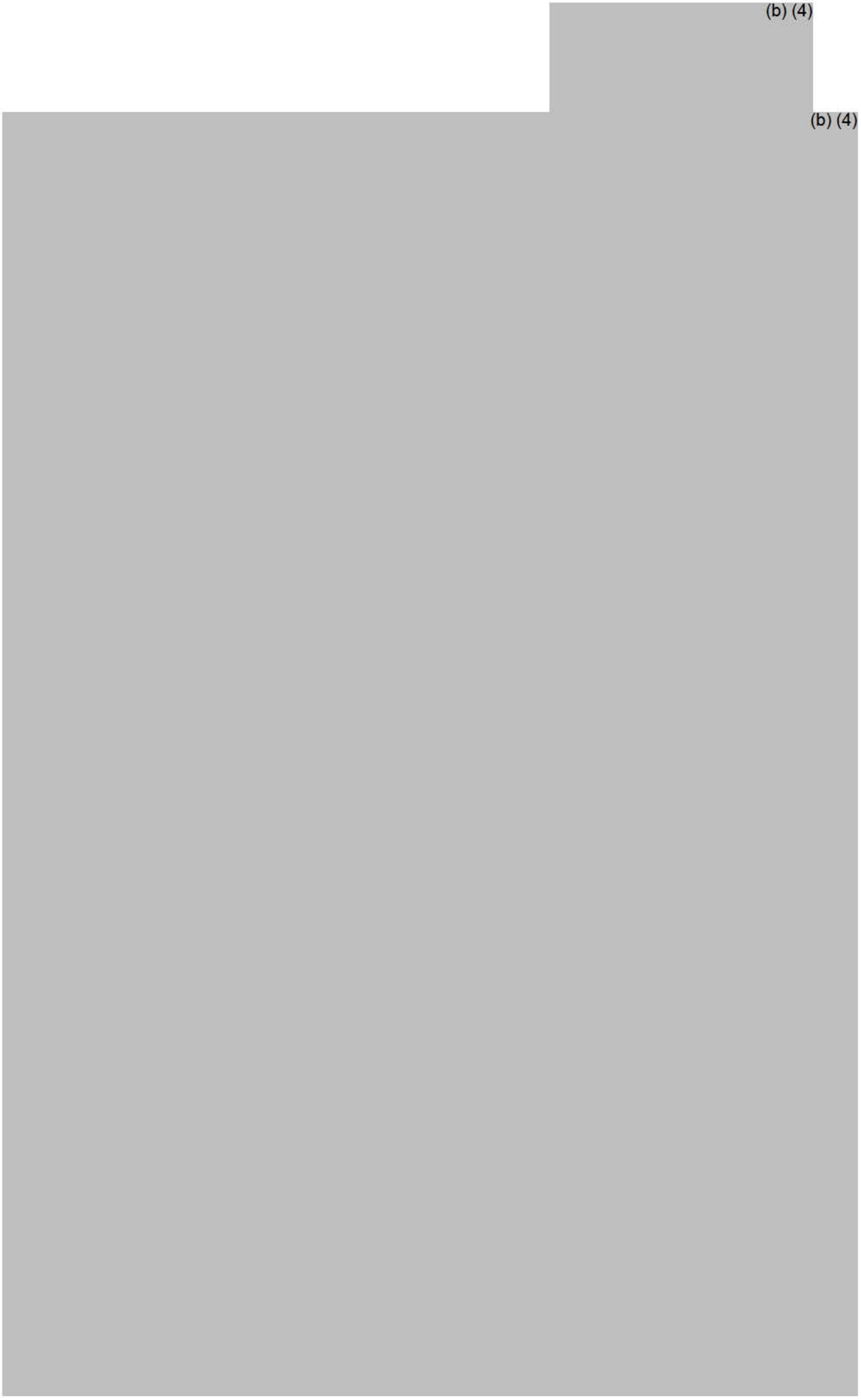
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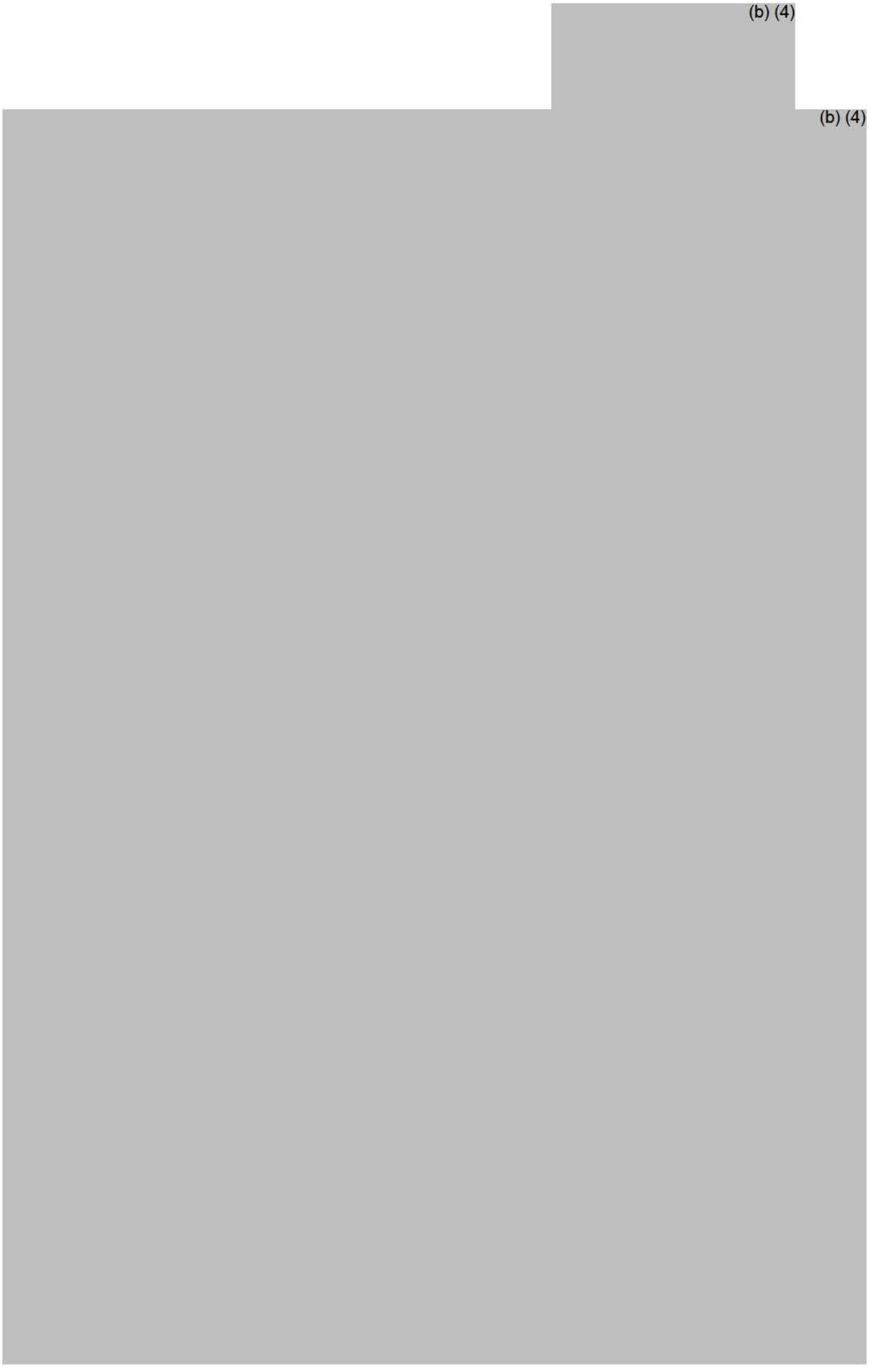
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A.4 Determination of Protease Activity

A.4.1 Protease

A kind of enzyme which can cut off the internal peptide bonds of protein molecules and turn the protein molecule to micro-molecule polypeptide and amino acid;

A.4.2 Protease activity

The protease activity is expressed as protease activity unit. One unit of enzyme activity is marked as U/g or U/mL, and defined as "1g or 1mL of enzyme hydrolyzes casein to get 1μg of tyrosine in 1 min under the condition of certain temperature and pH value".

A.4.3 Principle

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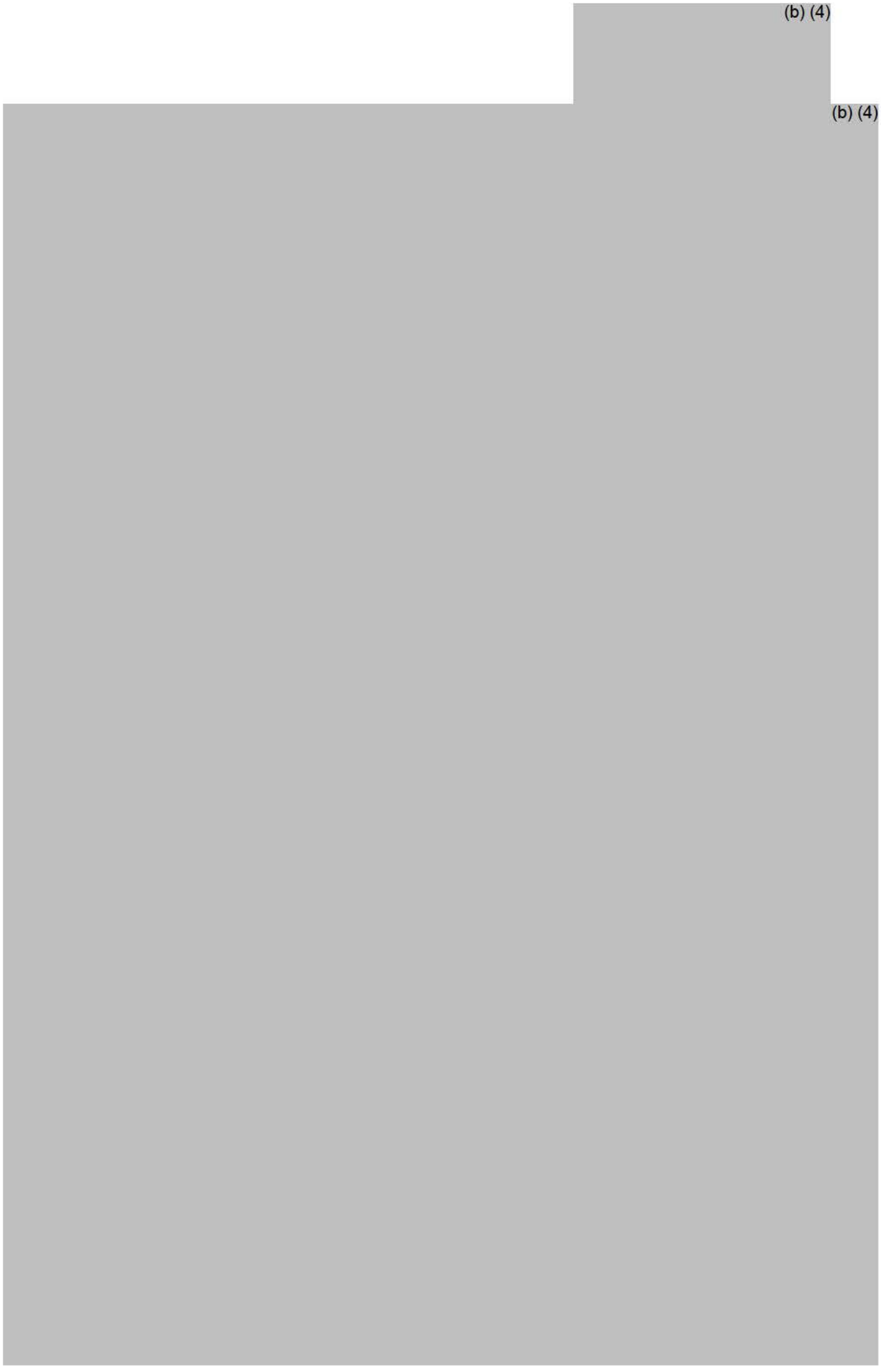
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A.5.1 Pectinase

A kind of enzyme which can hydrolyze the pectin and generate the products containing reducing groups;

A.5.2 Pectinase activity

One unit of enzyme activity is marked as U/g or U/mL, and defined as “1g of solid enzyme powder (or 1mL of liquid enzyme) decomposes pectin to get 1mg of galacturonic acid in 1h under the condition of 50℃ and pH 3.5”.

A.5.3 Principles

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A.5.4 Reagents and materials

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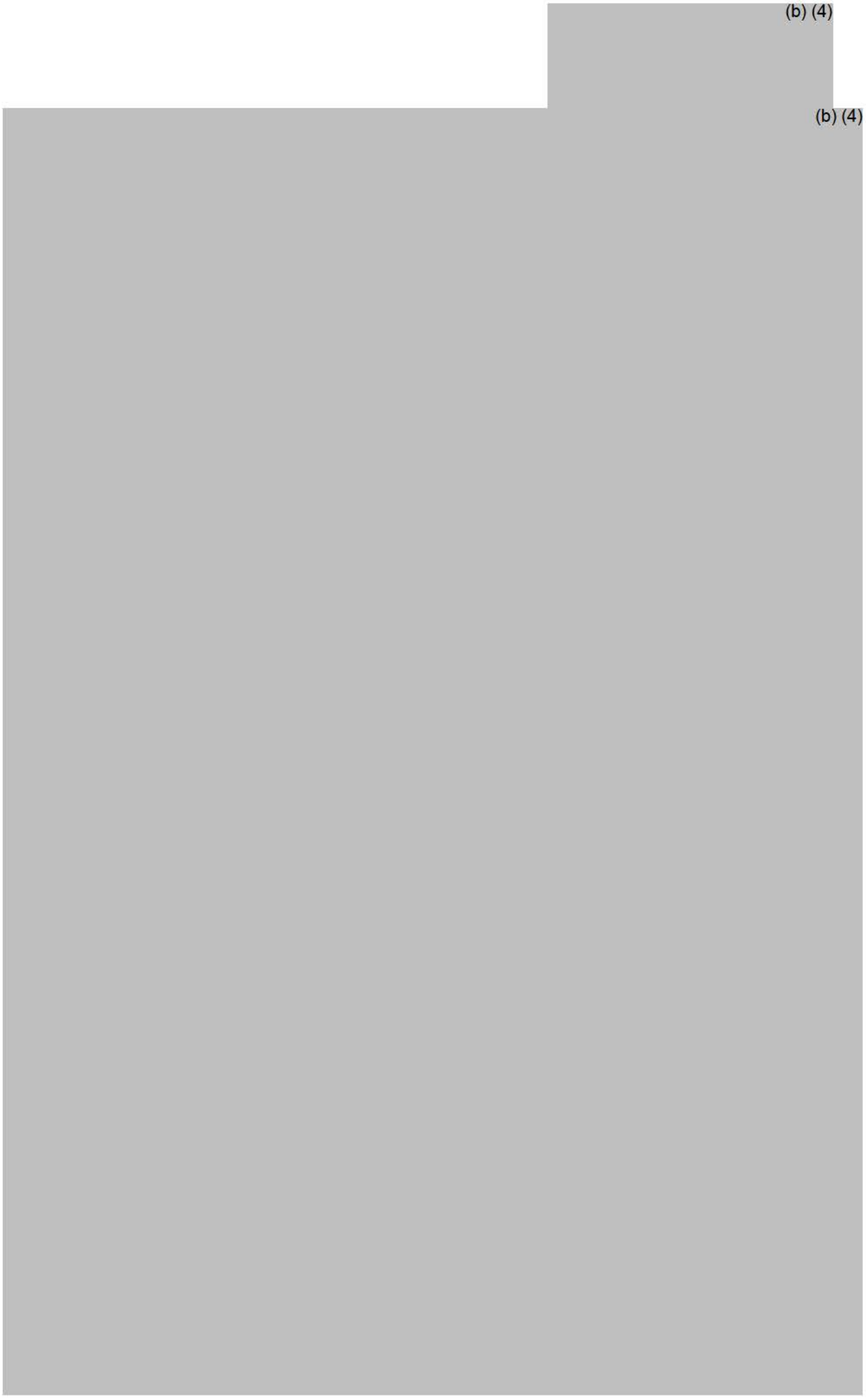
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A.6.3.2 Principles

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A.6.3.3 Reagents and materials

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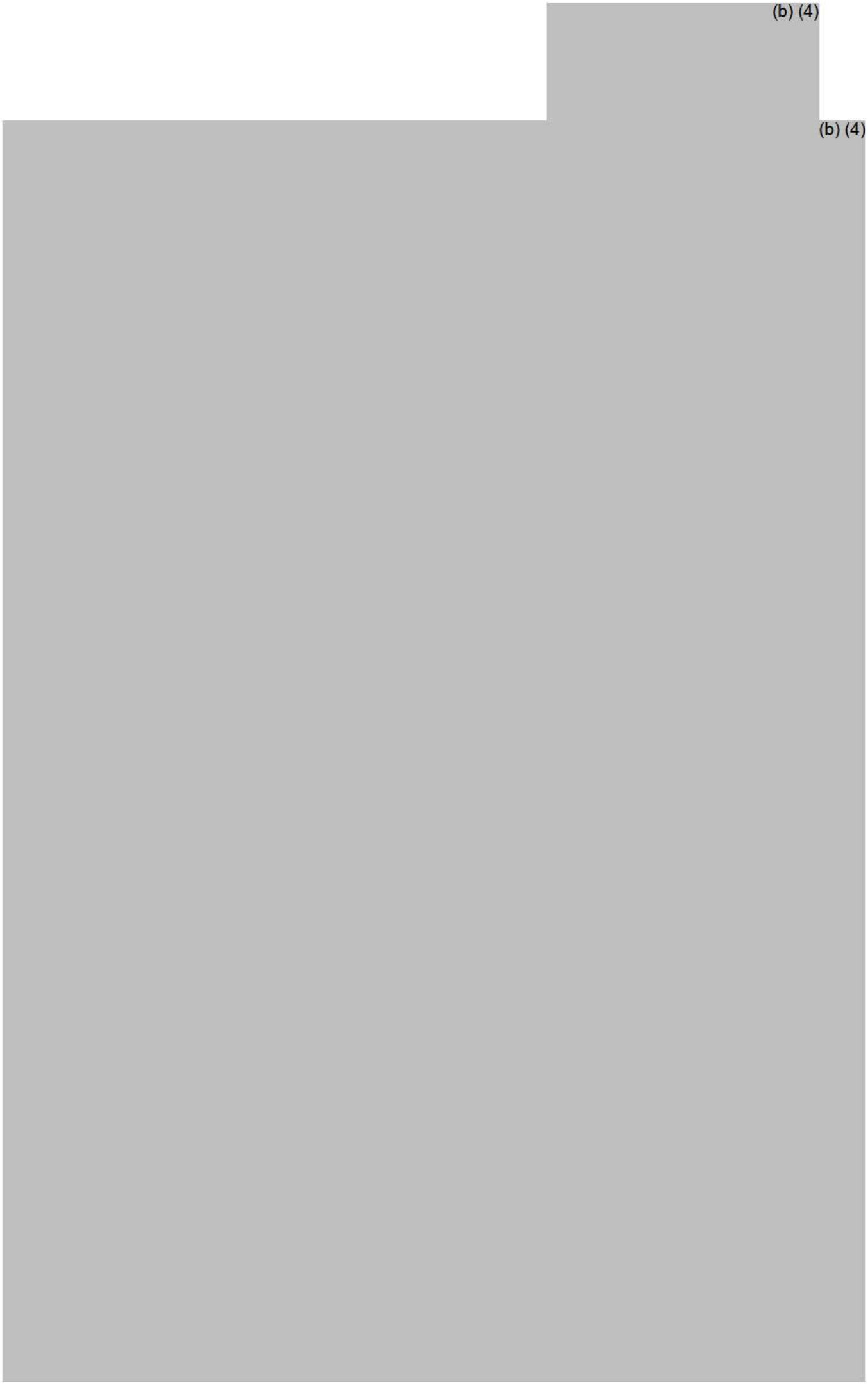
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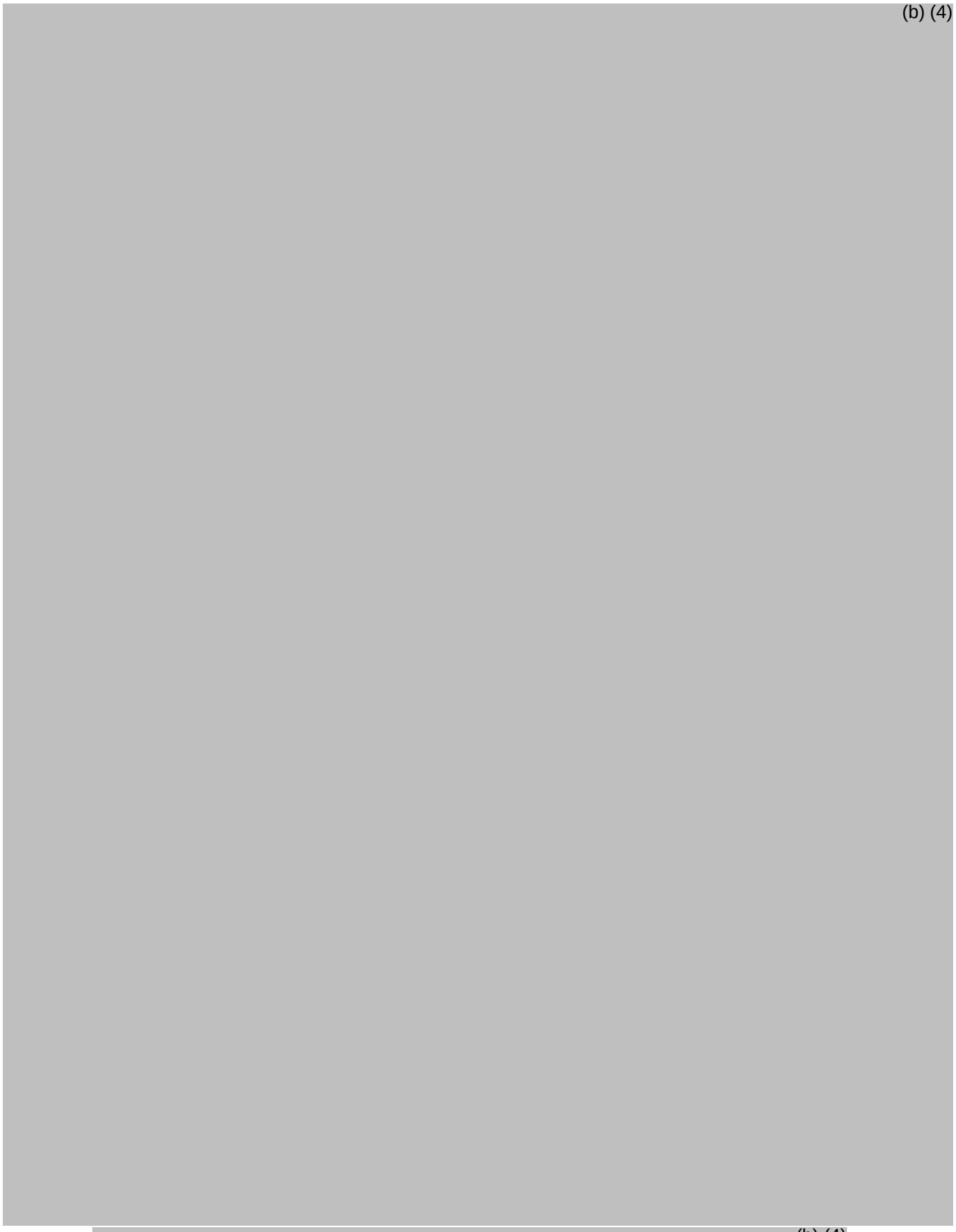

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A.7.3 Principles

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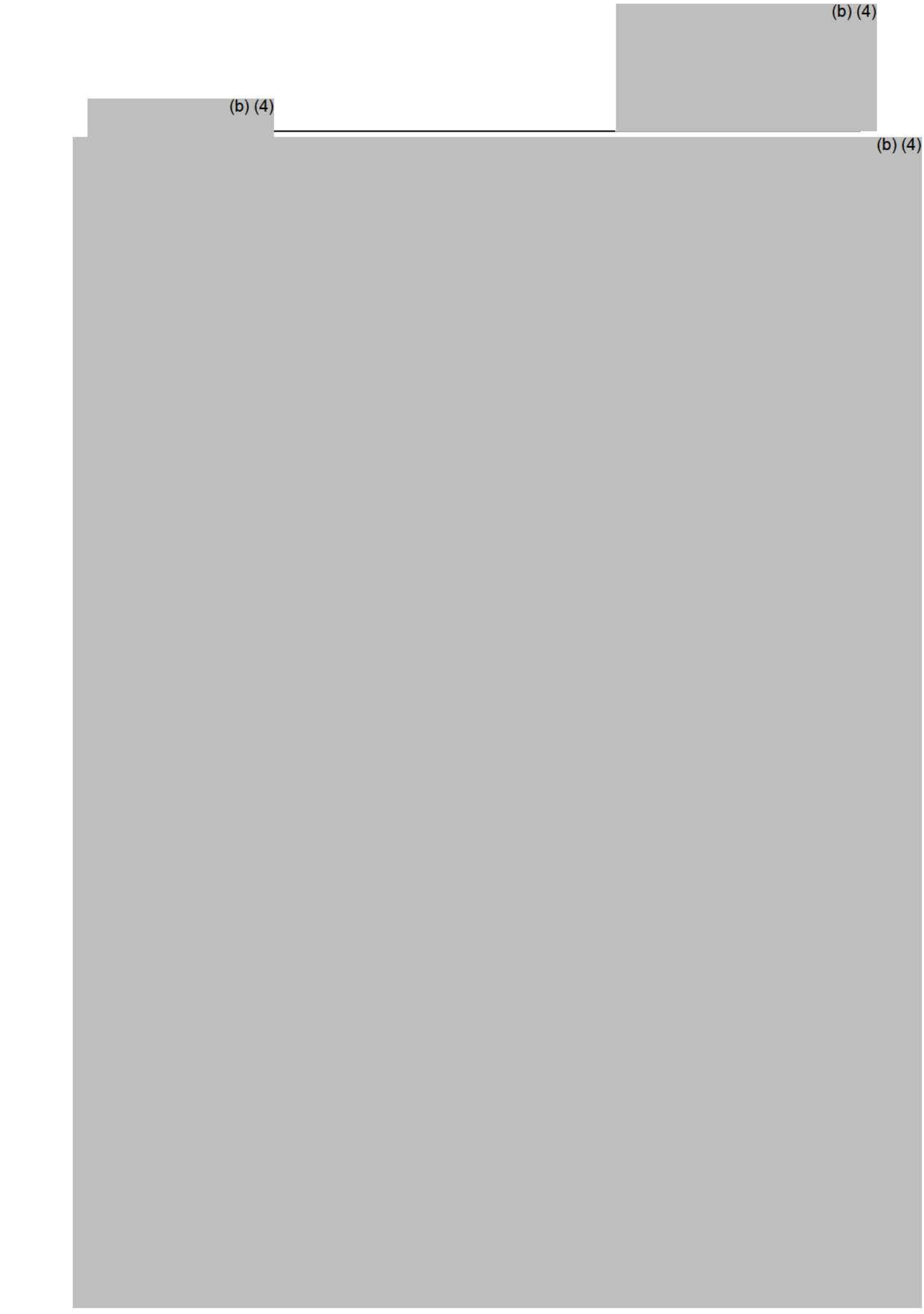
A.7.4 Reagents and materials

(b) (4)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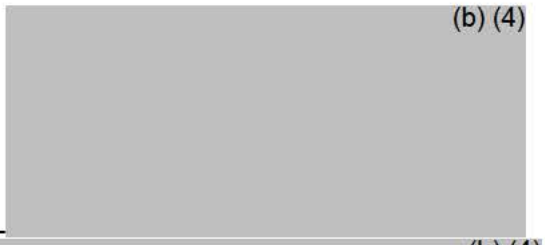

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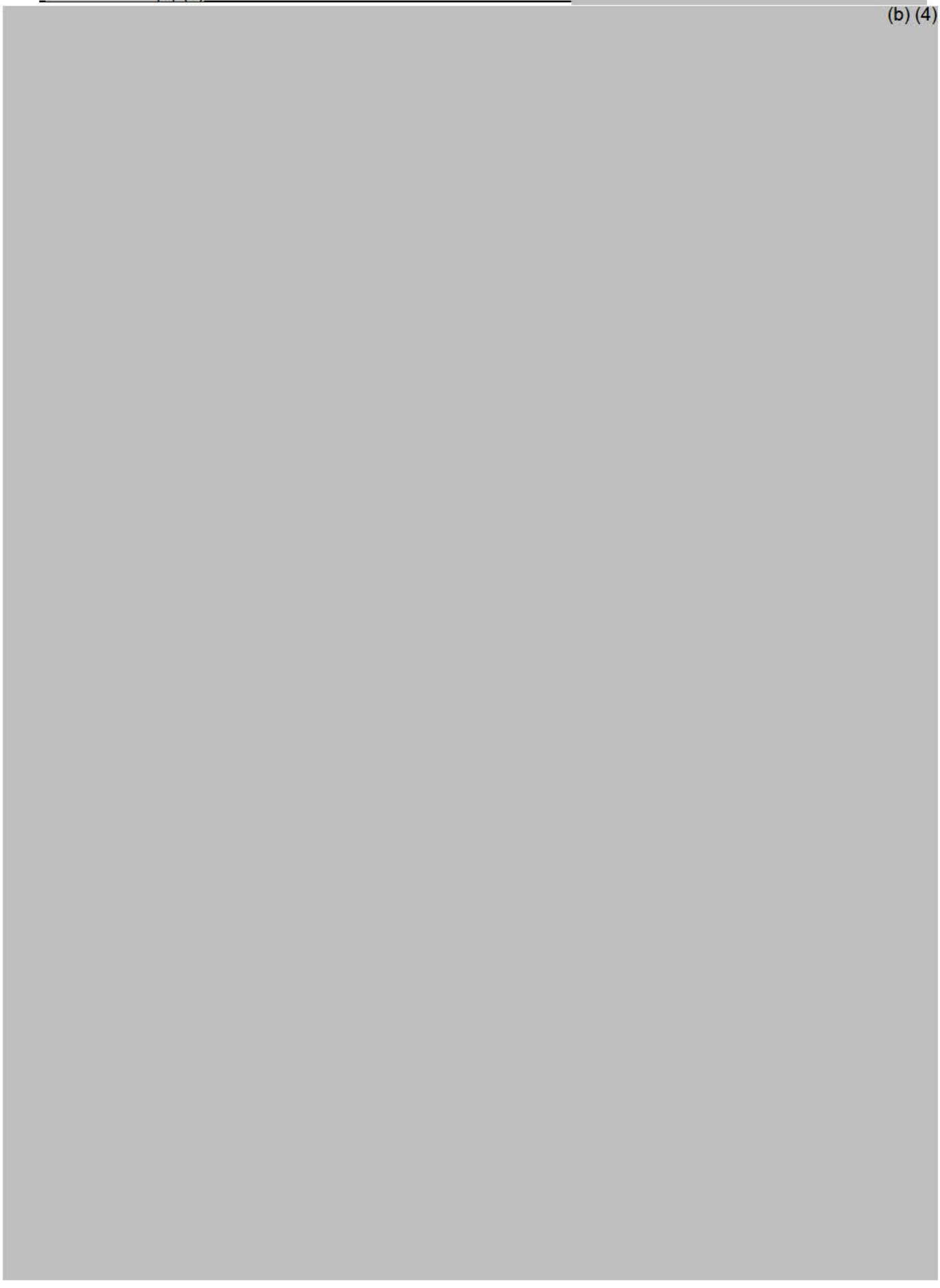

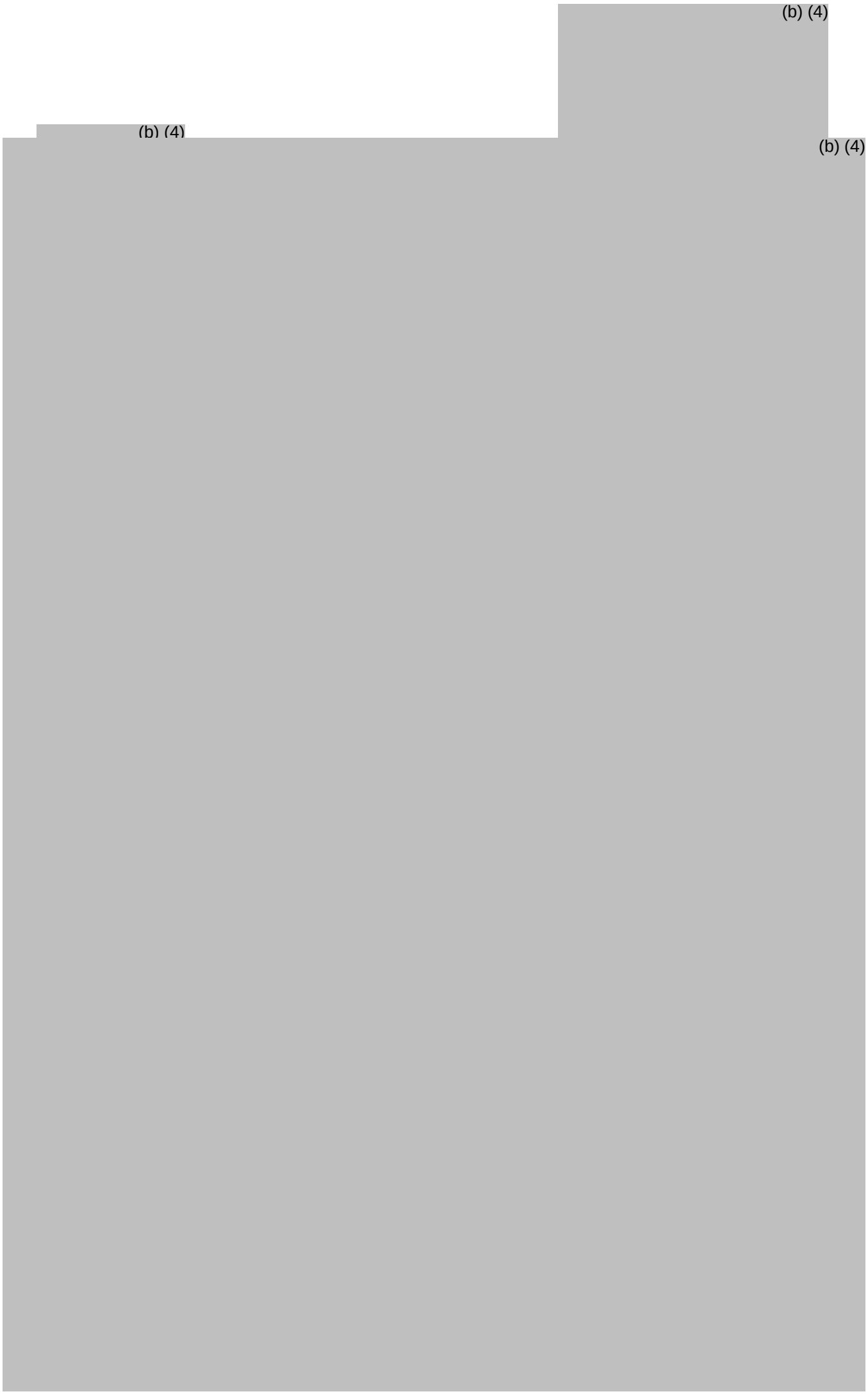
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A.9 Determination of Pullulanase Activity

A.9.1.1 Pullulanase

A kind of debranching enzyme which can exclusively cut off the α-1, 6-glucosidic bonds in amylopectin branch point, further cut off the whole side branch and then form the amylose.

A.9.1.2 Pullulanase activity (colorimetric method)

One unit of enzyme activity is marked as U/mL or U/g, and defined as "1g or 1mL of product reacts and generates 1μmol of glucose equivalent per minute under the given reaction condition".

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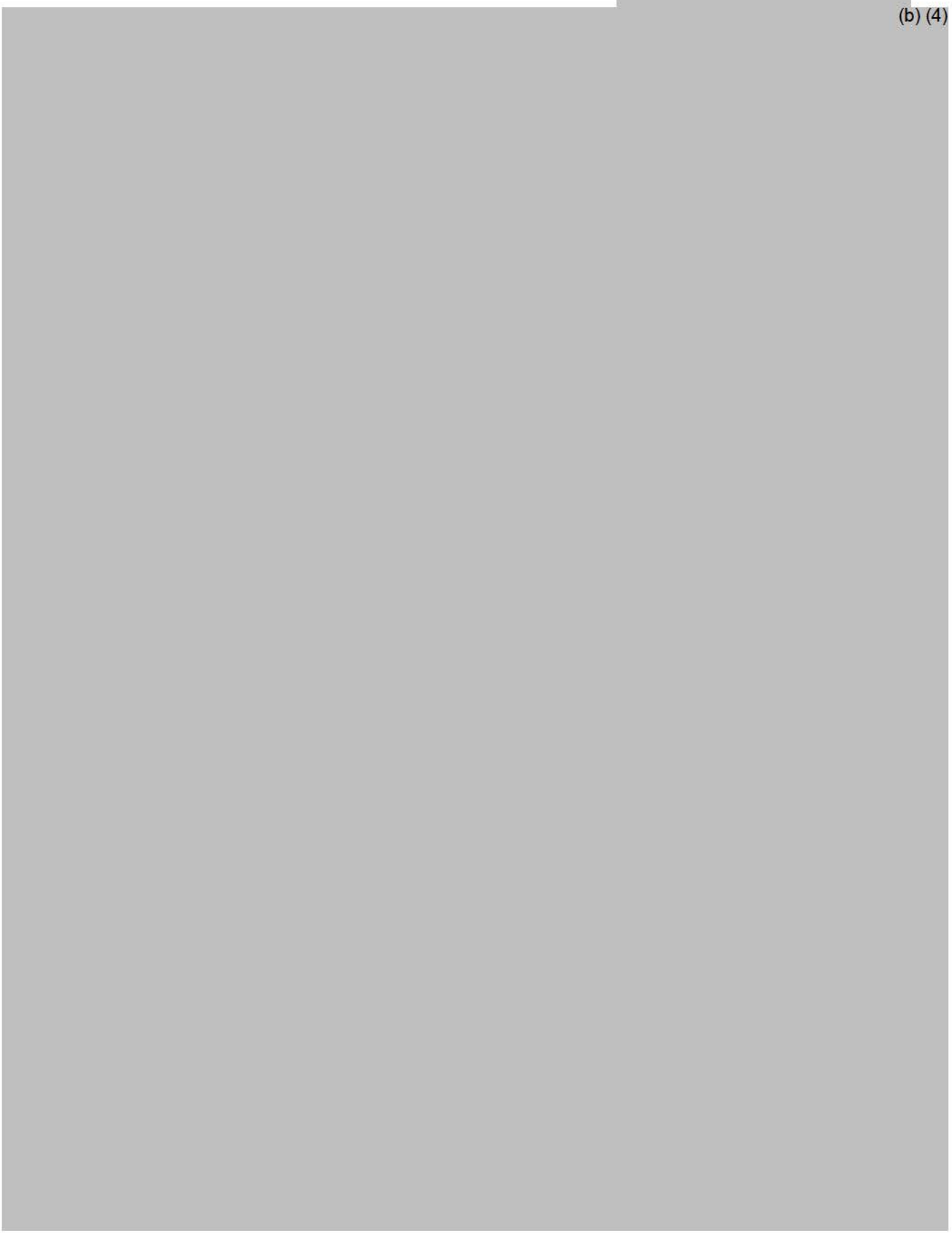
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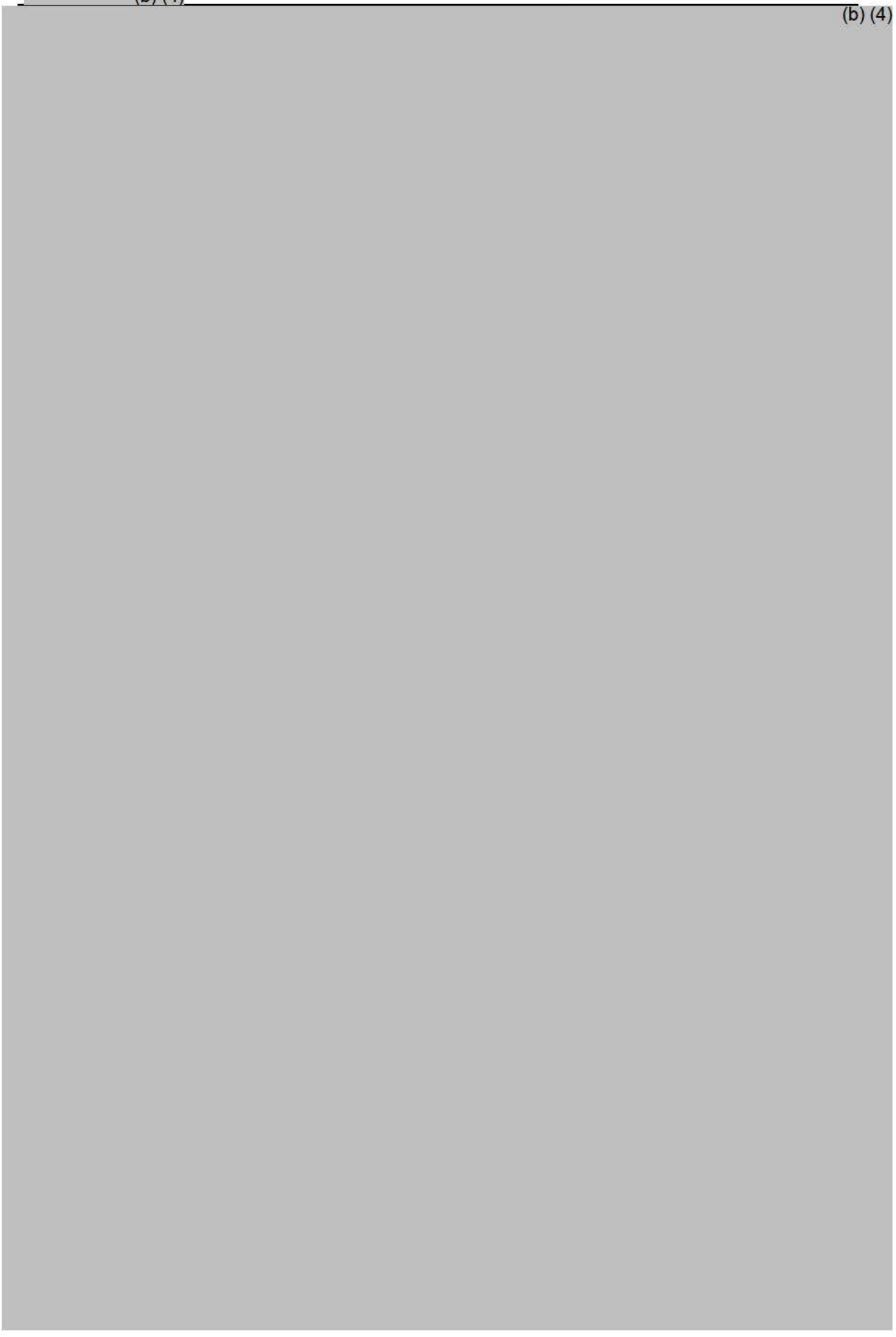
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Annex B

Comparison table for absorbance and α-amylase enzyme concentration

Table B.1 Comparison table for absorbance and α-amylase enzyme concentration

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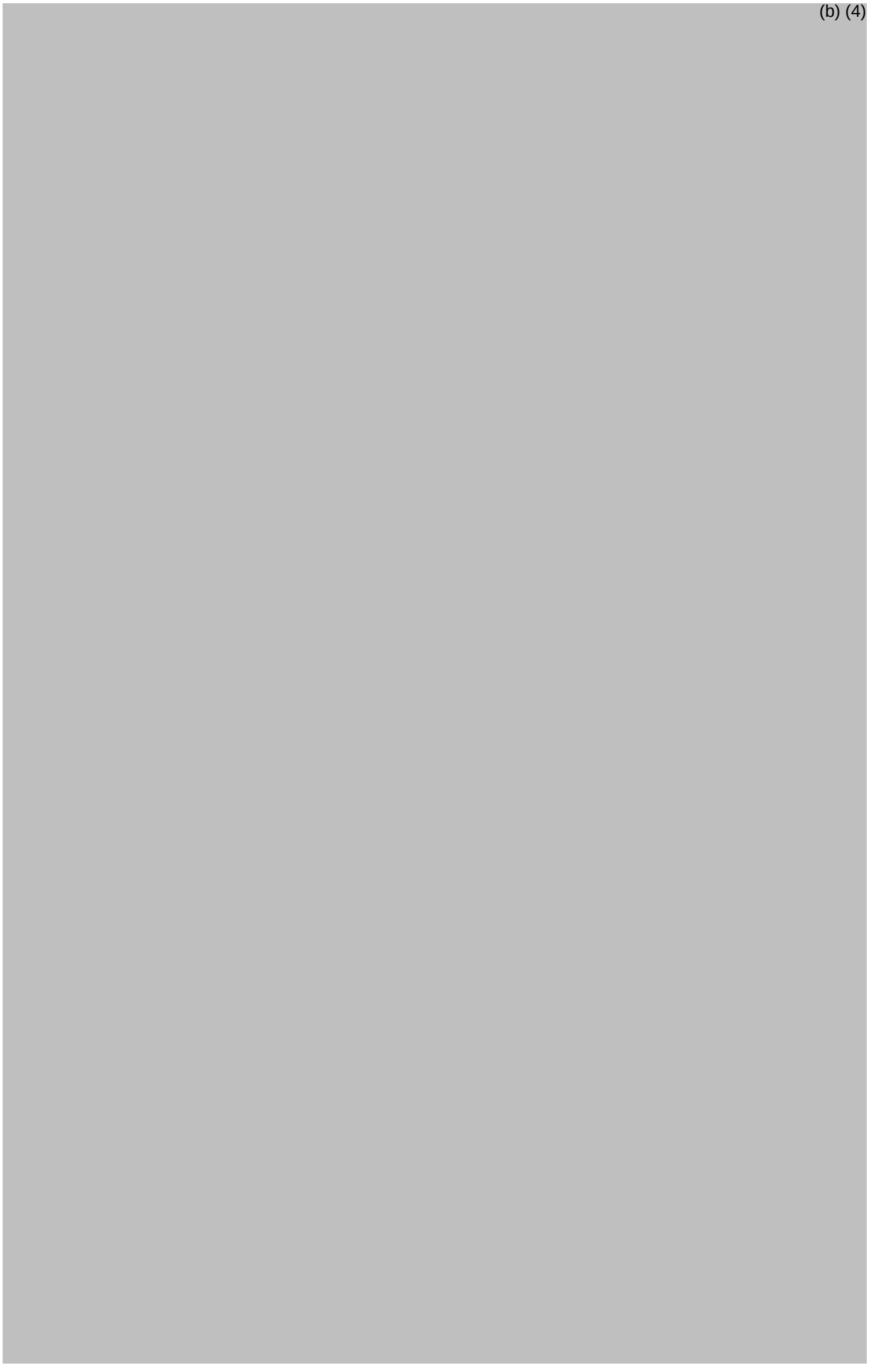

