ToxStrategies

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June 3, 2025

Dr. David Edwards
Director
Division of Animal Feeds (HFV- 220)
Center for Veterinary Medicine
Food and Drug Administration
7519 Standish Pl.
Rockville, MD 20855

+-!'$", Ā AGRN 73 GRAS Notice
Xylooligosaccharides in poultry, swine, and fish feed

()*,&%&$* Ā Rayonier Advanced Materials Inc
1301 Riverplace Blvd #2300,
Jacksonville, FL 32207

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On behalf of Rayonier Advanced Materials Inc, I am providing an updated version of AGRN 73. This update includes revisions dated February 12 and May 15, 2025, that were submitted in response to questions received on January 29 and May 9, 2025, and as discussed on May 29, 2025. One additional minor revision to the manufacturing process has also been included. The phrase (b) (4)" was changed to " (b) (4)" This change reflects typical (b) (4) This change does not affect the product specifications.

Should you have any questions on the filing, please contact me directly.

Sincerely,

(b) (6)

Jennifer Hinerman, PhD
ToxStrategies LLC
Consultant to Rayonier Advanced Materials Inc

cc: Larissa S. Fenn, PhD, Rayonier Advanced Materials Inc
Attachments: Rayonier XOS GRAS Notification - Amended

GRAS Notice for Xylooligosaccharides for Use in Poultry, Swine, and Fish Feed

July 24, 2024

Amended June 2, 2025

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July 24, 2024

Amended June 2, 2025

Prepared For:

Rayonier Advanced Materials Inc
1301 Riverplace Blvd #2300
Jacksonville, FL 32207

Prepared by:

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Acronyms

AAFCO	Association of American Feed Control Officials
bw	body weight
CAS	Chemical Abstracts Service
CFR	Code of Federal Regulations
cfu	colony forming unit
cGMP	current Good Manufacturing Practice
COA	Certificate of Analysis
DP	Degree of Polymerization (chain length)
EFSA	European Food Safety Authority
FDA	U.S. Food and Drug Administration
GMP	Good Manufacturing Practice
GRAS	Generally Recognized as Safe
HDP	High Degree of Polymerization
LD50	Median Lethal Dose
LDP	Low Degree of Polymerization
MDP	Medium Degree of Polymerization
OECD	Organization for Economic Co-operation and Development
XOS	Xylooligosaccharides

§ 570.225 Part 1, GRAS Notice: Signed Statements and Certification

(1) GRAS Notice Submission

Rayonier Advanced Materials Inc (RYAM), through its agent, ToxStrategies LLC, notifies the U.S. Food and Drug Administration (FDA) of its conclusion that the use of xylooligosaccharides in poultry, swine, and fish feed is generally recognized as safe.

(2) Name and Address

Rayonier Advanced Materials Inc
1301 Riverplace Blvd #2300
Jacksonville, FL 32207

(3) Name of Notified Substance

The name of the substance that is the subject of this GRAS determination is xylooligosaccharides.

(4) Intended Use in Food

The proposed intended use of the xylooligosaccharides is for swine, poultry, and fish feeds, at a dietary inclusion rate of up to 1% to provide a source of xylooligosaccharides.

(5) Statutory Basis for GRAS Determination

RYAM, through its agent, ToxStrategies, confirms that xylooligosaccharides, which meets the specifications described herein, has been determined to be GRAS through scientific procedures in accordance with 21 CFR § 570.30(a) and (b).

(6) Premarket Approval Statement

RYAM further asserts that the use of xylooligosaccharides as described herein is exempt from the pre-market approval requirements of the Federal Food, Drug, and Cosmetic Act, based on the conclusion that the substance is GRAS under the conditions of its intended use.

(7) Availability of Information

The data and information that serve as the basis for this GRAS determination will be sent on request or are available for the FDA's review and copying during customary business hours from the ToxStrategies main office, located in Katy, Texas.

(8) Data and Information Confidentiality Statement

RYAM has placed proprietary and confidential information in the following sections and appendices. Each section designated as [RYAM CONFIDENTIAL] either in its entirety or in part of the following sections is RYAM proprietary and confidential information:

§ 570.230 Part 2 E. Composition

§ 570.230 Part 2 F. Manufacturing Process

§ 570.230 Part 2 G. Product Specifications Tables 2 and 3

§ 570.230 Part 2 H. Stability Data Table 6

§ Part 507.250 Part 6 General Recognition of Safety

Appendix A

(9) GRAS Certification

To the best of our knowledge, the GRAS determination is a complete, representative, and balanced document. Rayonier Advanced Materials Inc is not aware of any information that would be inconsistent with a finding that the proposed uses and use levels of xylooligosaccharides in the animal feed intended for swine, poultry, and fish, meeting the appropriate specifications described herein and used according to current Good Manufacturing Practice (cGMP), is GRAS. Recent reviews of the scientific literature revealed no potential adverse health concerns.

(10) Name/Position of Notifier

(b) (6)

Larissa S. Fenn, PhD June 2, 2025
Director, New Products
Rayonier Advanced Materials Inc

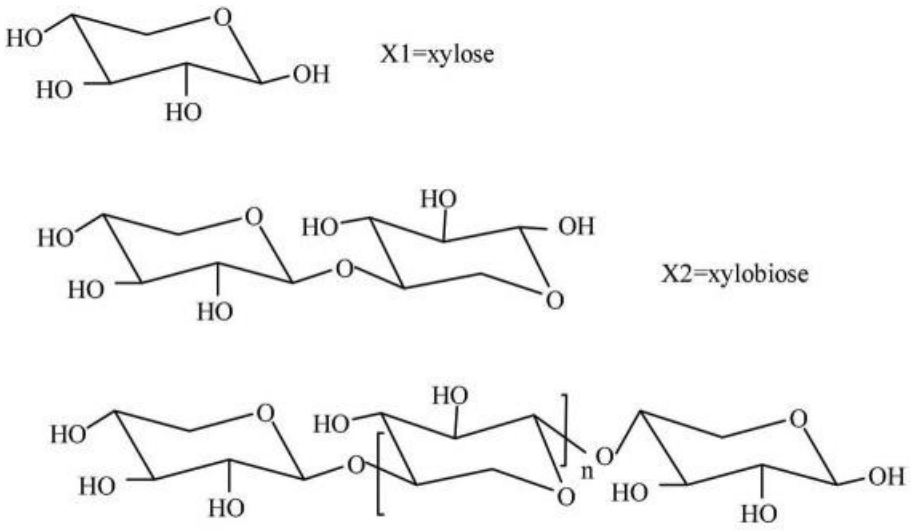
(11) FSIS Statement

Not applicable.

§ 570.230 Part 2, Identity, Method of Manufacture, Specifications, and Physical or Technical Effect

A. Identity

The proposed xylooligosaccharides (XOS) ingredient is described as xylooligosaccharides with a degree of polymerization (DP; chain length) ranging from 2 to 30 xylose units (Figure 1).



where
n=1: X3=xylotriose
n=2: X4=xylotetraose
n=3: X5=xylopentaose
n=4: X6=xylohexanose
n=5: X7=xyloheptaose
…
n=28: X30

Figure 1. The structure of xylose and xylooligosaccharides (Huang et al., 2022).

B. Common or Chemical Names

Xylooligosaccharides, xylo-oligosaccharides, and XOS where specific DP units may be known as xylobiose (DP2), xylotriose (DP3), xylotetrose or xylotetraose (DP4), xylopentose or xylopentaose (DP5), xylohexanose (DP6), xyloheptaose (DP7)

C. Chemical Abstracts Service (CAS) Registry Number

None[1]; 6860-47-5 for xylobiose

D. Trade Name

None

E. Composition

This XOS feed additive is typically comprised of:

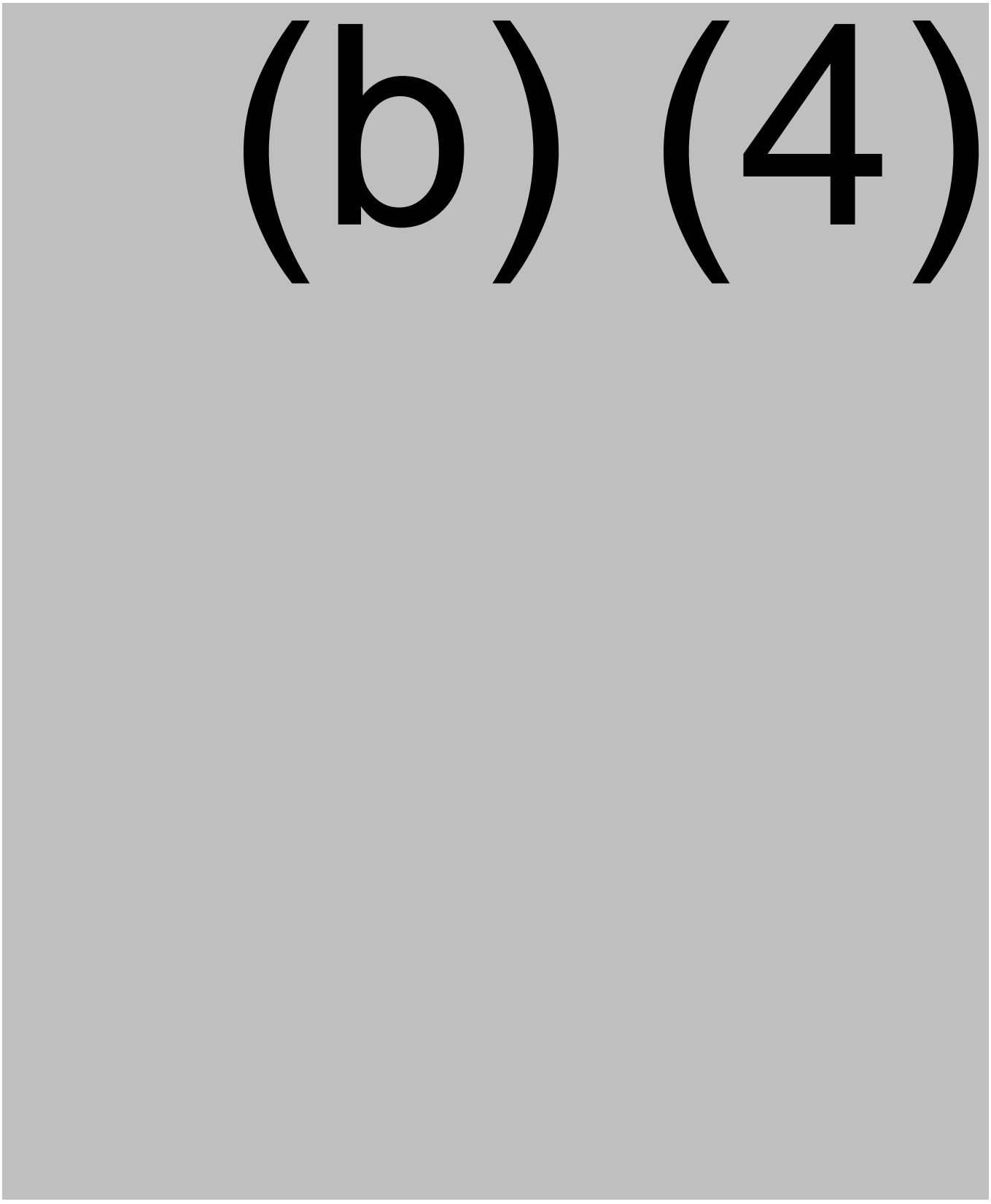
(b) (4)