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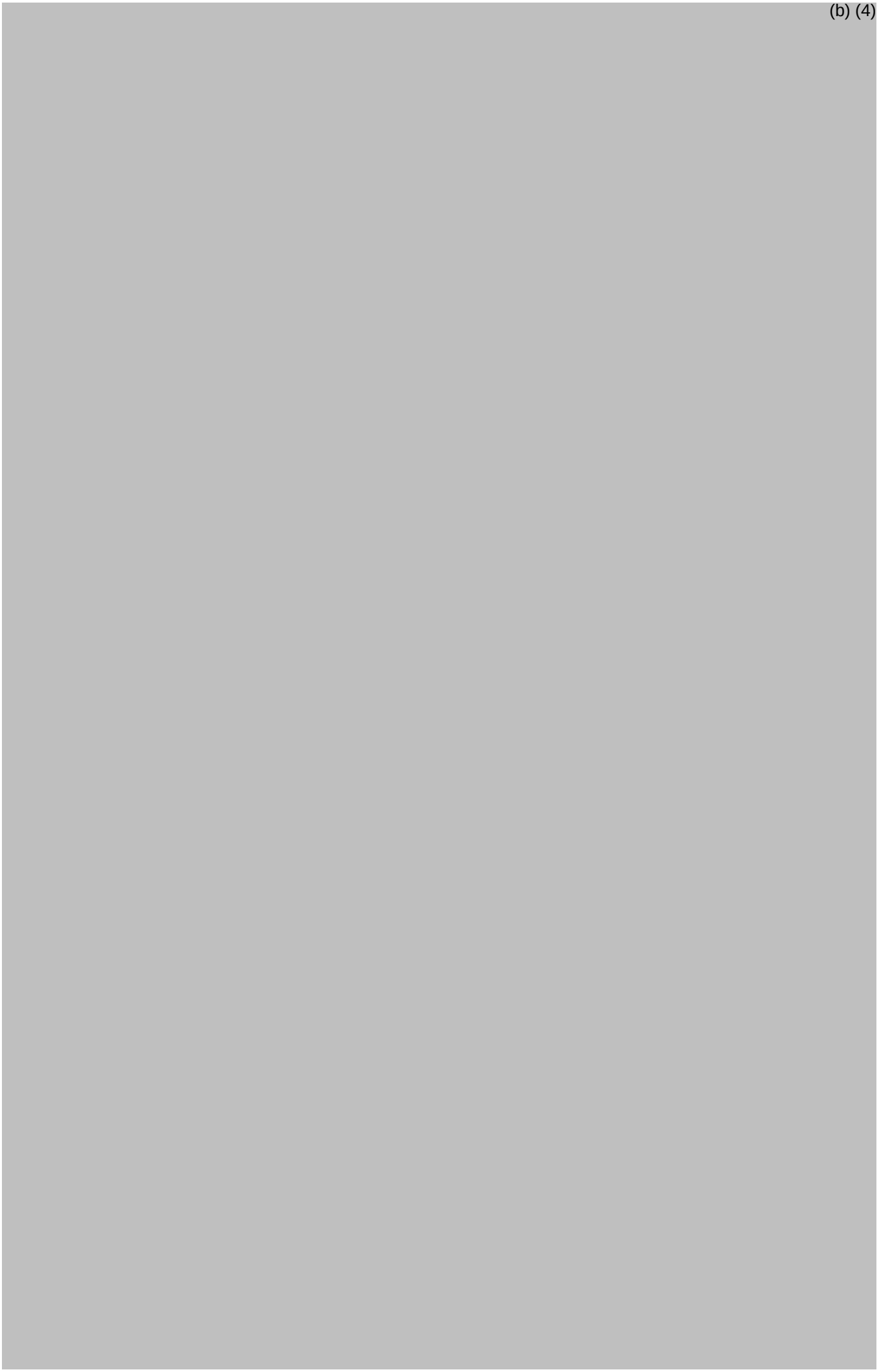

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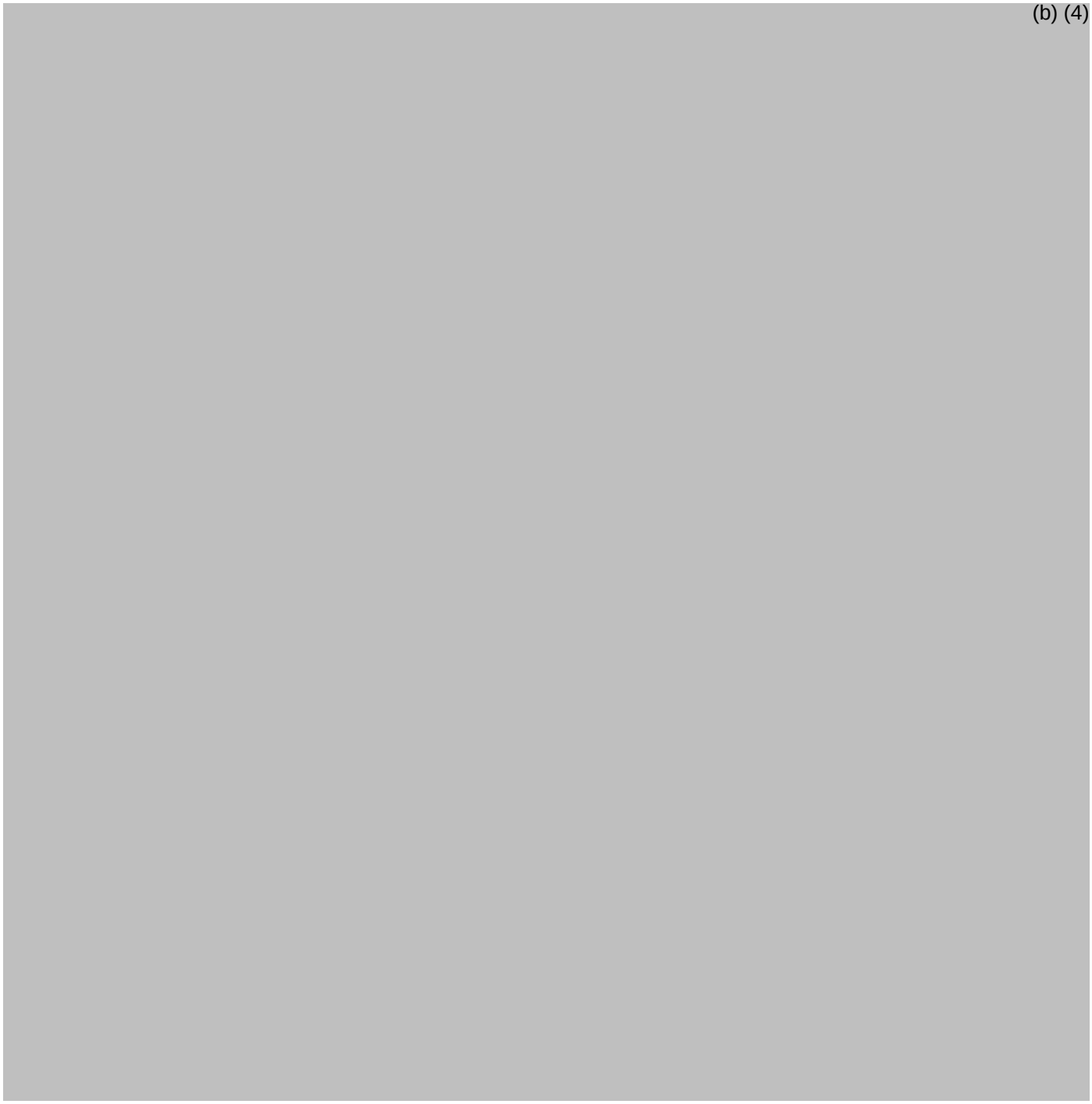
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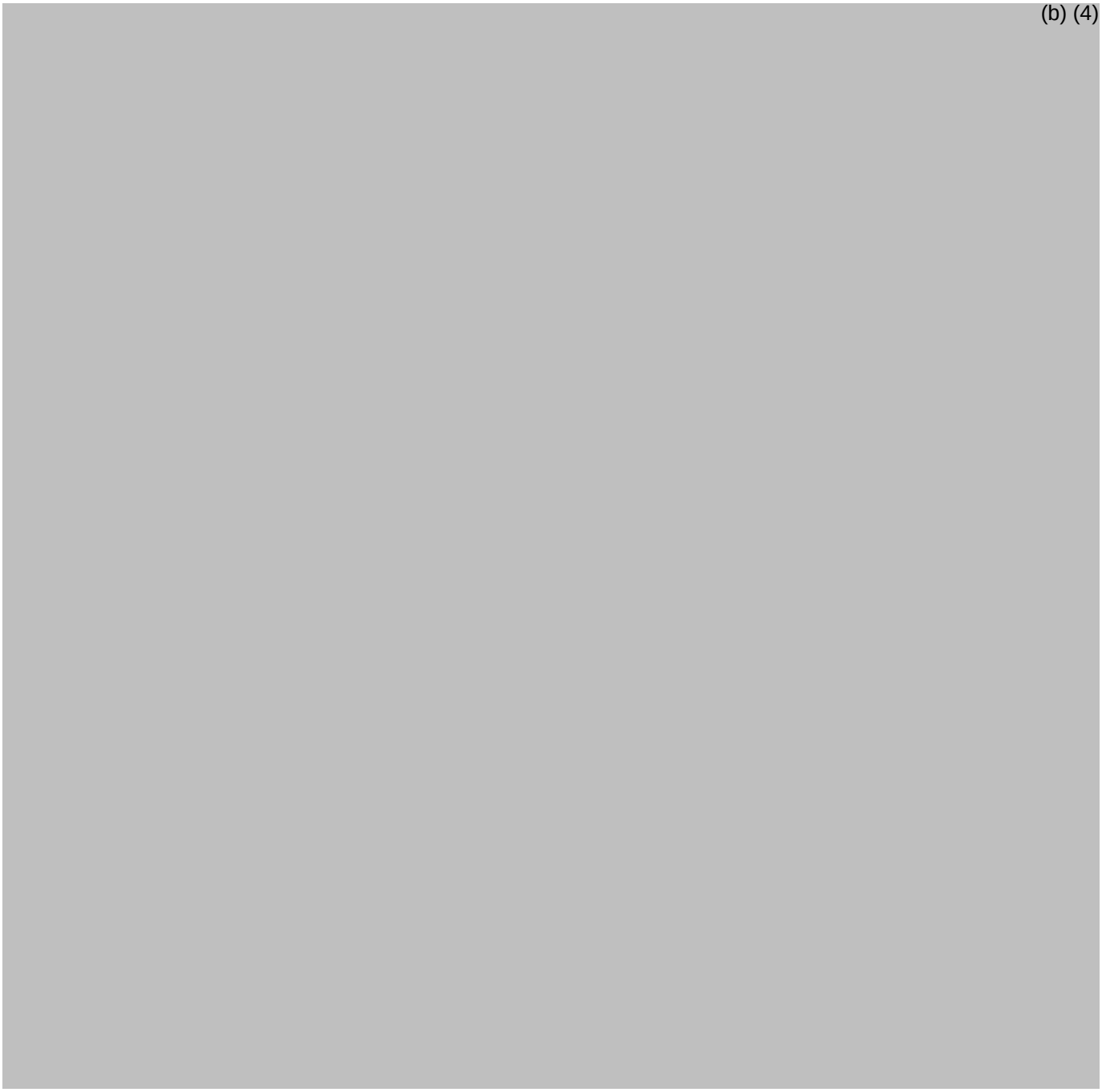

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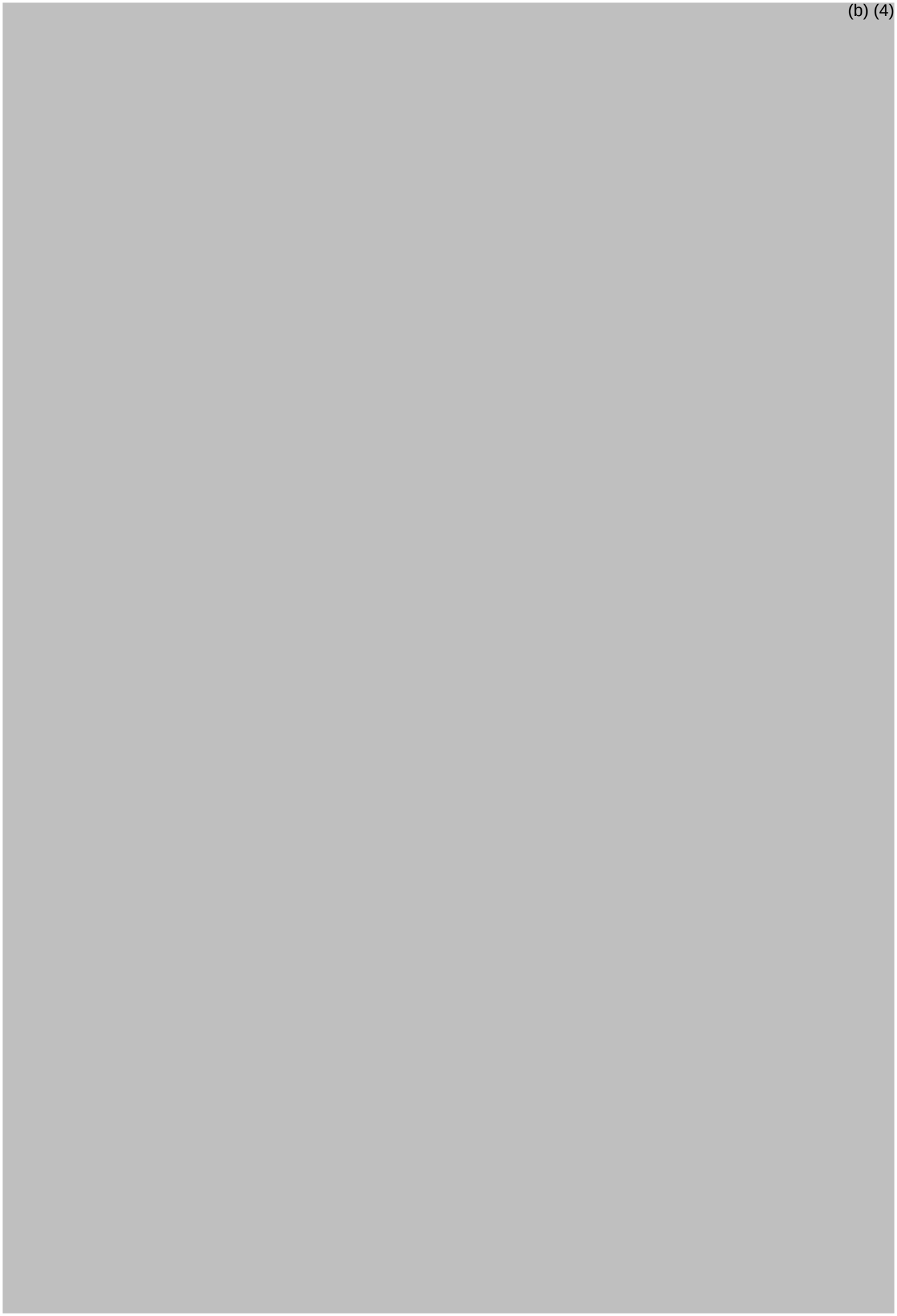

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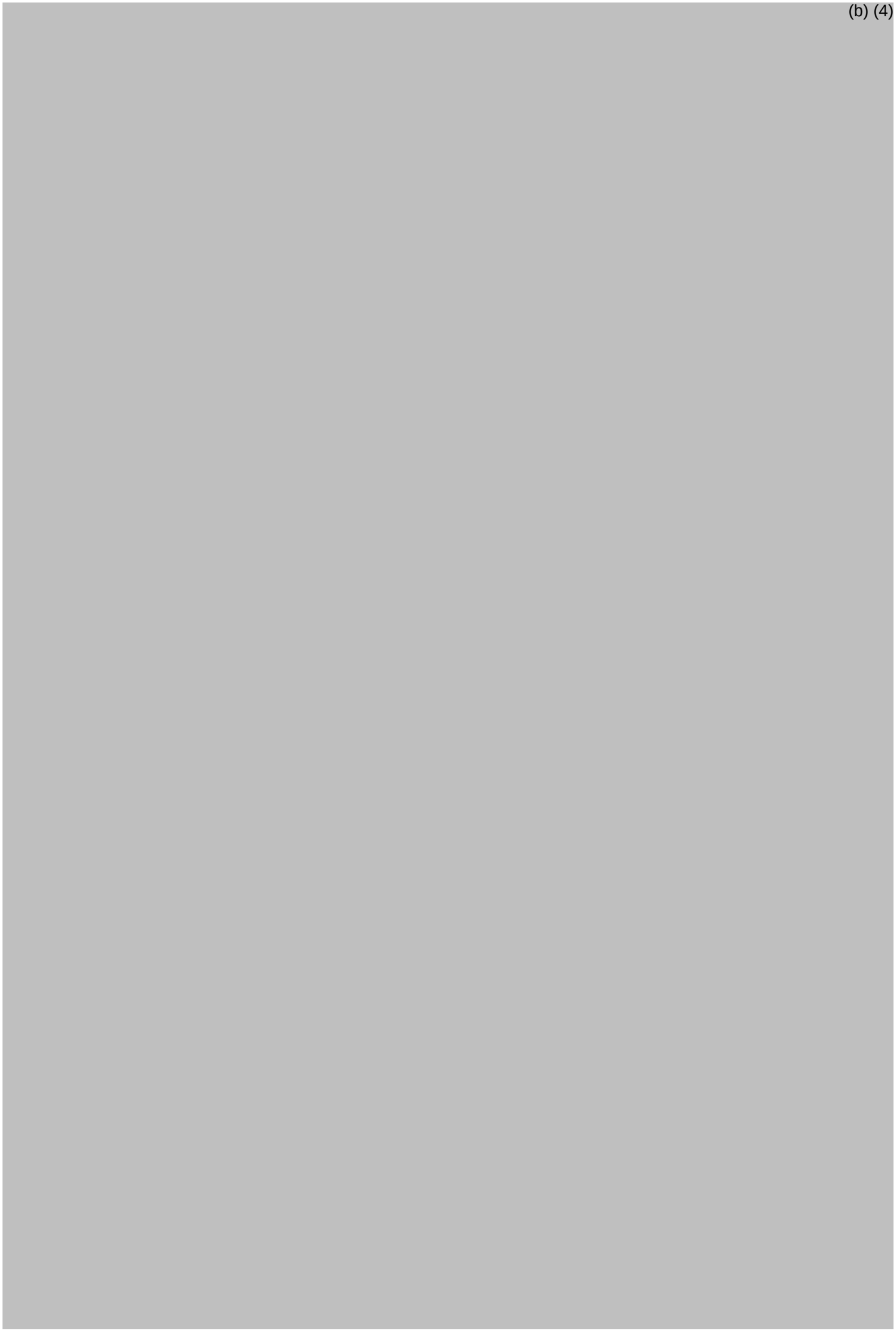

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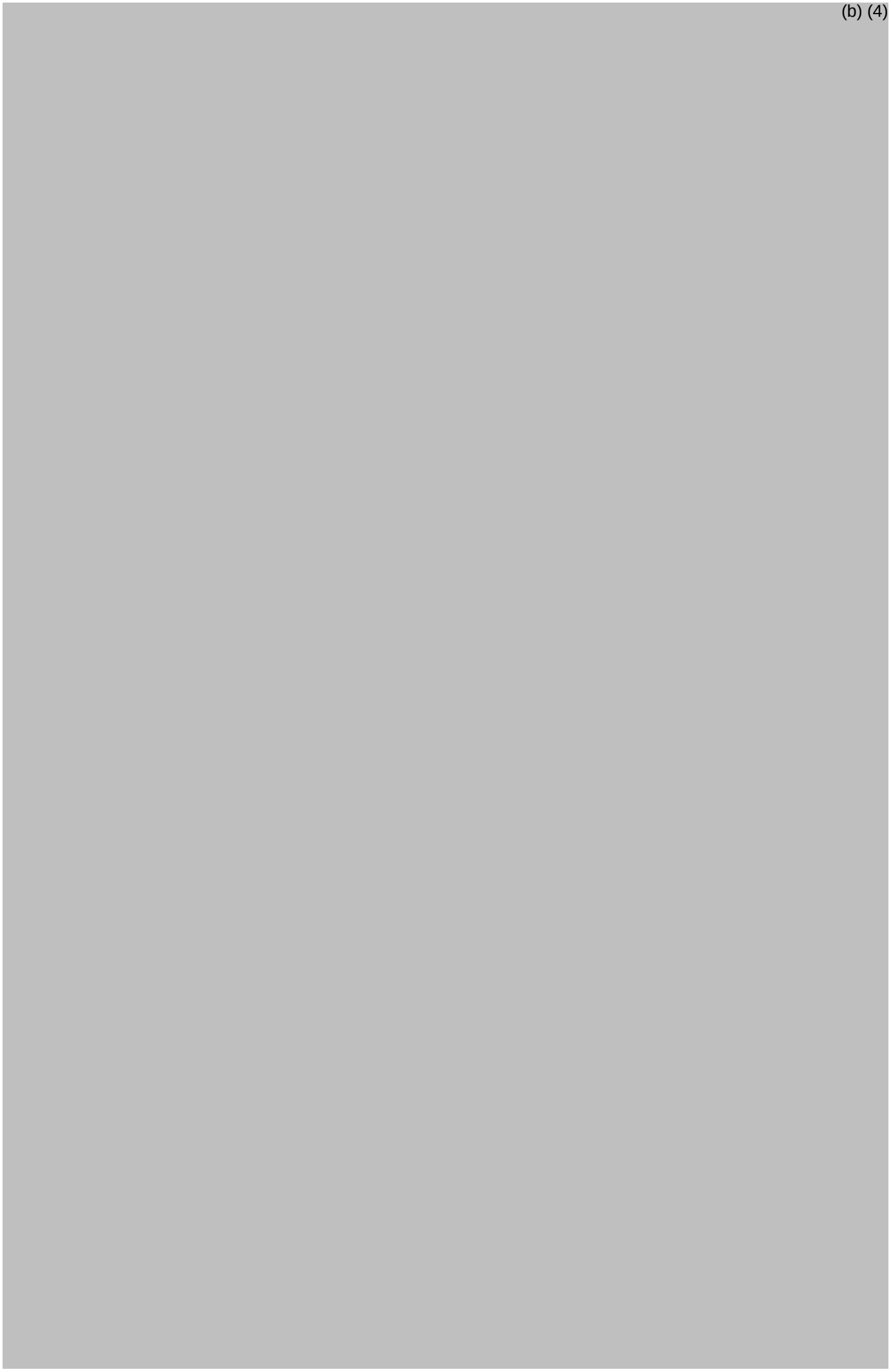

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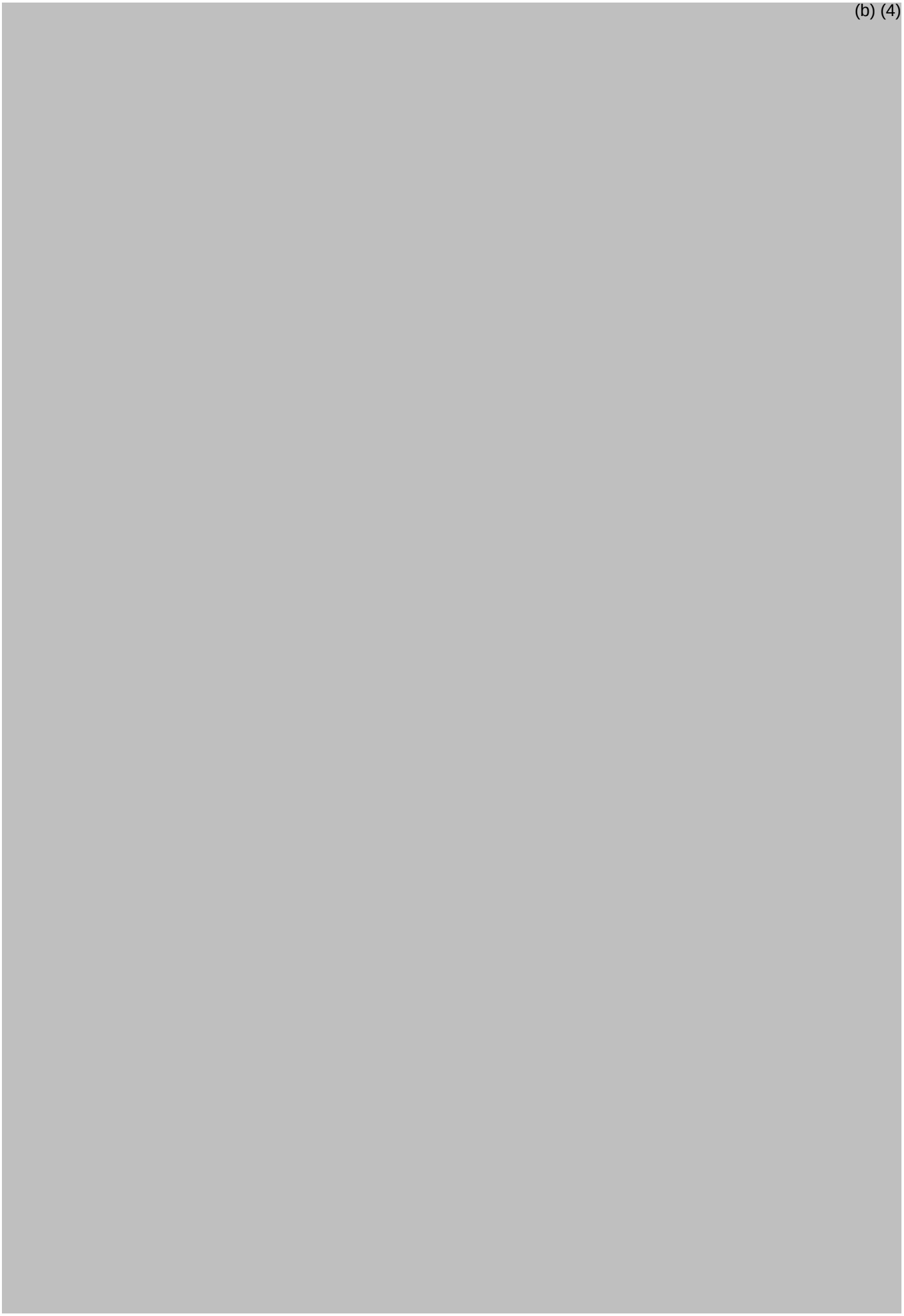
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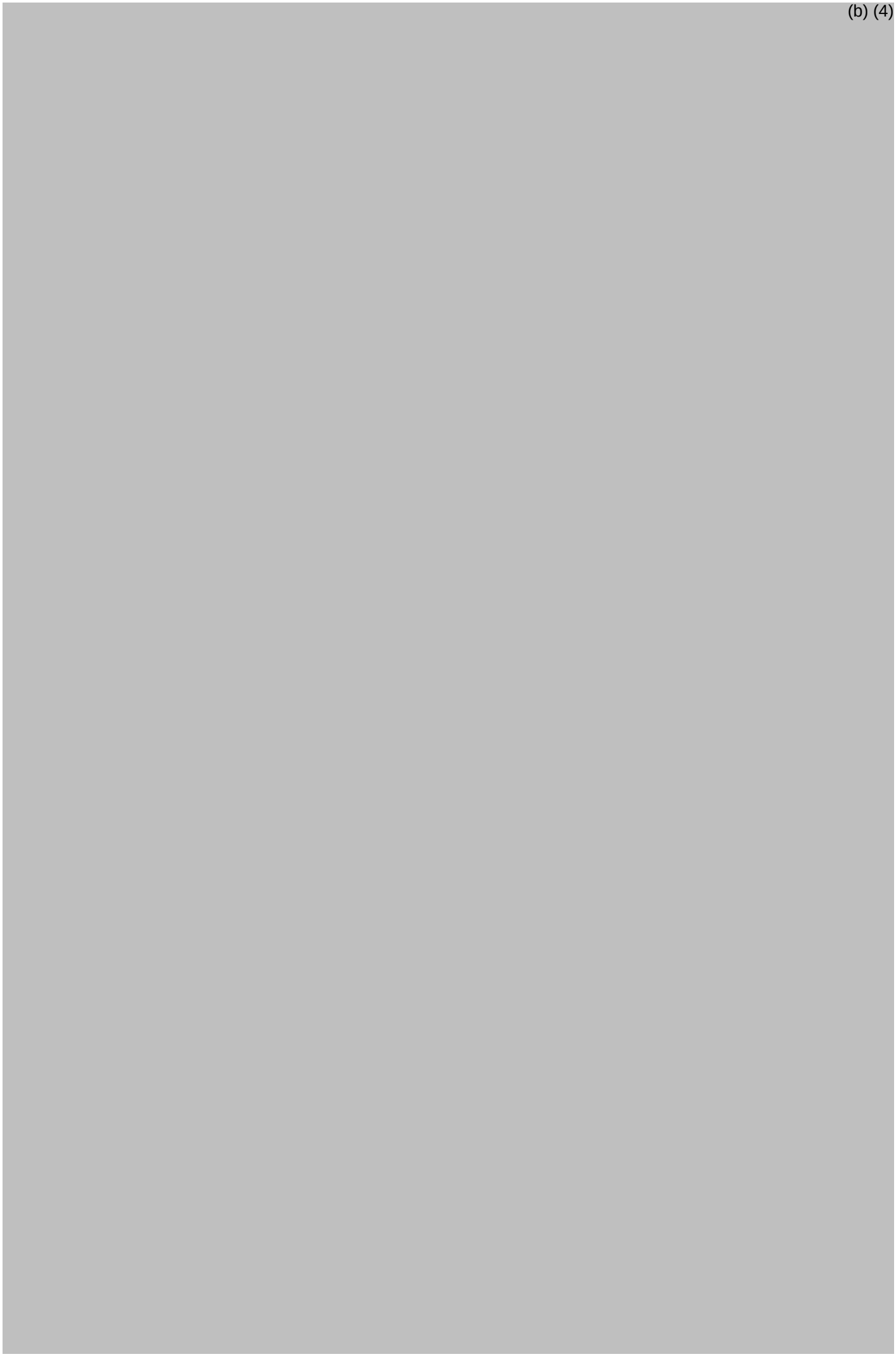

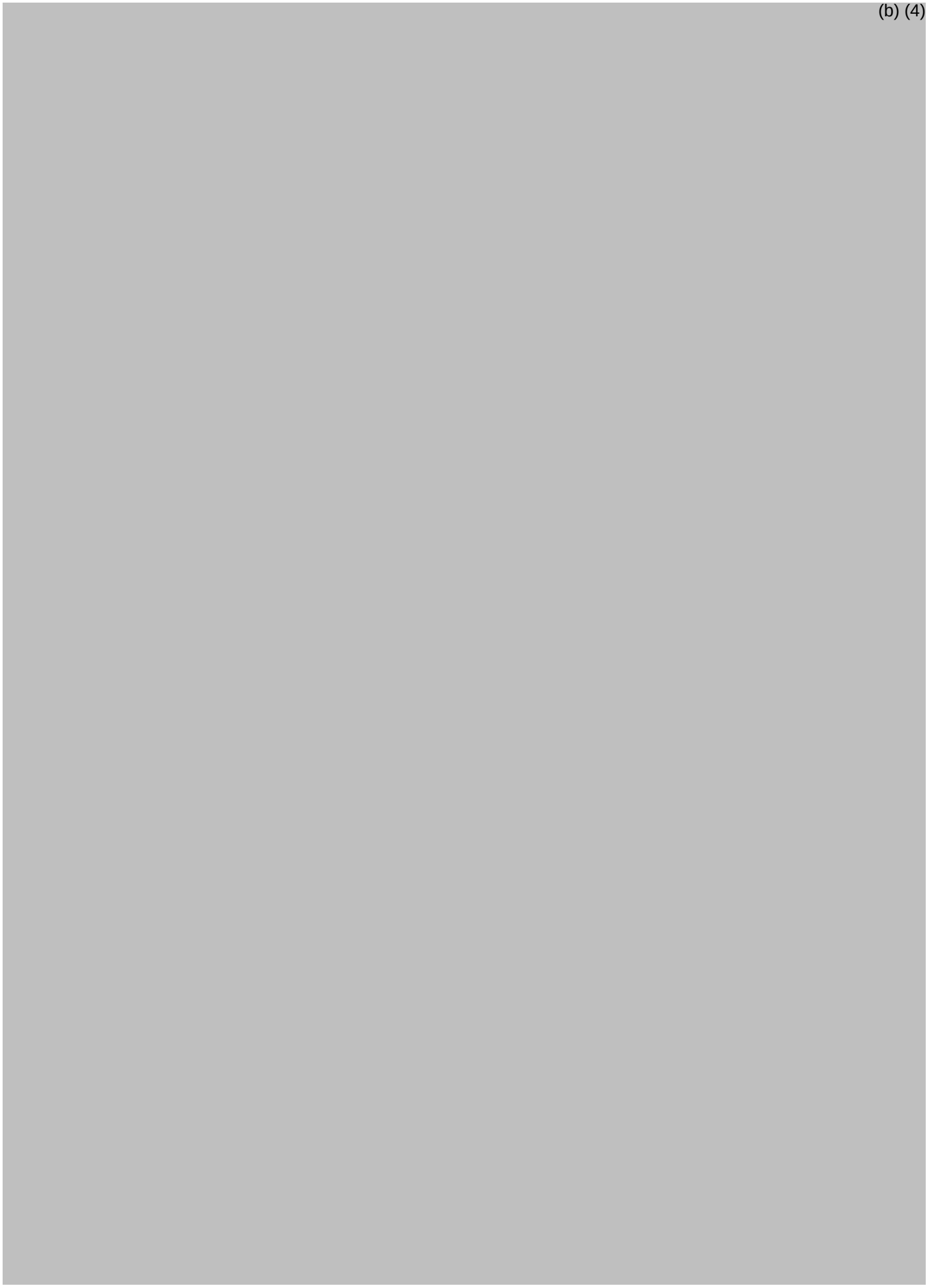
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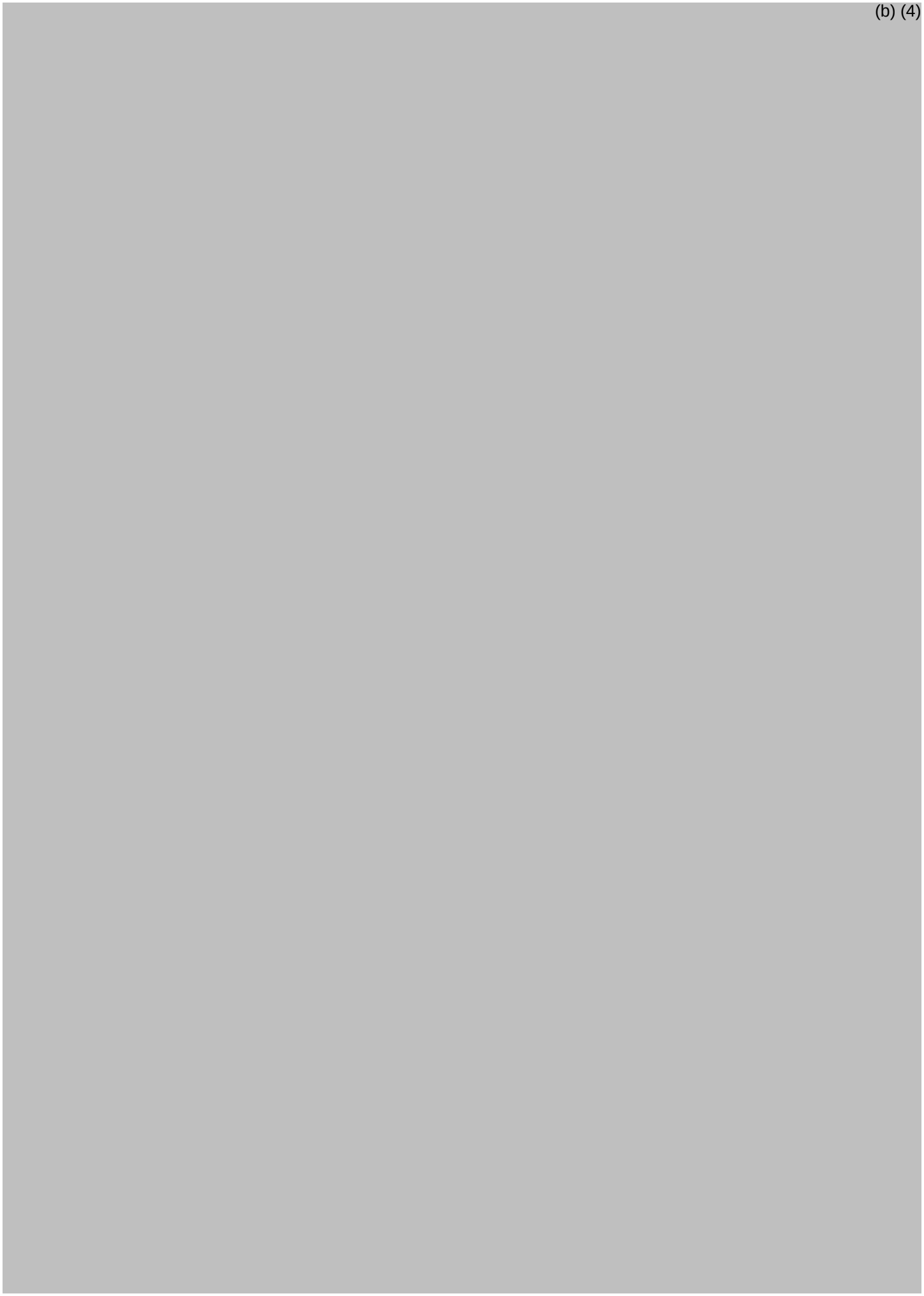

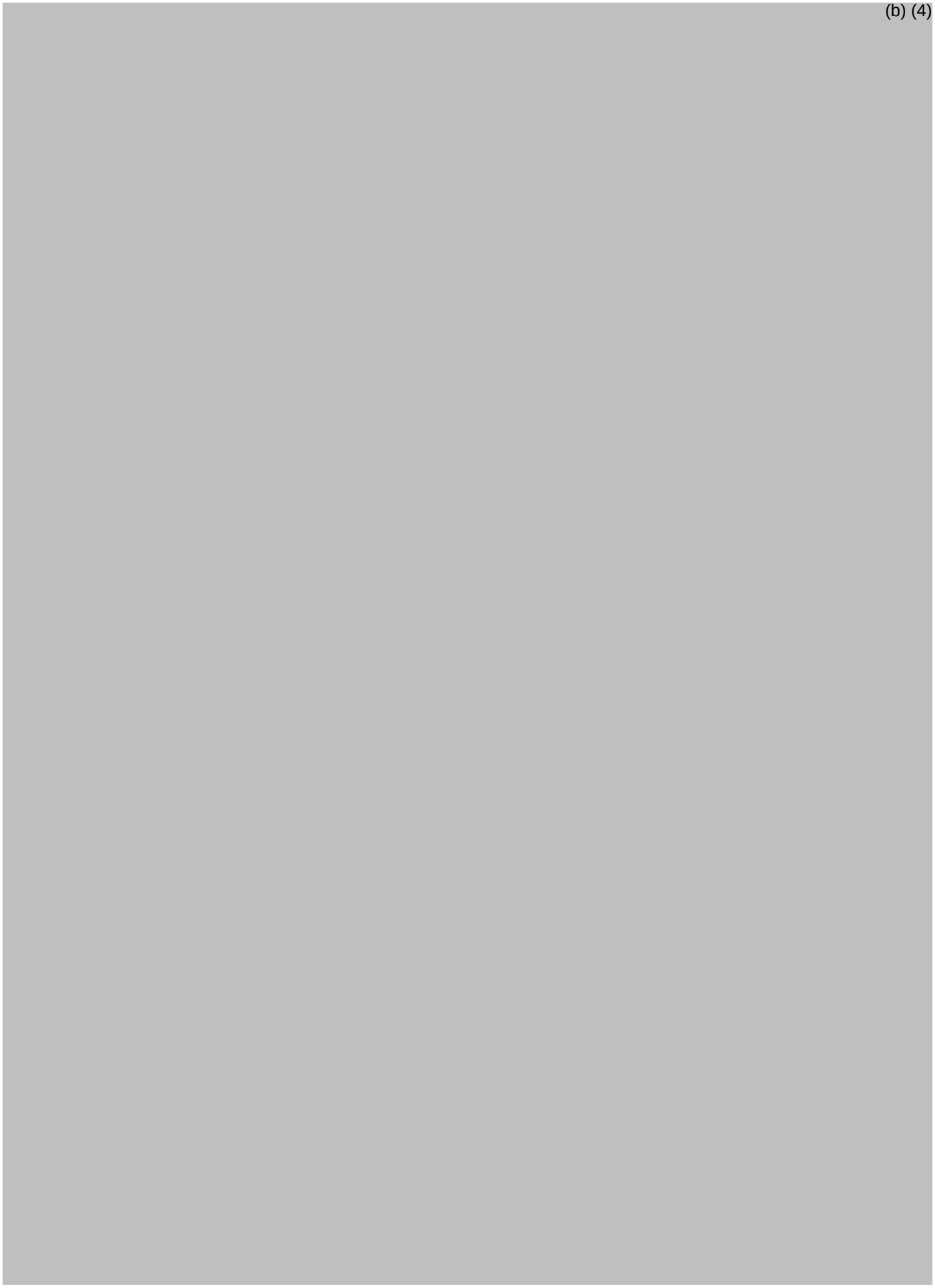
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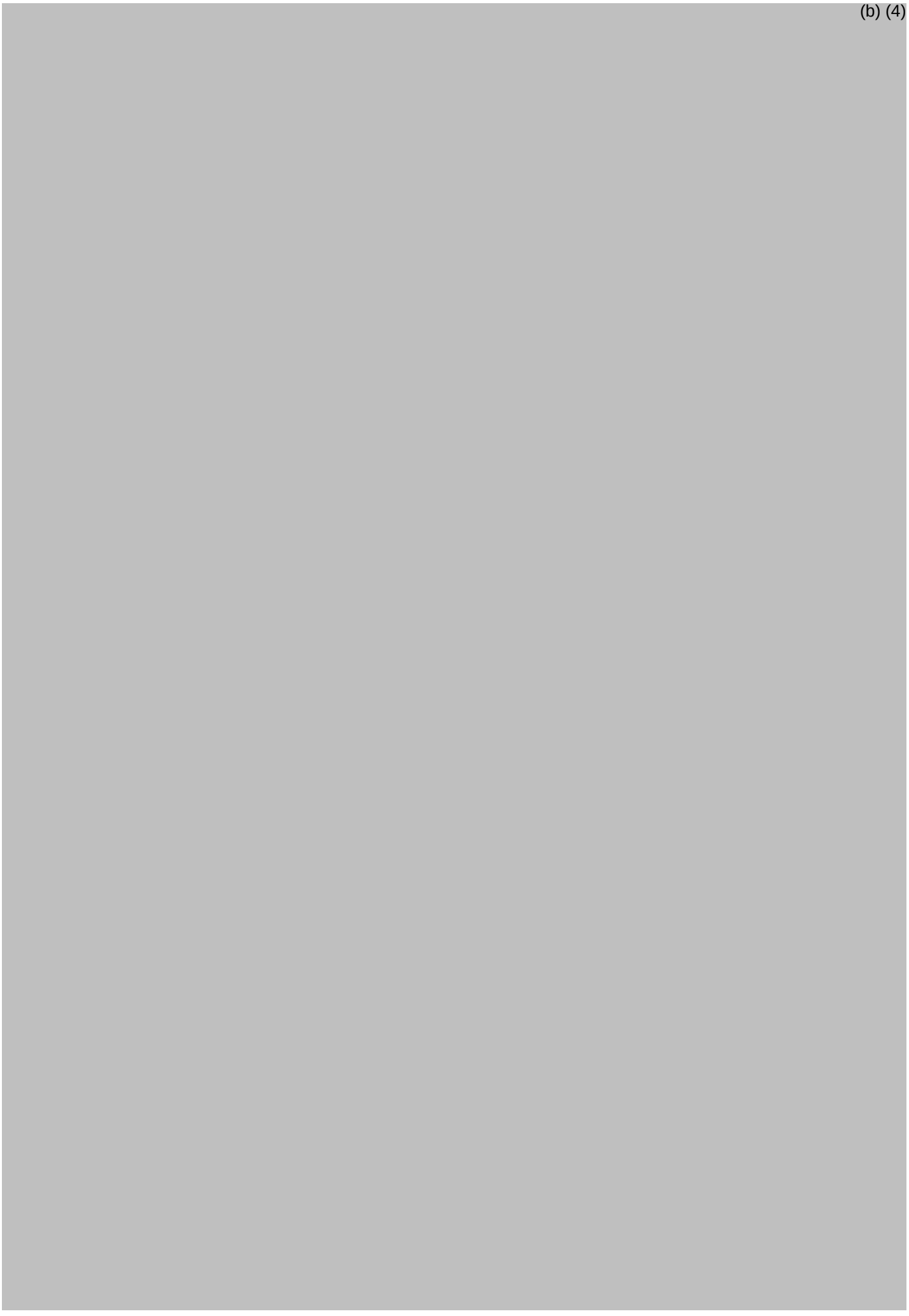

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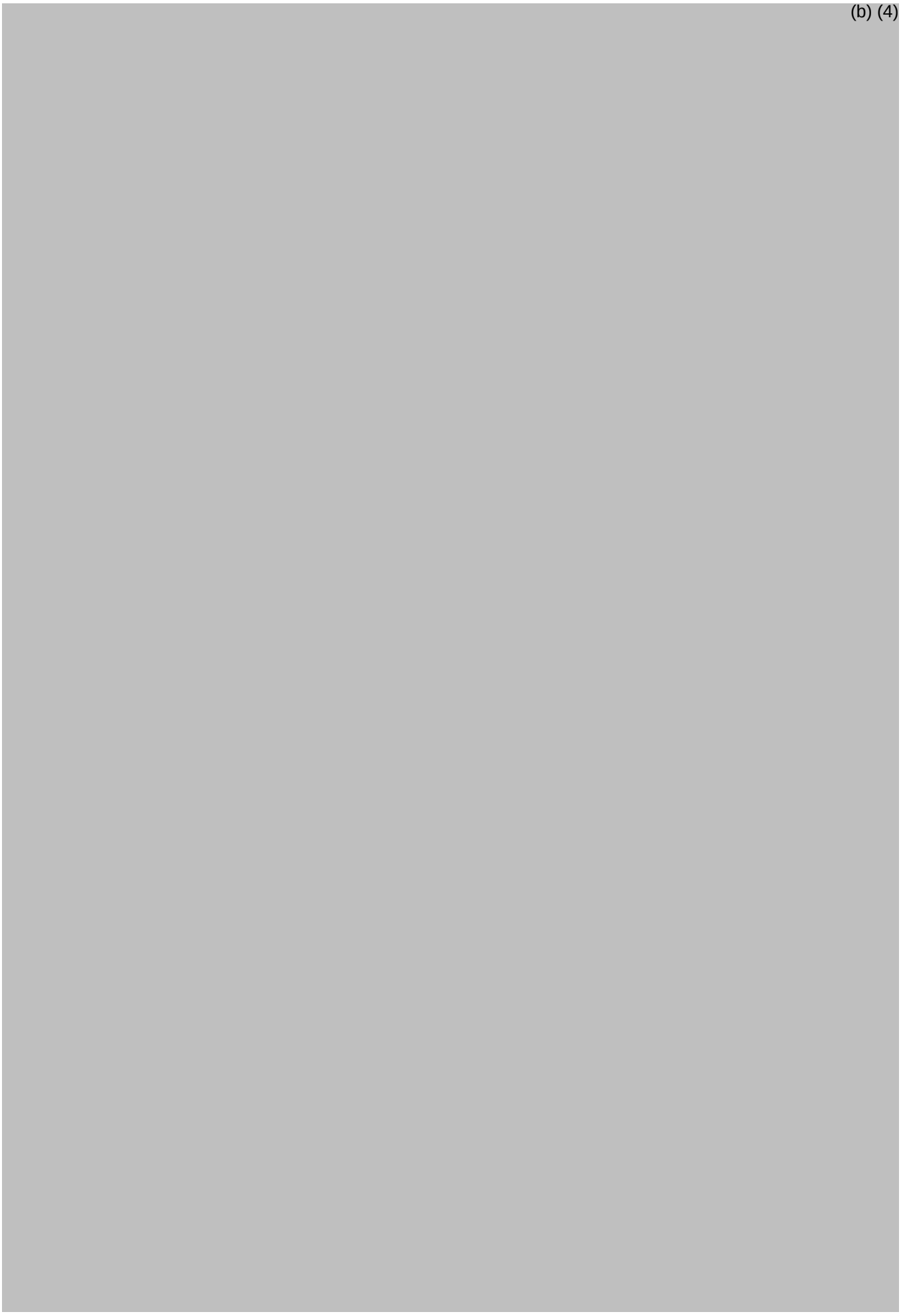
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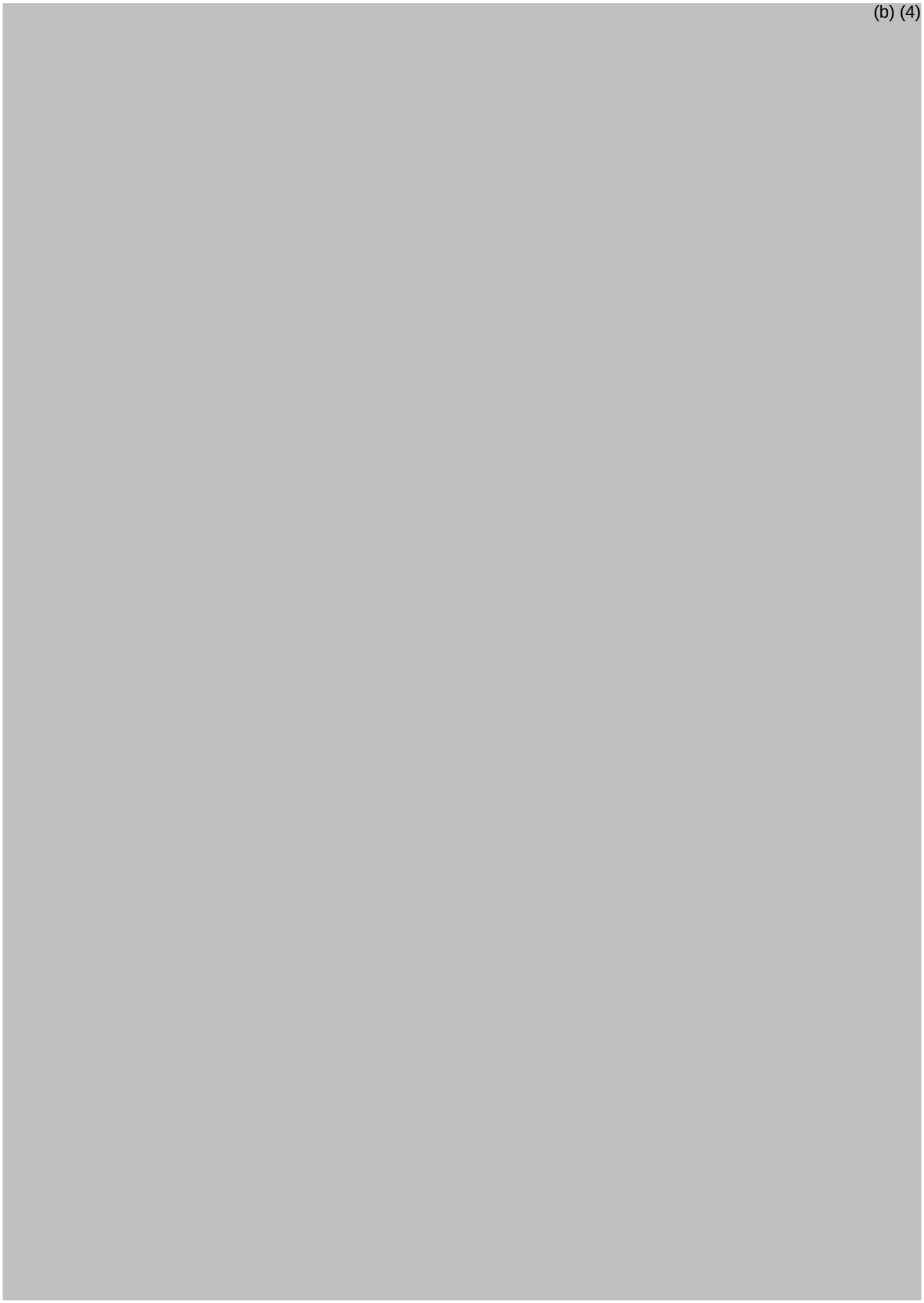

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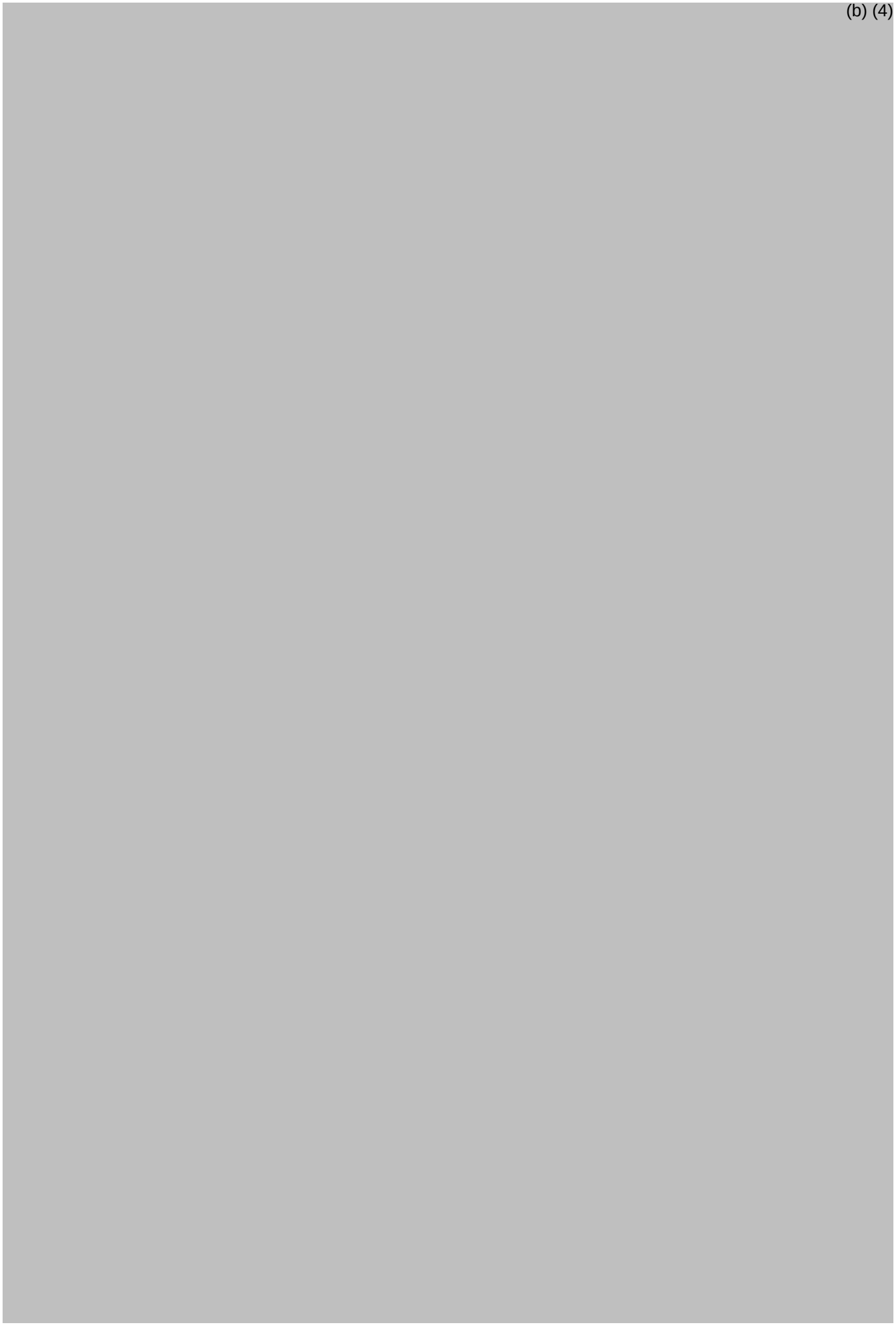
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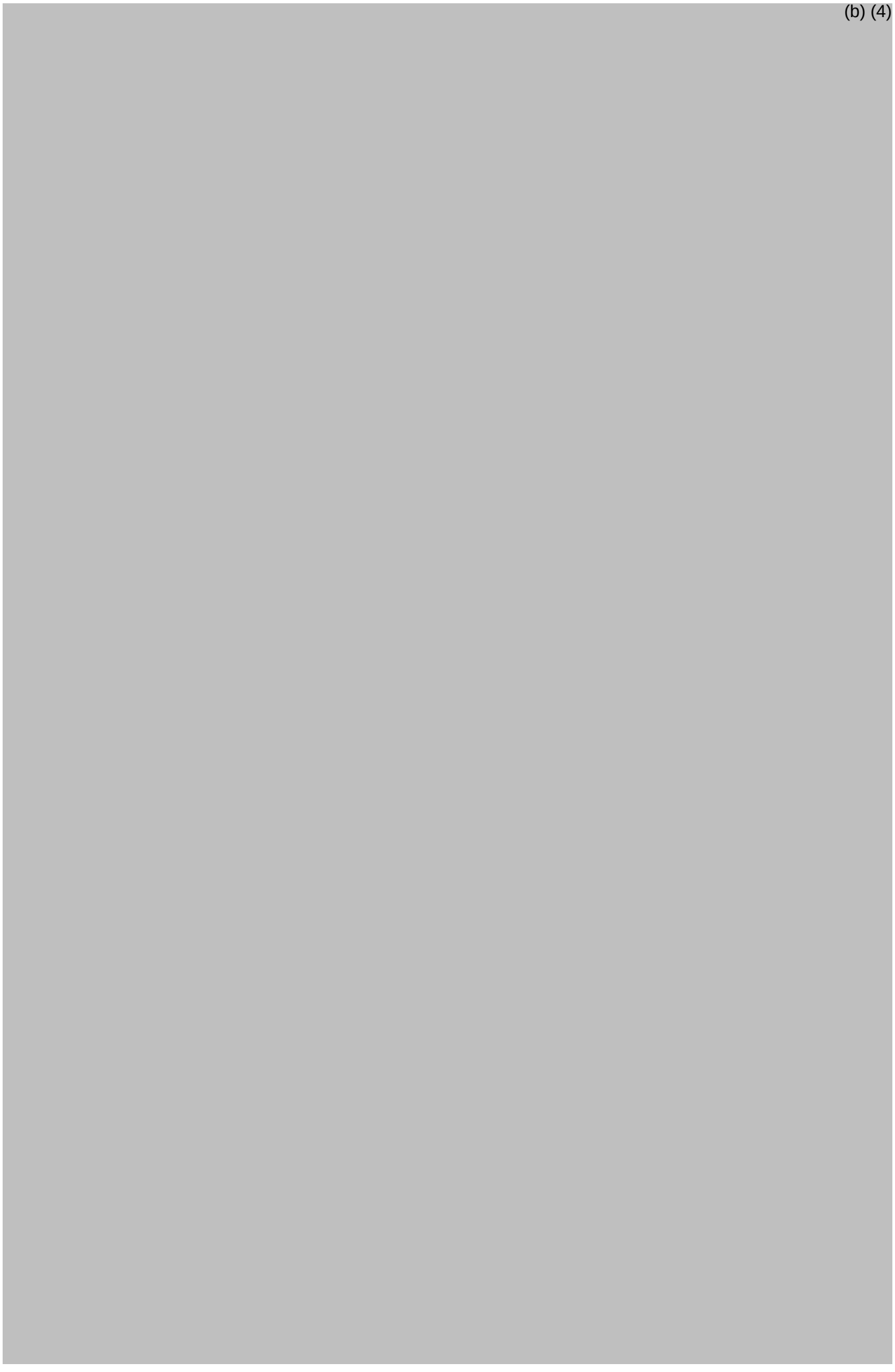
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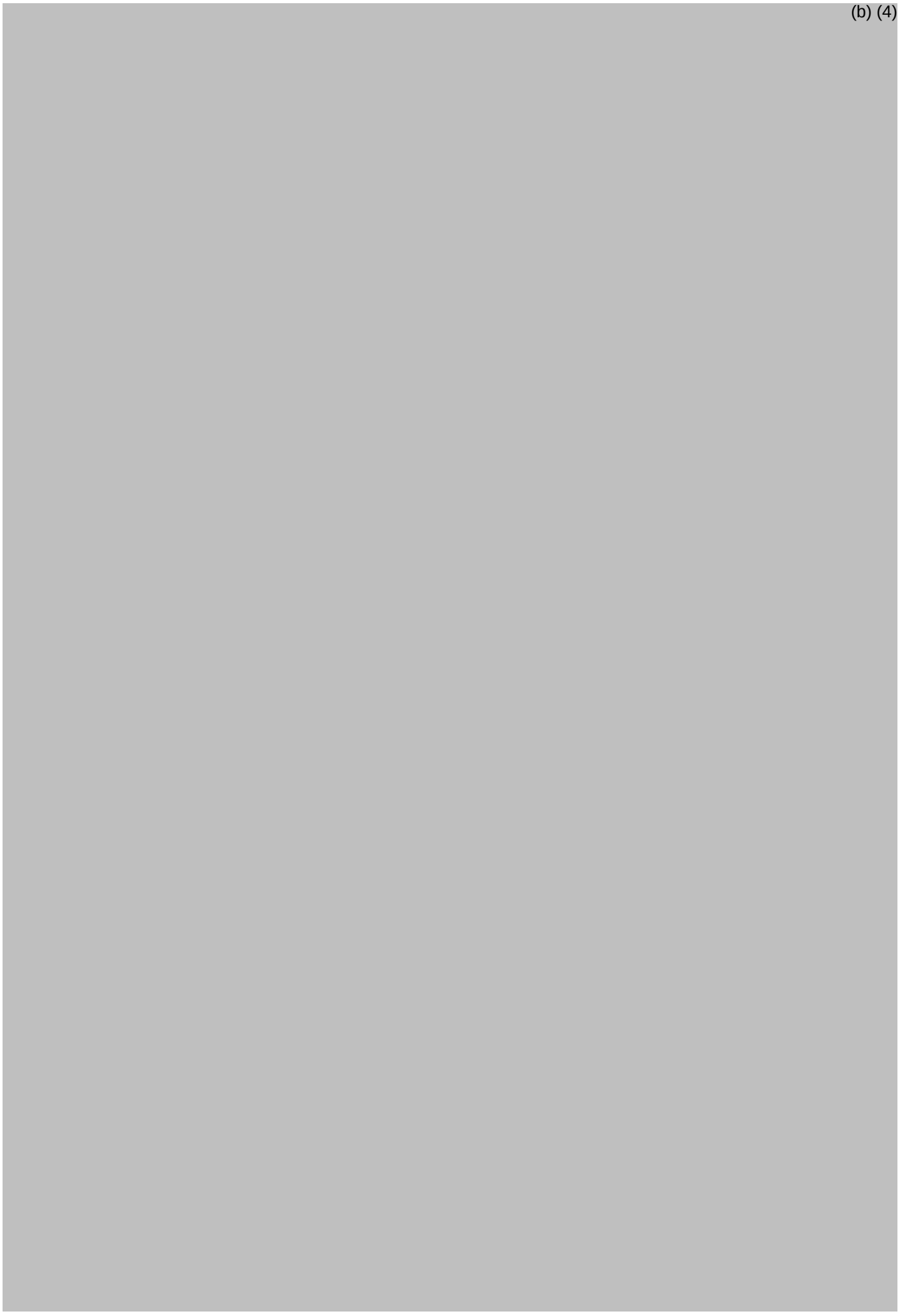
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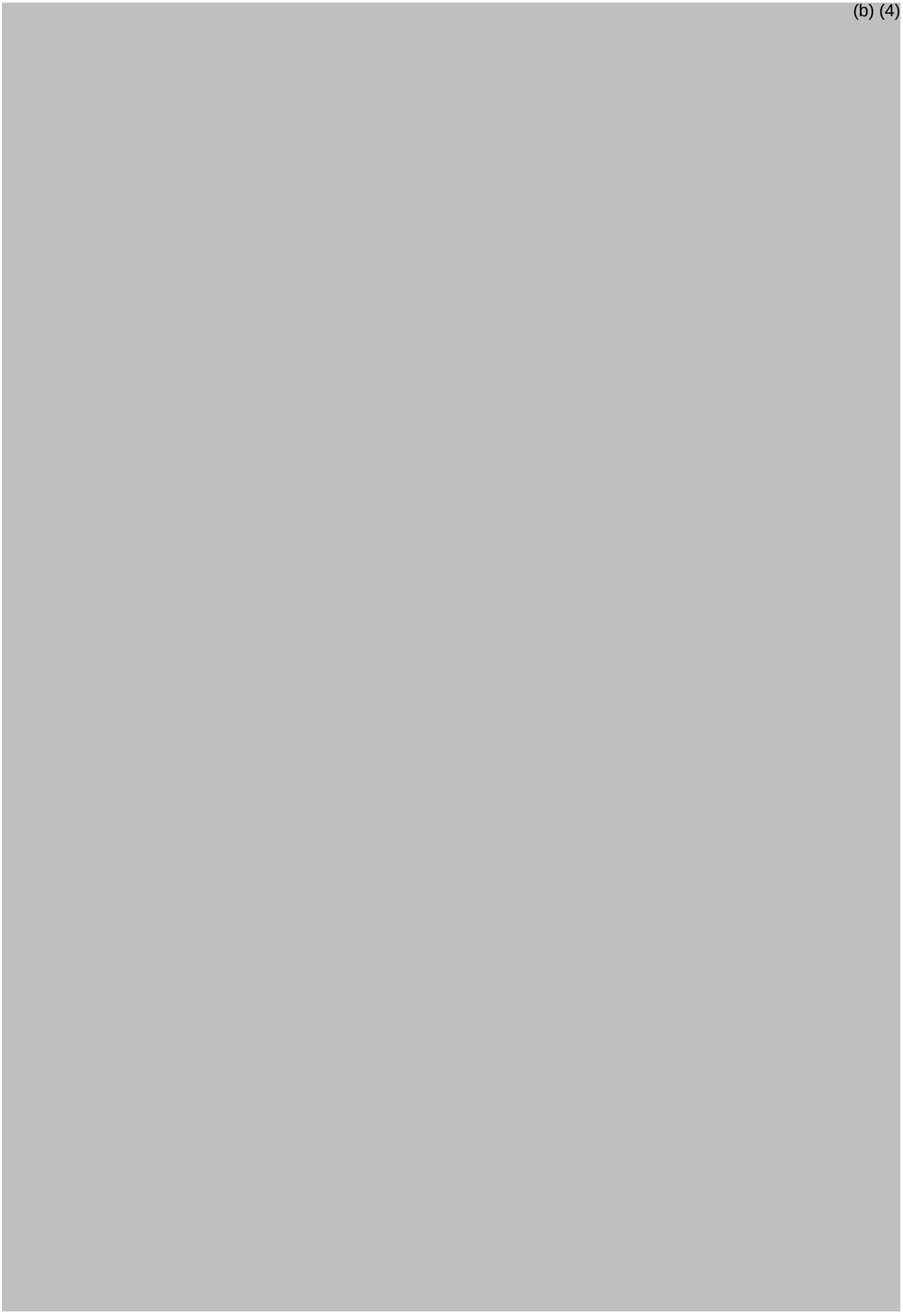
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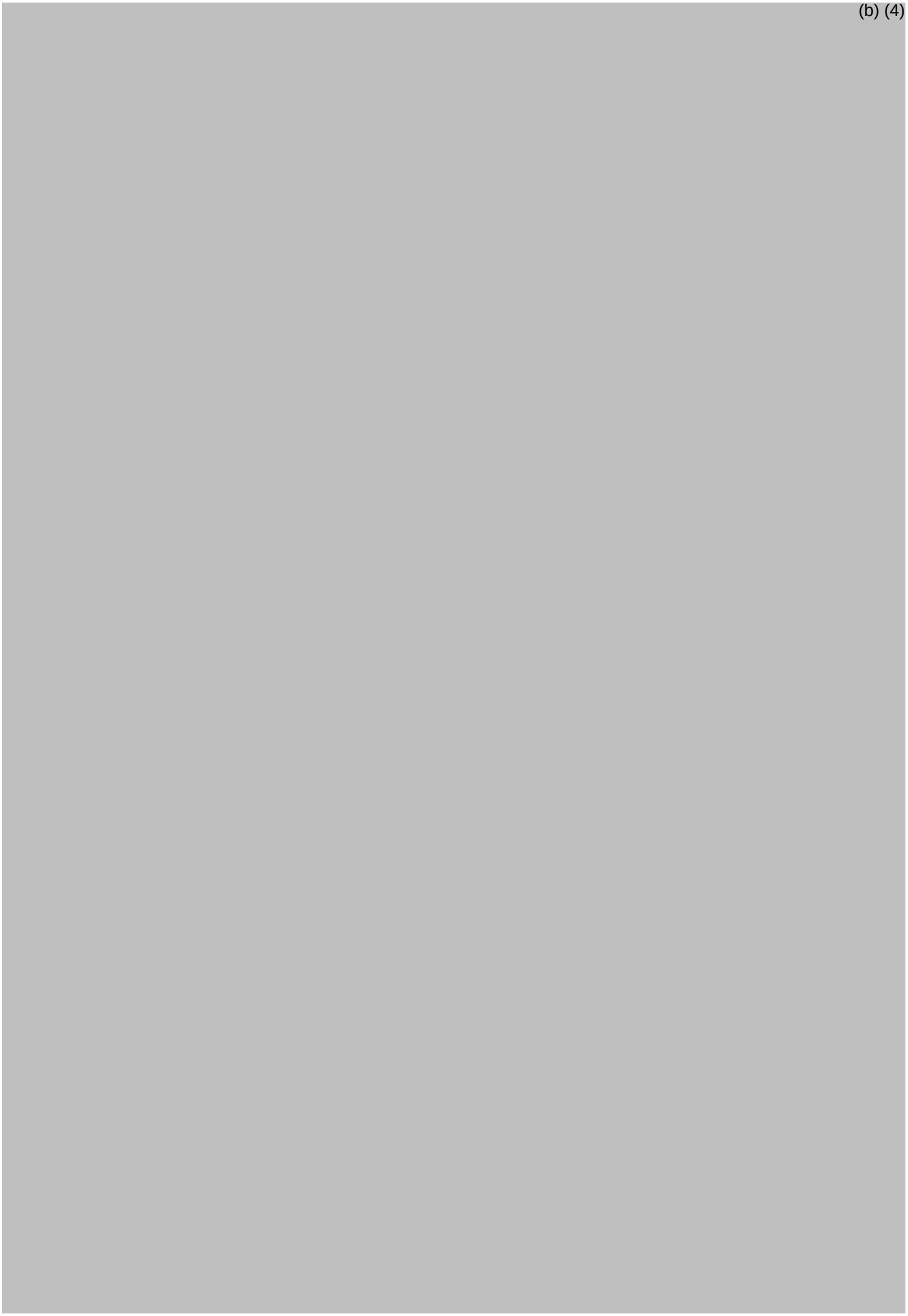

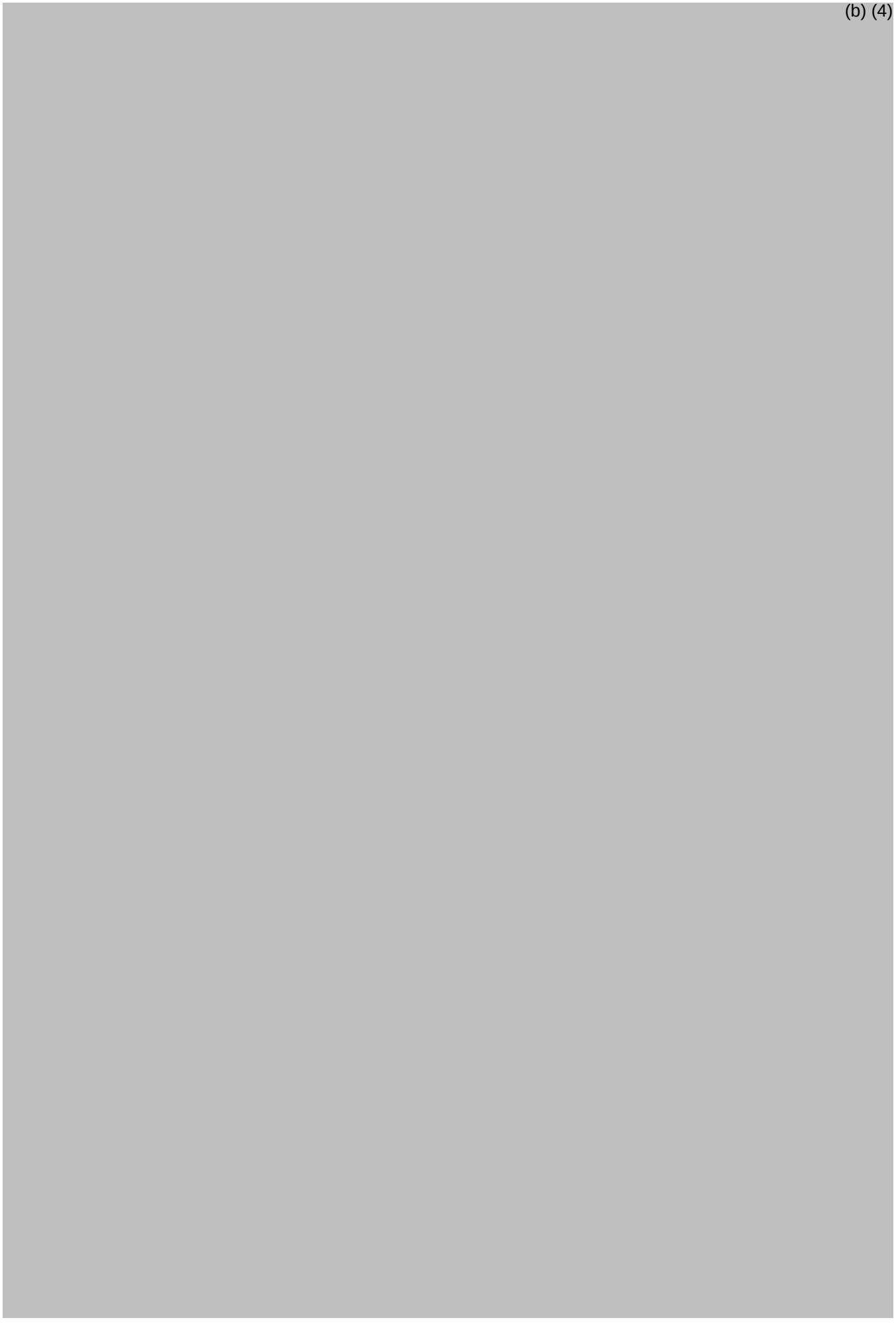
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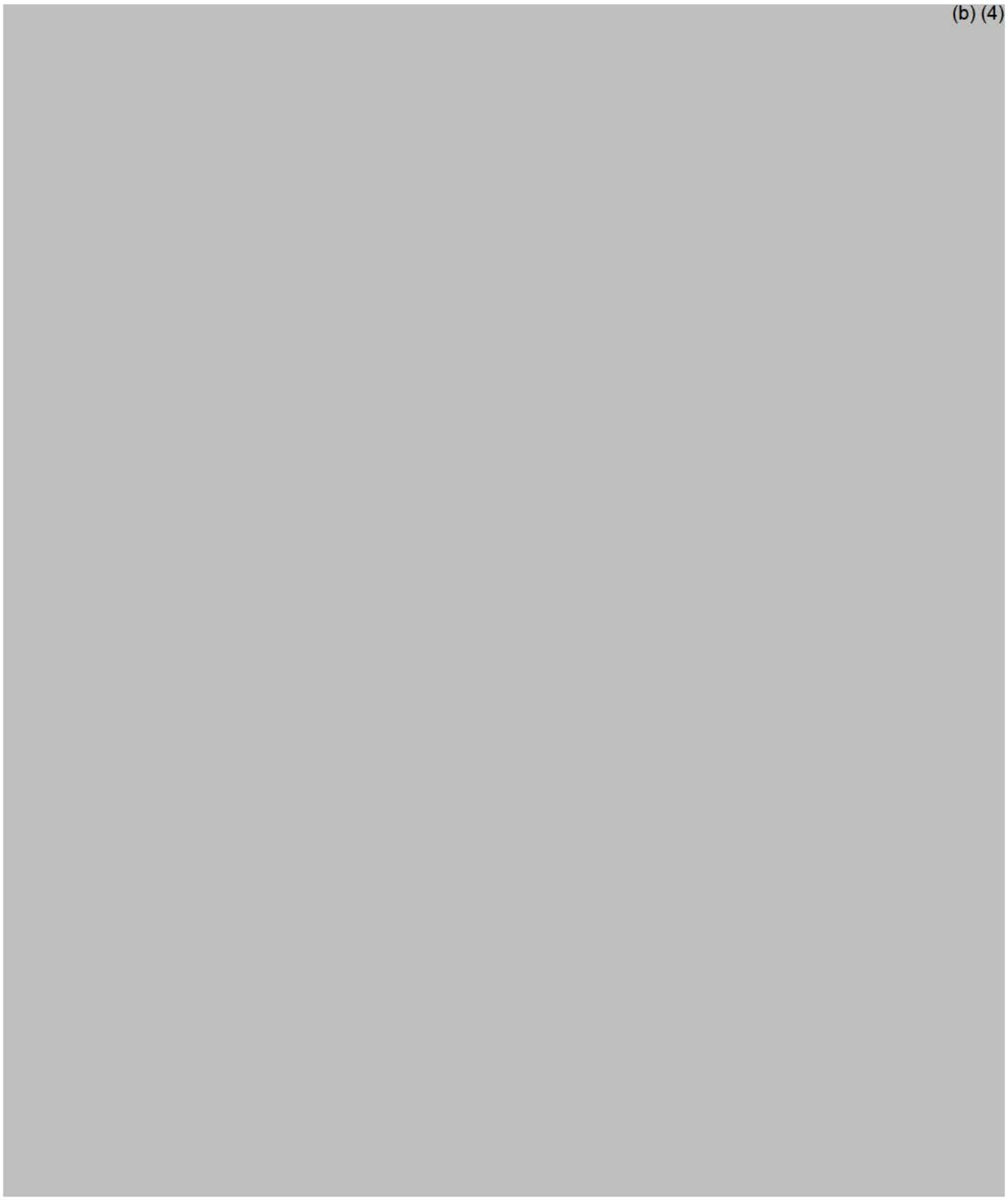
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National Health and Family Planning Commission of the People's Republic of China

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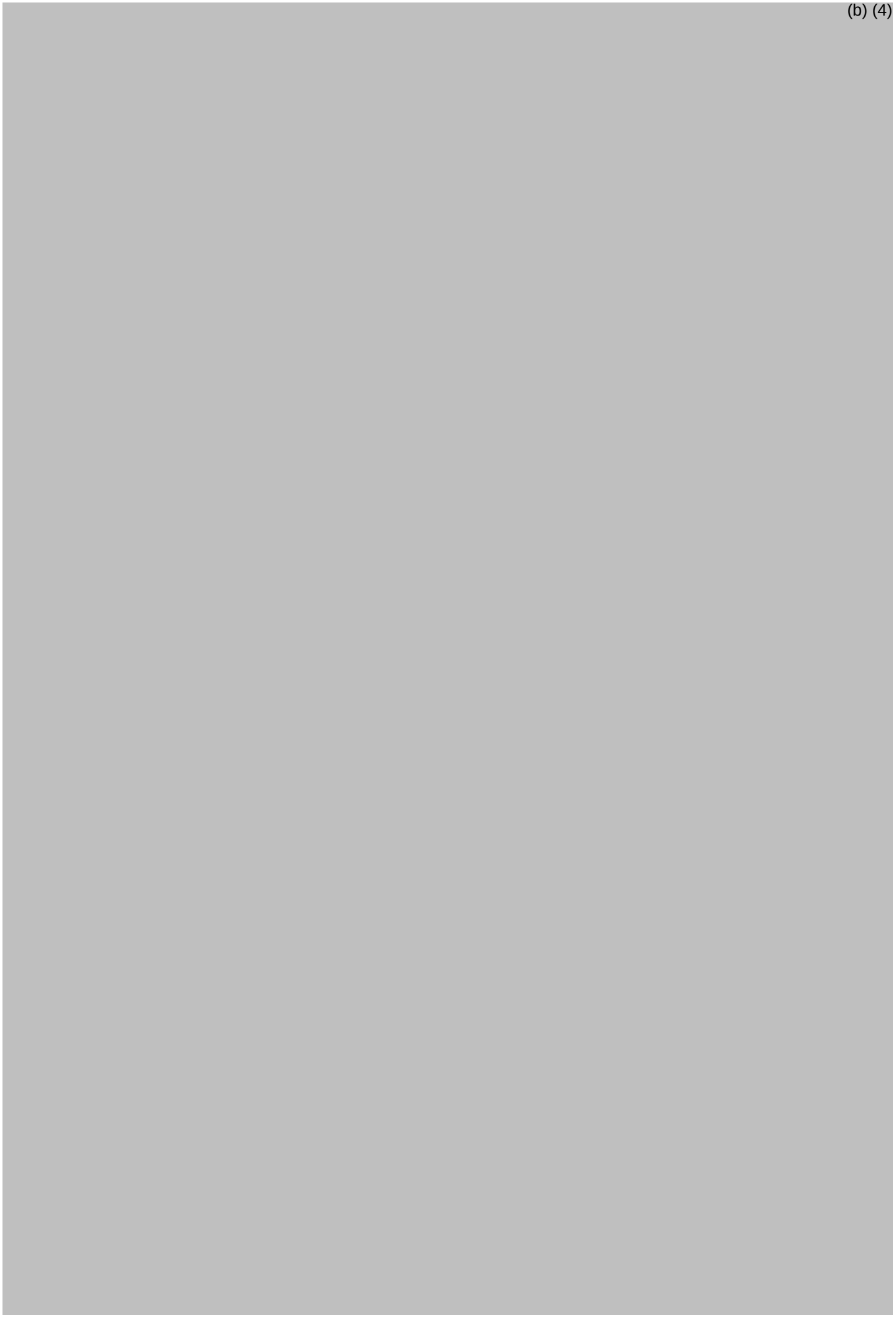

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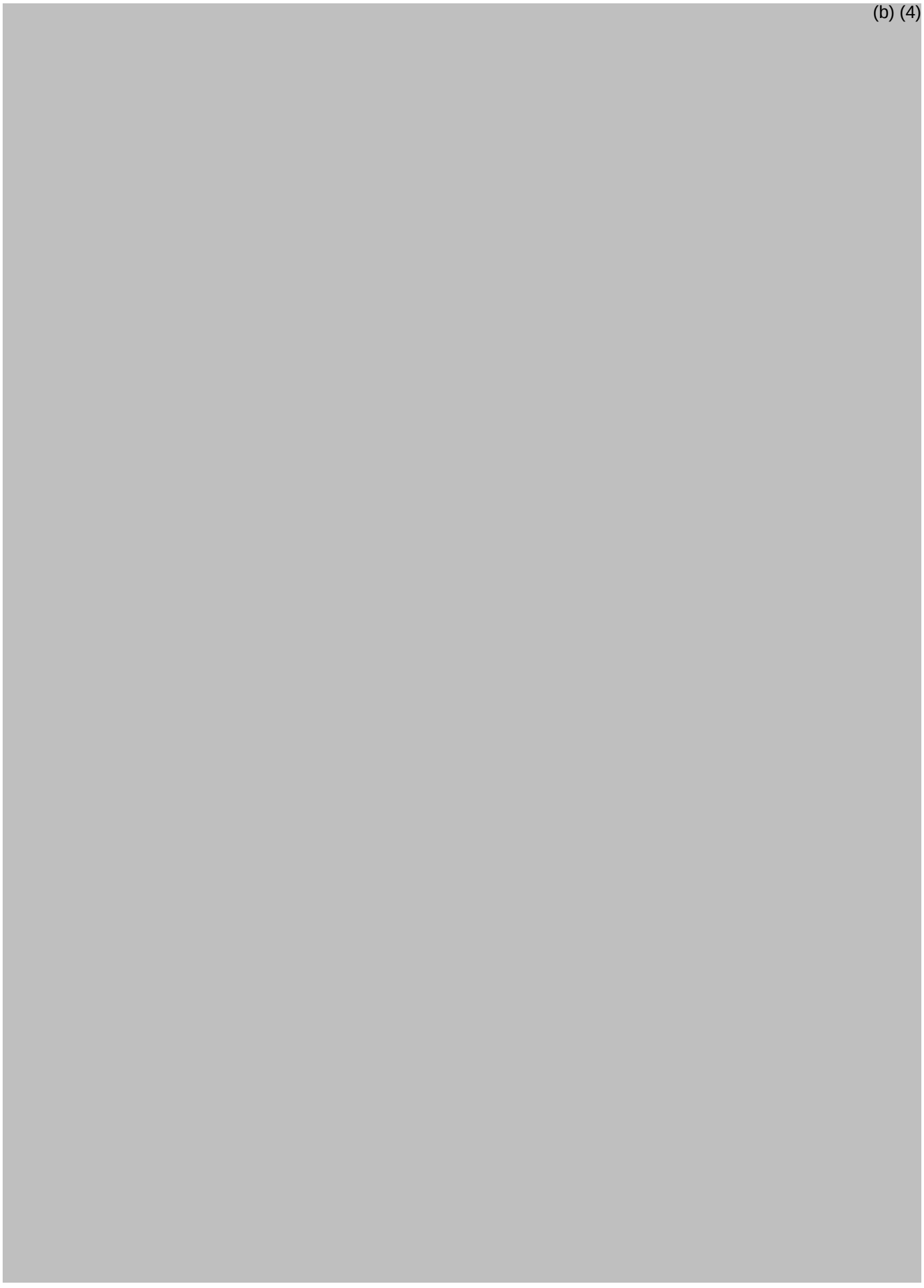
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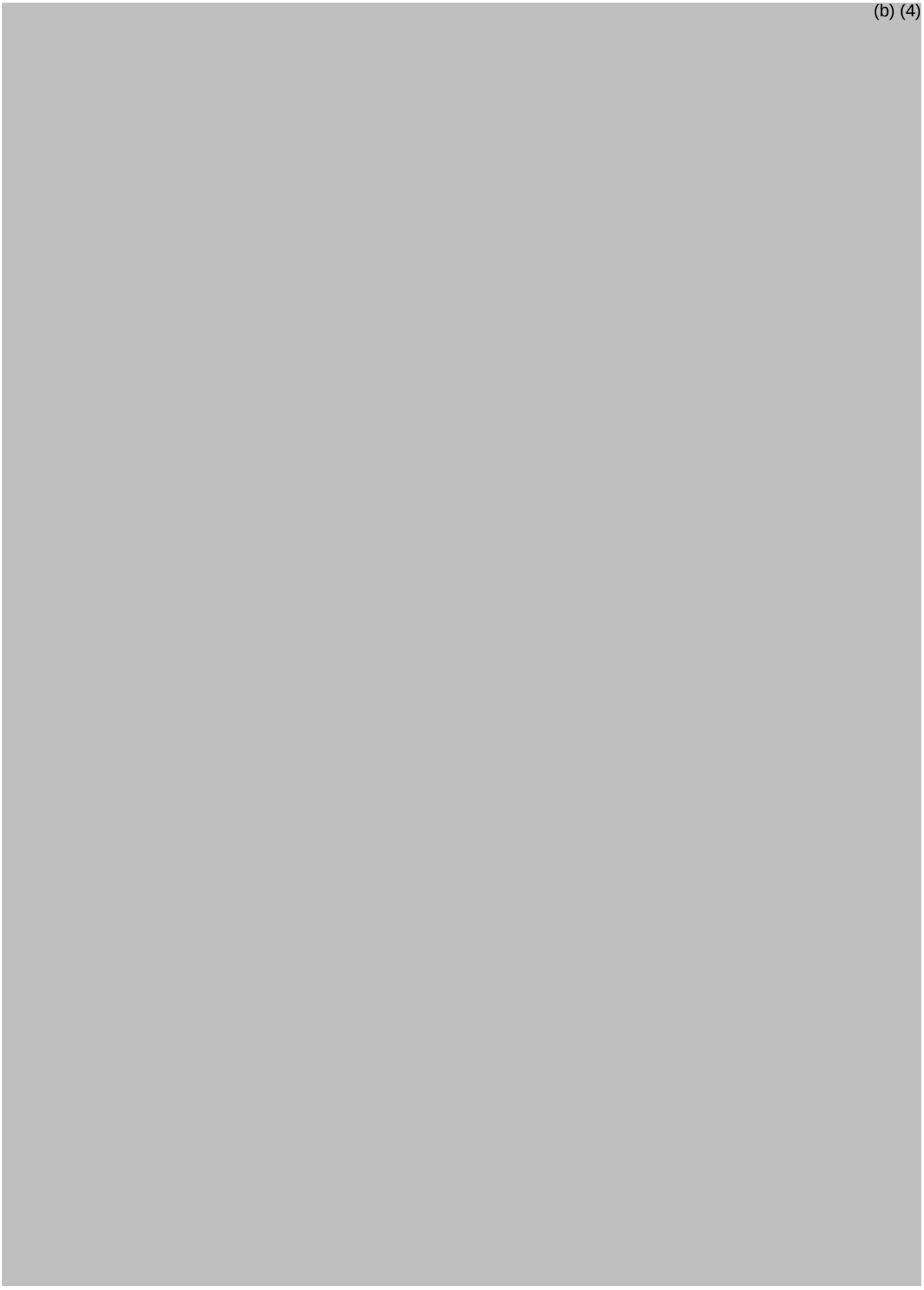
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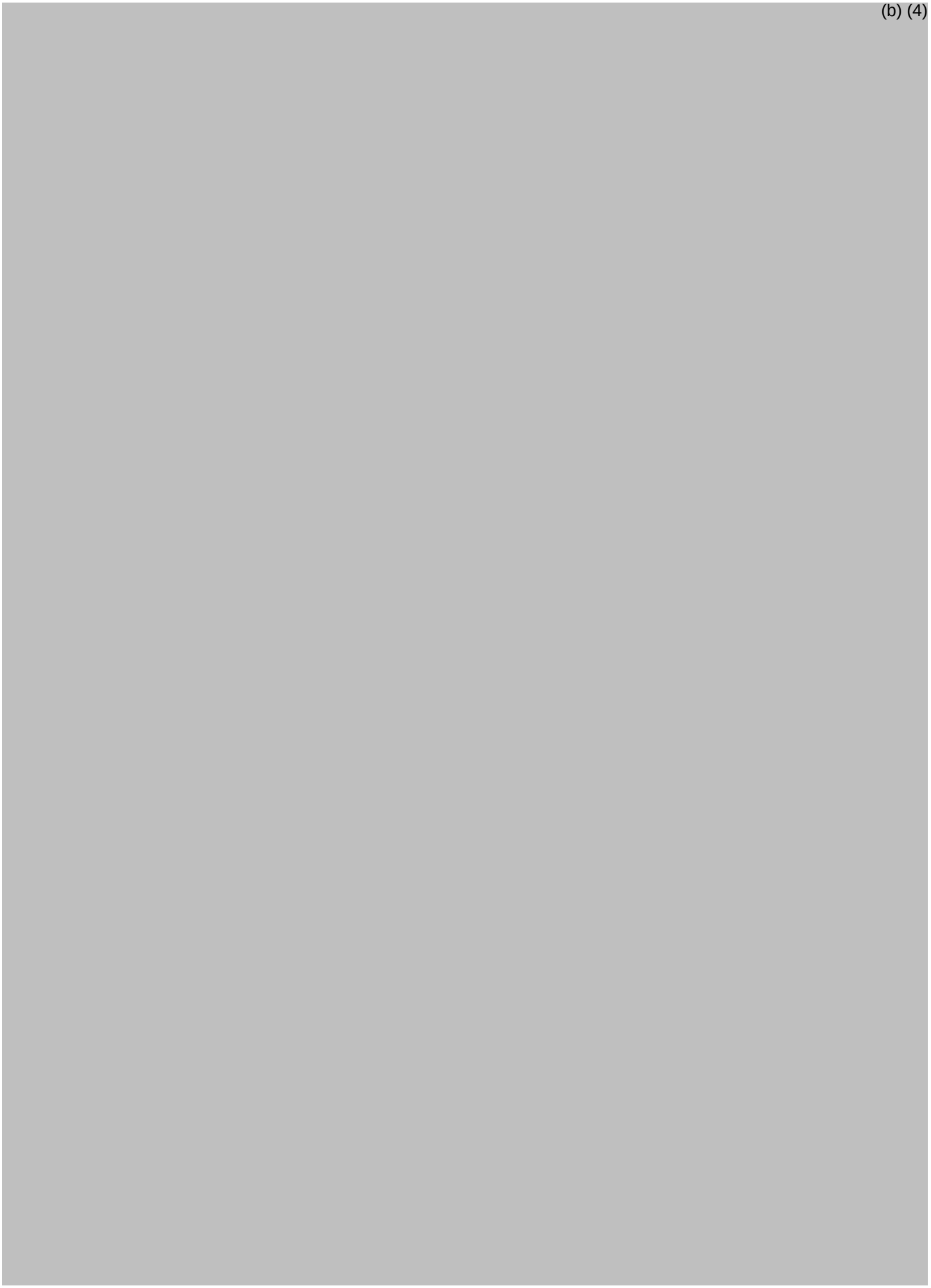