F. Manufacturing Process

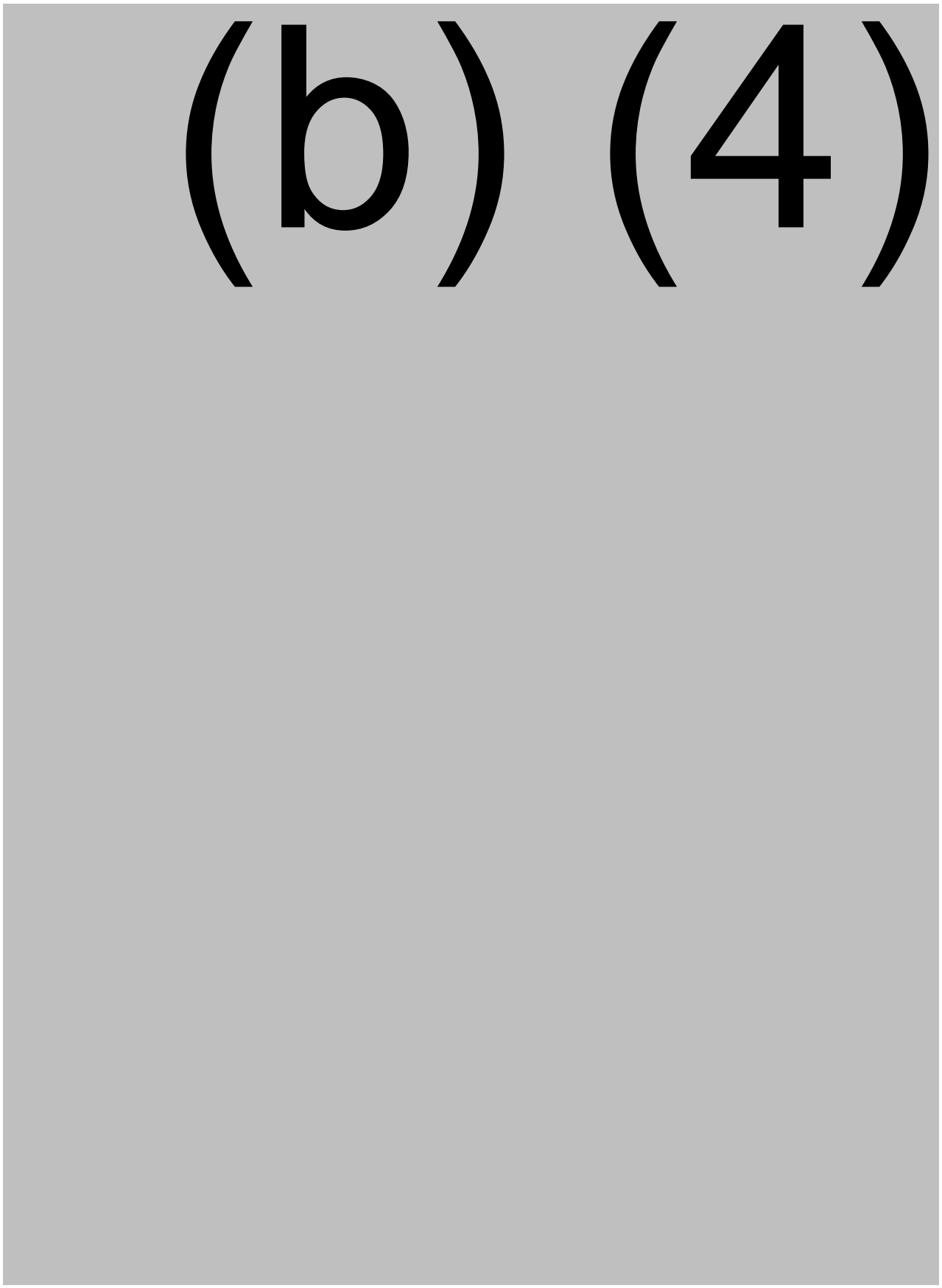
The starting material to produce XOS originates as a coproduct of RYAM's cellulose production. [RYAM CONFIDENTIAL to end of section]

(b) (4)

[1] Many companies use CAS 87-99-0 to describe xylooligosaccharides. However, CAS 87-99-0 is defined as xylitol (https://pubchem.ncbi.nlm.nih.gov/compound/Xylitol) which is chemically, compositionally, and physiologically different than xylooligosaccharides.



[Figure is RYAM CONFIDENTIAL]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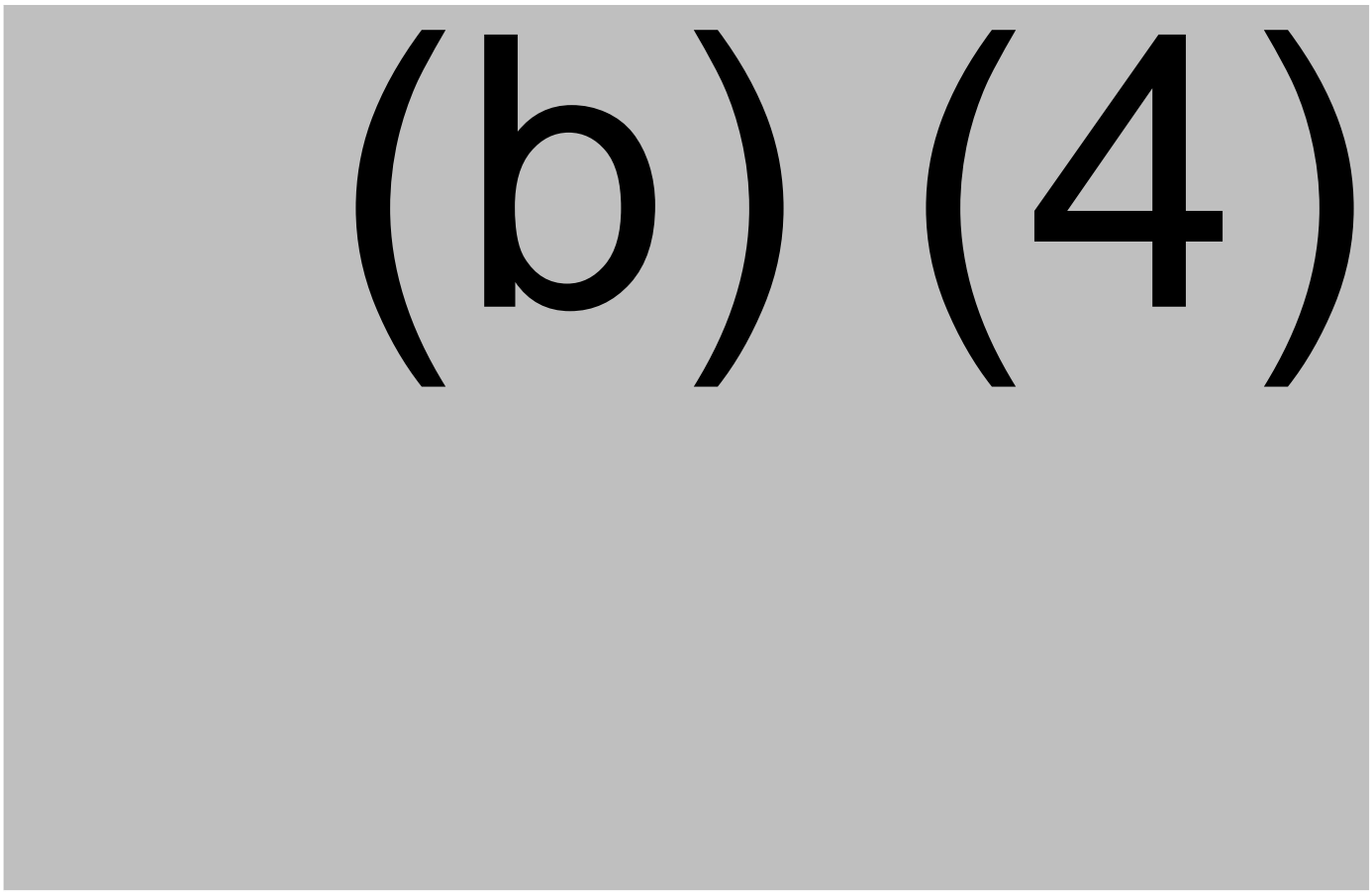

(b) (4)

Table 4. Contaminants for three non-consecutive lots of XOS*

Item	Specification	Lot	Lot	Lot	Method
				(b) (4)	
Aerobic plate count (cfu/g)	≤2000	(b) (4)			
Yeast (cfu/g)	≤50				
Mold count (cfu/g)	≤50				
Enterobacteriaceae (cfu/g)	≤10				
Salmonella (org/25g)	negative				
Aflatoxin summation, ppb	≤20				
Fumonisin summation, ppb	≤100				
Deoxynivalenol (DON), ppb	≤100				
Arsenic, ppm	≤0.5				
Cadmium, ppm	≤0.05				
Lead, ppm	≤0.2				
Mercury, ppm	≤0.05				

* n.d. = not detected. Values following a < symbol indicate the limit of detection for that analysis.