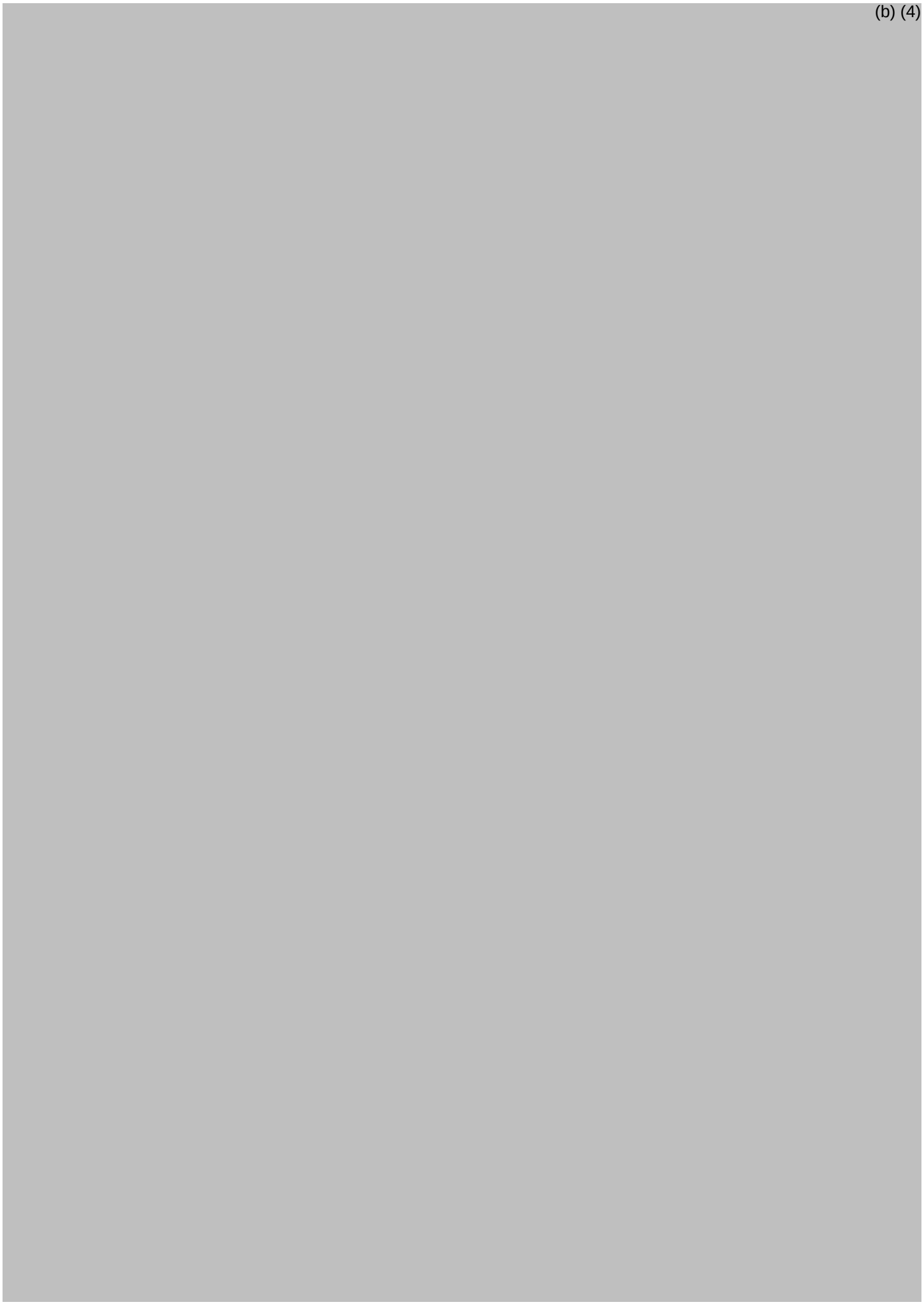

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National Standard of the People's Republic of China

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Food Additive – Potassium hydroxide

食品添加剂 – 氢氧化钾

Issue Date: Dec 21, 2010 Implementation Date: Feb 21, 2011

Issued by

Ministry of Health, People's Republic of China

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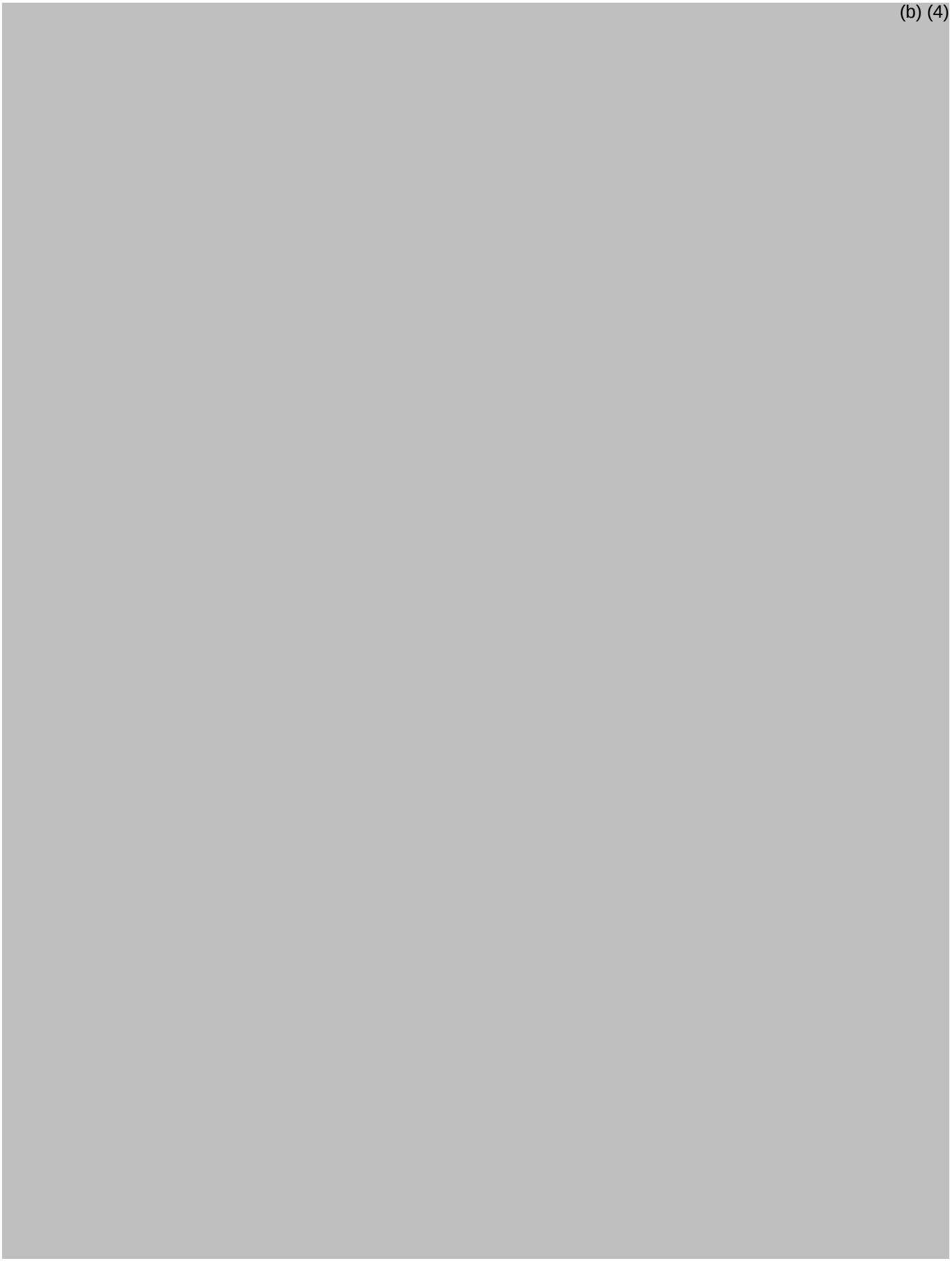
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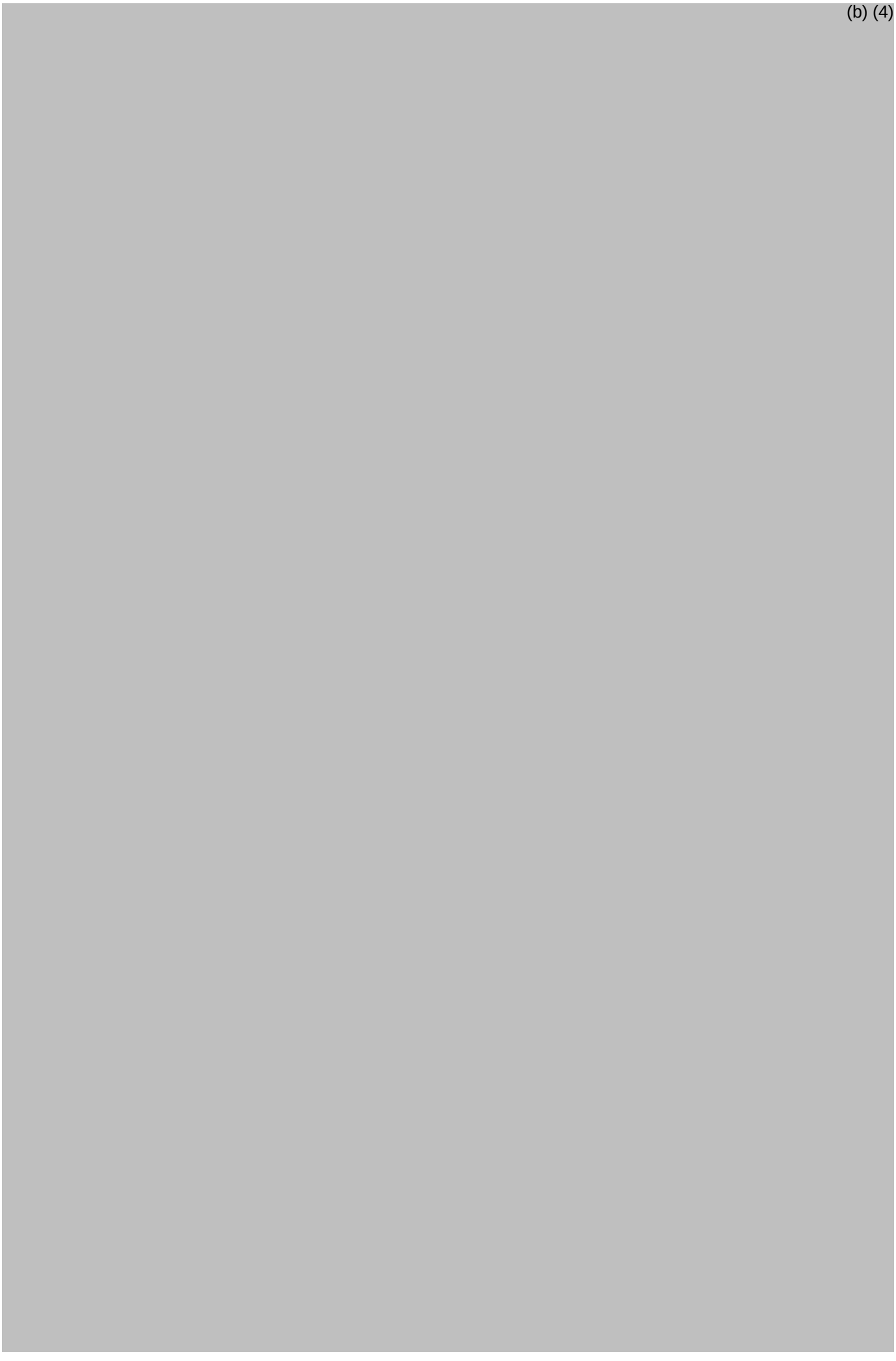

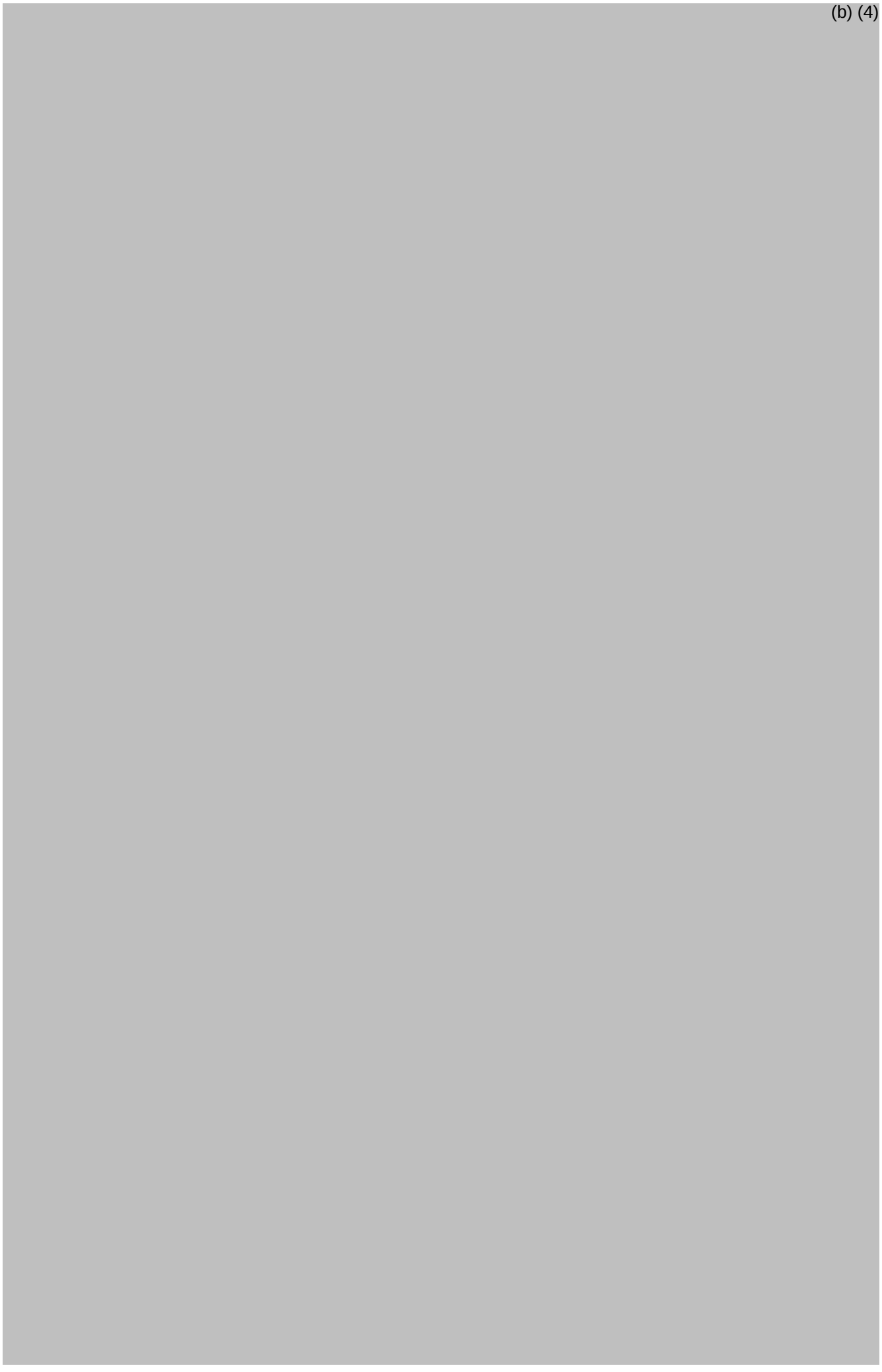
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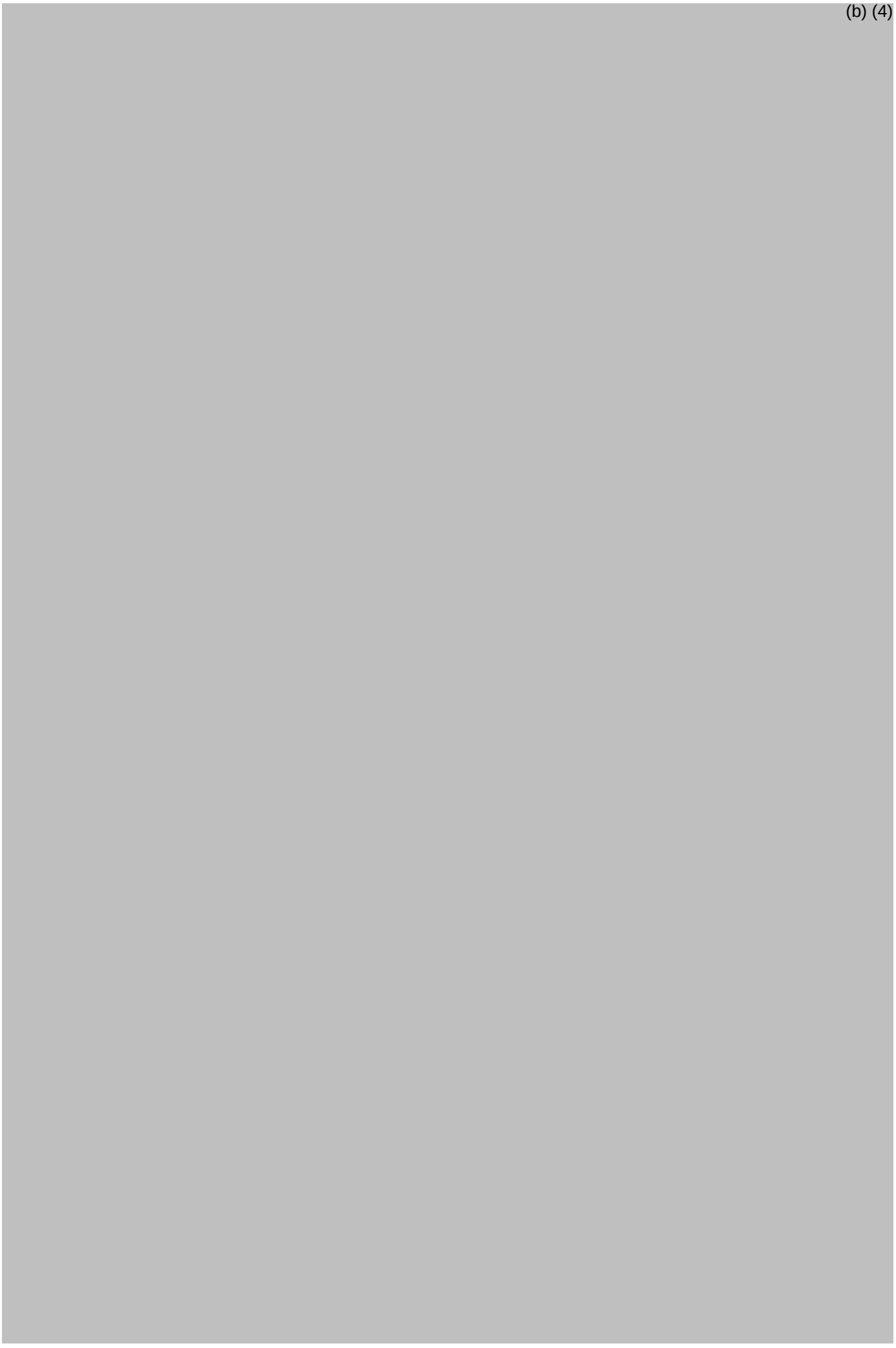

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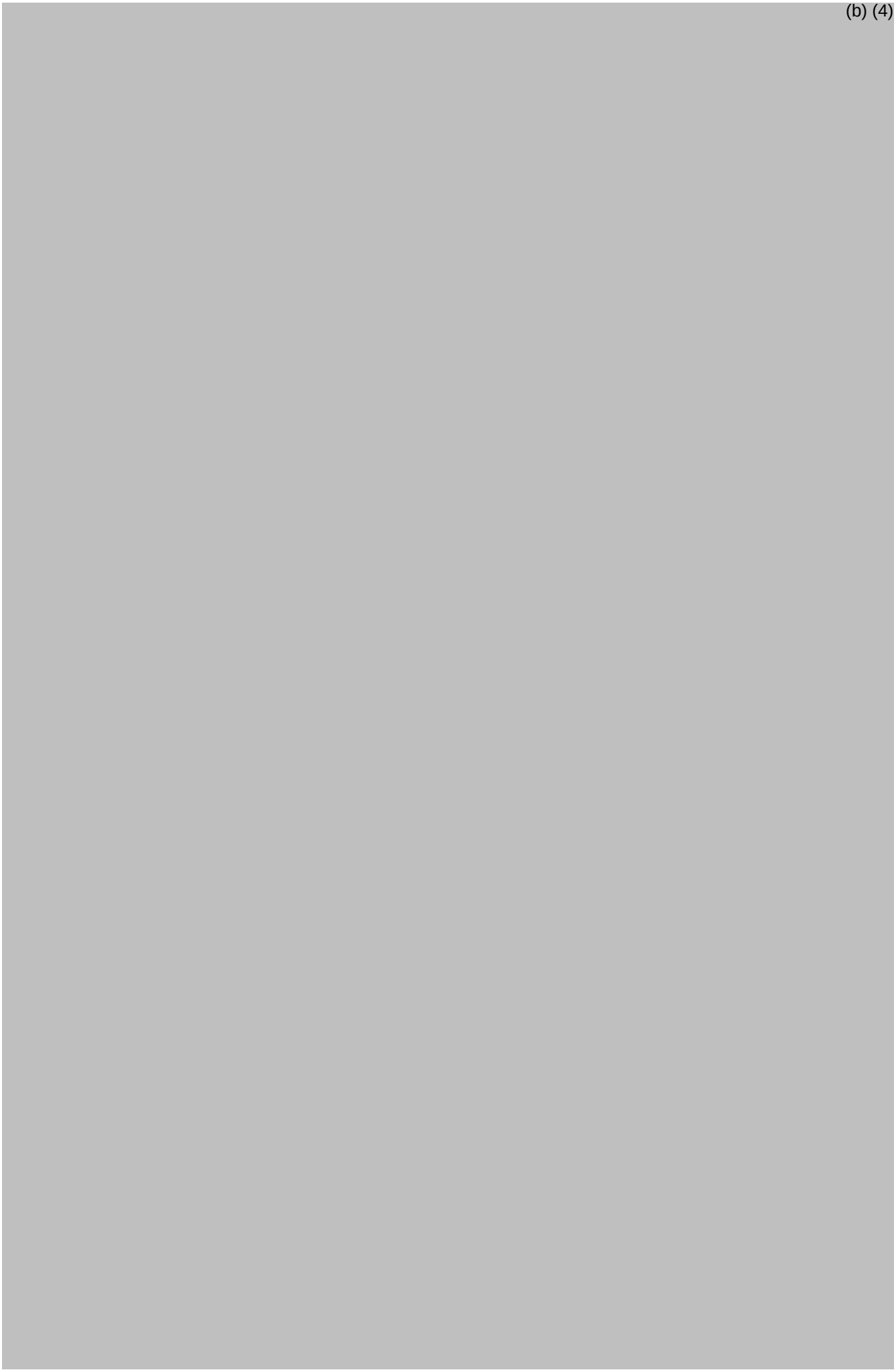

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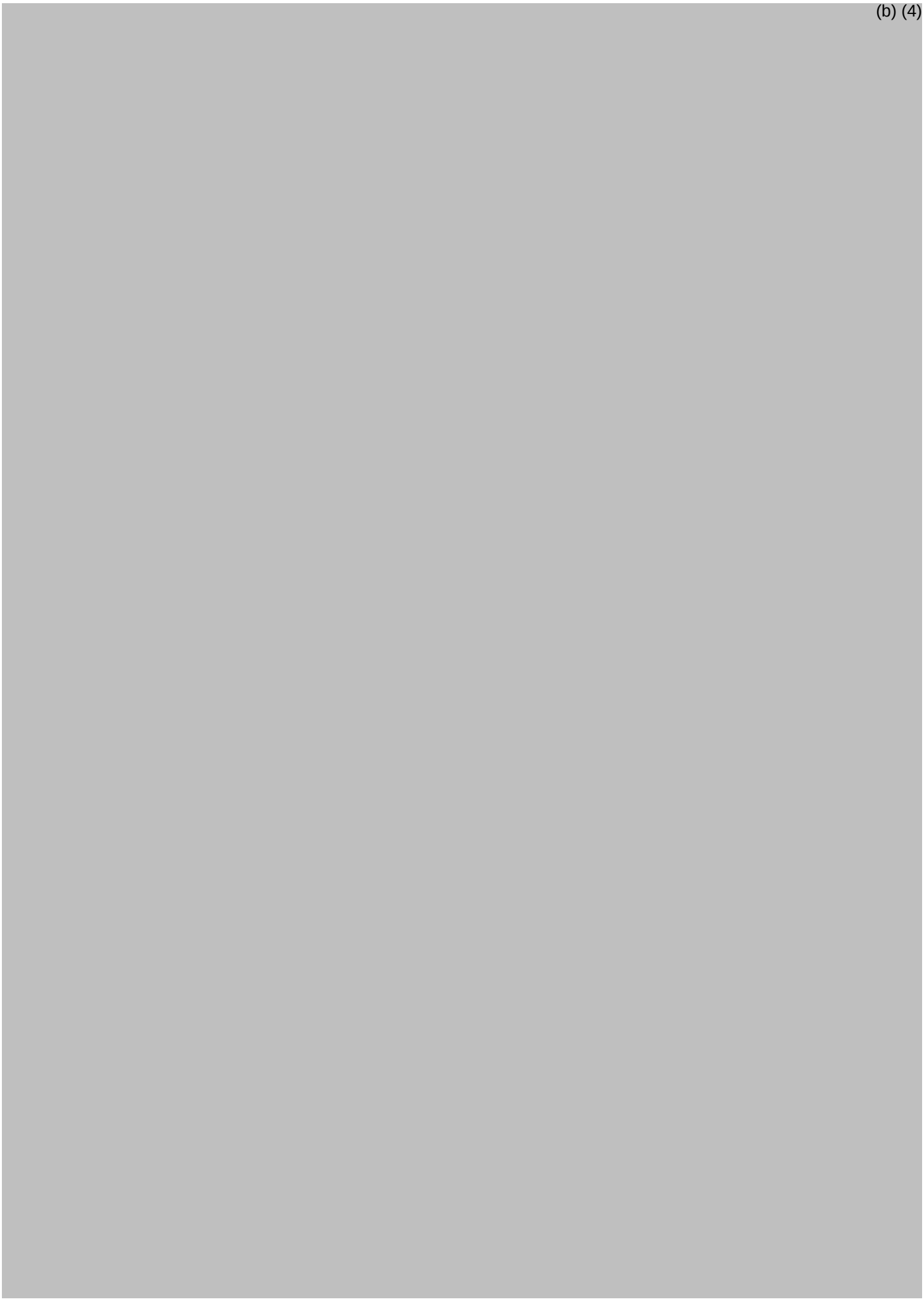

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GMP+International Registration Number: CI000006

(b) (4)

(b) (4) declares that there is justifiable confidence that the GMP+ scope(s) **Production of Feed Materials** at the GMP+ Certified Company (b) (4) (b) (4) comply with the applicable requirements and conditions of the GMP+ Feed Safety Assurance Module 2020.

(b) (4)

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For the validity can be checked on (b) (4)

This certificate issued on October 13, 2022, Expiry date October 9, 2025

(b) (4)

on behalf of (b) (4)

(b) (4)

(b) (4)

BUREAU VERITAS
Certification

(b) (4)

(b) (4)

Bureau Veritas Certification Holding SAS – UK Branch certifies that the Management System of the above organisation has been audited and found to be in accordance with the requirements of the management system standards detailed below

(b) (4)

Scope of certification

MANUFACTURE OF DRY YEAST, FRESH YEAST (COMPRESSED YEAST AND CREAM YEAST)

Product category: (b) (4)

Original cycle start date:	**15 October 2014**
Expiry date of previous cycle:	**14 October 2020**
Certification / Recertification Audit date:	**19 September 2020**
Certification / Recertification cycle start date:	**29 October 2020**

Subject to the continued satisfactory operation of the organization's Management System, this certificate expires on: **14 October 2023**

Certificate No. (b) (4) Version : **1** Revision date: **29 October 2020**

(b) (4)

UKAS MANAGEMENT SYSTEMS

0008

Further clarifications regarding the scope of this certificate and the applicability of the management system requirements may be obtained by consulting the organisation.
To check this certificate validity please call: +86 20 83073807

DETERMINATION OF MANNAN IN YEAST CELL WALLS

Version 1

I. DEFINITION AND APPLICATION AREA

(b) (4)

II. REAGENTS

(b) (4)

III. APPARATUS

(b) (4)

Phileo, a division of S.I.Lesaffre
137, rue Gabriel Péri - BP 3029 - 59703 Marcq-en-Baroeul cedex – France
Tel. +33 (0) 320 81 61 00 - Fax + 33 (0) 320 99 94 82 - info@phileo.lesaffre.com - www.phileo-lesaffre.com
SA au capital de 760 050€ - RSC Roubaix-Tourcoing 349 069 047 - Siret 349 069 047 00018 - TVA FR03 349069047

IV. WORKING METHOD

1. Preparation of reagent solutions

Phileo, a division of S.I.Lesaffre
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COM013	DETERMINATION OF BETA GLUCAN IN YEAST CELL WALLS
VERSION 1	20/08/2020

I. DEFINITION AND APPLICATION AREA

(b) (4)

II. REAGENTS

(b) (4)

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III. APPARATUS

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IV. WORKING METHOD

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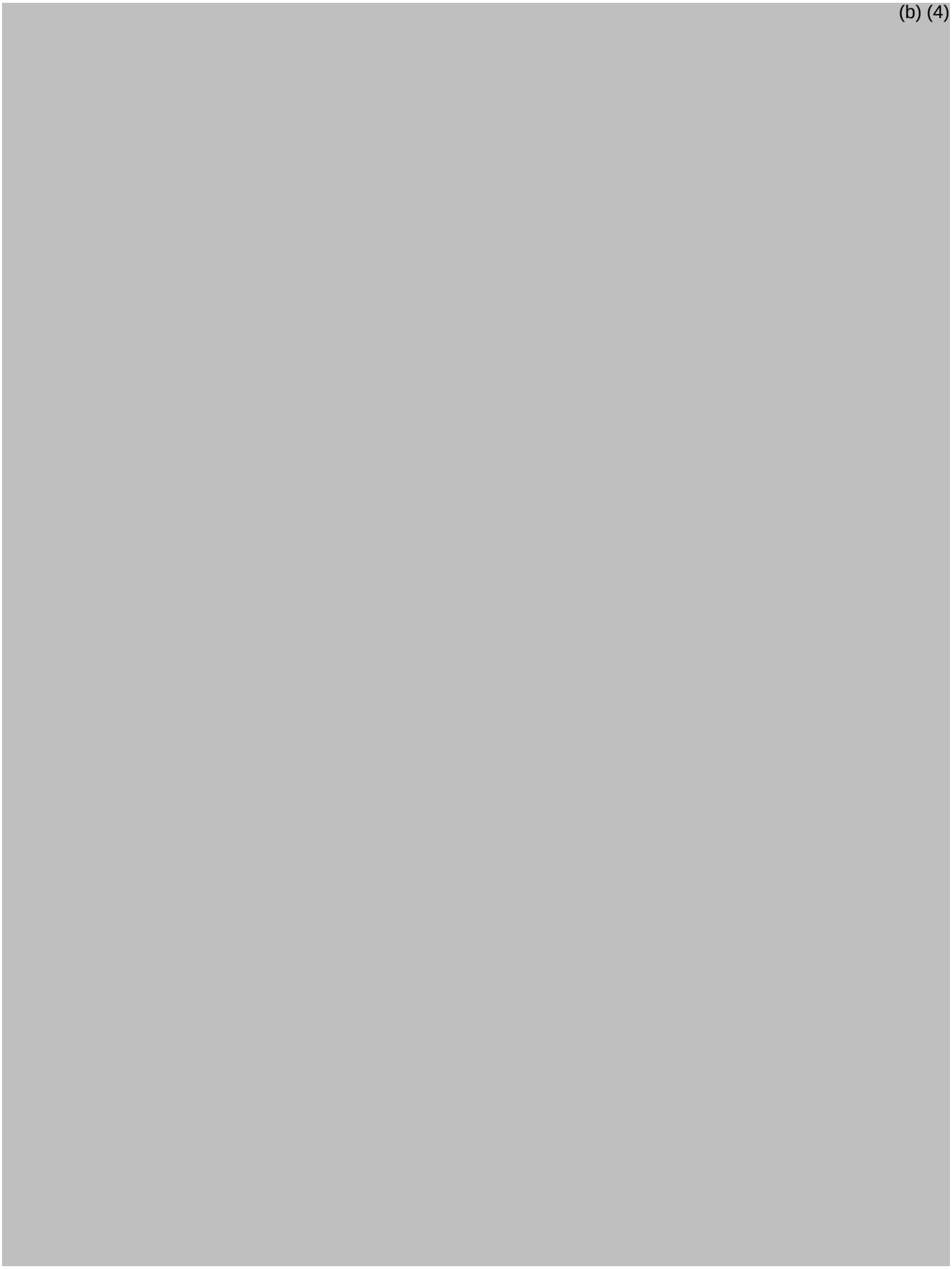

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Method used to analyze proteins: Kjeldhal method

Derived from COMMISSION REGULATION (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed

C. DETERMINATION OF THE CONTENT OF CRUDE PROTEIN

1. Purpose and scope

This method makes it possible to determine the crude protein content of feed on the basis of the nitrogen content, determined according to the Kjeldahl method.

2. Principle

The sample is digested by sulphuric acid in the presence of a catalyst. The acid solution is made alkaline with sodium hydroxide solution. The ammonia is distilled and collected in a measured quantity of sulphuric acid, the excess of which is titrated with a standard solution of sodium hydroxide.

Alternatively, the liberated ammonia is distilled into an excess of boric acid solution, followed by titration with hydrochloric acid or sulphuric acid solution.

3. Reagents

3.1. Potassium sulphate.

3.2. Catalyst: copper (II) oxide CuO or copper (II) sulphate pentahydrate, $CuSO_4$ $5H_2O$.

3.3. Granulated zinc.

3.4. Sulphuric acid, $\rho 20$ = 1,84 g/ml.

3.5. Sulphuric acid, standard volumetric solution, $c(H_2SO_4)$ = 0,25 mol/l.

3.6. Sulphuric acid, standard volumetric solution, $c(H_2SO_4)$ = 0,10 mol/l.

3.7. Sulphuric acid, standard volumetric solution, $c(H_2SO_4)$ = 0,05 mol/l.

3.8. Methyl red indicator; dissolve 300 mg of methyl red in 100 ml of ethanol, σ = 95 %-96 % (v/v).

3.9. Sodium hydroxide solution (Technical grade may be used) β = 40 g/100 ml (m/v: 40 %).

3.10. Sodium hydroxide, standard volumetric solution c(NaOH) = 0,25 mol/l.

3.11. Sodium hydroxide, standard volumetric solution c(NaOH) = 0,10 mol/l.

3.12. Granulated pumice stone, washed in hydrochloric acid and ignited.

3.13. Acetanilide (m.p. = 114 °C, N-content = 10,36 %).

3.14. Sucrose (nitrogen free).

3.15. Boric acid (H_3BO_3).

3.16. Methyl red indicator solution: dissolve 100 mg methyl red in 100 ml ethanol or methanol.

3.17. Bromocresol green solution: dissolve 100 mg bromocresol green in 100 ml ethanol or methanol.

3.18. Boric acid solution (10 g/l to 40 g/l depending on the apparatus used).

When colorimetric end-point detection is applied, methyl red and bromocresol indicators must be added to the boric acid solutions. If 1 litre of the boric acid solution is prepared, before adjusting to volume, 7 ml methyl red indicator solution (3.16) and 10 ml bromocresol green solution (3.17) shall be added.

Dependent on the water used, the pH of the boric acid solution might differ from batch to batch. Often an adjustment with a small volume of alkali is necessary to obtain a positive blank.

Note: The addition of about 3 ml to 4 ml of NaOH (3.11) into 1 litre of 10 g/l boric acid usually gives good adjustments. Store the solution at room temperature and protect the solution from light and sources of ammonia fumes during storage.

3.19. Hydrochloric acid standard volumetric solution c(HCl) = 0,10 mol/l.

Note: Other concentrations of volumetric solutions (3.5, 3.6, 3.7, 3.10, 3.11, and 3.19) can be used, if this is corrected for in the calculations. The concentrations shall always be expressed to four decimal places.

4. Apparatus

Apparatus suitable for performing digestion, distillation and titration according to the Kjeldahl procedure.

5. Procedure

5.1. *Digestion*

Weigh 1 g of the sample to the nearest 0,001 g and transfer the sample to the flask of the digestion apparatus. Add 15 g of potassium sulphate (3.1), an appropriate quantity of catalyst (3.2) (0,3 to 0,4 g of copper (II) oxide or 0,9 to 1,2 g of copper (II) sulphate pentahydrate), 25 ml of sulphuric acid (3.4) and if required, a few granules of pumice stone (3.12) and mix.

Heat the flask moderately at first, swirling from time to time if necessary until the mass has carbonised and the foam has disappeared; then heat more intensively until the liquid is boiling steadily. Heating is adequate if the boiling acid condenses on the wall of the flask. Prevent the sides from becoming overheated and organic particles from sticking to them.

When the solution becomes clear and light green continue to boil for another two hours, then leave to cool.

5.2. *Distillation*

Add carefully enough water to ensure complete dissolution of the sulphates. Allow to cool and then add a few granules of zinc (3.3), if required. Proceed according to 5.2.1 or 5.2.2.

5.2.1. Distillation into sulphuric acid

Place in the collecting flask of the distillation apparatus an exactly measured quantity of 25 ml of sulphuric acid (3.5) or (3.7) depending on the presumed nitrogen content. Add a few drops of methyl red indicator (3.8).

Connect the digestion flask to the condenser of the distillation apparatus and immerse the end of the condenser in the liquid contained in the collecting flask to a depth of at least 1 cm (see observation 8.3). Slowly pour 100 ml of sodium hydroxide solution (3.9) into the digestion flask without loss of ammonia (see observation 8.1). Heat the flask until the ammonia has distilled over.

5.2.2. Distillation into boric acid

Where titration of the ammonia content of the distillate is performed manually, the procedure mentioned below applies. Where the distillation unit is fully automated to include titration of the ammonia content of the distillate, follow the manufacturer's instructions for operation of the distillation unit.

Place a collecting flask containing 25 ml to 30 ml of the boric acid solution (3.18) under the outlet of the condenser in such a way that the delivery tube is below the surface of the excess boric acid solution. Adjust the distillation unit to dispense 50 ml of sodium hydroxide solution (3.9). Operate the distillation unit in accordance with the manufacturer's instructions and distil off the ammonia liberated by the addition of the sodium hydroxide solution. Collect distillate in the boric acid receiving solution. The amount of distillate (time of steam distillation) depends on the amount of nitrogen in the sample. Follow the instructions of the manufacturer.

Note: In a semi-automatic distillation unit, the addition of excess sodium hydroxide and the steam distillation are performed automatically.

5.3. *Titration*

Proceed according to 5.3.1 or 5.3.2.

5.3.1. Sulphuric acid

Titrate the excess sulphuric acid in the collecting flask with sodium hydroxide solution (3.10 or 3.11) depending on the concentration of the sulphuric acid used, until the end-point is reached.

5.3.2. Boric acid

Titrate the contents of the collecting flask with the hydrochloric acid standard volumetric solution (3.19) or with the sulphuric acid standard volumetric solution (3.6) using a burette and read the amount of titrant used.

When colorimetric end-point detection is applied, the end-point is reached at the first trace of pink colour in the contents. Estimate the burette reading to the nearest 0,05 ml. An illuminated magnetic stirrer plate or a photometric detector may aid visualisation of the end-point.

This can be done automatically using a steam distiller with automatic titration.

Follow the manufacturers' instructions for operation of the specific distiller or distiller/titrator.

Note: When an automatic titration system is used, titration begins immediately after distillation starts and the 1 % boric acid solution (3.18) is used.

Where a fully automatic distillation unit is employed, the automatic titration of the ammonia can also be carried out with end-point detection using a potentiometric pH system.

In this case an automatic titrator, with a pH-meter is used. The pH-meter shall be calibrated properly in the range of pH 4 to pH 7 following normal laboratory pH-calibration procedures.

The pH end-point of the titration is reached at pH 4,6, being the steepest point in the titration curve (inflection point).

5.4. *Blank test*

To confirm that the reagents are free from nitrogen, carry out a blank test (digestion, distillation and titration) using 1 g of sucrose (3.14) in place of the sample.

6. Calculation of results

Calculations are performed according to 6.1 or 6.2.

6. Calculation of results

Calculations are performed according to 6.1 or 6.2.

6.1. *Calculation for titration according to 5.3.1*

The content of crude protein, expressed as a percentage by weight, is calculated according to the following formula:

$$\frac{(V_0 - V_1) \times c \times 0{,}014 \times 100 \times 6{,}25}{m}$$

where:

V_o = is the volume (ml) of NaOH (3.10 or 3.11) used in the blank test,
V_1 = is the volume (ml) of NaOH (3.10 or 3.11) used in the sample titration,
c = is the concentration (mol/l) of sodium hydroxide (3.10 or 3.11),
m = is the weight (g) of sample.

6.2. *Calculation for titration according to 5.3.2*

6.2.1. Titration with hydrochloric acid

The content of crude protein, expressed as a percentage by weight, is calculated according to the following formula:

$$\frac{(V_1 - V_0) \times c \times 1{,}4 \times 6{,}25}{m}$$

where:

m = is the weight (g) of the test portion,
c = is the concentration (mol/l) of the standard volumetric solution of the hydrochloric acid (3.19),
V_0 = is the volume (in ml) of hydrochloric acid used for the blank test,
V_1 = is the volume (in ml) of hydrochloric acid used for the test portion.

6.2.2. Titration with sulphuric acid

The content of crude protein, expressed as a percentage by weight, is calculated according to the following formula:

$$\frac{(V_1 - V_0) \times c \times 2{,}8 \times 6{,}25}{m}$$

where:

m = is the weight (g) of the test portion,
c = is the concentration (mol/l) of the standard volumetric solution of sulphuric acid (3.6),
V_0 = is the volume (in ml) of sulphuric acid (3.6) used for the blank test,
V_1 = is the volume (in ml) of sulphuric acid (3.6) used for test portion.

7. Verification of the method

7.1. *Repeatability*

The difference between the results of two parallel determinations carried out on the same sample must not exceed:

— 0,2 % in absolute value, for crude protein contents of less than 20 %,

— 1,0 % relative to the higher value, for crude protein contents from 20 % to 40 %,

— 0,4 % in absolute value, for crude protein contents of more than 40 %.

7.2. *Accuracy*

Carry out the analysis (digestion, distillation and titration) on 1,5 to 2,0 g of acetanilide (3.13) in the presence of 1 g of sucrose (3.14); 1 g acetanilide consumes 14,80 ml of sulphuric acid (3.5). Recovery must be at least 99 %.

8. Observations

8.1. Apparatus may be of the manual, semi-automatic or automatic type. If the apparatus requires transference between the digestion and distillation steps, this transfer must be carried out without loss. If the flask of the distillation apparatus is not fitted with a dropping funnel, add the sodium hydroxide immediately before connecting the flask to the condenser, pouring the liquid slowly down the side.

8.2. If the digest solidifies, recommence the determination using a larger amount of sulphuric acid (3.4) than that specified above.

8.3. For products with a low nitrogen content, the volume of sulphuric acid (3.7) to be placed in the collecting flask may be reduced, if necessary, to 10 or 15 ml and made up to 25 ml with water.

8.4. For routine analysis, alternative methods of analysis can be applied for the determination of crude protein but the Kjeldahl method described in this Part C is the reference method. The equivalence of the results obtained with the alternative method (e.g. DUMAS) compared to the reference method must be demonstrated for each matrix individually. As the results obtained with an alternative method, even after having verified the equivalency, might deviate slightly from the results obtained with the reference method, it is necessary to mention in the analytical report the method of analysis used for the determination of crude protein.

3

Current Analytical Techniques for Food Lipids

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Abstract

The analysis of food lipids presents significant challenges due to the wide variety of sample matrices, large range of total fat contents, and complex compositions of fatty acids. This chapter reviews conventional analytical techniques for the quantification of total fat and fatty acids in foods and food ingredients, including the gravimetric determination of total fat, the calculation of fat and fatty acids using gas chromatography (GC), and the analysis of proximates content (i.e., fat, protein, carbohydrate, moisture, and ash) by Fourier transform infrared (FTIR) spectroscopy. Current official methods of analysis are evaluated and the use of certified reference materials and spike-recovery experiments for verifying method performance is discussed. Recent advances in automated and semi-automated sample preparation systems and rapid and portable spectroscopic devices are highlighted for their potential to significantly improve the speed by which accurate determinations of total fat and fatty acids may be achieved.

***Keywords*:** Fat, fatty acid, lipid analysis, official method of analysis, portable analyzers, standard reference material

3.1 Introduction

Lipids are a diverse class of compounds that contribute to the organoleptic, physiochemical, and nutritional aspects of foods and food ingredients. Food lipids provide a major source of energy in the diet. They also contribute

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Umile Gianfranco Spizzirri and Giuseppe Cirillo (eds.) Food Safety: Innovative Analytical Tools for Safety Assessment, (33–64) © 2017 Scrivener Publishing LLC

essential fatty acids and nutrients and serve as carriers for lipid-soluble vitamins. Food lipids are broadly divided into categories of fats and oils based on origin of the lipid substance and its physical state at room temperature. Fats are animal-based solids, such as lard and tallow, which remain solid at room temperature due to their high concentration of saturated fatty acids that allow for the close packing of triglycerides. Several vegetable-based oils, including palm and coconut oils and partially and fully hydrogenated oils, contain high concentrations of saturated and/or *trans* fatty acids that also produce a solid lipid matrix at room temperature. Most vegetable and seed oils, however, are found as liquids at room temperature owing to their high concentrations of monounsaturated and polyunsaturated fatty acids. From the consumer's perspective, information related to differences in the content and composition of total fat and fatty acids in foods and food ingredients is important for making educated, healthful food choices.