Table 5. Heavy metal comparison of XOS to approved ingredients and complete feed

Heavy Metal	XOS	Chromium propionate[1]	Selenomethionine hydroxy analogue[2]	EU Directive for Complete Feed
Arsenic, ppm	(b) (4)	≤1.0	≤2.0	≤2
Cadmium, ppm		≤1.0	≤1.0	≤2 [3]
Mercury, ppm		≤0.5	≤1.0	≤10 [4]
Lead, ppm		≤0.5	≤1.0	≤0.1 [5]

[1] 21 CFR 573.304 (c)

[2] 21 CFR 573.920 (h)(1)

[3] EU Directive 1275/2013/EC Cadmium Feed materials of mineral origin https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32013R1275

[4] EU Directive 2019/1869/EC Lead Feed materials https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32019R1869

[5] EU Directive 2019/1869/EC Mercury Feed materials https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32019R1869)

Heavy metal analysis of XOS confirms that heavy metals are not present at levels that would be of toxicological concern. For perspective, food additive specifications for chromium propionate and selenomethionine hydroxy analogue and EU Directive 2002/32/EC sets heavy metals limits for feed ingredients and complete feed (Table 3). Chromium propionate and selenomethionine hydroxy analogue are the only feed ingredients in 21 CFR Subchapter E with cadmium and mercury specifications. Complete feed maximum contents were selected from the EU Directive as the most conservative levels. The heavy metal analysis for XOS demonstrated levels in alignment with these specifications for each heavy metal.

The analytical composition for XOS and products containing XOS are summarized in Tables 3, 4, and 5 and were found in the Certificates of Analysis (COAs) in Appendix A.

These results confirm that the finished products meet the analytical specifications, demonstrate that the supplier's manufacturing process results in a consistently reproducible product, and confirm the lack of significant levels of impurities and/or contaminants.

H. Stability Data

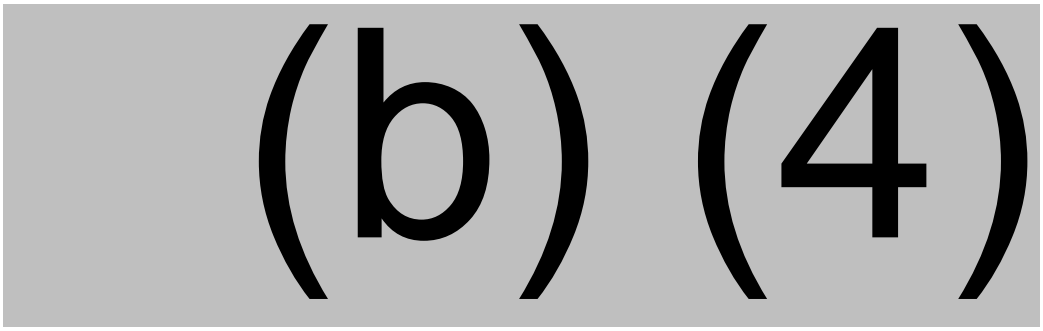
A real-time shelf-life study was performed on XOS and demonstrates XOS is shelf stable for 27 months (Table 6). The XOS will be commercially packaged in super sacks. To simulate the storage conditions of the super sacks during the shelf-life study, samples were collected and placed into small, sealed plastic bags which were stored in the laboratory

under controlled conditions. This approach ensures that the stability study accurately reflects the product's performance in its intended commercial packaging, as the primary material composition and protective properties were maintained.

For the shelf-life study, an approximately 500 g sample was collected and packaged in sealed, plastic bags and placed on the shelf of a laboratory. Two sample batches were tested in the shelf-life study. The temperature and relative humidity of the laboratory were 74°F and 48%, respectively, as measured using a hygrometer. The levels of moisture, xylose, and XOS, specifically the DP 2-6 fraction, were measured at study initiation and at a two- and three-year time period. Internal analytical methods were used to measure moisture (ISO1126-2015), xylose (RSTI 5099.0), and XOS DP 2-6 (RSTI 5099.0).

Table 6. Stability data to support shelf life of XOS

[Xylose, XOS, and Moisture results are RYAM CONFIDENTIAL]



* % error is ±10%

It acknowledged that the specifications for the shelf-life stability study are slightly different than the XOS specifications reported in Table 2. The XOS product used for the shelf-life stability study was one of the initial production lots which contained a relatively higher proportion of lower DP materials. These lower DP materials tend to be less stable and more hygroscopic resulting in a worst-case scenario for shelf-life testing. In addition, xylose formation has been significantly reduced over time through optimization of the processing conditions allowing a lower XOS product specification. This shelf-life study demonstrated that over the course of 27 months, xylose, DP 2–6 XOS, and moisture content was not changed.

I. Technical Effect

The XOS will provide a source of xylooligosaccharides. The utility of the notified substance as a source of xylooligosaccharides does not impact safety of the target animal when the substance is used at the proposed use rate. Shorter chain oligosaccharides of fructose, xylose, mannose, or galactose, etc. are fermentable and provide nutritive effects in monogastric animals (Singh and Kim, 2021) and a maximum of 40% longer chain DP has been established to limit any potential for nutrient dilution or gastrointestinal upset due to lower fermentability of these longer chain oligosaccharides.

570.235 Part 3, Target Animal and Human Exposure

The intended use of the XOS is for poultry, swine, and fish feeds at a dietary inclusion rate of up to 1% as a source of xylooligosaccharides to provide energy to and support of normal intestinal function and microbiota populations. Although exposure can be calculated as g/day or g/kg body weight/day, the safety studies for the target animal species discussed in Part 6 consistently present exposure as a % inclusion in the total feed ration.

Human Exposure

Data are available from the European Food Safety Authority (EFSA, 2018) and FDA GRAS Notices (GRN 343; GRN 458; GRN 816) relative to direct consumption of XOS in human foods. These authorities allow inclusion of XOS in a wide variety of foods up to 2.4 grams/serving. In addition, sodium chloride (21 CFR 182.1(a)) and sodium caseinate (21 CFR 182.1748) are generally recognized as safe for use in human foods when used in accordance with good manufacturing practice.

As part of an animal feed ingredient approval, potential human exposure through consumption of meat, milk, and eggs produced by animals consuming the proposed new ingredient must be assessed. However, as a feed additive for poultry, swine, and fish, the XOS will be fermented by the microbial flora in the intestinal tract to produce short-chain fatty acids. The fermentation products will be metabolized by the animal and their microbiota to intermediates and metabolic end-products typical of any food containing indigestible carbohydrates, negating any exposure via consumed meat or eggs. Similarly, the sodium chloride and sodium caseinate will be fully digested by the animal, absorbed, and metabolized, negating any exposure via consumed meat or eggs.

§ 570.240 Part 4, Self-Limiting Levels of Use

The use of XOS in poultry, swine, and fish feed is not considered to be self-limiting. There are no known characteristics of XOS that would limit the amount that can be added by making feed unpalatable or technically impractical. Recommended levels of up to 1% inclusion in poultry, swine, and fish feed should not be exceeded through application of formulation constraints and controls.

§ 570.245 Part 5, Experience Based on Common Use in Food

There are no approvals for the use of XOS in animal foods. The statutory basis for our conclusion of GRAS status for XOS is based on scientific procedures as a source of xylooligosaccharides in poultry, swine, and fish feed.

§ Part 507.250 Part 6, GRAS Narrative

General Safety Assessment of Xylooligosaccharides

Monogastric animals such as poultry, swine, and finfish do not produce the enzymes required for enzymatic hydrolysis of non-starch polysaccharides such as xylan and XOS (Baker et al., 2021; Saettone et al., 2020). Xylans are hydrolyzed to XOS either prior to dietary inclusion by a variety of methods including alkalization, as in the production of the XOS that is the subject of this dossier, or through dietary inclusion of xylanases (Baker et al., 2021). These XOS resist enzymatic hydrolysis by salivary, gastric, and pancreatic secretions and serve as a prebiotic that is fermented by the microbial community in the lower gut (Baker et al., 2021). Saettone et al. (2020) reviewed the intestinal microbiota communities across poultry, swine, and fish species and identified that use of prebiotics can improve intestinal microflora communities and subsequent intestinal health even though these host species represent feeding habits ranging from carnivorous through omnivorous to herbivorous. Functional oligosaccharides, such as XOS, positively affect intestinal microbial ecology (Baker et al., 2021). The microbial communities ferment the XOS as an energy source for microbial growth with a concomitant production of short-chain fatty acids such as acetate, propionate, and butyrate that are released into the lumen of the intestine (Baker et al., 2021). These short-chain fatty acids can be used by the host (e.g., poultry, swine, and finfish) as an energy source (e.g., up to 28% of the maintenance energy of a pig) and serve to reduce the pH in the lumen of the gut thereby promoting growth of known beneficial microbes (Baker et al., 2021).

Overall, there are sufficient safety data to support the proposed use of 1% dietary inclusion of XOS as a source of xylooligosaccharides to provide energy and support for normal intestinal health and microbiota populations in poultry, swine, and fish feed. Numerous studies in production animals indicate a lack of adverse effects and normal growth performance in animals consuming XOS-supplemented feed. Differences among treatments reported in this evaluation met a statistically significant threshold of $P < 0.05$. Data from traditional toxicity studies in research species also support the safety of XOS.

Safety of Xylooligosaccharides for Production Animals

Safety in Poultry

Broilers

A total of eighteen studies were identified with XOS used in broiler feed. Levels of XOS in feed ranged from 0.0002% (42 days) to 2% (59 days), and study duration ranged from 12 to 59 days. In all studies, broilers fed an XOS-supplemented feed had normal growth performance, and mortality was not different than controls. A few studies reported an increase in intestinal villus height, a decrease in crypt depth, and greater short-chain fatty acid concentrations with XOS supplementation that are considered indicative of positive intestinal health. Biochemical parameters and antibody titers were measured in several studies and, in general, were either not different from untreated controls or improved.

1. Yadav et al. (2024) conducted a dose-response study using 0, 0.05, 0.1 or 0.2% XOS that is the subject of this evaluation in feed from the day of hatch in Cobb 500 male broiler chicks (n=6 cages per group with 10 birds per replicate). Over the 21-day study, body weight, body weight gain, feed intake, and feed efficiency were similar to controls for all XOS groups. Villus height and crypt depth in the duodenum, jejunum, and ileum were also similar across all groups (Table 7). Cecal bacterial populations demonstrated a linear increase toward more Bifidobacterium with increasing XOS and decreased Clostridium perfringens with XOS inclusion (Table 8).

Table 7. Table 1 from Yadav et al. (2024)

Table 1. Effects of dietary supplementation of xylo-oligosaccharide (XOS) on growth performance and intestinal histomorphology of broilers during d 0 to 21.

Parameters[1]	Control	0.05% XOS	0.1% XOS	0.2% XOS	SEM	*P* value Linear	*P* value Quadratic
Wk 1 (0-7 d)							
BW, g	160.65	158.17	158.68	158.43	1.280	0.618	0.684
BWG, g	119.90	117.48	118.02	117.95	1.280	0.667	0.671
FI, g	123.78	116.87	120.37	121.87	1.610	0.879	0.213
FCR, g:g	1.03	1.00	1.02	1.03	0.006	0.550	0.047
Wk 2 (7-14 d)							
BW, g	445.73	451.65	450.45	437.12	2.860	0.291	0.099
BWG, g	285.08	293.48	291.77	278.68	2.590	0.305	0.040
FI, g	392.20	385.60	383.05	378.95	3.400	0.189	0.859
FCR, g:g	1.38	1.31	1.32	1.37	0.010	0.814	0.035
Wk 3 (14-21 d)							
BW, g	1006.52	986.05	988.78	988.23	7.700	0.477	0.543
BWG, g	560.78	534.40	538.33	551.10	8.570	0.756	0.284
FI, g	695.72	645.08	659.58	660.43	8.550	0.225	0.131
FCR, g:g	1.24	1.21	1.22	1.21	0.010	0.377	0.735
Wk 1-3 (0-21 d)							
BW, g	1006.52	986.05	988.78	988.23	7.700	0.477	0.543
BWG, g	965.77	945.36	948.11	947.74	7.690	0.484	0.541
FI, g	1211.7	1147.55	1162.99	1161.24	10.320	0.130	0.121
FCR, g:g	1.26	1.21	1.23	1.23	0.008	0.308	0.226
Duodenal histomorphology							
Villus height µm	2299.83	2598.50	2442.17	2502.50	56.830	0.377	0.299
Crypt depth µm	244.67	231.00	224.83	218.67	9.254	0.343	0.849
Villus:Crypt ratio	9.72	11.39	11.35	11.82	0.456	0.140	0.515
Jejunal histomorphology							
Villus height µm	1466.17	1547.00	1417.00	1519.83	41.116	0.936	0.899
Crypt depth µm	253.83	217.33	254.17	222.00	11.239	0.572	0.925
Villus:Crypt ratio	5.89	7.35	5.65	7.37	0.316	0.300	0.825
Ileal histomorphology							
Villus height µm	866.67	969.50	952.33	868.33	28.979	0.963	0.126
Crypt depth µm	173.67	190.67	203.67	186.33	8.077	0.501	0.3148
Villus:Crypt ratio	5.10	5.22	4.90	4.70	0.185	0.393	0.686

[1]BW = body weight (g); BWG = body weight gain (g); FI = feed intake (g); FCR = feed conversion ratio; all the BWG, FI, and FCR presented per bird for the period mentioned in Wk = week whereas BW was recorded at end of each period. Tendency among the treatment is shown at $0.05 \leq P \leq 0.1$. *N*=6.

Table 8. Table 2 from Yadav et al. (2024)

Table 2. Effects of dietary supplementation of xylo-oligosaccharide (XOS) on cecal bacterial count (Log_{10} CFU/g) of broiler on d 21.

Parameters, Log10 CFU[1]/g	Control	0.05% XOS	0.1% XOS	0.2% XOS	SEM	*P* value Linear	*P* value Quadratic
Lactobacillus	8.55	8.56	8.58	8.54	0.007	0.704	0.080
Bifidobacterium	7.05[d]	7.27[c]	7.46[b]	7.78[a]	0.058	<0.0001	0.203
Clostridium perfringens	7.73[a]	6.77[b]	6.78[b]	6.78[b]	0.086	<0.0001	<0.0001
E. coli	5.70	5.69	5.69	5.69	0.008	0.743	0.733

[1] CFU = colony forming unit. The error bars on the mean bar graph represent standard deviation (SD).
[a-d] Mean values in the same row with different superscripts differ significantly at $P < 0.05$. *N*=6.

2. Zhenping et al. (2013) studied the dose-response effects of 0, 0.5, 1, and 2% XOS in the diet (0, 5, 10, or 20 g XOS/kg feed) in male and female day-old Arbor Acres

broiler chicks for 59 days (n=6 wire cages per group with 8 birds per cage; 3 cages of males and 3 cages of females). Body weight gain, feed intake, and feed efficiency were similar to controls for most XOS groups, with the exception of increased body weight gain and improved feed efficiency for the 1% XOS group and increased feed intake for the 2% XOS group. Chicks in the 1% XOS group had higher day 59 body weight and improved feed efficiency; the 2% XOS group had higher feed intake. On day 44, the serum level of triiodothyronine of the 0.5% and 2% XOS groups were greater than control and the serum levels of thyroxine in all XOS groups were greater than control. In addition, the serum insulin levels in 0.5% and 2% XOS groups were greater than control. The antibody titers to H5N1 virus were elevated in the 1% XOS group at day 59.

3. De Maesschalck et al. (2015) used male and female Ross 308-day old broiler chicks in a dietary study of XOS (DP between 2 to 7 derived from corn-cobs) supplementation. Chicks were randomly assigned to 12 pens with 32 chicks/pen and 3 pens of male and 3 pens of females per group. The starter feed (fed from the first day of age until day 13) was supplemented with 0 or 0.2% XOS, and the grower feed (fed from day 14 until day 26) and finisher feed (fed from day 27 until day 39) were supplemented with 0 or 0.5% XOS. Feed efficiency was improved for study days 0 – 26 and 0 – 39. Body weight, feed intake, and body weight gain were not different from control for the XOS group. Supplementation of 0.5% XOS to the broiler feed increased the villus height in the ileum. Inclusion of XOS in broiler feed increased butyrate-producing bacteria and lactobacilli in the cecum and colon, respectively. These bacteria are known for their positive effect on intestinal health.
4. Ribeiro et al. (2018) reported three different experiments with XOS supplementation (DP between 2 to 12) in different basal diets. Day-old male Ross 308 broiler chicks were randomly assigned into 24 pens (n=6 pens per group with 15 birds per pen). In the first experiment, chicks were fed either the control (wheat-based diet) or a 0.06 g XOS/kg supplemented wheat-based diet (0.006% in diet) (data from additional treatment groups are not discussed) for 28 days. The XOS group had higher weight gain and improved feed efficiency compared to control. Feed intake and final body weight were not different.
5. In the second experiment (Riberio et al., 2018) with XOS supplementation (DP between 2 to 12), chicks were fed either the wheat-based control diet or that diet supplemented with 0.1 g/kg feed (0.01% in diet) or 1 g XOS/kg feed (0.1% in diet) for 42 days (data from additional treatment groups are not discussed). Final body weight and body weight gain of birds fed the basal diet supplemented with XOS, at the two levels of incorporation, was higher than those of birds fed the control diet. Differences in body weight were visible as early as day 7 but were particularly acute in the last 2 weeks of the trial, from days 28 to 42. All XOS-supplemented diets resulted in markedly higher feed intake than the control diet in the last week of the trial, although when the entire period of the experiment was considered, no differences among groups were observed. None of the supplements affected crop, gizzard, or liver relative weights, or the length of the small and large intestines.
6. The third experiment with XOS supplementation (DP between 2 to 12) utilized 0, 0.1, 1.0, or 10 g XOS/kg feed (0%, 0.01%, 0.1%, or 1% in the diet) for 42 days

(Riberio et al., 2018). The XOS had a beneficial effect on broiler weight, although the optimal benefit was observed only at lower incorporation rates (0.01% in diet); at higher incorporation levels, XOS had a marginal or no effect on animal performance. Authors noted that the addition of exogenous XOS resulted in a higher feed intake for birds receiving the oligosaccharide at an intermediate level when compared to control, yet there were no differences seen among the supplemented groups. In contrast, no differences in feed efficiency were observed among the four treatments. Animals receiving the XOS preparation displayed a similar gastrointestinal size as control birds.

7. Suo et al. (2015) conducted a 42-day XOS dose-response study (0, 25, 50, 75, or 100 mg XOS/kg diet); (0%, 0.0025%, 0.005%, 0.0075%, or 0.01% in diet) with day old male Arbor Acres broiler chicks (n=90/group; 6 replicates per cage with 15 chickens/cage). Over the 42 days, average daily gain, average daily feed intake, and mortality were similar across all groups. Feed efficiency was improved in the high dose XOS group compared to lower XOS groups and controls. Immune function, as measured via serum Newcastle disease antibody titers, serum H5N1 antibody titers, and blood T-lymphocyte proliferation, was similar between control and XOS groups. Duodenal morphology (villus height, villus width, crypt depth, and villus height to crypt depth) were similar for XOS groups and control with the exception of a lower crypt depth (considered a positive effect for intestinal health) for the 75 mg XOS/kg (0.0075% in diet) group.

8. Yuan et al. (2018) randomly assigned day old Arbor Acre broiler chicks (n=5 replicates per group with 10 chicks per replicate) to 0 or 2 mg XOS/kg feed (0% or 0.0002% in diet) for 42 days (additional treatment group data not discussed). XOS supplementation enhanced average daily feed intake and average daily gain but had no effect on feed efficiency. XOS did not affect the levels of plasma immunoglobulin G or interleukin-2. Cecal acetate and butyrate were increased with XOS supplementation while propionate was similar. Butyrate is a primary energy source for colonic cells and has other effects considered positive for intestinal health.