The Nutrition Labeling and Education Act (NLEA) of 1990 amended the Federal Food, Drug and Cosmetic Act (FD&C Act) to require mandatory nutrition labeling for packaged foods regulated by the FDA and the US Department of Agriculture (USDA) [1, 2]. The NLEA also gave the FDA authority to regulate health claims on food labels and in food labeling [3]. Under provisions of the NLEA, declarations for the content of total fat are to be expressed in triacylglycerol (TAG) equivalents, whereas those for saturated fat are expressed as free fatty acid equivalents [1, 2]. The contents of *cis*-monounsaturated and *cis*-polyunsaturated fatty acids are also permitted as voluntary declarations on product labels, except under certain conditions when a claim about fatty acids or cholesterol is made on the label or in the labeling of a food [1, 2]. More recently, the content of total *trans* fatty acids was added to the nutrition label of conventional foods and dietary supplements [1, 4]. FDA compliance programs help to ensure that the labels of foods and dietary supplements available in the US market contain accurate declarations of product composition and that they are truthful and not misleading.

A wide range of analytical techniques are currently available for the analysis of total fat and fatty acids in foods and food ingredients. Conventional analytical methods for the determination of total fat include the gravimetric determination of solvent-extracted lipids and the calculation of total fat based on the analyzed content of individual fatty acids in a test sample. Fourier transform infrared (FTIR) spectroscopic procedures are also available for the determination of total fat and other proximates (i.e., ash, protein, moisture, carbohydrate) in food commodities. FDA regulations do not specify particular methods of analysis, but the Agency accepts those that yield accurate results with satisfactory precision and are considered

appropriate for the analysis of specified nutrients and other food components. These methods, collectively referred to as official methods of analysis, are rigorously evaluated for method performance and validated in national or international collaborative studies by method-endorsing organizations such as AOAC INTERNATIONAL, the American Oil Chemists' Society (AOCS), and the International Organization for Standardization (ISO). Such methods are routinely applied to the analysis of foods and food ingredients in FDA field laboratories and independent contract laboratories that provide data for manufacturers for nutrition labeling purposes. FDA labeling regulations indicate that manufacturers may use any analytical method for determining nutrient contents, including the use of historical or nutrient database data. However, for compliance purposes, a product and its label are subject to analytical methods the agency considers appropriate (i.e., official methods) for verifying nutrient contents.

This chapter reviews conventional analytical techniques for the quantification of total fat and fatty acids in foods and food ingredients, as stated above. A schematic overview of these techniques is presented in Figure 3.1. Current official methods of analysis are presented, and the importance of method validation procedures, such as collaborative study testing and the use of certified reference materials and spike-recovery experiments, is critically discussed. Recent advances in the application of automated and semi-automated sample preparation systems and rapid

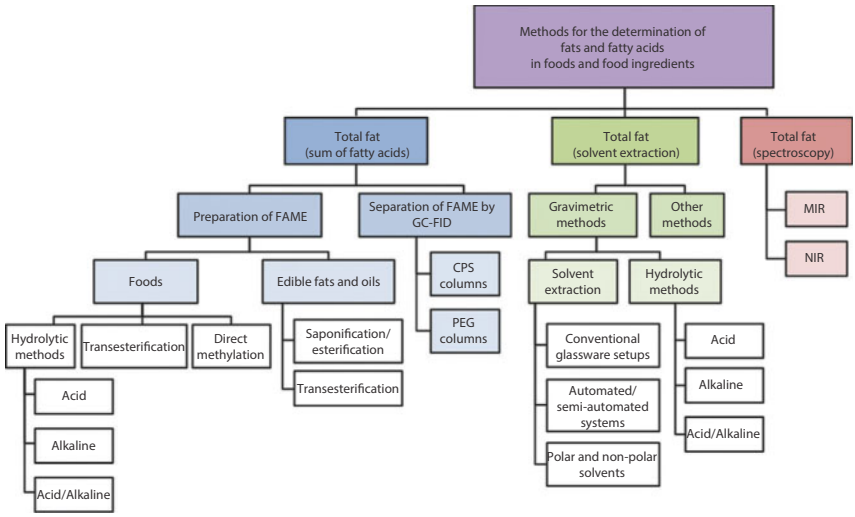

Figure 3.1 Schematic overview of conventional analytical methods for the determination of total fat and fatty acids in foods and food ingredients.

and portable spectroscopic devices are highlighted for their potential to significantly improve the speed by which accurate determinations of total fat and fatty acids may be achieved.

3.2 Official Methods for the Analysis of Fat in Foods

3.2.1 Importance of Official Methods of Analysis

Official methods of analysis are those which have been systematically evaluated and subsequently approved by a method-endorsing organization for routine use in regulatory and contract laboratories, among others. These methods are characterized by their scope, intended use, and applicable sample matrices. The approval of new official methods often includes the successful completion of a multi-laboratory validation (MLV) study to further investigate the performance of a candidate method. MLV studies are designed to test the clarity of the written protocol and the interlaboratory precision (repeatability and reproducibility), taking into account variability introduced from different analysts, laboratory environments, and analytical instruments [5]. These studies require a significant commitment of time and resources by study leaders and participants. As such, only qualified methods which have been previously investigated at the level of a single laboratory validation study are considered as appropriate candidate methods for an MLV study. These methods are defined for their performance specifications with regard to accuracy, precision, sensitivity, linearity, limits of detection and quantification, and robustness/ruggedness for determining one or more analytes in a specified matrix or matrices [5, 6]. Minimum criteria for successful completion of a quantitative MLV study include the analysis of five test materials, participation from a minimum of eight laboratories reporting valid data for each of the test materials, and the measurement of a minimum of one or two replicate samples, provided as blind replicates or split levels (Youden pairs) [5]. Acceptable ranges of analyte concentration are determined from the precision data, expressed as the reproducibility relative standard deviations (RSD_R) and/or the Horwitz ratio (Horrat) [7].

3.2.2 Official Methods for the Gravimetric Determination of Total Fat

The gravimetric approach provides a crude estimation of total fat content based on the mass of lipid extracted from a test sample. This approach tends to underestimate the caloric content of total fat by including in the

gravimetric determination the mass of non-fatty acid constituents, namely lipid-soluble vitamins, unsaponifiable material, and certain nonfat macromolecules that are also extracted [8]. The gravimetric determination of total fat content may be achieved by extraction of total lipids with nonpolar solvent under reflux conditions or by use of a combination of nonpolar and polar organic solvents to overcome interactions between lipids and the sample matrix, such as chloroform and methanol, which are recommended in the method of Bligh and Dyer [9]. Alternatively, the test sample may be subjected to a two-step hydrolytic procedure in which the matrix is first hydrolyzed and then extracted for total lipids with nonpolar solvent. Both approaches have been approved as official methods of analysis. A wide range of methods are now available for the crude determination of total fat content in foods and food ingredients. These protocols, which are listed in Table 3.1 (solvent extraction) and Table 3.2 (hydrolytic procedures), vary in applicable matrix and sample preparation procedure.

3.2.2.1 Solvent Extraction Procedures

Solvent extraction methods, such as the conventional Soxhlet procedure [10], involve the semi-continuous washing, or percolation, of dried and homogenized samples with organic solvent under reflux conditions using specific glassware. Ether (ethyl and/or petroleum) and hexanes are common solvents although they are reportedly inefficient for extracting polar lipids [11]. Solvent extraction methods tend to be straightforward and require minimal specialized training [10]. In addition, the organic solvent used to extract the test material requires no filtration prior to evaporation. However, these methods require the use of large volumes of organic solvent, which are costly to dispose of and hazardous to the environment. AOAC Official Method 948.22 [12] describes a solvent extraction procedure for the determination of crude total fat in nuts and nut products. Samples are extracted with ether in a Soxhlet-type extractor for 16 hours, then the lipid extract is evaporated to dryness at 95–100 °C and weighed. The Randall/Soxtec modification of the Soxhlet solvent extraction procedure, as recommended in AOAC 2003.05 and 2003.06 [13, 14], allows for a shorter duration extraction because the test portion is submerged in boiling solvent.

Many instrument manufacturers now offer automated or semi-automated systems, such as the Soxtec systems from FOSS (Hillerød, Denmark), the CEM Discover SP-X (Matthews, NC, USA), and the Buchi B-811 Extraction System (New Castle, DE, USA), for the extraction of food lipids with organic solvent. These instruments offer numerous advantages over

Table 3.1 Solvent extraction (SE) official methods for the gravimetric determination of crude total fat in foods and food ingredients.

Method	Applicable matrices	SE protocol	Organic solvent
920.39 [102] (also referred to as AOAC 945.38F)	Animal feed ingredients and mixed feeds	Soxhlet	Anhydrous EE
938.06 [103]	Butter	SE, indirect method	EE or PE
948.22 [12]	Nuts and nut products	Soxhlet	EE
960.39 [104]	Meat	Soxhlet	Anhydrous EE or PE
985.15 [15]	Meat and poultry products	Automated SE with microwave moisture analyzer	Methylene chloride
991.36 [105]	Meat and meat food products	Randall/Soxtec	PE
2003.05 [13]	Animal feeds, forages, and cereal grains	Automated/Semi-automated SE (Randall/Soxtec)	Anhydrous EE
2003.06 [14]	Animal feeds, forages, and cereal grains	Automated/Semi-automated SE (Randall/Soxtec)	Hexanes
AOCS Am 5-04 [16]	Oilseeds, meats, feeds, and foods	Automated/Semi-automated SE	PE

Abbreviations: EE, ethyl ether; PE, petroleum ether; SE, solvent extraction.

conventional glassware setups, including accelerated extraction durations and higher extraction efficiencies. Automated systems have also found their way into the official methods of analysis. AOAC Official Method 985.15 [15] describes a procedure for the determination of crude total fat in meat and poultry products. With this method, homogenized test samples are dried using a microwave moisture analyzer, extracted with methylene chloride in an automated solvent extractor, and then returned to the microwave moisture analyzer for drying, removal of residual solvent, and weighing. AOCS Official Method Am 5-04 [16] was approved for the rapid determination

Table 3.2 AOAC official methods for the hydrolytic determination of crude total fat in foods and food ingredients.

Method	Applicable matrices	Hydrolytic protocol	Organic solvent
983.23 [19]	Composite foods	Enzymatic digestion	Chloroform-methanol
922.06 [23]	Flour	Acid	EE/PE
925.12 [24]	Macaroni products	Acid	EE/PE
925.32 [26]	Eggs	Acid	EE/PE
935.38 [25]	Bread	Acid	EE/PE
945.44 [106]	Fig bars and raisin-filled crackers	Acid	EE/PE
948.15 [27]	Seafood	Acid	EE/PE
948.16 [107]	Fish meal	Acid	Acetone
950.54 [108]	Food dressings	Acid	PE
954.02 [109]	Pet food	Acid	EE
963.15 [110]	Cacao products	Acid	PE
920.111 [29]	Cream	Alkaline	EE/PE
920.115 [30]	Sweetened condensed milk	Alkaline	EE/PE
922.09 [31]	Malted milk	Alkaline	EE/PE
932.06 [32]	Milk powder	Alkaline	EE/PE
933.05 [33]	Cheese	Acid and alkaline	EE/PE
945.48 [34]	Evaporated milk (unsweetened)	Alkaline	EE/PE
952.06 [35]	Ice cream and frozen desserts	Alkaline	EE/PE
974.09 [36]	Whey cheese	Alkaline	EE/PE
986.25 [37]	Milk-based infant formula	Alkaline	EE/PE
989.05 [28]	Milk	Alkaline	EE/PE
995.19 [111]	Cream	Alkaline	EE/PE

Abbreviations: EE, ethyl ether; PE, petroleum ether.

of total fat content in oilseeds, meats, feeds, and foods. This method recommends the use of an automated or semi-automated extraction system, such as the XT10 and XT15 extractors (Ankom Technology, Macedon, NY, USA), with filter bag technology designed to reduce errors due to sample loss. The determination of crude total fat content using AOAC 985.15 [15] or AOCS Am 5-04 [16] is considered an indirect method of analysis in that quantification is based on the difference in sample weight before and after extraction [17]. In contrast, with direct methods the weight of the extracted lipid is measured directly and the content of total fat is calculated as the mass of the extracted lipid taken as a proportion of the test portion weight.

3.2.2.2 Hydrolytic Procedures

As an alternative to the solvent extraction methods, hydrolytic procedures involve a two-step process by which the sample is first treated with acid and/or alkaline reagents or an enzyme in order to breakdown the matrix prior to extraction with solvent. Hydrolytic procedures enable the disruption of lipid-carbohydrate bonds, proteins, polysaccharides, and plant cell walls. Such sample pretreatment is particularly necessary for dairy products in order to facilitate extraction of neutral lipids contained within the milk fat globule membranes [18]. A complete digestion or hydrolysis of the test material enables the extraction solvent to come in contact with all lipids contained within the test material. Thus, an exhaustive and quantitative extraction of total lipids is expected.

An enzymatic hydrolytic procedure for the determination of fat in foods is decribed in AOAC Official Method 983.23 [19]. It was developed by Daugherty and Lento [20] as a modification of the Bligh and Dyer method [9] and is applicable to the analysis of composite food samples. AOAC 983.23 [19] involves the enzymatic digestion of food samples using 1% amylase enzyme in 0.5 M sodium acetate solution, placed in a shaking water bath set at 45–50 °C for 60 min. Total lipids are then extracted by addition of chloroform, methanol, and water to cause separation of the aqueous and organic phases. The chloroform layer is transferred to a tared 100-mL beaker and evaporated to dryness. A modification of AOAC 983.23 by Phillips *et al.* [21] was proposed to simplify the established standard procedure and eliminate the requirement for enzymatic digestion. The authors found that the simplified method was less labor-intensive and permitted a higher rate of sample throughput than the standard procedure [21]. More recently, Phillips *et al.* [22] extended the application of their

simplified method to the quantitative determination of total fat in a variety of complex food matrices, including those in which the fat component was a constituent of low-fat foods (e.g., baked goods, salad dressing, and snack foods) and those in which the matrix was finely ground or homogenized (e.g., baby food, finely ground nuts and seeds, peanut butter, flour, and lyophilized oyster tissue).

A procedure for the determination of total fat in flour by acid hydrolysis is described in AOAC Official Method 922.06 [23]. Test samples are placed with ethanol and 8 M HCl in a 50-mL beaker and heated at 70–80 °C for 30–40 min with stirring. After cooling, more ethanol is added and the sample transferred to a Mojonnier fat-extraction apparatus for repeated extraction of total lipids with ethyl and petroleum ethers. The ether extracts are then combined and evaporated on a steam bath. The lipid residue is dried in a 100 °C oven to constant weight. Modifications of AOAC 922.06 [23] are available as AOAC official methods for the analysis of macaroni products [24], bread [25], eggs [26], and seafood [27] (Table 3.2).

A procedure for the determination of total fat in milk is described in AOAC Official Method 989.05 [28]. Test samples are weighed and mixed with concentrated ammonium hydroxide solution in order to neutralize any acid present, precipitate protein, and disrupt lipid-protein bonds of the milk fat globule membrane [18]. Three sequential extractions with ethanol and ethyl/petroleum ethers are performed. The ether extracts are decanted into weighing dishes and evaporated in a chemical fume hood on a hot plate set at 100 °C. The lipid residue is then dried to constant weight and cooled in a desiccator overnight. This procedure, commonly referred to as the Roese-Gottlieb method, has been adapted and approved as an AOAC official method for the analysis of cream [29], sweetened condensed milk [30], malted milk [31], milk powder [32], cheese [33], evaporated milk [34], ice cream and frozen desserts [35], whey cheese [36], and milk-based infant formula [37] (Table 3.2). Versions of the Roese-Gottlieb procedure are also available as ISO/IDF (International Dairy Federation) methods, including those specific for cream [38], evaporated milk [39], and milk-based infant foods [40], among others.

Several alternative methods have also been approved for the analysis of fat in milk, including the Babcock method (AOAC 989.04 [41]), the Gerber method (AOAC 2000.18 [42]), the rapid detergent method (AOAC 960.26 [43]), the automated turbidimetric methods (AOAC 969.16 [44] and 973.22 [45]), and the mid-infrared spectroscopic method (AOAC 972.16 [46]). These and other methods for the analysis of milk fat have been discussed elsewhere [47–50].

3.2.3 Official Methods for the Determination of Total Fat by GC

Interest in the content and composition of fatty acids in foods and food ingredients prompted the development and validation of novel analytical methods for the quantification of fatty acids by GC with flame ionization detection (FID) [51, 52]. As such, analytical methods for the gravimetric determination of total fat were modified to include protocols for the preparation and separation of fatty acid methyl esters (FAME) by GC-FID. The novel GC methods were found to yield comparable determinations of total fat content to those achieved using conventional gravimetric methods [52, 53].

The determination of total fat by summation of individual fatty acids allows for the accurate quantification of the nutritional content of total fat in a test sample. With this approach, fatty acids are derivatized to FAME and quantified by GC-FID. Preparation of other derivatives may be useful in specific applications, such as the use of benzyl esters for quantitative recovery of short-chain fatty acids from milk fat [54]. An internal standard is added during sample preparation to facilitate calculation of FAME on a mg/g basis. Conversion factors are applied to express the analyzed contents of FAME as TAG or free fatty acid equivalents for nutrition labeling purposes [55]. Several sample preparation procedures are available, including the two-step protocol involving total fat extraction followed by preparation of FAME, the direct transesterification procedure for analysis of liquid and reconstituted dairy products and infant formulas, and the direct methylation approach involving the *in-situ* digestion of test materials followed by derivatization. FAME derived from edible fats and oils may be prepared by transesterification or by using consecutive saponification and esterification reactions. Such approaches have been approved as official methods of analysis (Table 3.3).

3.2.3.1 Sample Preparation Procedures

A procedure for the determination of fat (total, saturated, and *cis* unsaturated) in cereal products containing 0.5–13% total fat is decribed in AOAC Official Method 996.01 [56]. Test samples are heated in glass extraction tubes with ethanol, 8 M HCl, and C13:0 TAG internal standard solution (5 mg/mL in chloroform) in a shaking water bath set at 80 °C for 40 min. Samples are subsequently cooled to room temperature and transferred with ethanol to Monjonnier fat extraction flasks for liquid-liquid extraction with ethyl and petroleum ethers. The aqueous phase is re-extracted twice with mixed ether. The ether extracts are then combined

J Assoc Off Anal Chem. May-Jun 1989;72(3):481-3.

Gravimetric determination of ash in foods: NMKL collaborative study

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Abstract

A gravimetric method for the determination of ash was collaboratively studied in 14 laboratories. The food is ashed at 550 degrees C to constant weight and the ash is determined by weighing. Seven samples of various food commodities with estimated ash contents varying between low and high (0.07-8.0 g/100 g) were included in the study. The relative standard deviations for reproducibility varied, ranging from 1.0 and 1.3 for ash contents of 7.2 and 8.0 g/100 g, to 11 +/- 1% for low ash contents of 0.07 and 0.27 g/100 g.

Related information

MedGen
PubChem Compound (MeSH Keyword)

LinkOut - more resources

Research Materials
NCI CPTC Antibody Characterization Program

Miscellaneous
NCI CPTAC Assay Portal

Method used to analyze moisture: dehydration

Derived from COMMISSION REGULATION (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed

A. DETERMINATION OF MOISTURE

1. Purpose and Scope

This method makes it possible to determine the moisture content of feed. In case of feed containing volatile substances, such as organic acids, it is to be observed that also significant amount of volatile substances are determined together with the moisture content.

It does not cover the analysis of milk products as feed materials, the analysis of mineral substances and mixtures composed predominantly of mineral substances, the analysis of animal and vegetable fats and oils or the analysis of the oil seeds and oleaginous fruit.

2. Principle

The sample is desiccated under specified conditions which vary according to the nature of the feed. The loss in weight is determined by weighing. It is necessary to carry out preliminary drying when dealing with solid feed which has high moisture content.

3. Apparatus

3.1. Crusher of non-moisture-absorbing material which is easy to clean, allows rapid, even crushing without producing any appreciable heating, prevents contact with the outside air as far as possible and meets the requirements laid down in 4.1.1 and 4.1.2 (e.g. hammer or water cooled micro-crushers, collapsible cone mills, slow motion or cog wheeled crushers).

3.2. Analytical balance, accurate to 1 mg.

3.3. Dry containers of non-corrodible metal or of glass with lids ensuring airtight closure; working surface allowing the test sample to be spread at about 0,3 g/cm^2.

3.4. Electrically heated isothermal oven (± 2 °C) properly ventilated and ensuring rapid temperature regulation ([1]).

3.5. Adjustable electrically heated vacuum oven fitted with an oil pump and either a mechanism for introducing hot dried air or a drying agent (e.g. calcium oxide).

3.6. Desiccator with a thick perforated metal or porcelain plate, containing an efficient drying agent.

4. Procedure

N.B. The operations described in this section must be carried out immediately after opening the packages of samples. Analysis must be carried out at least in duplicate.

4.1. *Preparation*

4.1.1. Feed other than those coming under 4.1.2 and 4.1.3

Take at least 50 g of the sample. If necessary, crush or divide in such a way as to avoid any variation in moisture content (see 6).

Method used to analyze moisture: dehydration

4.1.2. Cereals and groats

Take at least 50 g of the sample. Grind into particles of which at least 50 % will pass through a 0,5 mm mesh sieve and will leave no more than 10 % reject on a 1 mm round-meshed sieve.

(1) For the drying of cereals, flour, groats and meal, the oven must have a thermal capacity such that, when pre-set at 131 °C, it will return to that temperature in less than 45 minutes after the maximum number of test samples have been placed inside to dry simultaneously. Ventilation must be such that, when as many samples of common wheat as it can contain are dried for two hours, the results differ from those obtained after four hours of drying by less than 0,15 %.

4.1.3. Feed in liquid or paste form, feed predominantly composed of oils and fats

Take about 25 g of the sample, weigh to the nearest 10 mg, add an appropriate quantity of anhydrous sand weighed to the nearest 10 mg and mix until a homogeneous product is obtained.

4.2. *Drying*

4.2.1. Feed other than those coming under 4.2.2 and 4.2.3

Weigh a container (3.3) with its lid to the nearest 1 mg. Weigh into the weighed container, to the nearest 1 mg, about 5 g of the sample and spread evenly. Place the container, without its lid, in the oven preheated to 103 °C. To prevent the oven temperature from falling unduly, introduce the container as rapidly as possible. Leave to dry for four hours reckoned from the time when the oven temperature returns to 103 °C. Replace the lid on the container, remove the latter from the oven, leave to cool for 30 to 45 minutes in the desiccator (3.6) and weigh to the nearest 1 mg.

For feed composed predominantly of oils and fats, dry in the oven for an additional 30 minutes at 130 °C. The difference between the two weighings must not exceed 0,1 % of moisture.

4.2.2. Cereals, flour, groats and meal

Weigh a container (3.3) with its lid to the nearest 0,5 mg. Weigh into the weighed container, to the nearest 1 mg, about 5 g of the crushed sample and spread evenly. Place the container, without its lid, in the oven preheated to 130 °C. To prevent the oven temperature from falling unduly, introduce the container as rapidly as possible. Leave to dry for two hours reckoned from the time when the oven temperature returns to 130 °C. Replace the lid on the container, remove the latter from the oven, leave to cool for 30 to 45 minutes in the desiccator (3.6) and weigh to the nearest 1 mg.

4.2.3. Compound feed containing more than 4 % of sucrose or lactose: feed materials such as locust beans, hydrolysed cereal products, malt seeds, dried beet chips, fish and sugar solubles; compound feed containing more than 25 % of mineral salts including water of crystallisation.

Weigh a container (3.3) with its lid to the nearest 0,5 mg. Weigh into the weighed container, to the nearest 1 mg, about 5 g of the sample and spread evenly. Place the container, without its lid, in the vacuum oven (3.5) preheated to between 80 °C and 85 °C. To prevent the oven temperature from falling unduly, introduce the container as rapidly as possible.

Bring the pressure up to 100 Torr and leave to dry for four hours at this pressure, either in a current of hot, dry air or using a drying agent (about 300 g for 20 samples). In the latter instance, disconnect the vacuum pump when the prescribed pressure has been reached. Reckon drying time from the moment when the oven temperature returns to 80 °C to 85 °C. Carefully bring the oven back to atmospheric pressure. Open the oven, place the lid on the container immediately, remove the container from the oven, leave to cool for 30 to 45 minutes in the desiccator (3.6) and weigh to the nearest 1 mg. Dry for an additional 30 minutes in the vacuum oven at 80 °C to 85 °C and reweigh. The difference between the two weighings must not exceed 0,1 % of moisture.

Method used to analyze moisture: dehydration

4.3. *Preliminary drying*

4.3.1. Feed other than those coming under 4.3.2

Solid feed with a high moisture content which makes crushing difficult must be subjected to preliminary drying as follows:

Weigh 50 g of *uncrushed* sample to the nearest 10 mg (compressed or agglomerated feed may be roughly divided if necessary) in a suitable container (e.g. a 20 × 12 cm aluminium plate with a 0,5 cm rim). Leave to dry in an oven from 60 °C to 70 °C until the moisture content has been reduced to between 8 % and 12 %. Remove from the oven, leave to cool uncovered in the laboratory for one hour and weigh to the nearest 10 mg. Crush immediately as indicated in 4.1.1 and dry as indicated in 4.2.1 or 4.2.3 according to the nature of the feed.

4.3.2. Cereals

Grain with a moisture content of over 17 % must be subjected to preliminary drying as follows:

Weigh 50 g of unground grain to the nearest 10 mg in a suitable container (e.g. a 20 × 12 cm aluminium plate with a 0,5 cm rim). Leave to dry for 5 to 7 minutes in an oven at 130 °C. Remove from the oven, leave to cool uncovered in the laboratory for two hours and weigh to the nearest 10 mg. Grind immediately as indicated in 4.1.2 and dry as indicated in 4.2.2.

5. **Calculation of results**

The moisture content (X), as a percentage of the sample, is calculated by using the following formulae:

5.1. *Drying without preliminary drying*

$$X = \frac{(m - m_0)}{m} \times 100$$

where:

m = initial weight, in grammes, of the test sample,
m_0 = weight, in grammes, of the dry test sample.

5.2. *Drying with preliminary drying*

$$X_p = \left[\frac{(m_2 - m_0) \times m_1}{m_2} + m - m_1\right] \times \frac{100}{m} = 100 \times \left(1 - \frac{m_1 \times m_0}{m \times m_2}\right)$$

where:

m = initial weight, in grammes, of the test sample,
m_1 = weight, in grammes, of the test sample after preliminary drying,
m_2 = weight, in grammes, of the test sample after crushing or grinding,
m_0 = weight, in grammes, of the dry test sample.

5.3. *Repeatability*

The difference between the results of two parallel determinations carried out on the same sample shall not exceed 0,2 % of the absolute value of moisture.

6. Observation

If crushing proves necessary and if this is seen to alter the moisture content of the product, the results of the analysis of the components of the feed must be corrected on the basis of the moisture content of the sample in its initial state.

ISSN 0335-3931

French standard

NF EN ISO 4833-1
11 October 2013

Classification index: **V 08-011-1**

ICS: 07.100.30

Microbiology of the food chain — Horizontal method for the enumeration of microorganisms — Part 1: Colony count at 30 °C by the pour plate technique

F : Microbiologie de la chaîne alimentaire — Méthode horizontale pour le dénombrement des micro-organismes — Partie 1 : Comptage des colonies à 30 °C par la technique d'ensemencement en profondeur

D : Mikrobiologie von Lebensmitteln und Futtermitteln — Horizontales Verfahren zur Zählung von Mikroorganismen — Teil 1: Koloniezählverfahren bei 30 °C mittels Gussplattenverfahren

French standard approved

by decision of the Director General of AFNOR.

Together with part 2, replaces the approved standard NF EN ISO 4833 of May 2003.

Correspondence

The European standard EN ISO 4833-1:2013 has the status of French standard and reproduces in full the international standard ISO 4833-1:2013.

Summary

This document specifies a horizontal method for enumeration of microorganisms growing in a solid medium after aerobic incubation at 30 ºC.

It may be adapted to the analysis of certain fermented food and animal feeding stuffs is limited and other media and/or incubation conditions may be more appropriate.

However this method may be applied to such products even though the predominant microorganisms in those products may not be detected effectively.

For some matrices, the method specified in this document may give different results than those obtained with the method specified in NF EN ISO 4833-2.

Descriptors

Technical International Thesaurus: food products, human nutrition, animal feed, microorganisms, bacteria, fungi, yeasts, bacteria count methods, tests.

Modifications

With respect to the replaced document, division of the standard into 2 parts.

Corrections

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By nature, application of a standard is voluntary. When stipulated in a contract, it is binding on the parties. Legislation may require all or part of a standard to become compulsory.

The standard is a document defined by consensus within a standardisation body involving representatives of all stakeholders. It is submitted for public consultation prior to adoption.

The standard is regularly reviewed to assess its appropriateness over time.

Any standard is considered to be effective as from the date presented on the first page.

Understanding standards

The reader's attention is drawn to the following points:

Only the verbal form **shall** is used to express one or more requirements that shall be satisfied in order to comply with this document. Such requirements may be contained in the body of the standard or in a so-called "normative" annex. For test methods, the use of the imperative corresponds to a requirement.

Expressions involving the verbal form **should** are used to express a possibility that, while preferred, is not actually necessary in order to comply with this document. The verbal form **may** is used to express practical, but not mandatory advice or suggestions, or permission.

Furthermore, this document may provide additional information aimed at making certain elements easier to understand or use, or at clarifying how such elements are applied, but without actually defining a requirement. These elements are presented as **notes or informative annexes**.

Standardisation commission

Acting within a given field of activity, a standardisation commission features the necessary expertise for preparing French standards and defining France's positions in European and international draft standards. The commission may also prepare experimental standards and technical reports.

If you would like to provide feedback on this document, make suggested changes or take part in the document's revision, write to "norminfo@afnor.org".

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN ISO 4833-1

September 2013

ICS 07.100.30

Supersedes EN ISO 4833:2003

English Version

Microbiology of the food chain - Horizontal method for the enumeration of microorganisms - Part 1: Colony count at 30 degrees C by the pour plate technique (ISO 4833-1:2013)

Microbiologie de la chaîne alimentaire - Méthode horizontale pour le dénombrement des micro-organismes - Partie 1: Comptage des colonies à 30 degrés C par la technique d'ensemencement en profondeur (ISO 4833-1:2013)

Mikrobiologie von Lebensmitteln und Futtermitteln - Horizontales Verfahren zur Zählung von Mikroorganismen - Teil 1: Koloniezählverfahren bei 30 °C mittels Gussplattenverfahren (ISO 4833-1:2013)

This European Standard was approved by CEN on 26 July 2013.

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COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

CEN-CENELEC Management Centre: Avenue Marnix 17, B-1000 Brussels

Ref. No. EN ISO 4833-1:2013: E

EN ISO 4833-1:2013 (E)

Foreword

This document (EN ISO 4833-1:2013) has been prepared by Technical Committee ISO/TC 34 "Food products" in collaboration with Technical Committee CEN/TC 275 “Food analysis - Horizontal methods” the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by March 2014, and conflicting national standards shall be withdrawn at the latest by March 2014.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

This document supersedes EN ISO 4833:2003, together with EN ISO 4833-2:2013.

According to the CEN-CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

Endorsement notice

The text of ISO 4833-1:2013 has been approved by CEN as EN ISO 4833-1:2013 without any modification.

Contents

Page

INTERNATIONAL STANDARD

ISO 6611

IDF 94

Second edition
2004-10-15

Milk and milk products — Enumeration of colony-forming units of yeasts and/or moulds — Colony-count technique at 25 °C

Lait et produits laitiers — Dénombrement des unités formant colonie de levures et/ou moisissures — Comptage des colonies à 25 °C

Reference numbers
ISO 6611:2004(E)
IDF 94:2004(E)

ISO 6611:2004(E)
IDF 94:2004(E)

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Published in Switzerland

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 6611|IDF 94 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF), in collaboration with AOAC International. It is being published jointly by ISO and IDF and separately by AOAC International.

This edition of ISO 6611|IDF 94 cancels and replaces ISO 6611:1992, of which it constitutes a minor revision.

Foreword

IDF (the International Dairy Federation) is a worldwide federation of the dairy sector with a National Committee in every member country. Every National Committee has the right to be represented on the IDF Standing Committees carrying out the technical work. IDF collaborates with ISO and AOAC International in the development of standard methods of analysis and sampling for milk and milk products.

Draft International Standards adopted by the Action Teams and Standing Committees are circulated to the National Committees for voting. Publication as an International Standard requires approval by at least 50 % of the National Committees casting a vote.

ISO 6611|IDF 94 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF), in collaboration with AOAC International. It is being published jointly by ISO and IDF and separately by AOAC International.

All work was carried out by the Joint ISO/IDF/AOAC Group of Experts, *Enumeration of yeasts and moulds in dairy products* (E34), under the aegis of its chairman, Mr J.J. Devoyod (FR).

This edition of ISO 6611|IDF 94 cancels and replaces IDF 94B:1990, of which it constitutes a minor revision.

INTERNATIONAL STANDARD

ISO 6611:2004(E)
IDF 94:2004(E)

Milk and milk products — Enumeration of colony-forming units of yeasts and/or moulds — Colony-count technique at 25 °C

1 Scope

This International Standard specifies a method for the detection and enumeration of colony-forming units (CFU) of viable yeasts and/or moulds in milk and milk products by means of the colony-count technique at 25 °C.

The method is applicable to

— milk, liquid milk products,

— dried milk, dried sweet whey, dried buttermilk, lactose,

— cheese,

— acid casein, lactic casein, rennet casein,

— caseinate, acid whey powder,

— butter,

— frozen milk products (including edible ices),

— custard, desserts, fermented milk and cream.

NOTE This method is not suitable for a large number of thermolabile yeasts (in fresh cheese). In such cases the agar-surface-plating method is preferred.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 6887-1, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 1: General rules for the preparation of the initial suspension and decimal dilutions*

ISO 7218, *Microbiology of food and animal feeding stuffs — General rules for microbiological examinations*

ISO 8261|IDF 122:2001, *Milk and milk products — General guidance for the preparation of test samples, initial suspensions and decimal dilutions for microbiological examination*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
yeasts and moulds
microorganisms which at 25 °C form colonies in a selective medium under the conditions specified in this International Standard

(b) (4)

ISO 6611:2004(E)
IDF 94:2004(E)

ICS 07.100.30; 67.100.01

Price based on 8 pages

FA160467 ISSN 0335-3931

NF V 08-050

FRENCH STANDARDS

April 2009

Classification index: **V 08- 050**

ICS: 07.100.30

Food microbiology

Microbiology of food and animal feeding stuffs — Enumeration of presumptive coliforms by colony-count technique at 30 °C

EN: Microbiology of food and animal feeding stuffs — Enumeration of presumptive coliforms by colony-count technique at 30 °C

DE: Mikrobiologie von Lebensmitteln und Futtermitteln — Zählung der durch das Zählen der Kolonien nach Bebrütung bei 30 °C erwarteten Koliformen

French standards approval

By decision of the Director General of AFNOR, 25 March 2009 with effect from 25 April 2009.

Replaces approved French standard NF V 08-050, February 1999.

Correspondence

At the publication date for this document there exists no international or European work on this subject.

Analysis

This document is part of a suite of standards that define the methods applicable to testing and verification of microbiological criteria for human and animal foodstuffs, for use both by producers for the application of quality policy and by testing bodies for consumer protection.

This suite of standards comprises both horizontal, and specific and complementary methods for the microbiological inspection of products..

This document describes a method for the enumeration of presumptive coliforms in products destined for human consumption and animal feed by the colony-count in solid media after incubation at 30°C.

Descriptors

International Technical Thesaurus: microbiological analysis, food product, human foodstuffs, animal foodstuffs, enumeration, coliform bacteria, bacteria count, culture.

Amendments

Compared to previous version of document:

— Addition of the word *présumés* (presumptive) and deletion of the term *méthode de routine* (routine method) in title;

— Addition of control of media in 5.3.3;

— Harmonisation and compliance with standard NF EN ISO 7218 for expression of results (Article 10).

Corrections

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FE043856 ISSN 0335-3931

French standard

NF ISO 16649-2
July 2001

Classification index: **V 08-031-2**

ICS: 07.100.30

Microbiology of food and animal feeding stuffs

Horizontal method for the enumeration of β-glucuronidase-positive *Escherichia coli*

Part 2: Colony-count technique at 44 °C using 5-bromo-4-chloro-3-indolyl β-D-glucuronate

F : Microbiologie des aliments — Méthode horizontale pour le dénombrement des *Escherichia coli* β-glucuronidase positive — Partie 2 : Technique de comptage des colonies à 44 °C au moyen de 5-bromo-4-chloro-3-indolyl β-D-glucuronate

D : Microbiologie von Lebensmitteln — Horizontales Verfahren zur Zählung von *Escherichia coli* positiver β-Glukuronidase — Teil 2: Methode zum Auszählen der Kolonien bei 44 °C mit 5-bromo-4-chloro-3-indolyl β-D-Glukuronat

French standard approved

by decision of the Director General of AFNOR on July 20, 2001 taking effect on August 20, 2001.

Correspondence

This document reproduces in full the international standard ISO 16649-1:2001.

Analysis

This document describes a method for the enumeration of β-glucuronidase-positive *Escherichia coli* with a colony-count technique, after reviviscence by means of membranes and then incubation at 44 °C in a solid medium.

Descriptors

Technical International Thesaurus: food products, animal feeding products, microbiological analysis, bacteria count methods, coliform bacteria, preparation, procedure, computation.

Modifications

Corrections

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ISSN 0335-3931

French standard

NF EN ISO 6579-1
29 April 2017

Classification index: **V 08-013-1**

ICS: 07.100.30

Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella — Part 1: Detection of *Salmonella* spp.

F : Microbiologie de la chaîne alimentaire — Méthode horizontale pour la recherche, le dénombrement et le sérotypage des Salmonella — Partie 1 : Recherche des *Salmonella* spp.

D : Mikrobiologie der Lebensmittelkette — Horizontales Verfahren zum Nachweis, zur Zählung und zur Serotypisierung von Salmonellen — Teil 1: Nachweis von *Salmonella* spp.

French standard approved

by decision of the Director General of AFNOR.

Replaces the approved standard NF EN ISO 6785 (classification index: V 04-031), of April 2008 and the approved standard NF EN ISO 6579 (V 08-013) of December 2002 and its amendment A1 of October 2017.

Correspondence

The European standard EN ISO 6579-1:2017 has the status of French standard and reproduces in full the international standard ISO 6579-1:2017.

Summary

This document specifies a horizontal method for the detection of Salmonella. It is applicable to the following:

— products intended for human consumption and the feeding of animals;
— environmental samples in the area of food production and food handling;
— samples from the primary production stage such as animal faeces, dust, and swabs.

With this horizontal method, most of the Salmonella serovars are intended to be detected. For the detection of some specific serovars, additional culture steps may be needed. For Salmonella Typhi and Salmonella Paratyphi, the procedure is described in Annex D. The selective enrichment medium modified semi-solid Rappaport-Vassiliadis (MSRV) agar is intended for the detection of motile Salmonella and is not appropriate for the detection of non-motile Salmonella strains.

Descriptors

Technical International Thesaurus: human nutrition, food products, animal feed, animal feeding products, microbiological analysis, research, microorganisms, salmonella, sampling, samples, procedure.

Modifications

With respect to the replaced documents, consolidation into one standard.

Corrections

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EUROPEAN STANDARD
NORME EUROPÉENNE
EUROPÄISCHE NORM

EN ISO 6579-1

March 2017

ICS: 07.100.30

Supersedes EN ISO 6579:2002, EN ISO 6785:2007

English Version

Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella — Part 1: Detection of Salmonella spp. (ISO 6579-1:2017)

Microbiologie de la chaîne alimentaire — Méthode horizontale pour la recherche, le dénombrement et le sérotypage des Salmonella — Partie 1: Recherche des Salmonella spp. (ISO 6579-1:2017)

Mikrobiologie der Lebensmittelkette — Horizontales Verfahren zum Nachweis, zur Zählung und zur Serotypisierung von Salmonellen — Teil 1: Nachweis von Salmonella spp. (ISO 6579-1:2017)

This European Standard was approved by CEN on 3 February 2017.

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EUROPEAN COMMITTEE FOR STANDARDIZATION

Comité Européen de Normalisation
European Committee for Standardization

Management Centre: Avenue Marnix 17, B-1000 Brussels

EN ISO 6579-1:2017 (E)

European Foreword

This document (EN ISO 6579-1:2017) has been prepared by Technical Committee CEN/TC 275 “Food analysis - Horizontal methods”, the secretariat of which is held by DIN, in collaboration with Technical Committee ISO/TC 34 "Food products".

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by September 2017 and conflicting national standards shall be withdrawn at the latest by September 2017.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

This document supersedes EN ISO 6579:2002 and EN ISO 6785:2007.

This document has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association.

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Endorsement notice

The text of ISO 6579-1:2017 has been approved by CEN as EN ISO 6579-1:2017 without any modification.

Contents

Page

SafGlucan®

CERTIFICATE OF ANALYSIS

General information

Product name	SafGlucan®
Batch number	(b) (4)
Manufacturing date	30/03/2018
Best Before Date	29/03/2020

Product characteristics

	Values	Specifications	Method
Beta-glucans (%)	(b) (4)	≥50	In house Lesaffre used by Phileo method
Mannans (%)		≤5	In house Lesaffre used by Phileo method
Glycogen (%)		/	In house Lesaffre used by Phileo method

Remarks

Certified conform,

(b) (6)

SafGlucan®

CERTIFICATE OF ANALYSIS

General information	
Product name	SafGlucan®
Batch number	(b) (4)
Manufacturing date	15/03/2018
Best Before Date	14/03/2020

Product characteristics

	Values	Specifications	Method
Beta-glucans (%)	(b) (4)	≥50	In house Lesaffre used by Phileo method
Mannans (%)		≤5	In house Lesaffre used by Phileo method
Glycogen (%)		/	In house Lesaffre used by Phileo method

Remarks

Division of S.I.LESAFFRE
Phileo by Lesaffre
A LESAFFRE BUSINESS UNIT

Certified conform,

(b)(6)

SafGlucan®

CERTIFICATE OF ANALYSIS

General information	
Product name	SafGlucan®
Batch number	(b) (4)
Manufacturing date	27/04/2018
Best Before Date	26/04/2020

Product characteristics	Values	Specifications	Method
Beta-glucans (%)	(b) (4)	≥50	In house Lesaffre used by Phileo method
Mannans (%)		≤5	In house Lesaffre used by Phileo method
Glycogen (%)		/	In house Lesaffre used by Phileo method

Remarks

Certified conform,

(b) (6)

SafGlucan®

CERTIFICATE OF ANALYSIS

General information	
Product name	SafGlucan®
Batch number	(b) (4)
Manufacturing date	25/03/2019
Best Before Date	24/03/2021

Product characteristics	Values	Specifications	Method
Beta-glucans (%)	(b) (4)	≥50	In house Lesaffre used by Phileo method
Mannans (%)		≤5	In house Lesaffre used by Phileo method
Glycogen (%)		/	In house Lesaffre used by Phileo method

Remarks

Certified conform,

(b) (6)

SafGlucan®

CERTIFICATE OF ANALYSIS

General information	
Product name	SafGlucan®
Batch number	(b) (4)
Manufacturing date	04/03/2019
Best Before Date	03/03/2021

Product characteristics			
	Values	**Specifications**	**Method**
Beta-glucans (%)	(b) (4)	≥50	In house Lesaffre used by Phileo method
Mannans (%)		≤5	In house Lesaffre used by Phileo method
Glycogen (%)		/	In house Lesaffre used by Phileo method

Remarks

Certified conform,

(b) (6)

(b) (4)

Analytical report

Sample code Nr.	**370-2019-00240136**	**Report Date**	**27/08/2019**	**Page 1/1**
Analytical Report Nr.	**AR-19-AA-231472-01 / 370-2019-00240136**			

(b) (4)

Société Industrielle LESAFFRE

For the attention of (b)(6)
137, rue GABRIEL PERI
59700 Marcq en Baroeul
FRANCE

Email (b)(6)

Your contact for Customer Service (b)(6)

Our reference :	370-2019-00240136 / AR-19-AA-231472-01	**Type :**	EX
Client reference :	**Safglucan_batch** (b) (4)		
Sample described as :	levure		
Packaging :	NonCommercial 476g		
Your purchase order date :	20/08/2019	**Your purchase order reference :**	2019.07.15 LRH Safglucan US Chimie / (EOL
Sample reception date :	22/08/2019	**Analysis starting date :**	22/08/2019
Sampling/Transport :	DHL		
Analyses requested :	C0090 : Protéines AA001 : Humidité à 102-103°C (sur sable) A7367 : Matières grasses totales		
Best before	29/03/2020	**Batch Number**	(b) (4)
N° bon de commande	-	**Lettre**	-

Compositional analyses			Results (uncertainty)
C0090	**AA**	**Proteins Method : Internal, Kjeldahl (titrimetry)**	(b) (4)
	Total Nitrogen		
	Proteins (Nx6.25) (Kjeldahl)		
A7367	**AA**	**Total fat Method : Internal, Gravimetry**	
	Fat		
AA001	**AA**	**Moisture at 102-103°C on sand Method : Internal, Thermo-gravim**	
	Moisture		
	Total solids		

SIGNATURE (b)(6)

Report electronically validated by (b)(6)

(b) (4)

(b) (4)

(b) (4)

Analytical report

Sample code Nr.	**370-2019-00240137**	**Report Date**	**27/08/2019**	**Page 1/1**
Analytical Report Nr.	**AR-19-AA-231473-01 / 370-2019-00240137**			

(b) (4)

Société Industrielle LESAFFRE

For the attention of (b)(6)
137, rue GABRIEL PERI
59700 Marcq en Baroeul
FRANCE

Email (b)(6)

Your contact for Customer Service : (b) (4)

Our reference :	370-2019-00240137 / AR-19-AA-231473-01	**Type :**	EX
Client reference :	**Safglucan_batch** (b) (4)		
Sample described as :	levure		
Packaging :	NonCommercial 310g		
Your purchase order date :	20/08/2019	**Your purchase order reference :**	2019.07.15 LRH Safglucan US Chimie / (EOL
Sample reception date :	22/08/2019	**Analysis starting date :**	22/08/2019
Sampling/Transport :	DHL		
Analyses requested :	C0090 : Protéines AA001 : Humidité à 102-103°C (sur sable) A7367 : Matières grasses totales		

Best before	15/03/2021	**Batch Number**	(b) (4)
N° bon de commande	-	**Lettre**	-

Compositional analyses			Results (uncertainty)
C0090	**AA**	**Proteins Method : Internal, Kjeldahl (titrimetry)**	
	Total Nitrogen		(b) (4)
	Proteins (Nx6.25) (Kjeldahl)		
A7367	**AA**	**Total fat Method : Internal, Gravimetry**	
	Fat		
AA001	**AA**	**Moisture at 102-103°C on sand Method : Internal, Thermo-gravi**	
	Moisture		
	Total solids		

SIGNATURE (b)(6)

Report electronica

(b) (4)

(b) (4)

Analytical report

Sample code Nr.	**370-2019-00240135**	**Report Date**	**28/08/2019**	**Page 1/1**
Analytical Report Nr.	**AR-19-AA-232355-01 / 370-2019-00240135**			

(b) (4)

Société Industrielle LESAFFRE

For the attention of (b)(6)
137, rue GABRIEL PERI
59700 Marcq en Baroeul
FRANCE

Email (b)(6)

Your contact for Customer Service : (b)(6)

Our reference :	370-2019-00240135 / AR-19-AA-232355-01	**Type :**	EX
Client reference :	**Safglucan_batch** (b) (4)		
Sample described as :	levure		
Packaging :	NonCommercial 478g		
Your purchase order date :	20/08/2019	**Your purchase order reference :**	2019.07.15 LRH Safglucan US Chimie / (EOL
Sample reception date :	22/08/2019	**Analysis starting date :**	22/08/2019
Sampling/Transport :	DHL		
Analyses requested :	C0090 : Protéines AA001 : Humidité à 102-103°C (sur sable) A7367 : Matières grasses totales		

Best before	26/04/2020	**Batch Number**	(b) (4)
N° bon de commande	-	**Lettre**	-

Compositional analyses			Results (uncertainty)
C0090	**AA**	**Proteins Method : Internal, Kjeldahl (titrimetry)**	(b) (4)
	Total Nitrogen		
	Proteins (Nx6.25) (Kjeldahl)		
A7367	**AA**	**Total fat Method : Internal, Gravimetry**	
	Fat		
AA001	**AA**	**Moisture at 102-103°C on sand Method : Internal, Thermo-gravi**	
	Moisture		
	Total solids		

SIGNATURE (b)(6)

Report electronically validated by Caroline Deltel

(b) (4)

[redacted]

Analytical report

Sample code Nr.	**370-2019-00240139**	**Report Date**	**15/05/2020**	**Page 1/1**
Analytical Report Nr.	**AR-20-AA-123830-01 / 370-2019-00240139**			

[redacted]

Société Industrielle LESAFFRE

For the attention of [redacted]

137, rue GABRIEL PERI
59700 Marcq en Baroeul
FRANCE

Email [redacted]

Our reference :	370-2019-00240139 / AR-20-AA-123830-01	**Type :**	EX
Sample reception date :	22/08/2019		
Analysis starting date :	22/08/2019		
Sampling/Transport :	DHL		

Data provided by the customer

Packaging :	NonCommercial : 316g		
Your purchase order reference :	2019.07.15 LRH Safglucan US Chimie / (EOL) 006-105		
Your purchase order date :	20/08/2019	**Sample described as :**	levure
Client reference :	**Safglucan_batch** [redacted]		
Analyses requested :	C0090 : Protéines AA001 : Humidité à 102-103°C (sur sable) A7367 : Matières grasses totales		
Best before	24/03/2021	**Batch Number**	[redacted]
N° bon de commande	-	**Lettre**	-

Compositional analyses				Results (uncertainty)
C0090	**AA**	**Proteins**	**Method : Internal, Kjeldahl (titrimetry)**	[redacted]
	Total Nitrogen			
	Proteins (Nx6.25) (Kjeldahl)			
A7367	**AA**	**Total fat**	**Method : Internal, Gravimetry**	
	Fat			

CONCLUSION

Note : This report is an extract from the report AR-19-AA-232356-01/370-2019-00240139 dated 08/28/2019 which must be kept.