9. Akter and Akter (2021) conducted a two-week dose response study with Cobb 500 one day old broiler chicks. Chicks were randomly assigned to one of four treatment groups (n=4 replicates per group with 6 chicks per replicate). All chicks received the basal diet on days 1-12. On days 13-26, chicks received control, 0, 2.5, 5.0, or 7.5 g XOS/kg feed (0%, 0.25%, 0.5%, or 0.75% in diet). XOS supplementation had no effect on body weight gain, feed intake, or feed efficiency. There were no changes in serum biochemical parameters (triglycerides, total protein, glutamic pyruvic transaminase, glutamic oxaloacetic transaminase, cholesterol, creatinine, and triiodothyronine), except for glucose, which was increased in the 0.5% XOS treatment group versus all other treatments. Thyroid hormone levels were similar across groups, except thyroxine, which was increased in the 0.25% XOS treatment group versus all others. Upon visceral examination, it was noted that the 0.5% XOS treatment group increased proventriculus relative size over the 0.75% treatment group, but all were similar to the control group. The 0.75% XOS treatment group had reduced gizzard relative weight when compared to control group but not the

other XOS supplementation groups. Liver and pancreas relative weights were higher in the 0.25% XOS treatment group than in the 0.5% and 0.75% XOS treatment groups and, for pancreas relative weight, higher than the control group. However, liver relative weights with supplemental XOS were similar to the control group. There were no observed differences in weight for the small intestine, heart, spleen, and bursa between XOS treatment groups and control group. Carcass traits were also recorded, noting the 0.25% XOS treatment group showed a higher dressing percentage than other treatment groups. Thigh percentage increased and abdominal fat content decreased with the 0.25% and 0.5% XOS treatment groups, and the 0.25% XOS treatment group demonstrated an improved shank growth when compared to other treatment groups.

10. Singh et al. (2021) randomly assigned day old, unsexed Cobb 500 broiler chicks to 36 pens (n=4 pens per group with 8 birds per pen). Treatment groups included 0, 50, or 100 g XOS/metric tons feed (0, 0.005, or 0.01% in diet) and 3 levels of xylanase (0, 8,000, and 16,000 BXU/kg feed) inclusion in 3 x 3 factorial design for the 42-day study period (data from additional treatment groups are not discussed). Growth performance parameters were not different for XOS versus control. XOS supplementation had no effect on relative weight of the liver, gizzard, proventriculus, drumsticks, breast, or abdominal fat. XOS did not influence the villus height-to-crypt depth ratio in the ileum. The immune markers of T-cell (CD3) and B-cell (chB6) in the ileum were not different across treatments on day 42. Inclusion of 0.01% XOS resulted in a higher level of IL-4 (a T-cell differentiation cytokine) and IL-10 (a marker of anti-inflammatory cytokine) in the ileum compared to control. In all treatment groups, there was an increase in total short-chain fatty acids and acetate levels, which increased with higher supplementation with XOS in diet. There were no changes in propionate or butyrate levels when compared to the control group.

11. Wang et al. (2021) randomly assigned day old Ross 308 broiler chicks to a treatment group with 6 replicate pens per group with 8 chicks per pen. Chicks received control or 100 mg XOS/ kg feed (0.01% in diet) for 21 days (data from additional treatment groups are not discussed). XOS inclusion had no effect on average daily feed intake, average daily gain, or feed efficiency. Dietary XOS increased relative weight and length of the duodenum and increased the villus height and villus height-to-crypt depth ratio of all intestinal segments. Goblet cell numbers in the jejunum, but not in the duodenum or ileum, were increased with XOS in the diet. XOS inclusion had no effect on plasma diamine oxidase activity, but lowered plasma D-lactic acid levels when compared to the control, which the authors indicated meant that a healthy intestinal mucosal barrier function was present that would reduce D-lactic acid concentrations in the plasma.

12. Yang et al. (2022) studied the effects of 200 mg XOS/kg feed (0.02% in diet) in day old female Xiang Huang broiler chicks for 5 weeks. Each treatment group had 6 replicates with 10 birds per replicate (data from additional treatment groups are not discussed). XOS supplementation did not alter final body weight, average daily gain, average daily feed intake, or feed efficiency compared to controls. Jejunum and ileum villus height, but not crypt depths, were increased by XOS. Breast and

thigh muscles were increased in the XOS group when compared to controls with non-significant weight increases in liver and spleen. Serum total superoxide dismutase activity was higher in the XOS inclusion group, while copper superoxide dismutase activity was greater in the liver when compared to control group. Serum copper superoxide dismutase activity, glutathione peroxidase activity, and malondialdehyde levels were not affected. There was no effect on hepatic activities of total superoxide dismutase, glutathione peroxidase, or total antioxidant and no effect on malondialdehyde levels. There was only higher activity of glutathione peroxidase in breast muscle with no other differences observed. There were no effects on activities in the thigh muscle. Plasma total protein, albumin, alanine transaminase, glucose, triglycerides, total cholesterol, high density lipoprotein cholesterol, low density lipoprotein cholesterol, diamine oxidase, and immunoglobulin G levels were not affected by XOS. Aspartate transaminase and alkaline phosphatase were elevated by XOS compared to control. Authors attributed the increased liver weight and hepatic enzymes to heat stress experienced by the broilers in study week 5.

13. Morgan et al. (2022a) studied the effect of XOS supplementation (DP between 2-6) on Cobb 500 mixed sex broilers fed sorghum-soybean meal diet. After hatching, chicks were randomly assigned to a treatment group (n=8 replicates per treatment group with 10 or 11 birds per replicate with a total of 80 birds per treatment group) including control, 50, or 2000 mg XOS/kg feed (0%, 0.005%, or 0.2% in diet) for 35 days. Other treatment groups included wheat bran and xylanase in diets (data from additional treatment groups not discussed). Feed intake, body weight gain, feed efficiency corrected for mortality, and final body weight were not different when comparing the wheat bran and xylanase groups to both XOS groups. Short-chain fatty acids (acetic, propionic, isobutyric, butyric, isovaleric, valeric, lactate, and succinic acids) were not different in the XOS group when compared with wheat bran and xylanase groups.

14. Amir et al. (2023) studied the effects of XOS in 800 as-hatched Ross 308 broiler chicks fed a control diet or 0.1g XOS/kg (0.01%) diet for 35 days (n=2 pens per group with 200 birds per pen). XOS feeding improved some performance parameters (bird weight on d 7, 14, 21, and 28 and body weight gain d 7, 14, 21 and 35) compared to controls but body weight gain from days 0 to 35 was not different between the two groups.

15. Deng et al. (2023) randomly assigned one-day old Arbor Acres broilers to one of five treatment groups (8 replicates with 7 birds each) including controls (basal diet) and 100 mg XOS/kg (0.01% in diet) in feed for 21 days (data from the other treatment groups is not discussed). Growth performance parameters (final body weight gain, average daily gain, average daily feed intake, feed efficiency) were not different than controls for the XOS group. Ileum villus height-to-crypt-depth was increased with XOS supplementation compared to controls. Serum levels for immunoglobulin A, immunoglobulin G, and immunoglobulin M were similar between all groups. In serum, XOS inclusion did not affect total antioxidant capacity, superoxide dismutase activity or malondialdehyde levels when compared to the control. Interleukin-6 and tumor necrosis factor α were down-regulated, and

interleukin-10 and transforming growth factor β were up-regulated for XOS inclusion group. XOS inclusion increased butyric acid, valeric acid, and total short-chain fatty acids when compared to control group.

16. Dieryck et al. (2023) studied one day old male Ross 308 broiler chicks receiving a control basal diet or 0.13% XOS feed for days 1-8 and 0.32% XOS feed for days 9-15 (4 groups; n=2 replicates per group with 24 birds per replicate; 15 birds sampled per group). There was no difference in growth performance parameters between the control and XOS groups (body weight, body weight gain, daily feed intake, and mortality). An increase in *Bifidobacteria* was noted in the XOS group compared to control. Though propionic acid and valeric acid concentrations showed a decrease in the XOS group, total short-chain fatty acids concentrations were not statistically different from control group.
17. Li et al. (2023) randomly distributed one day old Arbor Acres chicks to a dose-response study with four XOS treatment groups (n=6 replicates per group with 10 birds per replicate) fed basal diet, 150, 300, or 450 mg XOS/kg feed (0%, 0.015%, 0.03%, or 0.045% in diet) for 42 days. Over the entire study, average daily gain, average daily feed intake, and feed efficiency were similar for all groups except for a higher average daily gain for the 150 mg XOS/kg (0.015% in diet) feed group. There were no differences in breast or thigh muscles in the XOS group when compared to control.
18. Lin et al. (2023) placed Cobb 500 off-sex male broiler chicks (n=49/group; allocated to 8 treatment groups with 7 replicates, with 7 birds per replicate) on a control or 0.5 g XOS/kg feed (0.05% in diet) diets starting at one day of age through 21 days (data from additional treatment groups and data from Eimeria challenge not discussed). *Eimeria* challenge (data not discussed) was performed on day 15 of study. Weight gain, feed intake, and feed efficiency were similar between the control and XOS groups during days 0-15 prior to the *Eimeria* challenge. Short-chain fatty acids were increased in the XOS-supplemented group when compared to control; however, isobutyrate and isovalerate levels were decreased in the XOS group when compared to control. Acetate and butyrate levels were increased, while propionate and valerate levels were slightly decreased in the XOS group when compared to control.

Layers

Four studies were identified with XOS added to the feed of layers. Levels of XOS in diet ranged from 0.0005% (56 days) to 0.6% (70 days), and study durations ranged from 8 to 12 weeks. In all studies, animals fed XOS-supplemented feed had normal egg production and egg quality parameters and normal or improved biochemical parameters. Villus height and villus-height:crypt-depth ratio were also increased in a few studies. When measured, immunoglobulin levels were increased with XOS. Two studies showed positive shifts in intestinal bacterial communities with increased presence *Bifidobacteria* and *Lactobacillus* and decreased presence of *Escherichia coli*, *Bacteroides*, and *Campylobacter*.

1. Ding et al. (2018) randomly assigned White Lohmann laying hens (28 weeks of age; n=6 replicates per group with 10 cages per replicate and 3 birds per cage) to

one of six diets (0, 0.01, 0.02, 0.03, 0.04, or 0.05% XOS supplementation) for 8 weeks. There was a linear improvement in jejunum villus height and villus-height:crypt-depth ratio as dietary XOS concentration increased. The relative length of the jejunum ($P<0.05$) was increased in diets with XOS. Dietary XOS did not affect the relative length of the duodenum or ileum, villus height, crypt depth, or villus-height:crypt-depth ratio. Plasma 1,25(OH)2D3 was quadratically increased as dietary XOS concentration increased up to 0.03% XOS. There were no differences in plasma calcitonin, immunoglobulin G, or parathyroid hormone. XOS supplementation increased plasma immunoglobulin A, immunoglobulin M, interleukin 2, and tumor necrosis factor-α. Cecal *Bifidobacteria* demonstrated an increase in the XOS group, with 0.04% XOS inclusion being the highest when compared to control. *Escherichia coli* showed a decrease in the 0.04% XOS group when compared to control but was higher than control at 0.01 and 0.02% XOS inclusion. Total cecal microbiota count and *Lactobacilli* were not different. Short-chain fatty acids, acetic acid, and butyrate acid were increased in the XOS group when compared to controls.

2. Zhou et al. (2021) fed 50-week-old Hy-Line Brown Laying hens one of three diets with 0, 200, or 400 mg/ XOS/kg (0%, 0.02%, or 0.04% in diet) for 12 weeks (n=8 replicates per group with 12 birds in 4 adjacent cages per replicate). Egg production, average egg weight, average daily feed intake, and feed efficiency were not different across treatment groups for the entire study period. Egg quality parameters were also similar across all the groups except for egg yolk color, valued by the Roche yolk color fan, for the XOS groups. Intestinal morphology (jejunum and ileum) was also similar across all groups with the exception of increased ileal villus-height:crypt-depth for the XOS groups compared to control. XOS inclusion increased the abundance of *Lactobacillus* and decreased the abundance of *Bacteroides* and *Campylobacter*.
3. Morgan et al. (2022b) studied the effects of XOS feed supplementation in Isa Brown layers (39 weeks of age). Hens (n=8 replicate cages per group with individual hens) were fed either a basal diet or basal diet supplemented with XOS at 50 or 2000 g/metric ton (0%, 0.005%, or 0.2% in diet) for 56 days. Other treatment groups included wheat bran and xylanase in diets (additional treatment group data not discussed). For the periods of days 0-14, days 14-28, and days 42-56, feed efficiency and body weight gain were similar between the XOS groups and control. From days 28-42, there was a dose-response-related decline in body weight gain with increasing levels of XOS supplementation. Measures of egg quality taken at day 56 (yolk color, yolk height, shell reflectivity, and shell thickness) were not different from control for both XOS groups. Day 56 shell breaking strength was greater for the 0.005% XOS group compared to the 0.2% XOS inclusion group and control. There was no effect on microbiota concentration in the ceca in the XOS group and there was no effect on short-chain fatty acid concentrations.
4. Wen et al. (2022) randomly assigned 72-week-old Hy-Line Brown laying hens to one of four treatment groups (6 replicates per treatment, and 12 hens per replicate): 0, 2, 4, or 6 g XOS/kg (0%, 0.2%, 0.4%, or 0.6%) in feed for 10 weeks. There were no differences in feed intake or mortality across groups. There were some scattered

differences in egg laying rate during the study and by weeks 81 and 84, the rate for the XOS groups was greater than control. Dietary treatments increased the grams of egg mass (g/hen) compared to controls during week 73 to 84, with no differences across the three XOS levels. Liver weight % was lower than control at 6 g/kg XOS (0.6% in diet) but not at other levels. XOS supplementation resulted in decreased body fat deposition. Levels of cholesterol and triglyceride at weeks 76, 80, and 84 were decreased in all XOS groups compared to controls. At 84 weeks, hens in the XOS groups had an increase in the number of large follicles and ovary weight as a percentage of body weight. Serum reproductive hormones were greater in XOS inclusion group when compared to control. There was an increase in luteinizing hormone at 80 and 84 weeks and follicle-stimulating hormone and progesterone at 76, 80, and 84 weeks for the XOS groups.

Safety in Swine

There were 12 studies with XOS use in swine feed. Levels of XOS in feed ranged from 0.01% (up to 56 days) to 3% (28 days), and study duration ranged from 28 – 84 days. In all studies, animals fed an XOS-supplemented feed had normal growth performance parameters and mortality was not different than controls. A few studies noted decreased diarrhea occurrence, improved intestinal morphology, and greater short-chain fatty acid concentrations with XOS supplementation. When evaluated, hematological and biochemical parameters and immunoglobin levels were somewhat improved or not different from untreated controls. XOS supplementation was also associated with positive shifts in intestinal bacterial communities with an increased presence of *Bifidobacteria* and *Lactobacillus* and a decreased presence of *Escherichia coli* and *Clostridium*.

1. Liu et al. (2018) randomly distributed weanling piglets ([Yorkshire x Landrace] x Duroc; 6.3±0.15 kg initial body weight) to a control or 200 mg XOS/kg diet (0% and 0.02%) (n=6 replicate pens per group with 5 castrated males and 5 females per pen) for a 28-day study that either included or did not include probiotics (*Enterococcus faecium* and *Bifidobacterium subtilis* endospores). Data without XOS inclusion are not reported. Average daily gain, average daily feed intake, and feed efficiency for the XOS group were greater, when compared to control. There was improved gain-to-feed ratio during days 15-28 and for the overall duration of the study. Serum clinical chemistry parameter values (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total protein, albumin, glucose, triglyceride, and total cholesterol) for the XOS groups were not significantly different from control. Intestinal morphology of the duodenum, jejunum, and ileum demonstrated an improvement in the villus-height:crypt-depth ratio of the jejunum. Concentration of jejunal mucosal enzymes, trypsin and amylase, were also greater in the XOS treatment groups when compared to probiotic inclusion with XOS and control. XOS inclusion increased *Lactobacilii* while decreasing *E. coli* counts on day 14, but these effects subsided by day 28.
2. Yin et al. (2019) randomly assigned male weaned piglets (Landrace × Large White, 7.44±0.47 kg initial body weight) to a control (n=9) and XOS-supplemented diet (n=10) for 28 days at 0.01% XOS in diet. Pigs were individually housed. Final body weight, body weight gain, feed intake, feed efficiency, and liver weights were not

affected by XOS. Spleen weight was decreased by XOS. XOS supplementation reduced red blood cell distribution width, but had no effect on white blood cell, hemoglobin, red blood cells, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelets, mean platelet volume, total protein, albumin, alanine transaminase, aspartate transaminase, alkaline phosphatase, blood urea nitrogen, creatinine, glucose, c-reactive protein, bile acid, or immunoglobulin G. Villus surface area was not changed by XOS.

3. Hou et al. (2020) examined the effects of XOS in nursery pigs (18.94±0.24 kg initial body weight). Ningxiang pigs were divided into three treatment groups (n=4 replicate pens per group and 6 pigs per replicate) after a 7-day acclimation period. Pigs were fed twice a day with the control basal diet, or a diet supplemented with 0.04% XOS (DP 2-4 comprising >35% of XOS) for 28 days (data from the third treatment group not discussed). Final body weight and average daily gain was higher for the XOS group versus the control. Average daily feed intake and feed efficiency were not different. Serum clinical chemistry parameters (total protein, albumin, alanine aminotransferase, aspartate aminotransferase, total cholesterol, amylase, and alkaline phosphatase) were similar between the control and XOS groups except for lower blood urea nitrogen and triglyceride with XOS supplementation. Glucose was significantly elevated, which can occur in high yield animals. Serum immunoglobulin G content and glutathione peroxidase were higher in the XOS group when compared to control. Antioxidant capacity, superoxide dismutase, and catalase were increased in concentration and malondialdehyde levels were decreased in the XOS inclusion group when compared to control which the authors categorized as an overall beneficial effect on *in vivo* antioxidant capacity.

4. Cai et al. (2021) studied the effects of XOS in Huanjiang mini-pigs. Weaned pigs (9.5±0.05 kg initial body weight) were divided into one of four treatment groups (n=8 replicate pens with 2 male and 2 female pigs per pen). One pig per pen was slaughtered on d 28, 56, and 84. Pigs in the control group received the basal diet or 0.02% XOS (xylose and DP 3-4 comprising > 35% XOS) in their diet for 84 days. Data from the other two groups are not discussed. Growth performance parameters (average daily feed intake, average daily gain, feed efficiency) were not different for the XOS group compared to control. *Longissimus dorsi* muscle examination saw a decrease in glutamate, isoleucine, leucine, methionine, tyrosine, valine, and essential amino acids on day 28, and on day 56 there was an increase in histidine, lysine, methionine, phenylalanine, and essential amino acids. At day 82, the XOS group had an increase in crude protein, alanine, arginine, aspartate, glutamate, isoleucine, leucine, lysine, methionine, proline, serine, threonine, tyrosine, valine, essential amino acids (defined by the author as His + Ile + Leu + Lys + Met + Phe + Thr + Val), flavor amino acids (defined by the author as Ala + Asp + Arg + Glu + Gly), non-essential amino acids (defined by the author as Arg + Asp + Ala + Glu + Gly + Pro + Ser + Tyr), and total amino acids (defined by the author as essential amino acids + non-essential amino acids). Carcass traits were similar between groups except for the XOS-supplemented group that had a higher carcass yield, lower muscle pH, and backfat thickness on day 84 when compared to control.

5. Chen et al. (2021a) exposed 180 (Duroc x Landrace x Large White) weaned piglets (8.84 ± 0.25 kg) divided into three treatment groups including control and 500 mg XOS/kg diet (0.05% in diet) for 28 days (positive control data are not discussed) following establishing 0.05% XOS (DP 2-7) as the optimal feed inclusion level (Chen et al., 2021b). The 0.05% group had improved body weight on day 28 and average daily gain and improved feed efficiency during days 1–28. The 0.05% group had increased villus height and villus height-to-crypt depth ratio in the ileum. Additionally, the 0.05% group had increased numbers of goblet cells in the crypt of the cecum and improved the bacterial diversity. *Lactobacillus* was higher and *Clostridum* levels were decreased in the XOS group when compared to the control and other treatment group. The 0.05% XOS group had increased total short-chain fatty acids, propionate, and butyrate concentrations and decreased acetate concentration in the cecum compared to the control.

6. Chen et al. (2021b) examined the dose-response effects of XOS in weaned piglets (Duroc x Landrace x Large White). Piglets (8.82 ± 0.05 kg) received diets with 0, 100, 500, or 1000 mg XOS/kg (0%, 0.01%, 0.05%, or 0.1% in diet; DP length 2-7) for 28 days (n=15 pigs/pen; 4 replicates). The 0.05% XOS group had improved body weight on day 28 and average daily gain and feed efficiency during days 1–28. The 0.05% supplementation resulted in increased villus height and villus height-to-crypt depth ratio in the jejunum and ileum. Performance data for the other two XOS groups were not different from controls. Serum immunoglobulin G concentration was greater for the 0.05% group compared to control with no differences seen for immunoglobulin A and immunoglobulin M. Ileal tissue was examined for intestinal epithelium immune-related genes (interleukin-1β, interleukin-6, interleukin-8, interferon-γ, interleukin-10, and tumor growth factor-β). The 0.05% XOS group's interleukin-10 mRNA expression level was higher when compared to other treatment groups and control. Similarly, the 0.05% XOS group had lower mRNA expression levels of interleukin-1β and interferon-γ when compared to other treatment groups and control. The authors interpreted the changes as indicative of improved intestinal health.