SIGNATURE [redacted]

Report electroni

EXPLANATORY NOTE

[redacted]

(b) (4)

Analytical report

Sample code Nr.	**370-2019-00240138**	**Report Date**	**15/05/2020**	**Page 1/1**
Analytical Report Nr.	**AR-20-AA-123829-01 / 370-2019-00240138**			

(b) (4)

Société Industrielle LESAFFRE

For the attention of (b)(6)

137, rue GABRIEL PERI
59700 Marcq en Baroeul
FRANCE

Email (b)(6)

Our reference :	370-2019-00240138 / AR-20-AA-123829-01	**Type :**	EX
Sample reception date :	22/08/2019		
Analysis starting date :	22/08/2019		
Sampling/Transport :	DHL		

Data provided by the customer

Packaging :	NonCommercial : 304g		
Your purchase order reference :	2019.07.15 LRH Safglucan US Chimie / (EOL) 006-105		
Your purchase order date :	20/08/2019	**Sample described as :**	levure
Client reference :	**Safglucan_batch** (b) (4)		
Analyses requested :	C0090 : Protéines AA001 : Humidité à 102-103°C (sur sable) A7367 : Matières grasses totales		
Best before	03/03/2021	**Batch Number**	(b) (4)
N° bon de commande	-	**Lettre**	-

Compositional analyses				Results (uncertainty)
C0090	**AA**	**Proteins**	**Method : Internal, Kjeldahl (titrimetry)**	(b) (4)
	Total Nitrogen			
	Proteins (Nx6.25) (Kjeldahl)			
A7367	**AA**	**Total fat**	**Method : Internal, Gravimetry**	
	Fat			

CONCLUSION

Note : This report is an extract from the report AR-19-AA-231474-01/370-2019-00240138 dated 08/27/2019 which must be kept.

SIGNATURE (b)(6)

Report electroni

(b) (4)

(b) (4)

Product name 产品名称: β-1,3-D-glucan β-1,3-D-葡聚糖
Model 型号: Safglucan 赛福葡聚糖
Batch number 批号: (b) (4)
Production date 生产日期: 2019-03-25
Test date 测试日期: 2019-03-26
Reporting date 报告日期: 2019-04-16
Best before 保质期: 2021-03-24

Test item 检验项目	Standard 标准	Test result 检验结果
Sensory 感官要求	Yellow powder with specific smell, no foreign smell and visible impurity 黄色粉末，无异味，无可见杂质，具有该产品特有气味	(b) (4)
Moisture, % 水分	≤6.0	
Crude protein / DM, % 粗蛋白（以干基计）	≤10.0	
β-1,3-D-glucan / DM, % β-1,3-D-葡聚糖（以干基计）	≥50.0%	
Salmonella, /25g 沙门氏菌	Absence 不得检出	

Conclusion 检验结论:

The product passes inspection and conforms to the requirements of (b) (4)

(b) (4)

(b) (4), (b) (9)

(b) (4)

(b) (4)

Product name 产品名称: β-1,3-D-glucan β-1,3-D-葡聚糖
Model 型号: Safglucan 赛福葡聚糖
Batch number 批号: (b) (4)
Production date 生产日期: 2019-03-04
Test date 测试日期: 2019-03-05
Reporting date 报告日期: 2019-04-09
Best before 保质期: 2021-03-03

Test item 检验项目	Standard 标准	Test result 检验结果
Sensory 感官要求	Yellow powder with specific smell, no foreign smell and visible impurity 黄色粉末，无异味，无可见杂质，具有该产品特有气味	(b) (4)
Moisture, % 水分	≤6.0	
Crude protein / DM, % 粗蛋白（以干基计）	≤10.0	
β-1,3-D-glucan / DM, % β-1,3-D-葡聚糖（以干基计）	≥50.0	
Salmonella, /25g 沙门氏菌	Absence 不得检出	

Conclusion 检验结论:

The product passes inspection and conforms to the requirements of (b) (4)

(b) (4)

(b) (4), (b)(6)

(b) (4)

Analytical report

Sample code Nr.	**370-2019-00241029**	**Report Date**	**27/08/2019**	**Page 1/1**
Analytical Report Nr.	**AR-19-AA-231461-01 / 370-2019-00241029**			

(b) (4)

Société Industrielle LESAFFRE

For the attention of (b)(6)

137, rue GABRIEL PERI
59700 Marcq en Baroeul
FRANCE

Email (b)(6)

Your contact for Customer Service : (b)(6)

Our reference :	370-2019-00241029 / AR-19-AA-231461-01	**Type :**	EX
Client reference :	**Safglucan_batch** (b) (4)		
Sample described as :	levure		
Packaging :	NonCommercial : 258g		
Your purchase order date :	21/08/2019	**Your purchase order reference :**	2019.08.21LRH Safglucan US Cendre / (EOL)
Sample reception date :	22/08/2019	**Analysis starting date :**	22/08/2019
Sampling/Transport :	Retour ELMO		
Analyses requested :	AA009 : Cendres		

Best before	29/03/2020	**Batch Number**	(b) (4)
Brand name	Safglucan	**N° bon de commande**	2019.08.21LRH
Lettre	-		

Compositional analyses	Results (uncertainty)
AA009 AA Ash Method : Internal, Gravimetry Crude ash	(b) (4)

(b)(6)

SIGNATURE

Report electroni

EXPLANATORY NOTE

(b) (4)

(b) (4)

Analytical report

Sample code Nr.	**370-2019-00241030**	**Report Date**	**27/08/2019**	**Page 1/1**
Analytical Report Nr.	**AR-19-AA-231462-01 / 370-2019-00241030**			

(b) (4)

Société Industrielle LESAFFRE

For the attention of (b)(6)

137, rue GABRIEL PERI
59700 Marcq en Baroeul
FRANCE

Email (b)(6)

Your contact for Customer Servic (b)(6)

Our reference :	370-2019-00241030 / AR-19-AA-231462-01	**Type :**	EX
Client reference :	**Safglucan_batch** (b) (4)		
Sample described as :	levure		
Packaging :	NonCommercial : 50g		
Your purchase order date :	21/08/2019	**Your purchase order reference :**	2019.08.21LRH Safglucan US Cendre / (EOL)
Sample reception date :	22/08/2019	**Analysis starting date :**	22/08/2019
Sampling/Transport :	Retour ELMO		
Analyses requested :	AA009 : Cendres		

Best before	15/03/2021	**Batch Number**	(b) (4)
Brand name	Safglucan	**N° bon de commande**	2019.08.21LRH
Lettre	-		

Compositional analyses	Results (uncertainty)
AA009 AA Ash Method : Internal, Gravimetry	
Crude ash	(b) (4)

SIGNATURE (b)(6)

Report electronica

EXPLANATORY NOTE

(b) (4)

(b) (4)

Analytical report

Sample code Nr.	**370-2019-00241028**	**Report Date**	**27/08/2019**	**Page 1/1**
Analytical Report Nr.	**AR-19-AA-231460-01 / 370-2019-00241028**			

(b) (4)

Société Industrielle LESAFFRE

For the attention of (b)(6)

137, rue GABRIEL PERI
59700 Marcq en Baroeul
FRANCE

Email (b)(6)

Your contact for Customer Service : (b)(6)

Our reference :	370-2019-00241028 / AR-19-AA-231460-01	**Type :**	EX
Client reference :	**Safglucan_batch** (b) (4)		
Sample described as :	levure		
Packaging :	NonCommercial : 258g		
Your purchase order date :	21/08/2019	**Your purchase order reference :**	2019.08.21LRH Safglucan US Cendre / (EOL)
Sample reception date :	22/08/2019	**Analysis starting date :**	22/08/2019
Sampling/Transport :	Retour ELMO		
Analyses requested :	AA009 : Cendres		

Best before	26/04/2020	**Batch Number**	(b) (4)
Brand name	Safglucan	**N° bon de commande**	2019.08.21LRH
Lettre	-		

Compositional analyses				Results (uncertainty)
AA009	**AA**	**Ash**	**Method : Internal, Gravimetry**	
	Crude ash			(b) (4)

SIGNATURE (b)(6)

Report electronica

EXPLANATORY NOTE

(b) (4)

(b) (4)

Analytical report

Sample code Nr.	**370-2019-00241032**	**Report Date**	**27/08/2019**	**Page 1/1**
Analytical Report Nr.	**AR-19-AA-231464-01 / 370-2019-00241032**			

(b) (4)

Société Industrielle LESAFFRE

For the attention of (b)(6)

137, rue GABRIEL PERI
59700 Marcq en Baroeul
FRANCE

Email (b)(6)

Your contact for Customer Service : (b)(6)

Our reference :	370-2019-00241032 / AR-19-AA-231464-01	**Type :**	EX
Client reference :	**Safglucan_batch** (b) (4)		
Sample described as :	levure		
Packaging :	NonCommercial : 50g		
Your purchase order date :	21/08/2019	**Your purchase order reference :**	2019.08.21LRH Safglucan US Cendre / (EOL)
Sample reception date :	22/08/2019	**Analysis starting date :**	22/08/2019
Sampling/Transport :	Retour ELMO		
Analyses requested :	AA009 : Cendres		

Best before	24/03/2021	**Batch Number**	(b) (4)
Brand name	Safglucan	**N° bon de commande**	2019.08.21LRH
Lettre	-		

Compositional analyses	Results (uncertainty)
AA009 AA Ash Method : Internal, Gravimetry Crude ash	(b)(6)

SIGNATURE (b)(6)

Report electronica

EXPLANATORY NOTE

(b) (4)

(b) (4)

Analytical report

Sample code Nr.	**370-2019-00241031**	**Report Date**	**27/08/2019**	**Page 1/1**
Analytical Report Nr.	**AR-19-AA-231463-01 / 370-2019-00241031**			

(b) (4)

Société Industrielle LESAFFRE

For the attention of (b)(6)

137, rue GABRIEL PERI
59700 Marcq en Baroeul
FRANCE

Email (b)(6)

Your contact for Customer Service : (b)(6)

Our reference :	370-2019-00241031 / AR-19-AA-231463-01	**Type :**	EX
Client reference :	**Safglucan_batch** (b) (4)		
Sample described as :	levure		
Packaging :	NonCommercial : 50g		
Your purchase order date :	21/08/2019	**Your purchase order reference :**	2019.08.21LRH Safglucan US Cendre / (EOL)
Sample reception date :	22/08/2019	**Analysis starting date :**	22/08/2019
Sampling/Transport :	Retour ELMO		
Analyses requested :	AA009 : Cendres		

Best before	03/03/2021	**Batch Number**	(b) (4)
Brand name	Safglucan	**N° bon de commande**	2019.08.21LRH
Lettre	-		

Compositional analyses	Results (uncertainty)
AA009 AA Ash Method : Internal, Gravimetry Crude ash	(b) (4)

SIGNATURE (b)(6)

Report electroni

EXPLANATORY NOTE

(b) (4)

(b) (4)

Supplementary Analytical Data: Crude Protein Content of Purified Yeast Cell Wall

Analytical data are presented in the GRAS notice for batches of purified yeast cell wall manufactured in 2018 and 2019. Phileo confirms that these data continue to be representative of the commercial product as intended to be marketed as a source of beta-glucans in animal feed in the U.S. However, in more recent batches of purified yeast cell wall, the protein content is observed to be slightly higher than that previously reported, with the results of 4 representative batches presented in Table S-1. The protein content in these 4 batches varies from 6.3 to 6.9% compared to 3.3 to 3.6% in the 5 batches presented in the GRAS notice. The slight variation in protein (~3% increase) is considered to be part of the normal variation within the manufacturing plant over time and the conditions employed continue to be those described within the GRAS notice (Appendix 01 – CONFIDENTIAL). As evidenced by the analytical results in Table S-1, the product continues to conform with the product specifications and the beta-glucans content remains within the range of that observed in earlier batches, i.e., 56.8 to 66.5% vs. 56.7 to 67.4%. On the basis of these analytical data, the maximum limit set by Phileo for crude protein is 8% by weight. Copies of the Certificates of Analysis for the 4 representative batches are enclosed herein.

Table S-1: Additional Analytical Data on Recent Production Batches of Purified Yeast Cell Wall

Parameter	Unit	Spec.	Analytical Data			
			Batch (b) (4)	Batch (b) (4)	Batch	Batch
Manufacturing date						
Appearance		Light beige powder				
Composition						
β-glucans	%	≥50				
Crude protein	%	≤8				
Moisture	%	≤6				
Microbiology						
Total plate count	CFU/g	<5 000				
Yeast	CFU/g	<100				
Molds	CFU/g					
Total coliforms	CFU/g	<100				
Escherichia coli	CFU/g	<10				
Salmonella	/25 g	Absent				

Note: the specifications laid down in the Certificates of Analysis are not those of the intended commercial feed ingredient for the U.S. market which will conform to that in the GRAS notice. Light beige and yellow are considered equivalent from the specification.

Phileo, a Division of S.I.Lesaffre
August, 2023

YPX_FSR_DQ_703

(b) (4)

Common name 通用名：β-1,3-D-glucan β-1,3-D-葡聚糖	Product name 产品名称：Safglucan 赛福葡聚糖
Product standard (b) (4)	Batch number (b) (4)
Production date 生产日期：2023-01-18	Shelf life 保质期：2 years2 年
Test date 测试日期：2023-01-19	Reporting date 报告日期：2023-02-17

Characteristics 指标	Specification 技术要求	Test result 检验结果
Sensory 感官要求	Yellow powder with specific smell, no foreign smell and visible impurity 黄色粉末，无异味，无可见杂质，具有该产品特有气味	(b) (4)
Moisture 水分，%	≤6.0	
Total nitrogen 总氮，%	≤1.6	
Crude protein 粗蛋白，%	≤10.0	
β-1,3-D-glucan β-1,3-D-葡聚糖，%	≥50.0	
Bacterial count 细菌总数，CFU/g	≤10000	
Coliforms 大肠菌群，CFU/g	≤100	
Wild yeasts 野生酵母，CFU/g	≤100	
Moulds 霉菌，CFU/g	≤100	
Salmonella 沙门氏菌，/25g	Negative 不得检出	
Escherichia coli 大肠埃希氏菌，CFU/g	Negative 不得检出	

(b) (4), (b)(6)

Conclusion 检验结论：

The product passes inspection and conforms to the requirements of C 格，符合 Q/YPX 05-2020 的要求。

Inspected by (b)(6) Reviewed by (b)(6)

YPX_FSR_DQ_703

(b) (4)

Common name 通用名：β-1,3-D-glucan β-1,3-D-葡聚糖	Product name 产品名称：Safglucan 赛福葡聚糖
Product standard (b) (4)	Batch number (b) (4)
Production date 生产日期：2023-02-08	Shelf life 保质期：2 years2 年
Test date 测试日期：2023-02-09	Reporting date 报告日期：2023-02-23

Characteristics 指标	Specification 技术要求	Test result 检验结果
Sensory 感官要求	Yellow powder with specific smell, no foreign smell and visible impurity 黄色粉末，无异味，无可见杂质，具有该产品特有气味	(b) (4)
Moisture 水分，%	≤6.0	
Total nitrogen 总氮，%	≤1.6	
Crude protein 粗蛋白，%	≤10.0	
β-1,3-D-glucan β-1,3-D-葡聚糖，%	≥50.0	
Bacterial count 细菌总数，CFU/g	≤10000	
Coliforms 大肠菌群，CFU/g	≤100	
Wild yeasts 野生酵母，CFU/g	≤100	
Moulds 霉菌，CFU/g	≤100	
Salmonella 沙门氏菌，/25g	Negative 不得检出	
Escherichia coli 大肠埃希氏菌，CFU/g	Negative 不得检出	

Conclusion 检验结论：

(b) (4), (b)(6)

The product passes inspection and conforms to the requirements o 符合 Q/YPX 05-2020 的要求。

Inspected by (b)(6)

Reviewed by (b)(6)

YPX_FSR_DQ_703

(b) (4)

Common name 通用名：β-1,3-D-glucan β-1,3-D-葡聚糖	Product name 产品名称：Safglucan 赛福葡聚糖
Product standard (b) (4)	Batch number 批号：(b) (4)
Production date 生产日期：2023-02-28	Shelf life 保质期：2 years2 年
Test date 测试日期：2023-03-01	Reporting date 报告日期：2023-03-17

Characteristics 指标	Specification 技术要求	Test result 检验结果
Sensory 感官要求	Yellow powder with specific smell, no foreign smell and visible impurity 黄色粉末，无异味，无可见杂质，具有该产品特有气味	(b) (4)
Moisture 水分，%	≤6.0	
Total nitrogen 总氮，%	≤1.6	
Crude protein 粗蛋白，%	≤10.0	
β-1,3-D-glucan β-1,3-D-葡聚糖，%	≥50.0	
Bacterial count 细菌总数，CFU/g	≤10000	
Coliforms 大肠菌群，CFU/g	≤100	
Wild yeasts 野生酵母，CFU/g	≤100	
Moulds 霉菌，CFU/g	≤100	
Salmonella 沙门氏菌，/25g	Negative 不得检出	
Escherichia coli 大肠埃希氏菌，CFU/g	Negative 不得检出	

Conclusion 检验结论：

The product passes inspection and conforms to the requirements of (b)(6)，符合 Q/YPX 05-2020 的要求。

Inspected by (b)(6) Reviewed by (b)(6)

YPX_FSR_DQ_703

(b) (4)

Common name 通用名：β-1,3-D-glucan β-1,3-D-葡聚糖	Product name 产品名称：Safglucan 赛福葡聚糖
Product standard 产品标准号：(b) (4)	Batch number 批号：(b) (4)
Production date 生产日期：2023-03-17	Shelf life 保质期：2 years2 年
Test date 测试日期：2023-03-18	Reporting date 报告日期：2023-04-04

Characteristics 指标	Specification 技术要求	Test result 检验结果
Sensory 感官要求	Yellow powder with specific smell, no foreign smell and visible impurity 黄色粉末，无异味，无可见杂质，具有该产品特有气味	(b) (4)
Moisture 水分，%	≤6.0	
Total nitrogen 总氮，%	≤1.6	
Crude protein 粗蛋白，%	≤10.0	
β-1,3-D-glucan β-1,3-D-葡聚糖，%	≥50.0	
Bacterial count 细菌总数，CFU/g	≤10000	
Coliforms 大肠菌群，CFU/g	≤100	
Wild yeasts 野生酵母，CFU/g	≤100	
Moulds 霉菌，CFU/g	≤100	
Salmonella 沙门氏菌，/25g	Negative 不得检出	
Escherichia coli 大肠埃希氏菌，CFU/g	Negative 不得检出	

Conclusion 检验结论：

(b)(6)

The product passes inspection and conforms to the requirements of 合 Q/YPX 05-2020 的要求。

Inspected by (b)(6)

Reviewed by (b)(6)

Molecular weight – Safglucan

On behalf of Phileo's request, Lesaffre R&D department analysed the product Safglucan.

The method used is the method SEC/GPC (Gel Permeation Chromatography).

This method is used for the separation of molecules based on their size and molecular shape. The amount of elution is determined using a known molecular weight marker and a standard curve is prepared by plotting the logarithm of the molecular weight on the vertical axis and the logarithm of the amount of elution on the horizontal axis (hold time) before analyzing the unknown sample.

Results of analysis on the product Safglucan are: (b) (4)

(b)(6)

Phileo, a division of S.I.Lesaffre
137, rue Gabriel Péri - BP 3029 - 59703 Marcq-en-Baroeul cedex – France
Tel. +33 (0) 320 81 61 00 - Fax + 33 (0) 320 99 94 82 - info@phileo.lesaffre.com - www.phileo-lesaffre.com
SA au capital de 760 050€ - RSC Roubaix-Tourcoing 349 069 047 - Siret 349 069 047 00018 - TVA FR03 349069047

(b) (4)

Rapport d'analyse

(b) (4)

Société Industrielle LESAFFRE
137, rue GABRIEL PERI
59700 Marcq en Baroeul

Reçu au laboratoire le *21/08/2019 11:37 à 18°C*

Analysé le *21/08/2019*

Echantillon N° (b) (4) **: Safglucan_batch** (b) (4) **- LEVURE**

Date et heure de prélèvement spécifique Client : 19/08/2019 13:51:00

Commande : 2019.08.19 LRH Safglucan US micro - EOL 10518-894340

Paramètres	Méthode	Résultats	Critères Européens	Critères spécifiques
		(b) (4), (b)(6)		
(a) Flore aérobie mésophile (30°C) /g	*NF EN ISO 4833-1*			
(a) Moisissures /g	*ISO 6611*			
(a) Levures /g	*ISO 6611*			
(a) Escherichia coli β-glucuronidase positive /g	*NF ISO 16649-2*			
(a) Coliformes totaux présumés (30°C) /g	*NF V08-050*			
(a) Salmonella (hors sérovars Typhi et Paratyphi) /25 g	*NF EN ISO 6579-1*			

(b) (4)

(b) (4)

Analysis performed by the Nantes site

Date received by the laboratory (see date on original)
Analysed on (see date on original)

Sample No. (b) (4) – Safglucan batch (number on original)

EUROFINS – translated Certificate of Analysis

Parameters	Method	Results	European criteria
(a) Aerobic mesophilic bacteria	See original	(b) (4)	Reg. 2073/2005 modified
(a) Mold	See original		
(a) Yeast	See original		
(a) *Escherichia coli* beta-glucuronidase positive	See original		
(a) *Coliforms* (presumed total)	See original		
(a) *Salmonella* (serovars tyhi and paratyphi)	See original		

Explanatory note:
Only the services identified by the symbol (a) are covered by the accreditation.

(b) (4)

Rapport d'analyse

(b) (4)

Société Industrielle LESAFFRE
137, rue GABRIEL PERI
59700 Marcq en Baroeul

Reçu au laboratoire le *21/08/2019 11:37 à 18°C*

Analysé le *21/08/2019*

Echantillon N° (b) (4) : Safglucan_batch (b) (4) - LEVURE

Date et heure de prélèvement spécifique Client : 19/08/2019 13:51:00

Commande : 2019.08.19 LRH Safglucan US micro - EOL 10518-894340

Paramètres	Méthode	Résultats	Critères Européens	Critères spécifiques
		(b) (4), (b)(6)		
(a) Flore aérobie mésophile (30°C) /g	*NF EN ISO 4833-1*			
(a) Moisissures /g	*ISO 6611*			
(a) Levures /g	*ISO 6611*			
(a) Escherichia coli β-glucuronidase positive /g	*NF ISO 16649-2*			
(a) Coliformes totaux présumés (30°C) /g	*NF V08-050*			
(a) Salmonella (hors sérovars Typhi et Paratyphi) /25 g	*NF EN ISO 6579-1*			

(b) (4)

(b) (4)

Analysis performed by the Nantes site

Date received by the laboratory (see date on original)
Analysed on (see date on original)

Sample No. (b) (4) – Safglucan batch (number on original)

EUROFINS – translated Certificate of Analysis

Parameters	Method	Results	European criteria
(a) Aerobic mesophilic bacteria	See original	(b) (4)	Reg. 2073/2005 modified
(a) Mold	See original		
(a) Yeast	See original		
(a) *Escherichia coli* beta-glucuronidase positive	See original		
(a) *Coliforms* (presumed total)	See original		
(a) *Salmonella* (serovars tyhi and paratyphi)	See original		

Explanatory note:
Only the services identified by the symbol (a) are covered by the accreditation.

(b) (4)

Rapport d'analyse

(b) (4)

Société Industrielle LESAFFRE
137, rue GABRIEL PERI
59700 Marcq en Baroeul

Code client : LN04225

Analyse réalisée sur le site de Nantes

Reçu au laboratoire le *21/08/2019 11:37 à 18°C*

Analysé le *21/08/2019*

Echantillon N° (b) (4) : Safglucan_batch (b) (4) - LEVURE

Date et heure de prélèvement spécifique Client : 19/08/2019 00:00:00

Commande : 2019.08.19 LRH Safglucan US micro - EOL 10518-894340

Paramètres	Méthode	Résultats	Critères Européens	Critères spécifiques
		(b) (4), (b)(6)		
(a) Flore aérobie mésophile (30°C) /g	*NF EN ISO 4833-1*			
(a) Moisissures /g	*ISO 6611*			
(a) Levures /g	*ISO 6611*			
(a) Escherichia coli β-glucuronidase positive /g	*NF ISO 16649-2*			
(a) Coliformes totaux présumés (30°C) /g	*NF V08-050*			
(a) Salmonella (hors sérovars Typhi et Paratyphi) /25 g	*NF EN ISO 6579-1*			

NOTE EXPLICATIVE
Seules les prestations repérées par le symbole (a) sont effectuées sous le couvert de l'accréditation.

(b) (4)

Analysis performed by the Nantes site

Date received by the laboratory (see date on original)
Analysed on (see date on original)

Sample No (b) (4) – Safglucan batch (number on original)

EUROFINS – translated Certificate of Analysis

Parameters	Method	Results	European criteria
(a) Aerobic mesophilic bacteria	See original	(b) (4)	Reg. 2073/2005 modified
(a) Mold	See original		
(a) Yeast	See original		
(a) *Escherichia coli* beta-glucuronidase positive	See original		
(a) *Coliforms* (presumed total)	See original		
(a) *Salmonella* (serovars tyhi and paratyphi)	See original		

Explanatory note:
Only the services identified by the symbol (a) are covered by the accreditation.

(b) (4)

Rapport d'analyse

(b) (4)

Société Industrielle LESAFFRE
137, rue GABRIEL PERI
59700 Marcq en Baroeul

Reçu au laboratoire le *21/08/2019 11:37 à 18°C*

Analysé le *21/08/2019*

Echantillon N° (b) (4) **: Safglucan_batch** (b) (4) **- LEVURE**

Date et heure de prélèvement spécifique Client : 19/08/2019 13:52:00

Commande : 2019.08.19 LRH Safglucan US micro - EOL 10518-894340

Paramètres	Méthode	Résultats	Critères Européens	Critères spécifiques
		(b) (4)	Régl 2073/2005 modifié	
(a) Flore aérobie mésophile (30°C) /g	*NF EN ISO 4833-1*			
(a) Moisissures /g	*ISO 6611*			
(a) Levures /g	*ISO 6611*			
(a) Escherichia coli β-glucuronidase positive /g	*NF ISO 16649-2*			
(a) Coliformes totaux présumés (30°C) /g	*NF V08-050*			
(a) Salmonella (hors sérovars Typhi et Paratyphi) /25 g	*NF EN ISO 6579-1*			

(b)(6)

NOTE EXPLICATIVE
**Présence de Moisissures mais avec moins de 40 ufc/g (Nombre estimé : 10 ufc/g).*
Seules les prestations repérées par le symbole (a) sont effectuées sous le couvert de l'accréditation.

(b) (4)

Analysis performed by the Nantes site

Date received by the laboratory (see date on original)
Analysed on (see date on original)

Sample No (b) (4) – Safglucan batch (number on original)

EUROFINS – translated Certificate of Analysis

Parameters	Method	Results	European criteria
(a) Aerobic mesophilic bacteria	See original	(b) (4)	Reg. 2073/2005 modified
(a) Mold	See original		
(a) Yeast	See original		
(a) *Escherichia coli* beta-glucuronidase positive	See original		
(a) *Coliforms* (presumed total)	See original		
(a) *Salmonella* (serovars tyhi and paratyphi)	See original		

Explanatory note:
Only the services identified by the symbol (a) are covered by the accreditation.

(b) (4)

Rapport d'analyse

(b) (4)

Société Industrielle LESAFFRE
137, rue GABRIEL PERI
59700 Marcq en Baroeul

Reçu au laboratoire le *21/08/2019 11:37 à 18°C*

Analysé le *21/08/2019*

Echantillon (b) (4) **: Safglucan_batch** (b) (4) **- LEVURE**

Date et heure de prélèvement spécifique Client : 19/08/2019 13:51:00

Commande : 2019.08.19 LRH Safglucan US micro - EOL 10518-894340

Paramètres	Méthode	Résultats	Critères Européens	Critères spécifiques
		(b) (4), (b)(6)		
(a) Flore aérobie mésophile (30°C) /g	*NF EN ISO 4833-1*			
(a) Moisissures /g	*ISO 6611*			
(a) Levures /g	*ISO 6611*			
(a) Escherichia coli β-glucuronidase positive /g	*NF ISO 16649-2*			
(a) Coliformes totaux présumés (30°C) /g	*NF V08-050*			
(a) Salmonella (hors sérovars Typhi et Paratyphi) /25 g	*NF EN ISO 6579-1*			

NOTE EXPLICATIVE
Seules les prestations repérées par le symbole (a) sont effectuées sous le couvert de l'accréditation.

(b) (4)

Analysis performed by the Nantes site

Date received by the laboratory (see date on original)
Analysed on (see date on original)

Sample No. (b) (4) – Safglucan batch (number on original)

EUROFINS – translated Certificate of Analysis

Parameters	Method	Results	European criteria
(a) Aerobic mesophilic bacteria	See original	(b) (4)	Reg. 2073/2005 modified
(a) Mold	See original		
(a) Yeast	See original		
(a) *Escherichia coli* beta-glucuronidase positive	See original		
(a) *Coliforms* (presumed total)	See original		
(a) *Salmonella* (serovars tyhi and paratyphi)	See original		

Explanatory note:
Only the services identified by the symbol (a) are covered by the accreditation.

(b) (4)

Analytical report

Sample code Nr.	**370-2019-00239997**	**Report Date 28/08/2019**	**Page 1/1**
Analytical Report Nr.	**AR-19-AA-233247-01 / 370-2019-00239997**		

(b) (4)

Société Industrielle LESAFFRE

For the attention of (b)(6)

137, rue GABRIEL PERI
59700 Marcq en Baroeul
FRANCE

Email (b)(6)

Your contact for Customer Service : (b)(6)

Our reference :	370-2019-00239997/ AR-19-AA-233247-01	**Type :**	EX
Client reference :	**Safglucan_batch** (b) (4)		
Sample described as :	levure		
Packaging :	NonCommercial : 518g+72g=590g		
Your purchase order date :	20/08/2019	**Your purchase order reference :**	2019.07.15 LRH Safglucan US ML / (EOI
Sample reception date :	22/08/2019	**Analysis starting date :**	23/08/2019
Sampling/Transport :	DHL		
Analyses requested :	PJJ3V : Métaux lourds : Pb, Cd, Hg, As		

Best before	29/03/2020	**Batch Number**	(b) (4)
Brand name	Safglucan	**N° bon de commande**	-
Lettre	-		

Elementary analysis			Results (uncertainty)
J8306	**JC**	**Lead(ICP-MS, food) Method : DIN EN 15763:2010 (2010-04), mod.**	
(a)	Lead (Pb)		(b) (4)
J8308	**JC**	**Cadmium (ICP-MS, food) Method : DIN EN 15763:2010 (2010-04), mod.**	
(a)	Cadmium (Cd)		(b) (4)
J8312	**JC**	**Arsenic(Food, ICP-MS) Method : DIN EN 15763:2010 (2010-04), mod.**	
(a)	Arsenic (As)		(b) (4)
JCHG2	**JC**	**Mercury (ICP-MS, food) Method : DIN EN 15763:2010 (2010-04), mod.**	
(a)	Mercury [Hg]		(b) (4)

SIGNATURE (b)(6)

Report electronically validated by (b)(6)

EXPLANATORY NOTE

(b) (4)

(b) (4), (b)(6)

(b) (4)

Analytical report

Sample code Nr.	**370-2019-00239998**	**Report Date**	**28/08/2019**	**Page 1/1**
Analytical Report Nr.	**AR-19-AA-232718-01 / 370-2019-00239998**			

Société Industrielle LESAFFRE

For the attention of (b)(6)

137, rue GABRIEL PERI
59700 Marcq en Baroeul
FRANCE

Email (b)(6)

Your contact for Customer Service (b)(6)

Our reference :	370-2019-00239998 / AR-19-AA-232718-01	**Type :**	EX
Client reference :	**Safglucan_batch** (b) (4)		
Sample described as :	levure		
Packaging :	NonCommercial : 374g+466g=840g		
Your purchase order date :	20/08/2019	**Your purchase order reference :**	2019.07.15 LRH Safglucan US ML / (EOL) 00
Sample reception date :	22/08/2019	**Analysis starting date :**	23/08/2019
Sampling/Transport :	DHL		
Analyses requested :	PJJ3V : Métaux lourds : Pb, Cd, Hg, As		

Best before	15/03/2021	**Batch Number**	(b) (4)
Brand name	Safglucan	**N° bon de commande**	-
Lettre	-		

Elementary analysis			Results (uncertainty)
J8306	**JC**	**Lead(ICP-MS, food) Method : DIN EN 15763:2010 (2010-04), mod.**	
(a)	Lead (Pb)		(b) (4)
J8308	**JC**	**Cadmium (ICP-MS, food) Method : DIN EN 15763:2010 (2010-04), mod.**	
(a)	Cadmium (Cd)		(b) (4)
J8312	**JC**	**Arsenic(Food, ICP-MS) Method : DIN EN 15763:2010 (2010-04), mod.**	
(a)	Arsenic (As)		(b)(6)
JCHG2	**JC**	**Mercury (ICP-MS, food) Method : DIN EN 15763:2010 (2010-04), mod.**	
(a)	Mercury [Hg]		(b) (4)

SIGNATURE (b)(6)

Report electronically validated by (b)(6)

EXPLANATORY NOTE

(b) (4), (b)(6)

(b) (4)

Analytical report

Sample code Nr.	**370-2019-00239996**	**Report Date**	**28/08/2019**	**Page 1/1**
Analytical Report Nr.	**AR-19-AA-232717-01 / 370-2019-00239996**			

(b) (4)

Société Industrielle LESAFFRE

For the attention of (b)(6)

137, rue GABRIEL PERI
59700 Marcq en Baroeul
FRANCE

Email (b)(6)

Your contact for Customer Service (b)(6)

Our reference :	370-2019-00239996 / AR-19-AA-232717-01	**Type :**	EX
Client reference :	**Safglucan_batch** (b) (4)		
Sample described as :	levure		
Packaging :	NonCommercial : 260g+518g=778g		
Your purchase order date :	20/08/2019	**Your purchase order reference :**	2019.07.15 LRH Safglucan US ML / (EOL) 00
Sample reception date :	22/08/2019	**Analysis starting date :**	23/08/2019
Sampling/Transport :	DHL		
Analyses requested :	PJJ3V : Métaux lourds : Pb, Cd, Hg, As		

Best before	26/04/2020	**Batch Number**	(b) (4)
Brand name	Safglucan	**N° bon de commande**	-
Lettre	-		

Elementary analysis			Results (uncertainty)
J8306	**JC**	**Lead(ICP-MS, food) Method : DIN EN 15763:2010 (2010-04), mod.**	
(a)	Lead (Pb)		(b) (4)
J8308	**JC**	**Cadmium (ICP-MS, food) Method : DIN EN 15763:2010 (2010-04), mod.**	
(a)	Cadmium (Cd)		(b) (4)
J8312	**JC**	**Arsenic(Food, ICP-MS) Method : DIN EN 15763:2010 (2010-04), mod.**	
(a)	Arsenic (As)		(b) (4)
JCHG2	**JC**	**Mercury (ICP-MS, food) Method : DIN EN 15763:2010 (2010-04), mod.**	
(a)	Mercury [Hg]		(b) (4)

SIGNATURE (b)(6)

Report electronically validated by (b)(6)

EXPLANATORY NOTE

(b) (4)

(b) (4), (b)(6)

(b) (4)

Analytical report

Sample code Nr.	**370-2019-00240000**	**Report Date**	**28/08/2019**	**Page 1/1**
Analytical Report Nr.	**AR-19-AA-232720-01 / 370-2019-00240000**			

(b) (4)

Société Industrielle LESAFFRE

For the attention of (b)(6)

137, rue GABRIEL PERI
59700 Marcq en Baroeul
FRANCE

Email (b)(6)

Your contact for Customer Service (b)(6)

Our reference :	370-2019-00240000 / AR-19-AA-232720-01	**Type :**	EX
Client reference :	**Safglucan_batch** (b) (4)		
Sample described as :	levure		
Packaging :	NonCommercial : 566g+268g=834g		
Your purchase order date :	20/08/2019	**Your purchase order reference :**	2019.07.15 LRH Safglucan US ML / (EOL) 00
Sample reception date :	22/08/2019	**Analysis starting date :**	23/08/2019
Sampling/Transport :	DHL		
Analyses requested :	PJJ3V : Métaux lourds : Pb, Cd, Hg, As		

Best before	24/03/2021	**Batch Number**	(b) (4)
Brand name	Safglucan	**N° bon de commande**	-
Lettre	-		

Elementary analysis			Results (uncertainty)
J8306	**JC**	**Lead(ICP-MS, food) Method : DIN EN 15763:2010 (2010-04), mod.**	
(a)	Lead (Pb)		(b) (4)
J8308	**JC**	**Cadmium (ICP-MS, food) Method : DIN EN 15763:2010 (2010-04), mod.**	
(a)	Cadmium (Cd)		(b) (4)
J8312	**JC**	**Arsenic(Food, ICP-MS) Method : DIN EN 15763:2010 (2010-04), mod.**	
(a)	Arsenic (As)		(b) (4)
JCHG2	**JC**	**Mercury (ICP-MS, food) Method : DIN EN 15763:2010 (2010-04), mod.**	
(a)	Mercury [Hg]		(b) (4)

SIGNATURE (b)(6)

Report electronically validated by (b)(6)

EXPLANATORY NOTE

(b) (4)

(b) (4)

Analytical report

Sample code Nr.	**370-2019-00239999**	**Report Date**	**28/08/2019**	**Page 1/1**
Analytical Report Nr.	**AR-19-AA-232719-01 / 370-2019-00239999**			

(b) (4)

Société Industrielle LESAFFRE

For the attention of (b)(6)

137, rue GABRIEL PERI
59700 Marcq en Baroeul
FRANCE

Email (b)(6)

Your contact for Customer Service : (b)(6)

Our reference :	370-2019-00239999 / AR-19-AA-232719-01	**Type :**	EX
Client reference :	**Safglucan_batch** (b) (4)		
Sample described as :	levure		
Packaging :	NonCommercial : 628g+216g=844g		
Your purchase order date :	20/08/2019	**Your purchase order reference :**	2019.07.15 LRH Safglucan US ML / (EOL) 00
Sample reception date :	22/08/2019	**Analysis starting date :**	23/08/2019
Sampling/Transport :	DHL		
Analyses requested :	PJJ3V : Métaux lourds : Pb, Cd, Hg, As		

Best before	03/03/2021	**Batch Number**	(b) (4)
Brand name	Safglucan	**N° bon de commande**	-
Lettre	-		

Elementary analysis			Results (uncertainty)
J8306	**JC**	**Lead(ICP-MS, food) Method : DIN EN 15763:2010 (2010-04), mod.**	
(a)	Lead (Pb)		(b) (4)
J8308	**JC**	**Cadmium (ICP-MS, food) Method : DIN EN 15763:2010 (2010-04), mod.**	
(a)	Cadmium (Cd)		(b) (4)
J8312	**JC**	**Arsenic(Food, ICP-MS) Method : DIN EN 15763:2010 (2010-04), mod.**	
(a)	Arsenic (As)		(b) (4)
JCHG2	**JC**	**Mercury (ICP-MS, food) Method : DIN EN 15763:2010 (2010-04), mod.**	
(a)	Mercury [Hg]		(b) (4)

SIGNATURE (b)(6)

Report electronically validated by (b)(6)

EXPLANATORY NOTE

(b) (4), (b)(6)

(b) (4)

Rapport d'analyse

Echantillon n°	**370-2019-00240457**	**Date**	**26/08/2019**	**Page 1/2**
Rapport d'analyse n°	**AR-19-AA-229871-01 / 370-2019-00240457**			

(b) (4)

Société Industrielle LESAFFRE

A l'attention de (b)(6)

137, rue GABRIEL PERI
59700 Marcq en Baroeul
FRANCE

Email (b)(6)

Coordinateur technique de votre dossier : (b)(6)

Notre référence :	370-2019-00240457 / AR-19-AA-229871-01	**Type :**	EX
Référence client :	**Safglucan_batch** (b) (4)		
Description de l'échantillon :	levure		
Conditionnement :	NonCommercial : 600g+284g=884g		
Votre date de commande :	21/08/2019	**Votre référence commande :**	2019.07.15 LRH Safglucan US min prof / (EOI
Date de réception :	22/08/2019	**Date de mise en analyse :**	23/08/2019
Prélèvement/Transport :	DHL		

Analyses demandées :
AAMSK : Calcium
AAMSB : Chrome
AAMS9 : Fer
AAMSM : Magnésium
AAMSL : Potassium
AAMS7 : Sélénium
AAMSJ : Sodium
AAMS8 : Zinc
AAMS0 : Minéralisation des échantillons à l'acide

DLC/DLUO	29/03/2020	**N° de lot**	(b) (4)
Marque	Safglucan	**N° bon de commande**	2019.07.15 LRH Safglucan US min prof
Lettre	-		

Analyses élémentaires	Résultats (incertitude)	Etiquetage
AAMS9 AA Fer Méthode : Interne, ICP/MS	(b) (4)	
(a) Fer (Fe)		na mg/kg
AAMSB AA Chrome Méthode : Interne, ICP/MS		
(a) Chrome (Cr)		
AAMS7 AA Sélénium Méthode : Interne, ICP/MS		
(a) Sélénium (Se)		
AAMS8 AA Zinc Méthode : Interne, ICP/MS		
(a) Zinc (Zn)		na mg/kg
AAMSJ AA Sodium Méthode : Interne, ICP/MS		
(a) Sodium		
AAMSK AA Calcium Méthode : Interne, ICP/MS		
(a) Calcium (Ca)		
AAMSL AA Potassium Méthode : Interne, ICP/MS		
(a) Potassium (K)		
AAMSM AA Magnésium Méthode : Interne, ICP/MS		
(a) Magnésium (Mg)		

(b) (4), (b)(6)

(b) (4)

Rapport d'analyse

Echantillon n°	**370-2019-00240457**	**Date**	**26/08/2019**	**Page 2/2**
Rapport d'analyse n°	**AR-19-AA-229871-01 / 370-2019-00240457**			

(b)(6)

Rapport validé électroniquement par

NOTE EXPLICATIVE

(b) (4), (b)(6)

(b) (4), (b)(6)

SAMPLE NUMBER – see original
ANALYTICAL REPORT NUMBER – see original

Our reference:
Client reference:
Sample description:
Condition:
Date of request:
Date of receipt:
Method of transport:
Analysis requested:
Request reference:
Date of analysis:

Cadmium
Chromium
Iron
Magnesium
Potassium
Selenium
Sodium
Zinc
Mineralisation of samples with acid

EUROFINS – translated Certificate of Analysis

Elemental analysis	**Results**	**Label/units**
Iron – internal method ICP/MS	See original certificate	mg/kg
Chromium – internal method ICP/MS	See original certificate	
Selenium – internal method ICP/MS	See original certificate	
Zinc – internal method ICP/MS	See original certificate	
Sodium – internal method ICP/MS	See original certificate	mg/kg
Calcium – internal method ICP/MS	See original certificate	
Potassium – internal method ICP/MS	See original certificate	
Magnesium – internal method ICP/MS	See original certificate	