7. Ding et al. (2021) studied the effects of XOS (DP 2-4) supplementation in Large White x Landrace castrated male piglets (7.84 ± 0.02 kg) that were fed a control diet for three days to acclimate and were then randomly assigned to one of four groups with 0, 250, or 500 g XOS/metric ton feed (0%, 0.025%, or 0.05% in diet) and fed for 42 days with or without the inclusion of probiotic *Bacillus subtilis* (data from treatments with *Bacillus subtilis* were not reported). Each had 8 replicates per group with 4 piglets per replicate. Piglets were fed nursery diets from days 1-21 and late nursery diets for days 22-42. Average daily feed intake, average daily gain, feed efficiency, and crypt depth were not changed by XOS supplementation. Ileal villus height and villus-height:crypt-depth ratio were increased in XOS treatment group when compared to the control. Short-chain fatty acids were measured in the ileum and colon, with ileal butyrate levels being higher in the XOS group when compared to control. Levels of acetate, propionate, valerate, colonic butyrate, isovalerate, and isobutyrate were similar among all groups. Microbiota examinations revealed that the XOS group had higher levels of *Acinetobacter*,

Herbaspirillum, *Xanthobacter*, *Sharpea*, *Bifidobacterium*, *Slackia*, and *Veillonella* when compared to control group.

8. Pang et al. (2021) studied XOS supplementation (0, 0.75, 1.5, or 3% of feed) in weaning (Duroc x Landrace x Large White) piglets (7.50 ±0.8 kg) for 28 days. Each group had six replicate pens with 8 piglets per pen. Diets supplemented with XOS at all levels had increased average daily gain and the 1.5% XOS group had increased average daily feed intake. Feed efficiency showed linear improvement with increasing XOS, and incidence of diarrhea was improved for the 0.75% and 1.5% XOS groups. Day 28 serum biochemical parameters (total protein, albumin, globulin, albumin:globulin ratio, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and blood urea nitrogen) were similar to controls for all XOS groups. There were no effects on the activity of total superoxide dismutase, catalase, or total antioxidant capacity. Total bacteria count was similar between 1.5% XOS group and control; however, *Bifidobacterium* and *Lactobacillus* were higher when compared to control (levels for 0.75% and 3% XOS were not reported). Total short-chain fatty acid levels in feces showed linear increases for the XOS groups compared to control. XOS treatment increased immunoglobulin M (at 0.75% and 1.5% XOS inclusion), immunoglobulin G (1.5% and 3% XOS inclusion), and immunoglobulin A (all XOS groups). The 1.5% XOS group had an increased interleukin-10 level compared to the control group. Serum levels of interleukin-6 decreased in XOS groups when compared to the control group. Interleukin-1β levels decreased for the 1.5 and 3% XOS groups when compared to the control group. The authors indicated that the shifts in interleukins were indicative of improved immune function.

9. Su et al. (2021) randomly assigned Duroc × Landrace × Large White weaned piglets (7.02 ± 0.05 kg) diets with 0, 100, 250, or 500 g XOS/metric ton (0%, 0.01%, 0.025%, or 0.05% in diet) for 56 days (n=6 replicates per group with 5 piglets per replicate). XOS was comprised of DP 2-4 at > 35%. The XOS-supplemented groups showed changes in jejunal villus height, crypt depth, and villus-height:crypt-depth ratio, when compared to control. The ileal villus height was also different in the 100 g/metric ton XOS group when compared to control. Fecal microbiota were measured on days 7, 21, and 56. *Lactobacillus* was lower on day 21 in the 250 and 500 g/metric ton XOS group but on day 56 was higher in the 100 g/metric ton XOS group. *Bifidobacterium* spp. was similar from day 7 through day 21 but was higher by day 56 in all XOS inclusion groups when compared to the control. Short-chain fatty acids were also measured, with acetic acid being higher in the XOS group for days 7 and 56 when compared to control. Isovaleric acid was higher in the 100 g/metric ton XOS inclusion group on day 21. Additionally, butyric acid was higher in XOS groups at day 56. Propionic acid, valeric acid, and isobutyric acid were similar among all XOS groups and control. Zonula occludens-1 (a scaffold protein that anchors tight junction strand proteins) mRNA levels were upregulated in the XOS groups when compared to controls.

10. Gao et al. (2022) studied the effects of 1 g XOS/kg (0.1%) feed supplementation in Duroc × Landrace × Large White weaned piglets (7.24 ± 0.19 kg) for 28 days. Piglets were assigned to one of four treatment groups (n=6 replicates per group with

4 piglets per sex per replicate; data from additional treatment groups are not discussed). Final body weight, average daily gain, average daily feed intake, and feed efficiency were similar between the control and XOS group. Incidence of diarrhea was decreased for the XOS group compared to control and ileal villus height was increased for the XOS group. Ileal lactate and acetate concentrations were higher in XOS group when compared to control. Colonic total short-chain fatty acid concentration was similar between XOS group and control, although lactate levels were lower in the XOS group and propionate was higher when compared to control. Lactate was proportionally lower in XOS group when compared to control. XOS increased the concentrations of secretory immunoglobulin A, interleukin-10, superoxide dismutase, glutathione peroxidase, and total antioxidant capacity while there was a reduced content of interleukin-6 and interleukin-12 in the ileal and colonic mucosa.

11. Sun et al. (2023) examined the effects of XOS (DP 2-4) on the growth performance of male and female weaned Duroc×(Landrace×LargeWhite) piglets (7.24 ± 0.19 kg). Piglets were randomly assigned to control or 1% XOS feed (n=48/group; 8 replicates per treatment with 3 barrows and 3 gilts per replicate) for 28 days (data from a third treatment group are not discussed). Average daily gain, average daily feed intake, and feed efficiency were not different between control and the XOS group. There was an increase in the digestibility of neutral detergent fiber and acid detergent fiber in the XOS group as compared to the control group and there were no effects on the apparent total tract digestibility of dry matter, crude protein, or gross energy. The incidence of diarrhea was decreased for the XOS group. Ileal villus height and colonic crypt depth were higher in XOS group when compared to control, while ileal crypt depth and villous crypt ratio had no effects. XOS inclusion increased the activity of superoxide dismutase and glutathione peroxidase in the ileum and increased activity of superoxide dismutase and total antioxidant capacity when compared to the control. There was a decrease in ileal and colonic interleukin-6 levels and colonic secretory immunoglobulin A and interleukin-10 concentrations in the XOS group as compared to the control group. As regards, *in vitro* fermentation, ileal *Lactobacillus* and *Bifidobacterium* concentrations were higher in the XOS group, and in the colon, *Bifidobacterium* was higher in the XOS group. Ileal acetate, lactate, and total organic acid concentrations were higher in the XOS group when compared to control, where colonic short-chain fatty acids were similar in concentration.

12. Wang et al. (2023) studied the effects of XOS supplementation in two different plant-based diets. Weanling piglets ([Landrace×Yorkshire]×Yorkshire; 7.6 ± 0.45 kg) were randomly divided into treatment groups with 8 pens per treatment with 2 barrows and 2 gilts per pen for the 28-day study. Animals received either a 68.3% plant-based protein diet (LP) or an 81.27% plant-based diet (HP) and each was further divided into 0.43% XOS-supplemented or non-XOS groups. Over 28 days, there was no difference in average daily feed intake, average daily gain, and feed efficiency across the groups. Villus height and crypt depth in the duodenum, jejunum, and ileum were similar for all four treatment groups. Cecal short-chain fatty acid concentrations for acetate, propionate, and valerate were similar between XOS and control. Cecal butyric acid was greater in the XOS group when compared

to control. Colonic short-chain fatty acids, butyrate and valerate, were higher in the XOS group when compared to control.

Safety in Fish

Fifteen published studies were found examining the effects of XOS feed supplementation in fish. Species studied included carp, sea bass, blunt snout bream, seabream, Caspian white fish, tilapia, rainbow trout, white shrimp, and grouper which represent a variety of feeding behaviors including herbivores, omnivores, carnivores, and detrivores. Feed supplementation ranged from 0.005% for 45 days to 3% XOS for 56 days. In all studies, animals fed an XOS-supplemented feed had normal to improved growth performance parameters. Survival rates were also similar to controls. Intestinal morphology was improved with XOS in feed.

1. Xu et al. (2009) reported on a dose-response study with XOS in allogynogenetic crucian carp (*Carassius auratus gibelio*). Juvenile carp underwent a two-week acclimation period on basal diet and were then divided into 12 aquaria at 16.88 – 17.56 g body weight (n=3 aquaria per group with 20 fish per aquaria). Treatment groups were 0, 50 mg, 100 mg, or 200 mg XOS/kg dry feed (0, 0.005, 0.01, or 0.02% in feed) provided three times a day for 45 days. Final body weight was higher in the 0.005% and 0.01% XOS groups compared to control and 0.02% XOS. Survival was 100% for all groups. Relative gain rate and daily weight gain were higher for all XOS groups compared to control. Additionally, protease activity of the hepatopancreas was higher in the 0.005% and 0.01% XOS groups and amylase activity was higher in the 0.01% XOS group.
2. Guerreiro et al. (2015) studied the effects of XOS on European sea bass (*Dicentrarchus labrax*). Two basal diets were formulated: one primarily fish meal (FM) and one with fish meal and plant protein (30:70). XOS was added at 1% to each of these basal diets. Juvenile sea bass were acclimated on commercial diet for 15 days before being randomly assigned to a treatment group at an initial body weight of 60 ± 0.01 g (n=three 100 L tanks per group with 20 fish per tank) for 7 weeks (data from additional treatment groups are not discussed). Addition of XOS to the fish meal diet had no effect on growth performance parameters. Addition of XOS to the plant protein diet improved final body weight and body weight gain compared to the plant protein diet control. There were no changes to plasma glucose, cholesterol, total lipids, or triglycerides with XOS supplementation to either diet. Liver hepatosomatic index, glycogen, and lipids were also similar across diets. Hepatic glucokinase levels were greater in fish meal supplemented with XOS when compared to control. XOS supplementation decreased the activity of glucose-6-phosphate dehydrogenase, fatty acid synthetase, and malic enzyme.
3. Abasubong et al. (2018a) studied the effects of XOS supplementation on blunt snout bream (*Megalobrama amblycephala*). Treatment groups included a control fish meal diet, a control rice protein concentrate diet, and rice protein concentrate diets supplemented with 0.5%, 1.5%, 2.3%, and 3% XOS. Young breams were acclimated for two weeks on a control fishmeal diet before being randomly distributed at 46.85 ± 0.34 g to 24 floating net cages (1x1x1 m) with 4 net cages

per group and 20 fish per net cage. Fish were hand-fed three times a day for eight weeks. Final body weight, feed intake, and weight gain were lower in the 0.5%, 2.3%, and 3% XOS supplement groups, and higher in the 1.5% XOS-supplemented group when compared to control. Efficiency was similar to fishmeal controls for the XOS groups.