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(b) (4)

Rapport d'analyse

Echantillon n°	**370-2019-00240458**	**Date**	**26/08/2019**	**Page 1/2**
Rapport d'analyse n°	**AR-19-AA-229872-01 / 370-2019-00240458**			

(b) (4)

Société Industrielle LESAFFRE

A l'attention de (b)(6)

137, rue GABRIEL PERI
59700 Marcq en Baroeul
FRANCE

Email (b)(6)

Coordinateur technique de votre dossier : (b)(6)

Notre référence :	370-2019-00240458 / AR-19-AA-229872-01	**Type :**	EX
Référence client :	**Safglucan_batch** (b) (4)		
Description de l'échantillon :	levure		
Conditionnement :	NonCommercial : 412g+480g=892g		
Votre date de commande :	21/08/2019	**Votre référence commande :**	2019.07.15 LRH Safglucan US min prof / (EOI
Date de réception :	22/08/2019	**Date de mise en analyse :**	23/08/2019
Prélèvement/Transport :	DHL		
Analyses demandées :	AAMSK : Calcium AAMSB : Chrome AAMS9 : Fer AAMSM : Magnésium AAMSL : Potassium AAMS7 : Sélénium AAMSJ : Sodium AAMS8 : Zinc AAMS0 : Minéralisation des échantillons à l'acide		

DLC/DLUO	15/03/2021	**N° de lot**	(b) (4)
Marque	Safglucan	**N° bon de commande**	2019.07.15 LRH Safglucan US min prof
Lettre	-		

Analyses élémentaires	Résultats (incertitude)	Etiquetage
AAMS9 AA Fer Méthode : Interne, ICP/MS	(b) (4)	
(a) Fer (Fe)		na mg/kg
AAMSB AA Chrome Méthode : Interne, ICP/MS		
(a) Chrome (Cr)		
AAMS7 AA Sélénium Méthode : Interne, ICP/MS		
(a) Sélénium (Se)		
AAMS8 AA Zinc Méthode : Interne, ICP/MS		
(a) Zinc (Zn)		na mg/kg
AAMSJ AA Sodium Méthode : Interne, ICP/MS		
(a) Sodium		
AAMSK AA Calcium Méthode : Interne, ICP/MS		
(a) Calcium (Ca)		
AAMSL AA Potassium Méthode : Interne, ICP/MS		
(a) Potassium (K)		
AAMSM AA Magnésium Méthode : Interne, ICP/MS		
(a) Magnésium (Mg)		

SIGNATURE (b)(6)

(b) (4)

(b) (4)

Rapport d'analyse

Echantillon n°	**370-2019-00240458**	**Date**	**26/08/2019**	**Page 2/2**
Rapport d'analyse n°	**AR-19-AA-229872-01 / 370-2019-00240458**			

(b)(6)

Rapport validé électroniquement par

NOTE EXPLICATIVE

(b) (4), (b)(6)

(b) (4), (b)(6)

SAMPLE NUMBER – see original
ANALYTICAL REPORT NUMBER – see original

Our reference:
Client reference:
Sample description:
Condition:
Date of request:
Date of receipt:
Method of transport:
Analysis requested:
Request reference:
Date of analysis:

Cadmium
Chromium
Iron
Magnesium
Potassium
Selenium
Sodium
Zinc
Mineralisation of samples with acid

EUROFINS – translated Certificate of Analysis

Elemental analysis	**Results**	**Label/units**
Iron – internal method ICP/MS	See original certificate	mg/kg
Chromium – internal method ICP/MS	See original certificate	
Selenium – internal method ICP/MS	See original certificate	
Zinc – internal method ICP/MS	See original certificate	
Sodium – internal method ICP/MS	See original certificate	mg/kg
Calcium – internal method ICP/MS	See original certificate	
Potassium – internal method ICP/MS	See original certificate	
Magnesium – internal method ICP/MS	See original certificate	

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(b) (4)

Rapport d'analyse

Echantillon n°	**370-2019-00240456**	**Date**	**26/08/2019**	**Page 1/2**
Rapport d'analyse n°	**AR-19-AA-229870-01 / 370-2019-00240456**			

(b) (4)

Société Industrielle LESAFFRE

A l'attention de (b)(6)

137, rue GABRIEL PERI
59700 Marcq en Baroeul
FRANCE

Email (b)(6)

Coordinateur technique de votre dossier (b)(6)

Notre référence :	370-2019-00240456 / AR-19-AA-229870-01	**Type :**	EX
Référence client :	**Safglucan_batch** (b) (4)		
Description de l'échantillon :	levure		
Conditionnement :	NonCommercial : 582g+322g=904g		
Votre date de commande :	21/08/2019	**Votre référence commande :**	2019.07.15 LRH Safglucan US min prof / (EOI
Date de réception :	22/08/2019	**Date de mise en analyse :**	23/08/2019
Prélèvement/Transport :	DHL		
Analyses demandées :	AAMSK : Calcium AAMSB : Chrome AAMS9 : Fer AAMSM : Magnésium AAMSL : Potassium AAMS7 : Sélénium AAMSJ : Sodium AAMS8 : Zinc AAMS0 : Minéralisation des échantillons à l'acide		

DLC/DLUO	26/04/2020	**N° de lot**	(b) (4)
Marque	Safglucan	**N° bon de commande**	2019.07.15 LRH Safglucan US min prof
Lettre	-		

Analyses élémentaires	Résultats (incertitude)	Etiquetage
AAMS9 AA Fer Méthode : Interne, ICP/MS	(b) (4)	
(a) Fer (Fe)		na mg/kg
AAMSB AA Chrome Méthode : Interne, ICP/MS		
(a) Chrome (Cr)		
AAMS7 AA Sélénium Méthode : Interne, ICP/MS		
(a) Sélénium (Se)		
AAMS8 AA Zinc Méthode : Interne, ICP/MS		
(a) Zinc (Zn)		na mg/kg
AAMSJ AA Sodium Méthode : Interne, ICP/MS		
(a) Sodium		
AAMSK AA Calcium Méthode : Interne, ICP/MS		
(a) Calcium (Ca)		
AAMSL AA Potassium Méthode : Interne, ICP/MS		
(a) Potassium (K)		
AAMSM AA Magnésium Méthode : Interne, ICP/MS		
(a) Magnésium (Mg)		

SIGNATURE (b)(6)

(b) (4), (b)(6)

(b) (4)

Rapport d'analyse

Echantillon n°	**370-2019-00240456**	**Date**	**26/08/2019**	**Page 2/2**
Rapport d'analyse n°	**AR-19-AA-229870-01 / 370-2019-00240456**			

(b)(6)

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NOTE EXPLICATIVE

(b) (4)

(b) (4)

SAMPLE NUMBER – see original
ANALYTICAL REPORT NUMBER – see original

Our reference:
Client reference:
Sample description:
Condition:
Date of request:
Date of receipt:
Method of transport:
Analysis requested:
Request reference:
Date of analysis:

Cadmium
Chromium
Iron
Magnesium
Potassium
Selenium
Sodium
Zinc
Mineralisation of samples with acid

EUROFINS – translated Certificate of Analysis

Elemental analysis	**Results**	**Label/units**
Iron – internal method ICP/MS	See original certificate	mg/kg
Chromium – internal method ICP/MS	See original certificate	
Selenium – internal method ICP/MS	See original certificate	
Zinc – internal method ICP/MS	See original certificate	
Sodium – internal method ICP/MS	See original certificate	mg/kg
Calcium – internal method ICP/MS	See original certificate	
Potassium – internal method ICP/MS	See original certificate	
Magnesium – internal method ICP/MS	See original certificate	

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(b) (4)

Rapport d'analyse

Echantillon n°	**370-2019-00240460**	**Date**	**26/08/2019**	**Page 1/2**
Rapport d'analyse n°	**AR-19-AA-229873-01 / 370-2019-00240460**			

(b) (4)

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A l'attention de (b)(6)

137, rue GABRIEL PERI
59700 Marcq en Baroeul
FRANCE

Email (b)(6)

Coordinateur technique de votre dossier (b)(6)

Notre référence :	370-2019-00240460 / AR-19-AA-229873-01	**Type :**	EX
Référence client :	**Safglucan_batch** (b) (4)		
Description de l'échantillon :	levure		
Conditionnement :	NonCommercial : 244g+582g=826g		
Votre date de commande :	21/08/2019	**Votre référence commande :**	2019.07.15 LRH Safglucan US min prof / (EOI
Date de réception :	22/08/2019	**Date de mise en analyse :**	23/08/2019
Prélèvement/Transport :	DHL		
Analyses demandées :	AAMSK : Calcium AAMSB : Chrome AAMS9 : Fer AAMSM : Magnésium AAMSL : Potassium AAMS7 : Sélénium AAMSJ : Sodium AAMS8 : Zinc AAMS0 : Minéralisation des échantillons à l'acide		

DLC/DLUO	24/03/2021	**N° de lot**	(b) (4)
Marque	Safglucan	**N° bon de commande**	2019.07.15 LRH Safglucan US min prof
Lettre	-		

Analyses élémentaires	Résultats (incertitude)	Etiquetage
AAMS9 AA Fer Méthode : Interne, ICP/MS	(b) (4)	
(a) Fer (Fe)		na mg/kg
AAMSB AA Chrome Méthode : Interne, ICP/MS		
(a) Chrome (Cr)		
AAMS7 AA Sélénium Méthode : Interne, ICP/MS		
(a) Sélénium (Se)		
AAMS8 AA Zinc Méthode : Interne, ICP/MS		
(a) Zinc (Zn)		na mg/kg
AAMSJ AA Sodium Méthode : Interne, ICP/MS		
(a) Sodium		
AAMSK AA Calcium Méthode : Interne, ICP/MS		
(a) Calcium (Ca)		
AAMSL AA Potassium Méthode : Interne, ICP/MS		
(a) Potassium (K)		
AAMSM AA Magnésium Méthode : Interne, ICP/MS		
(a) Magnésium (Mg)		

SIGNATURE (b)(6)

(b) (4), (b)(6)

(b) (4)

Rapport d'analyse

Echantillon n°	**370-2019-00240460**	**Date**	**26/08/2019**	**Page 2/2**
Rapport d'analyse n°	**AR-19-AA-229873-01 / 370-2019-00240460**			

(b)(6)

Rapport validé électroniquement par

NOTE EXPLICATIVE

(b) (4), (b)(6)

(b)(6)

SAMPLE NUMBER – see original
ANALYTICAL REPORT NUMBER – see original

Our reference:
Client reference:
Sample description:
Condition:
Date of request:
Date of receipt:
Method of transport:
Analysis requested:
Request reference:
Date of analysis:

Cadmium
Chromium
Iron
Magnesium
Potassium
Selenium
Sodium
Zinc
Mineralisation of samples with acid

EUROFINS – translated Certificate of Analysis

Elemental analysis	**Results**	**Label/units**
Iron – internal method ICP/MS	See original certificate	mg/kg
Chromium – internal method ICP/MS	See original certificate	
Selenium – internal method ICP/MS	See original certificate	
Zinc – internal method ICP/MS	See original certificate	
Sodium – internal method ICP/MS	See original certificate	mg/kg
Calcium – internal method ICP/MS	See original certificate	
Potassium – internal method ICP/MS	See original certificate	
Magnesium – internal method ICP/MS	See original certificate	

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(b) (4)

Rapport d'analyse

Echantillon n°	**370-2019-00240459**	**Date**	**24/08/2019**	**Page 1/2**
Rapport d'analyse n°	**AR-19-AA-229737-01 / 370-2019-00240459**			

(b) (4)

Société Industrielle LESAFFRE

A l'attention de (b)(6)

137, rue GABRIEL PERI
59700 Marcq en Baroeul
FRANCE

Email (b)(6)

Coordinateur technique de votre dossier : (b)(6)

Notre référence :	370-2019-00240459 / AR-19-AA-229737-01	**Type :**	EX
Référence client :	**Safglucan_batch** (b) (4)		
Description de l'échantillon :	levure		
Conditionnement :	NonCommercial : 612g+236g=848g		
Votre date de commande :	21/08/2019	**Votre référence commande :**	2019.07.15 LRH Safglucan US min prof / (EOI
Date de réception :	22/08/2019	**Date de mise en analyse :**	23/08/2019
Prélèvement/Transport :	DHL		
Analyses demandées :	AAMSK : Calcium AAMSB : Chrome AAMS9 : Fer AAMSM : Magnésium AAMSL : Potassium AAMS7 : Sélénium AAMSJ : Sodium AAMS8 : Zinc AAMS0 : Minéralisation des échantillons à l'acide		

DLC/DLUO	03/03/2021	**N° de lot**	(b) (4)
Marque	Safglucan	**N° bon de commande**	2019.07.15 LRH Safglucan US min prof
Lettre	-		

Analyses élémentaires	Résultats (incertitude)	Etiquetage
AAMS9 AA Fer Méthode : Interne, ICP/MS	(b) (4)	
(a) Fer (Fe)		na mg/kg
AAMSB AA Chrome Méthode : Interne, ICP/MS		
(a) Chrome (Cr)		
AAMS7 AA Sélénium Méthode : Interne, ICP/MS		
(a) Sélénium (Se)		
AAMS8 AA Zinc Méthode : Interne, ICP/MS		
(a) Zinc (Zn)		na mg/kg
AAMSJ AA Sodium Méthode : Interne, ICP/MS		
(a) Sodium		
AAMSK AA Calcium Méthode : Interne, ICP/MS		
(a) Calcium (Ca)		
AAMSL AA Potassium Méthode : Interne, ICP/MS		
(a) Potassium (K)		
AAMSM AA Magnésium Méthode : Interne, ICP/MS		
(a) Magnésium (Mg)		

SIGNATURE (b)(6)

(b) (4)

(b) (4)

Rapport d'analyse

Echantillon n°	**370-2019-00240459**	**Date**	**24/08/2019**	**Page 2/2**
Rapport d'analyse n°	**AR-19-AA-229737-01 / 370-2019-00240459**			

(b)(6)

Rapport validé électroniquement par

NOTE EXPLICATIVE

(b) (4)

(b) (4), (b)(6)

SAMPLE NUMBER – see original
ANALYTICAL REPORT NUMBER – see original

Our reference:
Client reference:
Sample description:
Condition:
Date of request:
Date of receipt:
Method of transport:
Analysis requested:
Request reference:
Date of analysis:

Cadmium
Chromium
Iron
Magnesium
Potassium
Selenium
Sodium
Zinc
Mineralisation of samples with acid

EUROFINS – translated Certificate of Analysis

Elemental analysis	**Results**	**Label/units**
Iron – internal method ICP/MS	See original certificate	mg/kg
Chromium – internal method ICP/MS	See original certificate	
Selenium – internal method ICP/MS	See original certificate	
Zinc – internal method ICP/MS	See original certificate	
Sodium – internal method ICP/MS	See original certificate	mg/kg
Calcium – internal method ICP/MS	See original certificate	
Potassium – internal method ICP/MS	See original certificate	
Magnesium – internal method ICP/MS	See original certificate	

Explanatory note
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STABILITY STUDY

A study was designed to test the stability of 4 batches of Safglucan stored at room temperature. The characteristics of the product are regularly analyzed (at production date, after 3 months, after 6 months after 12 months, after 18 months and after 24 months).

Description of the batches

	Batches			
				(b) (4)
Production date	03/25/2019	03/04/2019	03/15/2018	03/30/2018
Expiration date	03/24/2021	03/03/2021	03/15/2020	03/29/2020

Description of batch preparation

100 g of each batch is put in a PE bag (100*250 mm). The bag is then sealed under air. The samples are put in a sample bank with monitored temperature and relative humidity.

The samples are not modified for the dry matter, B-glucans and crude protein analysis. For the microbiology, the samples are prepared according to the methods mentioned thereafter.

Description of analysis

Dry matter

The dry matter is measured with the following protocol:

- An empty aluminum cup is weighed (m0),
- 2 g of the products are put in the cup and weighed again (m1),
- The product is put at 103°C for 16 h,
- After cool-down in a desiccator, the cup with the product is weighed again (m2),
- The dry matter is then determined with the following formula:

$$\frac{(m_2 - m_0)}{(m_1 - m_0)} * 100 = \% MS$$

Crude protein

The crude protein content is measured using the Dumas total nitrogen method, on the Rapid Max N Exceed ELEMENTAR (N/protein analyzer).

The Dumas principle relies on quantitative conversion of the sample into well-defined gaseous species at 950°C in an oxygen enriched environment. During the combustion phase, all nitrogen in the sample is converted to nitrogen oxides, which are reduced to nitrogen gas and quantified with a thermal conductivity detector. All other combustion gases, including excess oxygen, are trapped or absorbed prior to the quantification.

Glucans

Whole glucans: Hydrolysis with trifluoracetic acid and enzymatic measurement of glucose and mannose. Home method (Megazyme)

β – glucan

(b) (4)

Microbiology

Parameters	**Methods**
Total viable count /g	NF EN ISO 4833-1
Molds /g	ISO 6611
Yeasts /g	ISO 6611
β-glucuronidase positive *Escherichia coli* /g	NF ISO 16649-2
Total coliforms (30°C) /g	NF V08-050
Listeria monocytogenes /25 g	NF EN ISO 11290-1
Salmonella /25 g	NF EN ISO 6579-1
Coagulase positive *Staphilococcus*	NF EN ISO 6888-3

Results

The results are provided in the following attached Certificate of Analysis of 4 significant batches.

Phileo by Lesaffre

Certificate of Analysis

Product	Safglucan®
Timepoint	T0 (08/2019)

	Batches			
	(b) (4)			
Production date	30/03/2018	15/03/2018	04/03/2019	25/03/2019
Expiration date	29/03/2020	14/03/2020	03/03/2021	24/03/2021

Parameters	Method	Specification
Dry matter	2g of product 103°C during 16h MEMMERT UF30 Oven	> 94%
Crude protein	Dumas total nitrogen method Rapid Max N Exceed ELEMENTAR	≤ 10 %
β-glucan	(b) (4)	≥ 50 %
Microbiology	NF EN ISO 4833-1 ISO 6611 NF ISO 16649-2 NF V08-050 NF EN ISO 11290-1 NF EN ISO 6579-1 NF EN ISO 6888-3	

Results

- **Dry matter**

Batch	Date of analysis	Result (%)
BATCH (b) (4)	21/08/2019	(b) (4)
BATCH	21/08/2019	
BATCH	21/08/2019	
BATCH	21/08/2019	

- **Crude protein**

Batch	Date of analysis
BATCH (b) (4)	22/08/2019
BATCH	22/08/2019
BATCH	22/08/2019
BATCH	22/08/2019

- **β-glucan**

Batch	Date of analysis
BATCH (b) (4)	19/08/2019
BATCH	19/08/2019
BATCH	19/08/2019
BATCH	19/08/2019

- **Microbiology**

Batch	Date of analysis
BATCH (b) (4)	03/09/2019
BATCH	03/09/2019
BATCH	03/09/2019
BATCH	03/09/2019

(b)(6)

Certificate of Analysis

Product	Safglucan®
Timepoint	T3 (11/2019)

	Batches			
	(b) (4)			
Production date	30/03/2018	15/03/2018	04/03/2019	25/03/2019
Expiration date	29/03/2020	14/03/2020	03/03/2021	24/03/2021

Parameters	Method	Specification
Dry matter	2g of product 103°C during 16h MEMMERT UF30 Oven	> 94%
Crude protein	Dumas total nitrogen method Rapid Max N Exceed ELEMENTAR	≤ 10 %
β-glucan	(b) (4)	≥ 50 %

Results

- **Dry matter**

Batch		Date of analysis	Result (%)
BATCH	(b) (4)	28/11/2019	(b) (4)
BATCH		28/11/2019	
BATCH		28/11/2019	
BATCH		28/11/2019	

- **Crude protein**

Batch		Date of analysis
BATCH	(b) (4)	29/11/2019
BATCH		29/11/2019
BATCH		29/11/2019
BATCH		29/11/2019

- **β-glucan**

Batch		Date of analysis
BATCH	(b) (4)	02/12/2019
BATCH		02/12/2019
BATCH		02/12/2019
BATCH		02/12/2019

(b)(6)

Phileo by Lesaffre

Certificate of Analysis

Product	Safglucan®
Timepoint	T6 (02/2020)

	Batches			
	(b) (4)			
Production date	30/03/2018	15/03/2018	04/03/2019	25/03/2019
Expiration date	29/03/2020	14/03/2020	03/03/2021	24/03/2021

Parameters	Method	Specification
Dry matter	2g of product 103°C during 16h MEMMERT UF30 Oven	> 94%
Crude protein	Dumas total nitrogen method Rapid Max N Exceed ELEMENTAR	≤ 10 %
β-glucan	(b) (4)	≥ 50 %

Results

- **Dry matter**

Batch		Date of analysis	Result (%)
BATCH	(b) (4)	17/02/2020	(b) (4)
BATCH		17/02/2020	
BATCH		17/02/2020	
BATCH		17/02/2020	

- **Crude protein**

Batch		Date of analysis
BATCH	(b) (4)	20/02/2020
BATCH		20/02/2020
BATCH		20/02/2020
BATCH		20/02/2020

- **β-glucan**

Batch		Date of analysis
BATCH	(b) (4)	28/02/2020
BATCH		28/02/2020
BATCH		28/02/2020
BATCH		28/02/2020

(b)(6)

Phileo by Lesaffre

Certificate of Analysis

Product	Safglucan®
Timepoint	T12 (08/2020)

	Batches			
	(b) (4)			
Production date	30/03/2018	15/03/2018	04/03/2019	25/03/2019
Expiration date	29/03/2020	14/03/2020	03/03/2021	24/03/2021

Parameters	Method	Specification
Dry matter	2g of product 103°C during 16h MEMMERT UF30 Oven	> 94%
Crude protein	Dumas total nitrogen method Rapid Max N Exceed ELEMENTAR	≤ 10 %
β-glucan	(b) (4)	≥ 50 %

Results

- **Dry matter**

Batch	Date of analysis	Result (%)
BATCH (b) (4)	24/08/2020	(b) (4)
BATCH	24/08/2020	
BATCH	24/08/2020	
BATCH	24/08/2020	

- **Crude protein**

Batch	Date of analysis
BATCH (b) (4)	26/08/2020
BATCH	26/08/2020
BATCH	26/08/2020
BATCH	26/08/2020

- **β-glucan**

Batch	Date of analysis
BATCH (b) (4)	10/09/2020
BATCH	10/09/2020
BATCH	10/09/2020
BATCH	10/09/2020

(b)(6)

Phileo by Lesaffre

Certificate of Analysis

Product	Safglucan®
Timepoint	T18 (03/2021)

	Batches			
	(b) (4)			
Production date	30/03/2018	15/03/2018	04/03/2019	25/03/2019
Expiration date	29/03/2020	14/03/2020	03/03/2021	24/03/2021

Parameters	Method	Specification
Dry matter	2g of product 103°C during 16h MEMMERT UF30 Oven	> 94%
Crude protein	Dumas total nitrogen method Rapid Max N Exceed ELEMENTAR	≤ 10 %
β-glucan	(b) (4)	≥ 50 %

Results

- **Dry matter**

Batch		Date of analysis	Result (%)
BATCH	(b) (4)	03/03/2021	(b) (4)
BATCH		03/03/2021	
BATCH		03/03/2021	
BATCH		03/03/2021	

- **Crude protein**

Batch		Date of analysis
BATCH	(b) (4)	03/03/2021
BATCH		03/03/2021
BATCH		03/03/2021
BATCH		03/03/2021

- **β-glucan**

Batch		Date of analysis
BATCH	(b) (4)	29/03/2021
BATCH		29/03/2021
BATCH		29/03/2021
BATCH		29/03/2021

Division of S.I.L.

(b)(6)

Phileo by Lesaffre

Certificate of Analysis

Product	Safglucan®
Timepoint	T24 (08/2021)

	Batches			
	[redacted] (b) (4)			
Production date	30/03/2018	15/03/2018	04/03/2019	25/03/2019
Expiration date	29/03/2020	14/03/2020	03/03/2021	24/03/2021

Parameters	Method	Specification
Dry matter	2g of product 103°C during 16h MEMMERT UF30 Oven	> 94%
Crude protein	Dumas total nitrogen method Rapid Max N Exceed ELEMENTAR	≤ 10 %
β-glucan	[redacted] (b) (4)	≥ 50 %
Microbiology	NF EN ISO 4833-1 ISO 6611 NF ISO 16649-2 NF V08-050 NF EN ISO 11290-1 NF EN ISO 6579-1 NF EN ISO 6888-3	

Results

- **Dry matter**

Batch		Date of analysis	Result (%)
BATCH	(b) (4)	18/08/2021	(b) (4)
BATCH		18/08/2021	
BATCH		18/08/2021	
BATCH		18/08/2021	

- **Crude protein**

Batch		Date of analysis
BATCH	(b) (4)	18/08/2021
BATCH		18/08/2021
BATCH		18/08/2021
BATCH		18/08/2021

- **β-glucan**

Batch		Date of analysis
BATCH	(b) (4)	27/10/2021
BATCH		27/10/2021
BATCH		27/10/2021
BATCH		27/10/2021

- **Microbiology**

Batch		Date of analysis
BATCH	(b) (4)	21/10/2021
BATCH		21/10/2021
BATCH		21/10/2021
BATCH		21/10/2021

(b)(6)

Time = 0 months

(b) (4)

Rapport d'analyse

(b) (4)

Société Industrielle LESAFFRE
137, rue GABRIEL PERI
59700 Marcq en Baroeul

Reçu au laboratoire le *03/09/2019 11:55 à 18°C*
Analysé le *03/09/2019*

Echantillon N° 854-2019-07420132 : Safglucan_ (b) (4) - PAROIS DE LEVURE

Commande : 2019-08-23_CGH8stab - EOL 10518-895753

Paramètres	Méthode	Résultats	Critères Européens	Critères spécifiques
		(b) (4), (b)(6)		
(a) Flore aérobie mésophile (30°C) /g	NF EN ISO 4833-1			
(a) Moisissures /g	ISO 6611			
(a) Levures /g	ISO 6611			
(a) Escherichia coli β-glucuronidase positive /g	NF ISO 16649-2			
(a) Coliformes totaux présumés (30°C) /g	NF V08-050			
(a) Listeria monocytogenes /25 g	NF EN ISO 11290-1			
(a) Salmonella (hors sérovars Typhi et Paratyphi) /25 g	NF EN ISO 6579-1			
(a) Staphylocoques à coagulase positive - Recherche /g	NF EN ISO 6888-3			

NOTE EXPLICATIVE
Seules les prestations repérées par le symbole (a) sont effectuées sous le couvert de l'accréditation.

(b) (4), (b)(6)

Translation of the results table into English:

Parameters	**Methods**	**Results**	**European criteria**	**Specific criteria**
			Regulation 2073/2005 modified	
Total viable count (30°C) /g	NF EN ISO 4833-1	(b) (4)		
Molds /g	ISO 6611			
Yeasts /g	ISO 6611			
β-glucuronidase positive Escherichia coli /g	NF ISO 16649-2			
Total coliforms (30°C) /g	NF V08-050			
Listeria monocytogenes /25 g	NF EN ISO 11290-1			
Salmonella (excluding tiphy & paratiphy serovars) / 25 g	NF EN ISO 6579-1			
Coagulase positive Staphilococcus (searching/g)	NF EN ISO 6888-3			

(b) (4)

Rapport d'analyse

(b) (4)

Société Industrielle LESAFFRE
137, rue GABRIEL PERI
59700 Marcq en Baroeul

Reçu au laboratoire le *03/09/2019 11:55 à 18°C*
Analysé le *03/09/2019*

Echantillon N° 854-2019-07420133 : Safglucan_(b) (4)_PAROIS DE LEVURE

Commande : 2019-08-23_CGH8stab - EOL 10518-895753

Paramètres	Méthode	Résultats	Critères Européens	Critères spécifiques
		(b) (4), (b)(6)		
(a) Flore aérobie mésophile (30°C) /g	NF EN ISO 4833-1			
(a) Moisissures /g	ISO 6611			
(a) Levures /g	ISO 6611			
(a) Escherichia coli β-glucuronidase positive /g	NF ISO 16649-2			
(a) Coliformes totaux présumés (30°C) /g	NF V08-050			
(a) Listeria monocytogenes /25 g	NF EN ISO 11290-1			
(a) Salmonella (hors sérovars Typhi et Paratyphi) /25 g	NF EN ISO 6579-1			
(a) Staphylocoques à coagulase positive - Recherche /g	NF EN ISO 6888-3			

NOTE EXPLICATIVE
Seules les prestations repérées par le symbole (a) sont effectuées sous le couvert de l'accréditation.

(b) (4), (b)(6)

Translation of the results table into English:

Parameters	Methods	Results	European criteria	Specific criteria
			Regulation 2073/2005 modified	
Total viable count (30°C) /g	NF EN ISO 4833-1	(b) (4)		
Molds /g	ISO 6611			
Yeasts /g	ISO 6611			
β-glucuronidase positive Escherichia coli /g	NF ISO 16649-2			
Total coliforms (30°C) /g	NF V08-050			
Listeria monocytogenes /25 g	NF EN ISO 11290-1			
Salmonella (excluding tiphy & paratiphy serovars) / 25 g	NF EN ISO 6579-1			
Coagulase positive Staphilococcus (searching/g)	NF EN ISO 6888-3			

(b) (4)

Rapport d'analyse

(b) (4)

Société Industrielle LESAFFRE
137, rue GABRIEL PERI
59700 Marcq en Baroeul

Reçu au laboratoire le *03/09/2019 11:55 à 18°C*
Analysé le *03/09/2019*

Echantillon N° 854-2019-07420130 : Safglucan_ (b) (4) - PAROIS DE LEVURE

Commande : 2019-08-23_CGH8stab - EOL
10518-895753

Paramètres	Méthode	Résultats	Critères Européens	Critères spécifiques
		(b) (4), (b)(6)		
(a) Flore aérobie mésophile (30°C) /g	NF EN ISO 4833-1			
(a) Moisissures /g	ISO 6611			
(a) Levures /g	ISO 6611			
(a) Escherichia coli β-glucuronidase positive /g	NF ISO 16649-2			
(a) Coliformes totaux présumés (30°C) /g	NF V08-050			
(a) Listeria monocytogenes /25 g	NF EN ISO 11290-1			
(a) Salmonella (hors sérovars Typhi et Paratyphi) /25 g	NF EN ISO 6579-1			
(a) Staphylocoques à coagulase positive - Recherche /g	NF EN ISO 6888-3			

NOTE EXPLICATIVE
Seules les prestations repérées par le symbole (a) sont effectuées sous le couvert de l'accréditation.

(b) (4), (b)(6)

Translation of the results table into English:

Parameters	Methods	Results	European criteria	Specific criteria
			Regulation 2073/2005 modified	
Total viable count (30°C) /g	NF EN ISO 4833-1	(b) (4)		
Molds /g	ISO 6611			
Yeasts /g	ISO 6611			
β-glucuronidase positive Escherichia coli /g	NF ISO 16649-2			
Total coliforms (30°C) /g	NF V08-050			
Listeria monocytogenes /25 g	NF EN ISO 11290-1			
Salmonella (excluding tiphy & paratiphy serovars) / 25 g	NF EN ISO 6579-1			
Coagulase positive Staphilococcus (searching/g)	NF EN ISO 6888-3			

(b) (4)

Rapport d'analyse

(b) (4)

Société Industrielle LESAFFRE
137, rue GABRIEL PERI
59700 Marcq en Baroeul

Code client : LN04225
Analyse réalisée sur le site de Nantes

Reçu au laboratoire le *03/09/2019 11:55 à 18°C*
Analysé le *03/09/2019*

Echantillon N° 854-2019-07420131 : Safglucan_(b) (4)_PAROIS DE LEVURE

Commande : 2019-08-23_CGH8stab - EOL
10518-895753

Paramètres	Méthode	Résultats	Critères Européens	Critères spécifiques
		(b) (4), (b)(6)		
(a) Flore aérobie mésophile (30°C) /g	NF EN ISO 4833-1			
(a) Moisissures /g	ISO 6611			
(a) Levures /g	ISO 6611			
(a) Escherichia coli β-glucuronidase positive /g	NF ISO 16649-2			
(a) Coliformes totaux présumés (30°C) /g	NF V08-050			
(a) Listeria monocytogenes /25 g	NF EN ISO 11290-1			
(a) Salmonella (hors sérovars Typhi et Paratyphi) /25 g	NF EN ISO 6579-1			
(a) Staphylocoques à coagulase positive - Recherche /g	NF EN ISO 6888-3			

NOTE EXPLICATIVE
Seules les prestations repérées par le symbole (a) sont effectuées sous le couvert de l'accréditation.

(b) (4), (b)(6)

Translation of the results table into English:

Parameters	Methods	Results	European criteria	Specific criteria
			Regulation 2073/2005 modified	
Total viable count (30°C) /g	NF EN ISO 4833-1	(b) (4)		
Molds /g	ISO 6611			
Yeasts /g	ISO 6611			
β-glucuronidase positive Escherichia coli /g	NF ISO 16649-2			
Total coliforms (30°C) /g	NF V08-050			
Listeria monocytogenes /25 g	NF EN ISO 11290-1			
Salmonella (excluding tiphy & paratiphy serovars) / 25 g	NF EN ISO 6579-1			
Coagulase positive Staphilococcus (searching/g)	NF EN ISO 6888-3			

(b) (4)

Rapport d'analyse

(b) (4)

Société Industrielle LESAFFRE
137, rue GABRIEL PERI
59700 Marcq en Baroeul

Echantillon N° 854-2021-07519013 : PHILEO (b) (4) - PAROIS DE LEVURES

Données fournies par le client

Commande : EOL 10518-1342453 - 4500654283

Paramètres	Méthode	Résultats	Critères Européens	Critères spécifiques client
(a) Salmonella (hors sérovars Typhi et Paratyphi) /25 g	*NF EN ISO 6579-1*	(b) (4), (b)(6)		

NOTE EXPLICATIVE

Seules les prestations repérées par le symbole (a) sont effectuées sous le couvert de l'accréditation.
Le laboratoire est exonéré de responsabilité dans le cas d'informations fournies par le client et pouvant affecter la validité des résultats.
Dans le cas où le laboratoire n'est pas en charge de l'étape d'échantillonnage, les résultats s'appliquent à l'échantillon tel qu'il a été reçu ou pris en charge.

(b) (4), (b)(6)

(b) (4)

Rapport d'analyse

(b) (4)

Société Industrielle LESAFFRE
137, rue GABRIEL PERI
59700 Marcq en Baroeul

Echantillon N° 854-2021-07519014 : PHILEO (b) (4) - PAROIS DE LEVURES

Données fournies par le client

Commande : EOL 10518-1342453 - 4500654283

Paramètres	Méthode	Résultats	Critères Européens	Critères spécifiques client
(a) Salmonella (hors sérovars Typhi et Paratyphi) /25 g	*NF EN ISO 6579-1*	(b) (4), (b)(6)		

NOTE EXPLICATIVE

Seules les prestations repérées par le symbole (a) sont effectuées sous le couvert de l'accréditation.
Le laboratoire est exonéré de responsabilité dans le cas d'informations fournies par le client et pouvant affecter la validité des résultats.
Dans le cas où le laboratoire n'est pas en charge de l'étape d'échantillonnage, les résultats s'appliquent à l'échantillon tel qu'il a été reçu ou pris en charge.

(b) (4), (b)(6)

(b) (4)

Rapport d'analyse

(b) (4)

Société Industrielle LESAFFRE
137, rue GABRIEL PERI
59700 Marcq en Baroeul

Echantillon N° 854-2021-07519015 : PHILEO_058CRTT243 - PAROIS DE LEVURES

Données fournies par le client

Commande : EOL 10518-1342453 - 4500654283

Paramètres	Méthode	Résultats	Critères Européens	Critères spécifiques client
(a) Salmonella (hors sérovars Typhi et Paratyphi) /25 g	*NF EN ISO 6579-1*	(b) (4), (b)(6)		

NOTE EXPLICATIVE

Seules les prestations repérées par le symbole (a) sont effectuées sous le couvert de l'accréditation.
Le laboratoire est exonéré de responsabilité dans le cas d'informations fournies par le client et pouvant affecter la validité des résultats.
Dans le cas où le laboratoire n'est pas en charge de l'étape d'échantillonnage, les résultats s'appliquent à l'échantillon tel qu'il a été reçu ou pris en charge.

(b) (4), (b)(6)

(b) (4)

Rapport d'analyse

(b) (4)

Société Industrielle LESAFFRE
137, rue GABRIEL PERI
59700 Marcq en Baroeul

Echantillon N° 854-2021-07519016 : PHILEO (b) (4) - PAROIS DE LEVURES

Données fournies par le client

Commande : EOL 10518-1342453 - 4500654283

Paramètres	Méthode	Résultats	Critères Européens	Critères spécifiques client
(a) Salmonella (hors sérovars Typhi et Paratyphi) /25 g	*NF EN ISO 6579-1*	(b) (4), (b)(6)		

NOTE EXPLICATIVE

Seules les prestations repérées par le symbole (a) sont effectuées sous le couvert de l'accréditation.
Le laboratoire est exonéré de responsabilité dans le cas d'informations fournies par le client et pouvant affecter la validité des résultats.
Dans le cas où le laboratoire n'est pas en charge de l'étape d'échantillonnage, les résultats s'appliquent à l'échantillon tel qu'il a été reçu ou pris en charge.

(b) (4), (b)(6)

SafGlucan®

CERTIFICATE OF ANALYSIS

General information

Product name	SafGlucan®
Batch number	(b) (4)
Manufacturing date	25/03/2019
Best Before Date	24/03/2021
Date of analysis	Timepoint 24 months

Product characteristics

	Values	Specifications	Method
Total viable count (CFU /g)	(b) (4)	<5000	NF EN ISO 4833-1
Molds (CFU /g)		<100	ISO 6611
Yeasts (CFU /g)		<100	ISO 6611
β-glucuronidase positive Escherichia coli (CFU /g)		<10	NF ISO 16649-2
Total coliforms (CFU /g)		<100	NF V08-050
Listeria monocytogenes (/25 g)		Absence	NF EN ISO 11290-1
Coagulase positive Staphilococcus (/g)		Absence	NF EN ISO 6888-3

Remarks

Certified conform,
(b)(6)

Division of S.I.LESAFFRE
Phileo by Lesaffre
A LESAFFRE BUSINESS UNIT

SafGlucan®

CERTIFICATE OF ANALYSIS

General information	
Product name	SafGlucan®
Batch number	(b) (4)
Manufacturing date	04/03/2019
Best Before Date	03/03/2021
Date of analysis	Timepoint 24 months

Product characteristics

	Values	Specifications	Method
Total viable count (CFU /g)	(b) (4)	<5000	NF EN ISO 4833-1
Molds (CFU /g)		<100	ISO 6611
Yeasts (CFU /g)		<100	ISO 6611
β-glucuronidase positive Escherichia coli (CFU /g)		<10	NF ISO 16649-2
Total coliforms (CFU /g)		<100	NF V08-050
Listeria monocytogenes (/25 g)		Absence	NF EN ISO 11290-1
Coagulase positive Staphilococcus (/g)		Absence	NF EN ISO 6888-3

Remarks

(b)(6)

Certified conform,

Division of S.I.LESAFFRE
Phileo by Lesaffre
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SafGlucan®

CERTIFICATE OF ANALYSIS

General information	
Product name	SafGlucan®
Batch number	(b) (4)
Manufacturing date	15/03/2018
Best Before Date	14/03/2020
Date of analysis	Timepoint 24 months

Product characteristics			
	Values	**Specifications**	**Method**
Total viable count (CFU /g)	(b) (4)	<5000	NF EN ISO 4833-1
Molds (CFU /g)		<100	ISO 6611
Yeasts (CFU /g)		<100	ISO 6611
β-glucuronidase positive Escherichia coli (CFU /g)		<10	NF ISO 16649-2
Total coliforms (CFU /g)		<100	NF V08-050
Listeria monocytogenes (/25 g)		Absence	NF EN ISO 11290-1
Coagulase positive Staphilococcus (/g)		Absence	NF EN ISO 6888-3

Remarks

(b)(6)

Certified conform,

Division of S.I.LESAFFRE
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SafGlucan®

CERTIFICATE OF ANALYSIS

General information	
Product name	SafGlucan®
Batch number	(b) (4)
Manufacturing date	30/03/2018
Best Before Date	29/03/2020
Date of analysis	Timepoint 24 months

Product characteristics			
	Values	**Specifications**	**Method**
Total viable count (CFU /g)	(b) (4)	<5000	NF EN ISO 4833-1
Molds (CFU /g)		<100	ISO 6611
Yeasts (CFU /g)		<100	ISO 6611
β-glucuronidase positive Escherichia coli (CFU /g)		<10	NF ISO 16649-2
Total coliforms (CFU /g)		<100	NF V08-050
Listeria monocytogenes (/25 g)		Absence	NF EN ISO 11290-1
Coagulase positive Staphilococcus (/g)		Absence	NF EN ISO 6888-3

Remarks

Certified conform,

(b)(6)

Division of S.I. LESAFFRE

Phileo by Lesaffre

A LESAFFRE BUSINESS UNIT

Method of Analysis: Mannans and Beta-Glucans

Phileo has previously used the enclosed published method for the analysis of mannans and beta-glucans in purified yeast cell wall which is also that used by an external laboratory (when samples are analyzed externally as a verification of internal methods). All analysis of batches for the assessment of stability over 25 months has been conducted using this analysis. However, more recently alternative methods have been developed by Phileo and verified internally to yield comparable/consistent results to the published method. Commercial purified yeast cell wall placed on the market in the U.S. will be tested using the new test methods and analytical data using these methods is provided in Table 2.4 (analytical data on 4 representative batches of purified yeast cell wall).

Method of Analysis: Mannans and Beta-Glucans

Original test method (Freimund *et al*., 2005)

Optimised quantification method for yeast-derived 1,3-beta-D-glucan and alpha-D-mannan

Author(s):
Freimund, Stefan; Janett, Sandro; Arrigoni, Eva; Amadò, Renato

Publication date:
2005-01

Permanent link:
https://doi.org/10.3929/ethz-b-000033813

Originally published in:
European Food Research and Technology 220(1), https://doi.org/10.1007/s00217-004-1008-0

Eur Food Res Technol (2005) 220:101 105
DOI 10.1007/s00217-004-1008-0

ORIGINAL PAPER

Stefan Freimund · Sandro Janett · Eva Arrigoni · Renato Amadò

Optimised quantification method for yeast-derived 1,3-β-D-glucan and α-D-mannan

Received: 1 June 2004 / Revised: 19 July 2004 / Published online: 15 September 2004

Abstract The polysaccharides 1,3-β-D-glucan and α-D-mannan show numerous beneficial effects for the health of humans and animals. For several years, an increasing number of glucan- and mannan-containing products intended for food and feed applications are commercially available. For the determination of glucan and mannan contents, however, widely accepted methods have not yet been established. We have developed a practicable and reliable quantification method characterised by acidic hydrolysis with trifluoroacetic acid and subsequent determination of released D-glucose and D-mannose. The unavoidable loss of the monosaccharides due to the acidic conditions was minimised by optimisation of the hydrolysis parameters. The best conditions found were compared with literature methods in order to demonstrate the suitability. Finally, glucan and mannan contents of various commercial products were determined and compared to the specifications given by the manufacturers.

Keywords 1,3-β-D-Glucan · α-D-Mannan · Acidic hydrolysis · Quantification · MOS

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Introduction

1,3-β-D-Glucan and α-D-mannan comprise two classes of polysaccharides which occur in numerous bacteria, fungi, mushrooms, algae and higher plants. In the last decades, both compounds have been described to show many benefits for the health of humans and animals [1, 2, 3, 4, 5, 6]. Accordingly, the number of commercial products containing 1,3-β-D-glucan—usually named 'β-glucan' or 'glucan'—or α-D-Mannan ('mannan' will be used in the following text) have increased in the last years. Glucan is primarily obtained from bacteria (curdlan [7]), baker's and brewer's yeast [8], fungi (scleroglucan [9]), and edible mushrooms (lentinan [10], shizophyllan [11]). It is used as a health food ingredient or in tablets and capsules for supporting the immune system, and curdlan has found use as a gelling agent and thickener in puddings, jellies and salad dressings. Mannan is often named MOS meaning 'mannan oligosaccharides'. MOS products are derived from common yeast and are used in the feed industry to prevent the colonisation of pathogens in the intestinal tract of farm animals [12, 13].

The marketing of glucan and MOS is often supported by certificates of analysis which are intended to prove a high content or purity of glucan and mannan. However, widely accepted quantification methods have not yet been introduced. One great obstacle concerns the more or less distinctive insolubility of many glucans, particularly of yeast glucans. Yeast glucans with a molecular weight of more than 10^5 kDa show a low solubility in dimethyl sulfoxide and are insoluble in other organic solvents and in water [14]. Therefore, direct analytical methods have predominantly been reported for well-soluble glucans. In the case of water-soluble β-1,3–1,4-mixed glucans occurring in cereals, complexes with fluorescent dyes like calcofluor white [15] and congo red [16] are used for quantification [17]. Recently described methods by means of NMR [18] or of an enzyme-linked immunosorbent assay [19] also require a solubility in dimethyl sulfoxide or in water. This is sometimes difficult or impossible if the glucan—as for example in yeast cell walls—is as-

DETERMINATION OF MANNAN IN YEAST CELL WALLS

Version 1

I. DEFINITION AND APPLICATION AREA

(b) (4)

II. REAGENTS

(b) (4)

III. APPARATUS

(b) (4)

Phileo, a division of S.I.Lesaffre
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SA au capital de 760 050€ - RSC Roubaix-Tourcoing 349 069 047 - Siret 349 069 047 00018 - TVA FR03 349069047

IV. WORKING METHOD

1. Preparation of reagent solutions

(b) (4)

(b) (4)

2. Hydrolysis

(b) (4)

Pre-solubilization:

(b) (4)

Phileo, a division of S.I.Lesaffre
137, rue Gabriel Péri - BP 3029 - 59703 Marcq-en-Baroeul cedex – France
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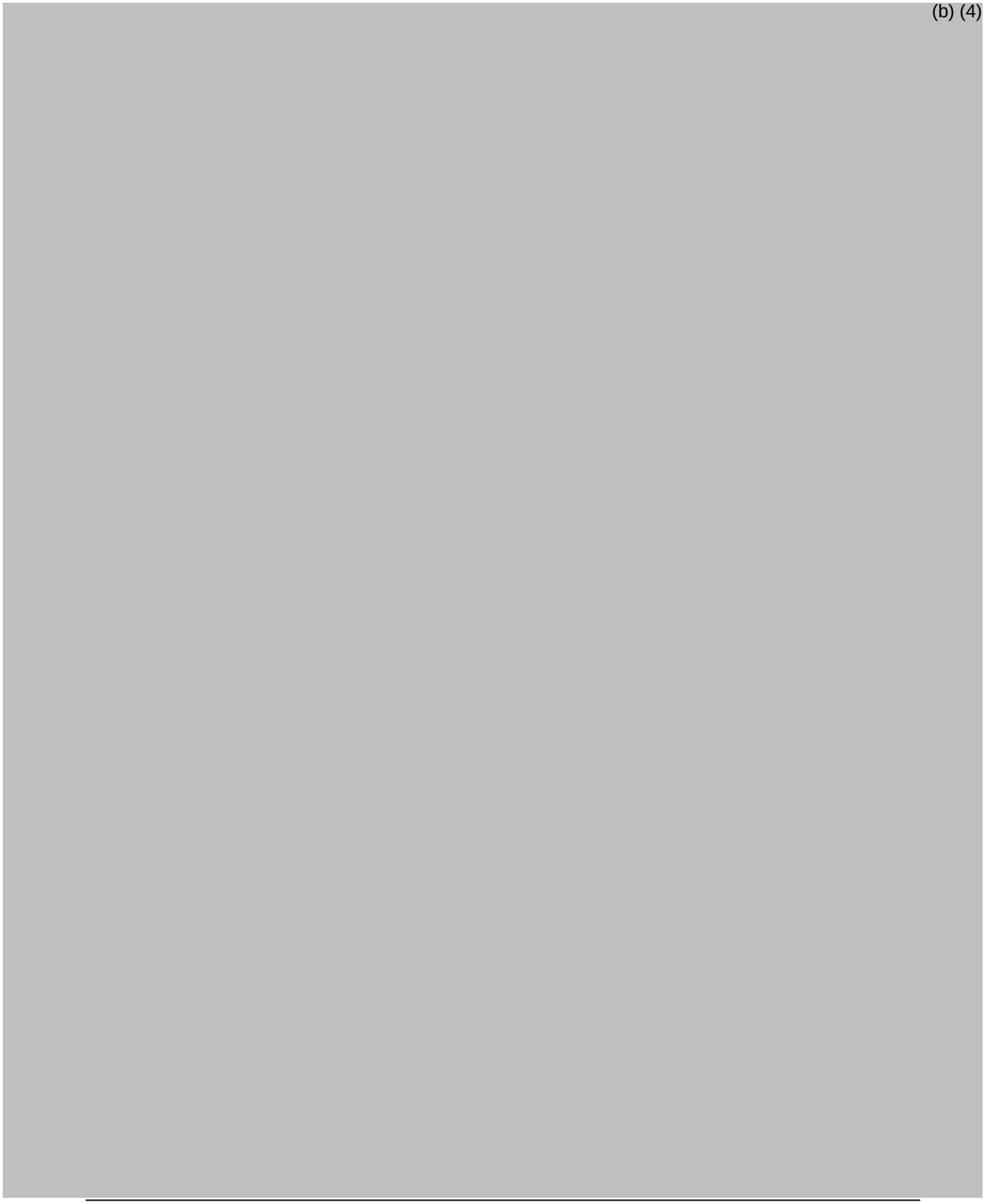
SA au capital de 760 050€ - RSC Roubaix-Tourcoing 349 069 047 - Siret 349 069 047 00018 - TVA FR03 349069047

3. Determination of Mannose by HPLC

(b) (4)



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Phileo Internal Method for Analysis of Beta-Glucans (Existing Method)

COM013	DETERMINATION OF BETA GLUCAN IN YEAST CELL WALLS
VERSION 1	20/08/2020

I. DEFINITION AND APPLICATION AREA

(b) (4)

II. REAGENTS

(b) (4)

(b) (4)

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III. APPARATUS

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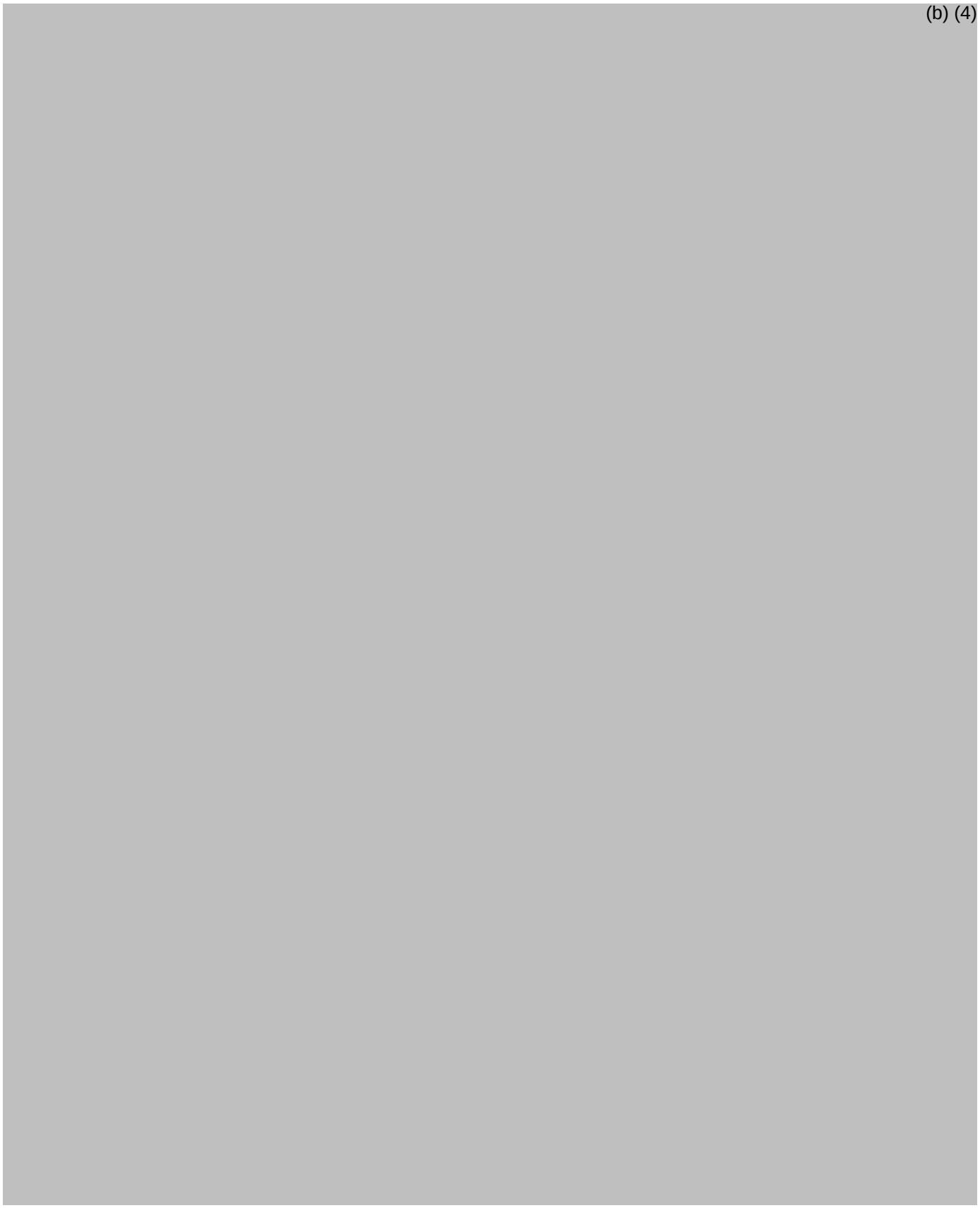

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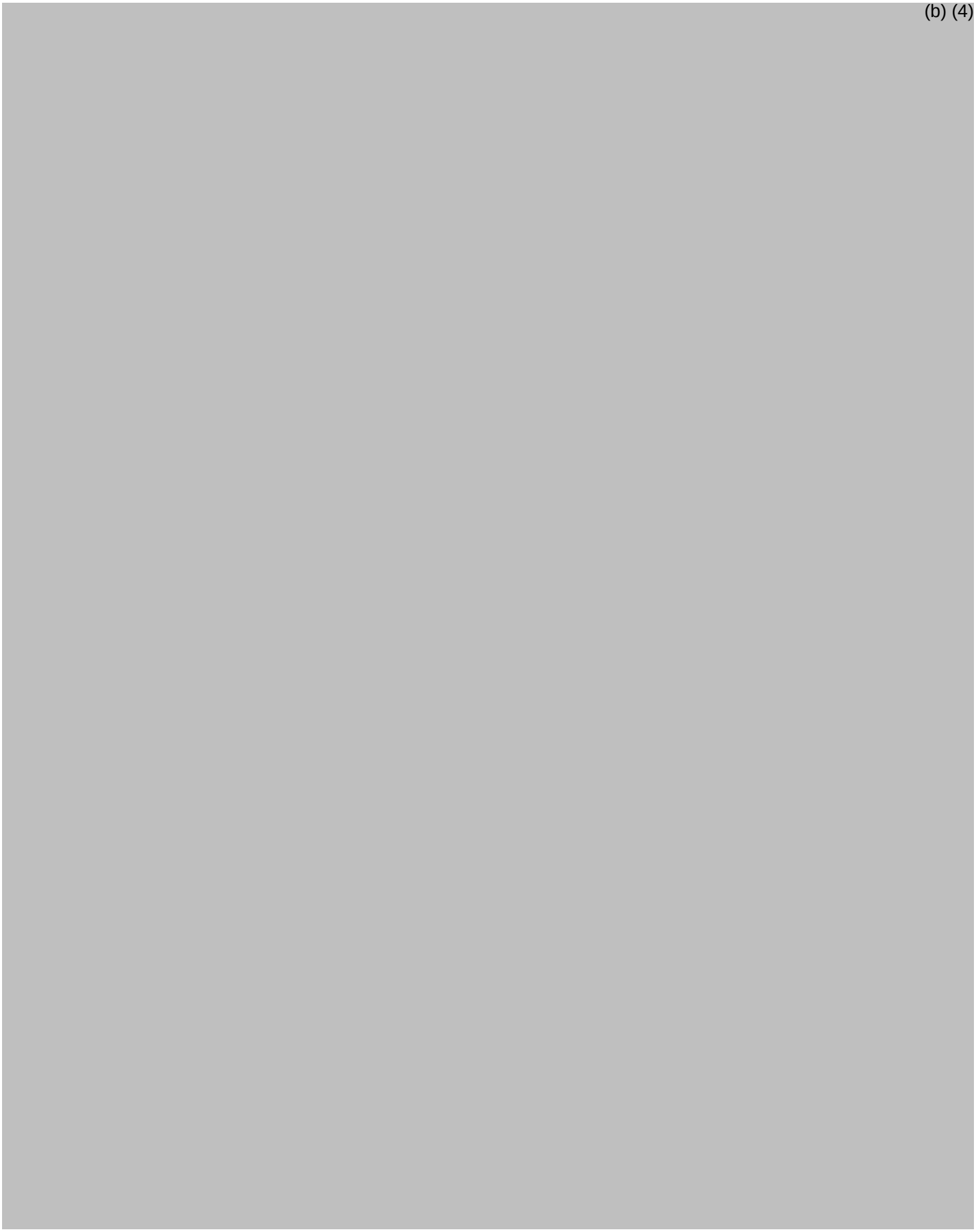

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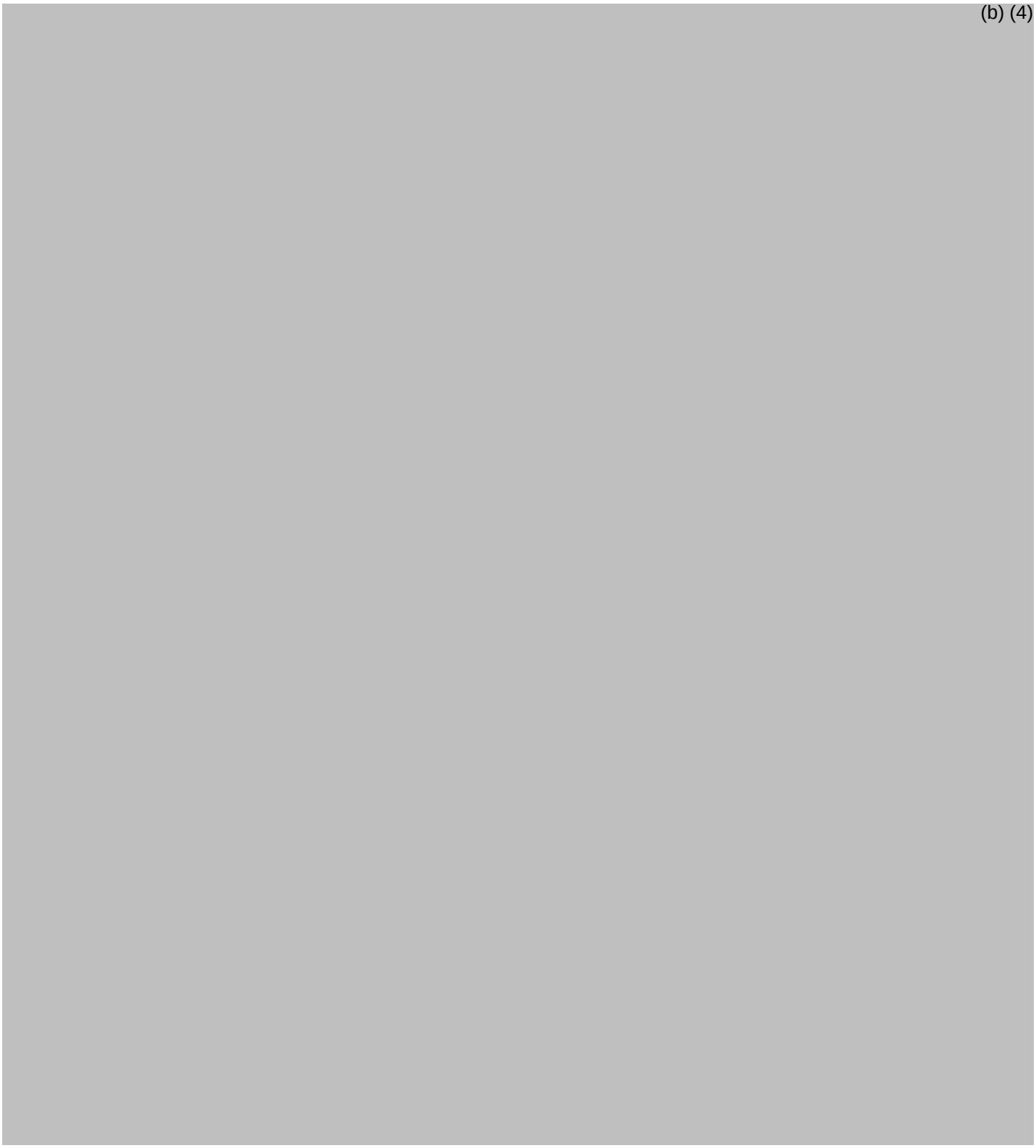

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Comparative study of water intake of Safglucan in commercial packaging versus packaging used for stability studies

Table des matières

Introduction - Objectives of the report

The stability studies conducted for registration dossier are usually carried out in ICH conditions, in climate chambers. The design of the study includes different timepoints up to 2 years shelf life, as well as the monitoring of different parameters such as product composition or microbiological content.

This specific design makes it technically impossible to use commercial-sized samples for each timepoint and each analysis during the study. The size of the samples is thus reduced to the quantity of product that will be necessary to carry out the analysis during the study.

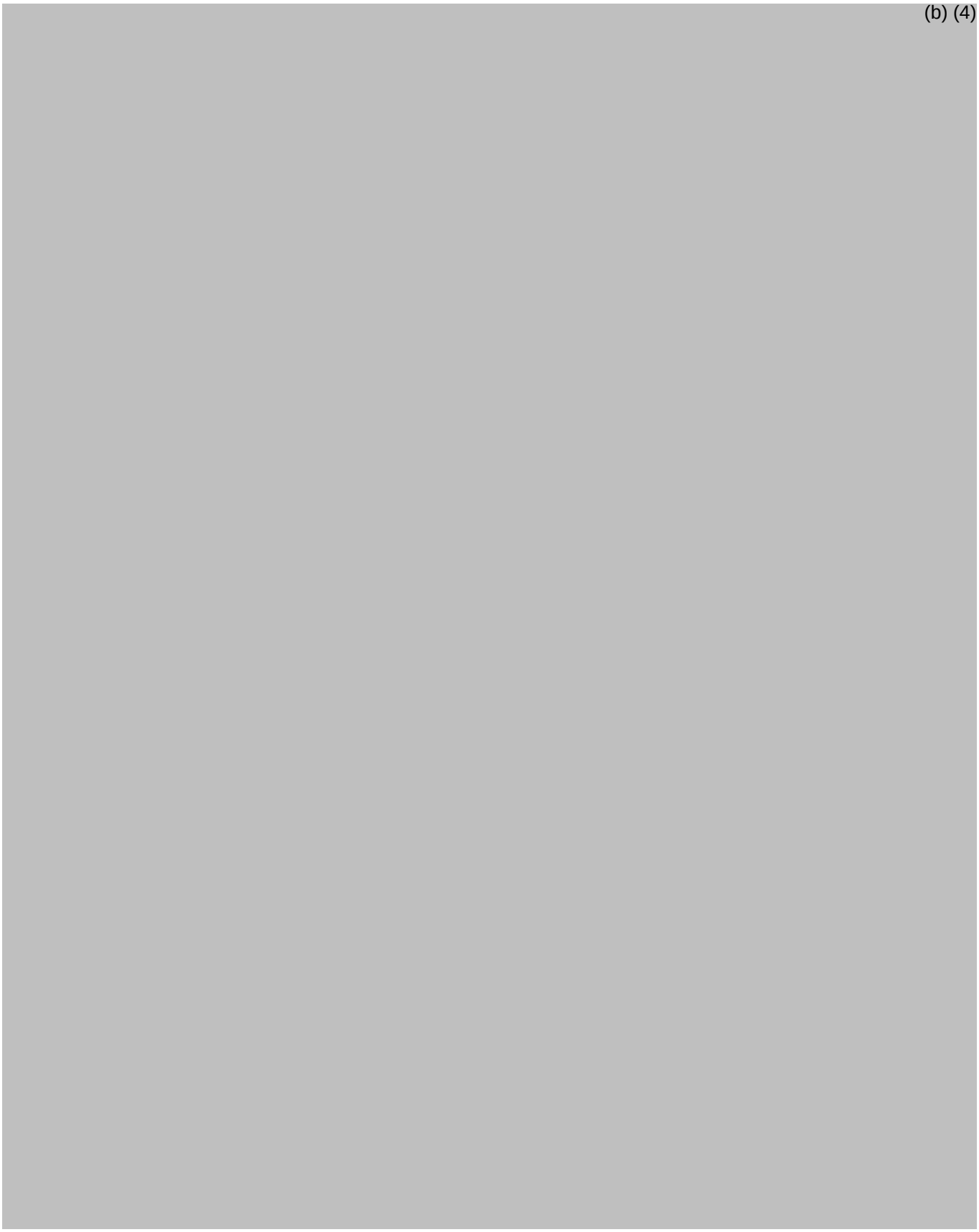
The objective of this report is to provide data to justify the differences that may be observed between the water intake in a commercial bag of Safglucan versus a smaller-sized packaging designed for stability studies.

1. Study of PE packaging permeability

The objective of this study is to understand the behaviour of the PE packaging under different relative humidities of its environment to be able to link it

1.1. Measurement of PE permeability

(b) (4)



1.1.2. Results

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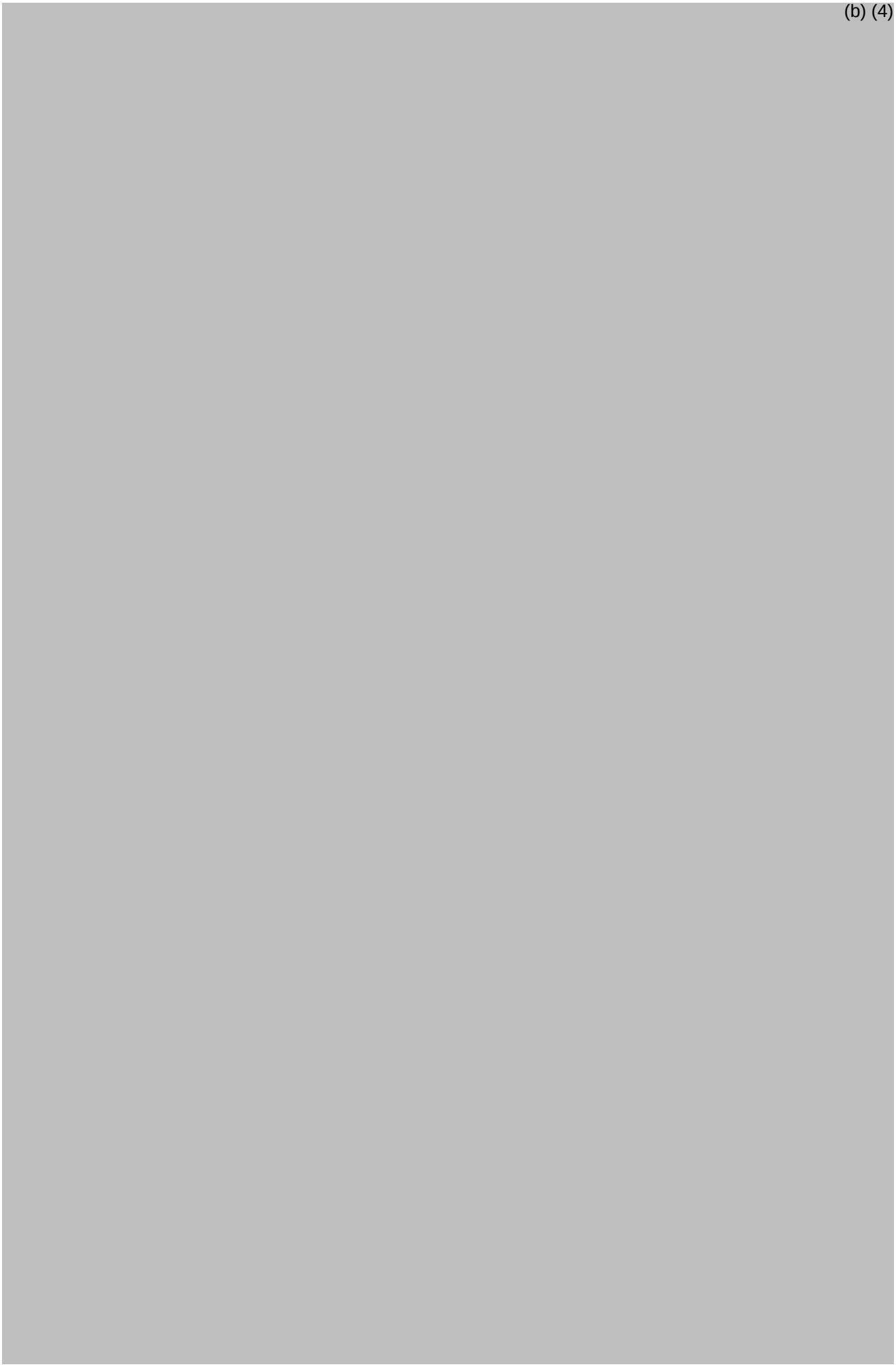
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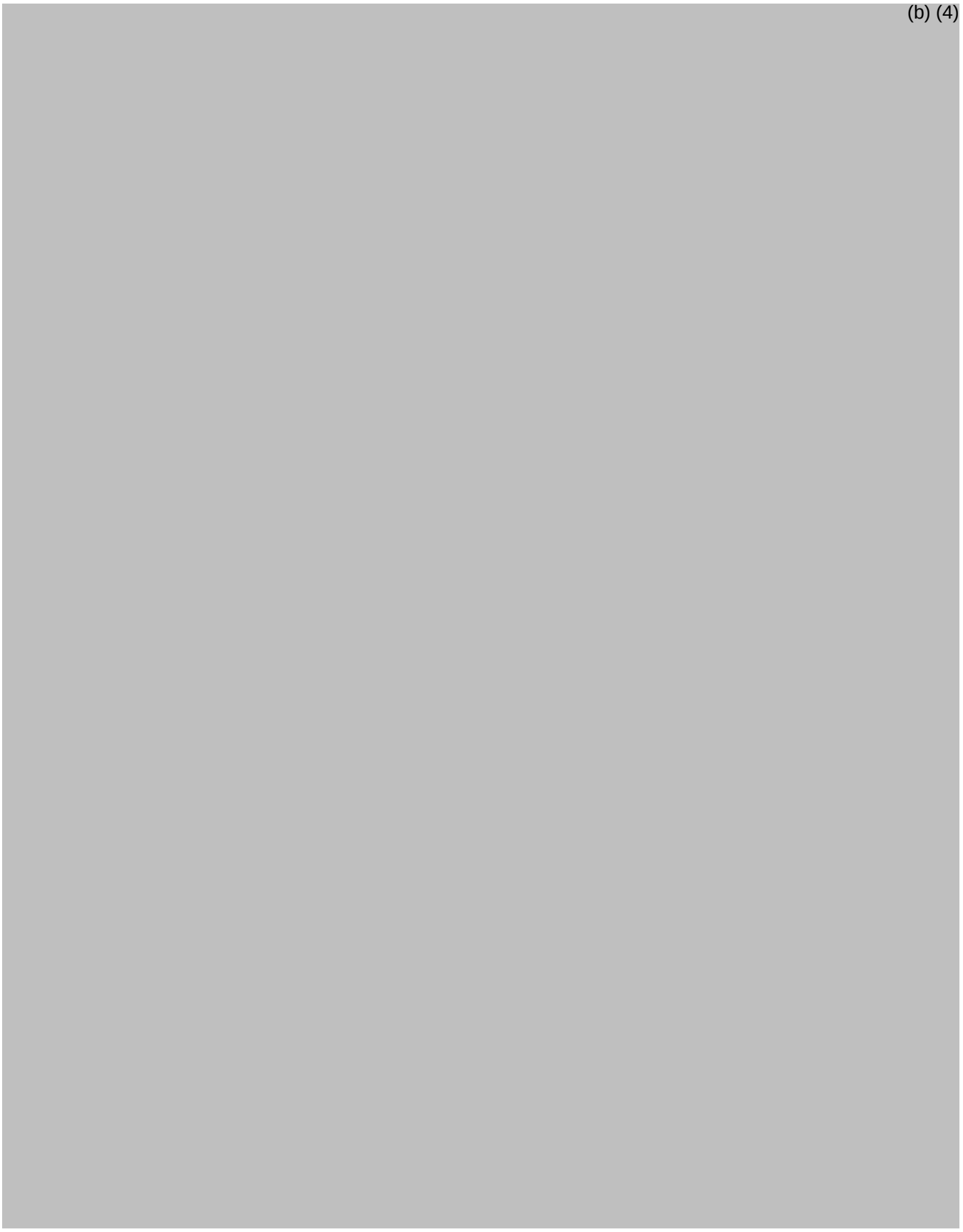
(b) (4)



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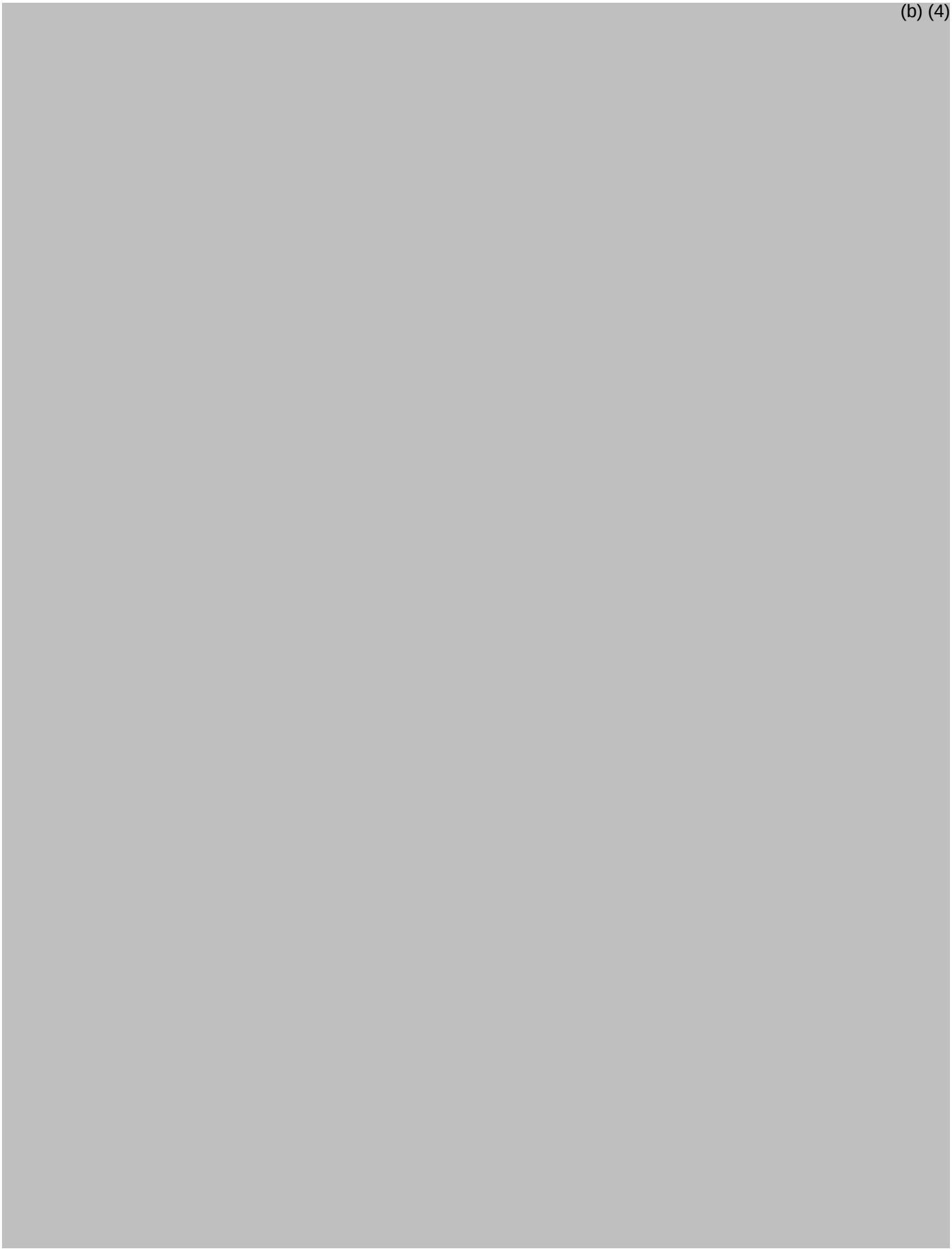

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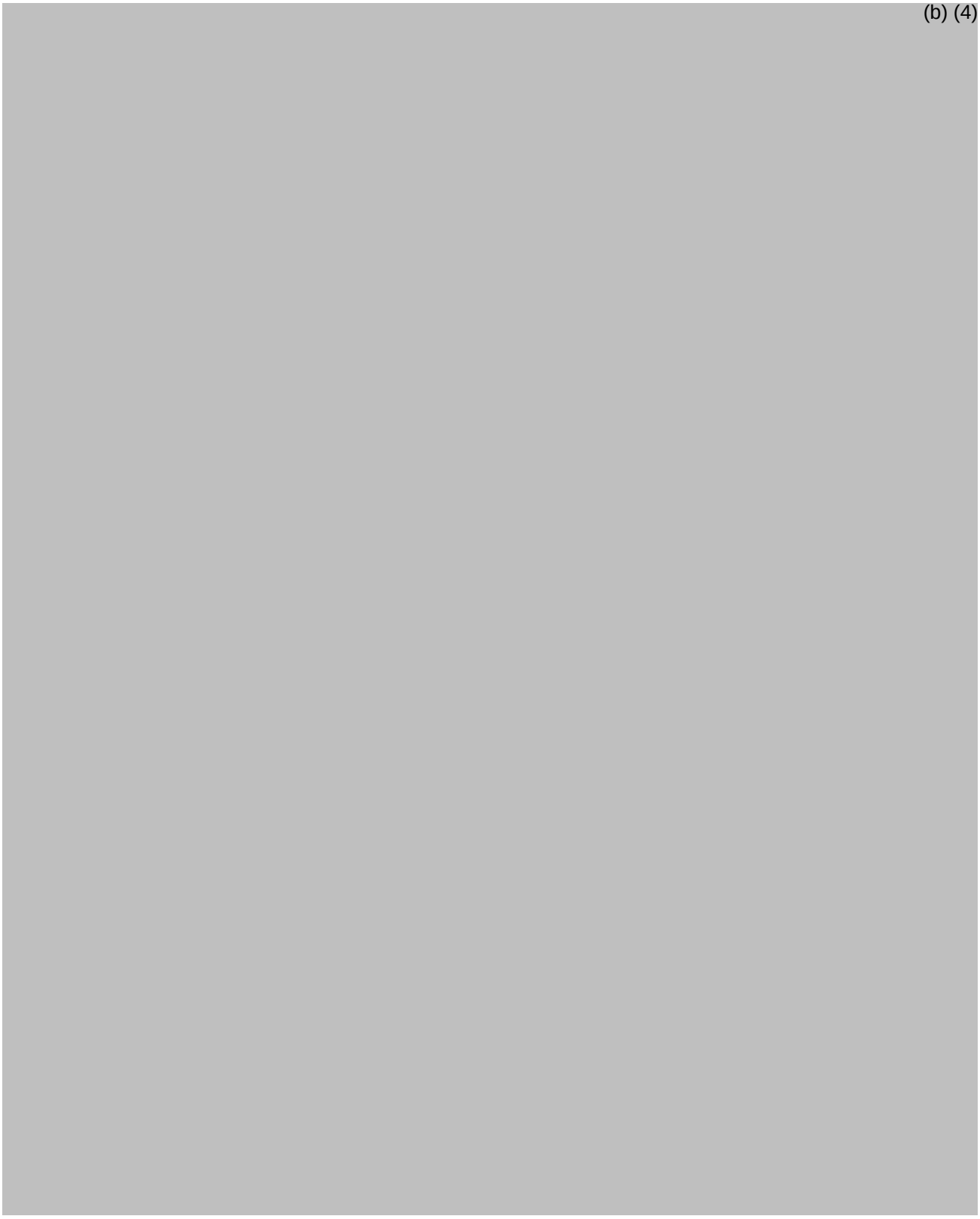

4. Discussion


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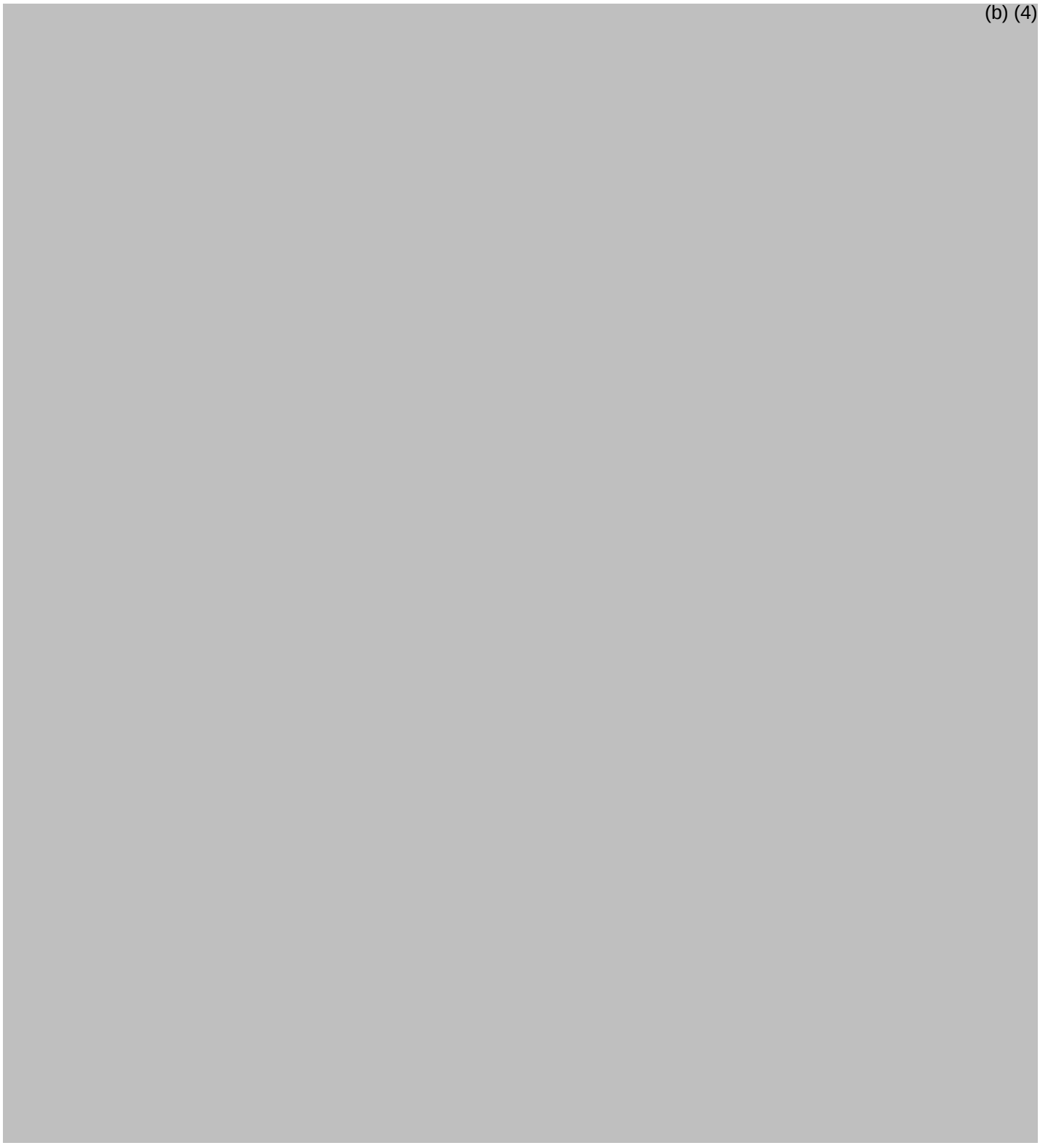
(b) (4)

Annex III – COA of last time point (24 months) of stability study at room temperature

Phileo by Lesaffre

Certificate of Analysis

Product	Safglucan®
Timepoint	T24 (08/2021)

	Batches			
	(b) (4)			
Production date	30/03/2018	15/03/2018	04/03/2019	25/03/2019
Expiration date	29/03/2020	14/03/2020	03/03/2021	24/03/2021

Parameters	Method	Specification
Dry matter	2g of product 103°C during 16h MEMMERT UF30 Oven	> 94%
Crude protein	Dumas total nitrogen method Rapid Max N Exceed ELEMENTAR	≤ 10 %
β-glucan	(b) (4)	≥ 50 %

Results

- Dry matter

Batch		Date of analysis	Result (%)
BATCH	(b) (4)	18/08/2021	94.3
BATCH		18/08/2021	93.4
BATCH		18/08/2021	90.2
BATCH		18/08/2021	92.6

- Crude protein

Batch		Date of analysis	Result (%)
BATCH	(b) (4)	18/08/2021	(b) (4)
BATCH		18/08/2021	
BATCH		18/08/2021	
BATCH		18/08/2021	

- β-glucan

Batch		Date of analysis
BATCH	(b) (4)	27/10/2021
BATCH		27/10/2021
BATCH		27/10/2021
BATCH		27/10/2021

- Microbiology

Batch		Date of analysis
BATCH	(b) (4)	21/10/2021
BATCH		21/10/2021
BATCH		21/10/2021
BATCH		21/10/2021

Stability of Safglucan – 24 months – supporting evidence – moisture specification

Phileo wants to market its product Safglucan with a 24 months expiry date.

The below results of analysis towards moisture parameter support our position to market our product with a 24 months shelf life. Phileo already markets the product Safglucan in Europe and was able to gather samples of the products being stored in real conditions for over 2 years. After analysis of those gathered samples, the results below show that Safglucan is still compliant with product specification even after 24 months. Indeed, the moisture parameter is below 6% for both batches.

Product	Batch number	Production date	Expiry date	Date of analysis	Humidity (%)	Specification (%)	Status
Safglucan	(b) (4)	22/08/2019	22/08/2021	15/03/2023	4,7	≤ 6	(b) (4)
Safglucan		02/02/2021	02/02/2023	15/03/2023	5,2	≤ 6	
Safglucan		03/03/2019	03-03-2021	04/26/2023	6,0	$\leq$ 6	

SafGlucan®

CERTIFICATE OF ANALYSIS

General information	
Product name	SafGlucan®
Batch number	(b) (4)
Manufacturing date	22/08/2019
Best Before Date	22/08/2021
Date of analysis	15/03/2023

Product characteristics		
	Values	**Specifications**
Moisture	(b) (4)	≤ 6 %

Remarks

Certified conform,

(b) (6)

FOR REGISTRATION PURPOSES

Phileo LESAFFRE ANIMAL CARE

(b) (4)

(b) (4)

Product name 产品名称： β-1,3-D-glucan β-1,3-D-葡聚糖
Model 型号： Safglucan 赛福葡聚糖
Batch number 批号： (b) (4)
Production date 生产日期： 2019-08-23
Test date 测试日期： 2019-08-24
Reporting date 报告日期： 2019-12-02
Best before 保质期： 2021-08-22

Characteristics 指标	Specification 技术要求	Test result 检验结果
Sensory 感官要求	Yellow powder with specific smell, no foreign smell and visible impurity 黄色粉末，无异味，无可见杂质，具有该产品特有气味	(b) (4)
Moisture, % 水分	≤6.0	
Total nitrogen, % 总氮	≤1.12	
Crude protein/DM, % 粗蛋白（以干基计）	≤10.0	
β -1,3-D-glucan/DM, % β -1,3-D-葡聚糖（以干基计）	≥50.0%	
Aerobic plate count, CFU/g 菌落总数	≤10000	
Coliforms, CFU/g 大肠菌群	≤100	
Wild Yeasts, CFU/g 野生酵母	≤100	
Moulds, CFU/g 霉菌	≤100	
Escherichia coli, CFU/g 大肠埃希氏菌	Negative 不得检出	
Salmonella, /25g 沙门氏菌	Negative 不得检出	

Conclusion 检验结论：

The product passes inspection and conforms to the requirements of Q/YPX 05.

本批次产品检验合格，符合 Q/YPX 05 的要求。

(b)(6)

SafGlucan®

CERTIFICATE OF ANALYSIS

General information	
Product name	SafGlucan®
Batch number	(b) (4)
Manufacturing date	02/02/2021
Best Before Date	02/02/2023
Date of analysis	15/03/2023

Product characteristics		
	Values	**Specifications**
Moisture	(b) (4)	≤ 6 %

Remarks

Certified conform,

(b) (6)

FOR REGISTRATION PURPOSES

(b) (4)

(b) (4)

Product name 产品名称： β-1,3-D-glucan β-1,3-D-葡聚糖

Model 型号： Safglucan 赛福葡聚糖

Batch number 批号： (b) (4)

Production date 生产日期： 2021-02-02

Test date 测试日期： 2021-02-03

Reporting date 报告日期： 2021-02-20

Best before 保质期： 2023-02-01

Characteristics 指标	Specification 技术要求	Test result 检验结果
Sensory 感官要求	Yellow powder with specific smell, no foreign smell and visible impurity 黄色粉末，无异味，无可见杂质，具有该产品特有气味	(b) (4)
Moisture, % 水分	≤6.0	
Total nitrogen, % 总氮	≤1.12	
Crude protein, % 粗蛋白	≤10.0	
β -1,3-D-glucan, % β -1,3-D-葡聚糖	≥50.0%	
Aerobic plate count, CFU/g 菌落总数	≤10000	
Coliforms, CFU/g 大肠菌群	≤100	
Wild Yeasts, CFU/g 野生酵母	≤100	
Moulds, CFU/g 霉菌	≤100	
Escherichia coli, CFU/g 大肠埃希氏菌	Negative 不得检出	
Salmonella, /25g 沙门氏菌	Negative 不得检出	

Conclusion 检验结论：

The product passes inspection and conforms to the requirements of Q/YPX 05.

本批次产品检验合格，符合 Q/YPX 05 的要求。

(b)(6)

SafGlucan®

CERTIFICATE OF ANALYSIS

General information	
Product name	SafGlucan®
Batch number	(b) (4)
Manufacturing date	03/03/2019
Best Before Date	03/03/2021
Date of analysis	26/04/2023

Product characteristics		
	Values	**Specifications**
Moisture	(b) (4)	≤ 6 %

Remarks

Certified conform,

(b) (6)

FOR REGISTRATION PURPOSES

(b) (4)

Product name 产品名称： β-1,3-D-glucan β-1,3-D-葡聚糖
Model 型号： Safglucan 赛福葡聚糖
Batch number 批号： (b) (4)
Production date 生产日期： 2019-03-04
Test date 测试日期： 2019-03-05
Reporting date 报告日期： 2019-04-09
Best before 保质期： 2021-03-03

Test item 检验项目	Standard 标准	Test result 检验结果
Sensory 感官要求	Yellow powder with specific smell, no foreign smell and visible impurity 黄色粉末，无异味，无可见杂质，具有该产品特有气味	(b) (4)
Moisture, % 水分	≤6.0	
Crude protein / DM, % 粗蛋白（以干基计）	≤10.0	
β -1,3-D-glucan / DM, % β -1,3-D-葡聚糖（以干基计）	≥50.0	
Salmonella, /25g 沙门氏菌	Absence 不得检出	

Conclusion 检验结论：

The product passes inspection and conforms to the requirements of (b) (4)

本批次产品检验合格，符合 (b) (4) 的要求。

(b)(6)

SafMannan

Description

Safmannan® is a premium yeast fraction rich in mannan-oligosaccharides and ß-glucans (1,3 and 1,6). **Safmannan®** is obtained by autolysis of *Saccharomyces cerevisiae* proprietary bakery strains. Batch-to-batch consistency and high concentration in active ingredients allow **Safmannan®** to achieve repeatable excellent performance.

Typical profile

Nutritional

Mannan	**≥ 20%**
ß-1,3 glucan, ß-1,6 glucan	**≥ 20%**
Moisture	≤ 6%
Crude protein	≤ 25%

Microbiological

Salmonella/25g	Negative

Physical characteristics

Color	Light beige
Odor	Typical yeast odor
Appearance	Fine powder

Recommendation

Ruminants	2 - 10 g/day/animal
Swine	250 - 500 g/T of feed
Poultry	125 - 500 g/T of feed
Aquaculture	500 - 2000 g/T of feed

Incorporation rate to be adapted to specific needs.

Packaging and Storage

Packaging: 25 kg polyethylene bag. 850 kg or 1650 lbs polypropylene woven big bag with inside polyethylene liner.
Shelf life: 2 years from production date, in original packaging.
Storage: keep in a dry and cool place for optimum preservation.

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