4. Van Doan et al. (2020) studied the effects of XOS feed supplementation in Nile Tilapia (*Oreochromis niloticus*). Fingerling fish (~5 g) were acclimated for two weeks and randomly distributed to sixteen 150 L glass tanks (20 fish/tank). Fish received control or 10 g XOS/kg (1%) diet twice a day for 12 weeks (data from two other treatment groups not discussed). Growth performance parameters, including final body weight, weight gain, specific growth weight, and feed efficiency were all improved for the XOS-fed fish compared to control. XOS also improved skin mucus enzyme activity compared to control, a measure of immune function. Serum lysozyme activity was higher in the XOS group when compared to control. Serum peroxidase, alternative complement, and phagocytosis activity were similar between XOS group and control.

5. Abasubong et al. (2022) conducted an XOS dose response study (0, 0.5%, 1%, 2%, or 3% XOS) with common carp (*Cyprinus carpio*) fed a high fat diet. Juvenile carp (19.61 ± 0.96 g) were acclimated for 2 weeks on a control diet and then randomly assigned to one of 24 culture tanks (2 x 1 x 1 m) (n=4 tanks per group with 8 fish per tank). A basal control (lower fat) diet was also included in the study. Diets were fed three times a day for 56 days. Body mass index was higher for the high fat diet + 1% XOS group compared to both basal diet and high fat diet controls. Feed efficiency was improved for the high fat diet + 1% XOS group compared to the high fat control but not the basal diet control. Mid-intestine villus length was greater for the high fat diet + 1% XOS group compared to both controls, while villus thickness was greater than both controls for the high fat diet + 1% XOS and high fat diet + 2% XOS groups. Compared to the high fat diet control, XOS treatment did not affect plasma glucose, total protein, albumin, aspartate transaminase, or cortisol. Alanine transaminase was significantly decreased compared to the high fat diet control. Protease and alkaline phosphatase activity were significantly increased compared to the high fat diet control. Plasma biochemical parameters for up to 3% XOS in feed were similar to the basal control. The high fat diet control had higher aspartate aminotransaminase, alanine transaminase, and cortisol levels than the basal diet control and these same parameters were similar to the basal diet control for the high fat diet + 1% and high fat diet +2% XOS groups (aspartate aminotransaminase and cortisol) and all XOS groups for alanine transaminase. Immunoglobulin M levels were higher for the high fat diet + 1% XOS and high fat diet+2% XOS groups compared to both controls.

6. Zhu et al. (2023) evaluated the effects of XOS in the juvenile hybrid grouper (*Epinephelus lanceolatus*♀×*Epinephelus fuscoguttatus*♂). Grouper (27.48 ± 0.63 g) were acclimated for two weeks on basal diet and then randomly assigned to 300 L tanks (n=3 tanks per group with 35 fish per tank). Fish were fed twice a day for 4 weeks with control or 0.05% XOS (data from four other treatment groups not discussed). Weight gain and specific growth rate were higher for the XOS group

versus controls and feed efficiency was not different from control. Intestinal villi length and muscle thickness were higher in the XOS group compared to control, while the villi width was similar between the XOS and control groups. XOS supplementation had an impact on antioxidant levels, being higher for glutathione peroxidase, catalase, and superoxide dismutase. Malondialdehyde was lower in the XOS group when compared to control. The XOS group also had an increased survival rate in a 96-hour ammonia-nitrogen stress test, a crowding stress test, and after a *Vibrio harveyi* bacterial challenge.

7. Abasubong et al. (2018b) investigated the effects of XOS on common carp (*Cyprinus carpio*). Carp (19 ± 0.96 g) were randomly allocated into 6 testing groups (n=4 tanks per group with 8 fish per tank). Carp were fed a commercial diet, three times per day, for two weeks to acclimate to test conditions. Fish were fed a control diet, a low-fat diet, and a high fat diet that was delineated into 4 supplemented high fat diets with XOS inclusion at 5, 10, 20, and 30 g/kg XOS (0.5%, 1%, 2%, and 3% XOS inclusion) for 8 weeks. Weight gain, specific growth rate, and final weights were higher in the 1% XOS inclusion group when compared to control and high-fat diet without XOS, while feed conversion ratio was improved in the 1% XOS inclusion when compared to the high-fat diet without XOS. There was a decrease in the hepatosomatic index in the 1% and 2% XOS inclusion when compared to the high-fat diet while there was an increase in the hepatosomatic index in the 3%XOS inclusion when compared to the control group. XOS inclusion at 0.5 and 3.0% had increases in total cholesterol and triglyceride levels as compared to the control group, while only inclusion of 1% XOS had an effect on the high-density lipoprotein level. The low-density lipoprotein and non-esterified fatty acid levels decreased in all XOS inclusion groups as compared to the high-fat diet group.

8. Morshedi et al. (2018) evaluated the effects of XOS inclusion on juvenile seabream (*Sparidentex hasta*). Seabream were acclimated to test conditions for 2 weeks on a commercial diet, ending acclimation with an average initial body weight of 35.64 g. Seabream were separated into fifteen, 250 L tanks (triplicate groups per dietary treatment), at 20 fish per tank. Diets were supplemented with 5 and 10 g/kg XOS (0.5% or 1% XOS inclusion), at two feedings per day, for 56 days. There were no effects on growth and feeding performances (i.e., final weights, weight gains, specific growth rate, feed conversion ratio or protein efficiency ratio) in the XOS inclusion groups. Hematological parameters showed no effects on red blood cell count, hematocrit content, and hemoglobin concentration between XOS inclusion groups and control. White blood cell count had differences between control and XOS inclusion groups, with 0.5% having a higher white blood cell count than control or 1% XOS inclusion, and 1% XOS inclusion being lower than control or 0.5% XOS inclusion.

9. Sun et al. (2019) conducted a trial to evaluate the effects of XOS inclusion on juvenile white shrimp (*Litopenaeus vannamei*). Shrimp were acclimated for 2 weeks to experimental conditions, with a total of 600 shrimp (initial body weights: 0.1 g) divided into 40 shrimp per tank, run in triplicate groups. Diets were supplemented with 0.1%, 0.2%, 0.4%, and 0.6% XOS inclusion, fed four times daily, for 60 days. Final body weights and weight gain were similar between XOS

inclusion groups and control. Feeding efficiency and survival rate were significantly higher in the XOS inclusion groups when compared to controls (with the exception of no effect on the 0.2% inclusion group). XOS inclusion showed higher values of intestinal villi length and villi area when compared to control. Resistance to *Vibriosis alginolyticus* was significantly higher in the 0.2% XOS inclusion group and higher but was similar between 0.1% inclusion and control. There were no effects on albumin and lysozyme levels after a 72 h challenge. There was an increase in superoxide dismutase levels in the 0.4% XOS inclusion group and an increase in phenoloxidase levels in the 0.6% XOS inclusion group as compared to the control group. Comparing immune parameter levels at 0 h and 72 h challenges demonstrated effects on levels of albumin, superoxide dismutase, and lysozyme in the 0.1% XOS inclusion and decreases in levels of phenoloxidase and lysozyme at 0.2 and 0.4% XOS inclusion.

10. Hoseinifar et al. (2014) conducted a study to investigate the effects of XOS inclusion on Caspian white fish (*Rutilius frisii kutum*). Caspian white fish were acclimated for 2 weeks prior to study start: thereafter, they were stocked in twelve 60 L tanks (n = 30/tank). Fish were fed XOS inclusion diets of 1%, 2%, and 3%, twice a day, for 8 weeks, with an initial body weight of 1.54 g. Intestinal microbiotas (microbiota and autochthonous LAB) were increased in all XOS inclusion groups when compared to control. Intestinal histomorphology, via villus and enterocyte height, were similar among XOS inclusion groups and control group. There were no significant differences in growth performance (ie. final body weight, weight gain, growth rate, and feed conversion ratio) in XOS inclusion groups when compared to controls.

11. Zhang et al. (2020) investigated the effects of XOS inclusion on juvenile grass carp (*Ctenopharyngodon idella*). Juvenile carp were acclimated for 2 weeks prior to study start and were thereafter divided into eighteen 280 L tanks (n = 30/tank, 3 replicates per diet). Fish were fed XOS inclusion diets of 0.05%, 0.1%, 0.2%, 0.4%, and 0.6% XOS, fed twice daily, for 8 weeks, with an initial body weight of 3.05 g. 0.05%, 0.1%, and 0.2% XOS inclusion groups had an increase in final body weights, weight gain, and specific growth rate when compared to the control group and the 0.6% XOS group. Feed conversion ratio and survival rate were similar across all inclusion groups and control. Morphological examinations of intraperitoneal fat, viscerosomatic index, and hepatosomatic index were similar between inclusion groups and control. There were decreases in alkaline phosphatase levels in the 0.05, 0.1, 0.4, and 0.6% XOS inclusion groups and decreases in alanine aminotransferase levels in the 0.05, 0.2, and 0.6% XOS inclusion groups. There were decreases in aspartate aminotransferase levels in the 0.05 and 0.4% XOS inclusion groups and decreases in triglyceride levels in the 0.05, 0.1, and 0.6% XOS inclusion groups. There were no effects on cholesterol levels. Immunity was measured via lysozyme activity and malondialdehyde content, where the 0.1% XOS inclusion group had increased lysozyme activity and decreased malondialdehyde plasma levels.

12. Wang et al. (2022) investigated the effects of XOS inclusion on juvenile rainbow trout (*Oncorhynchus mykiss*). Fish were acclimated for 14 days prior to study

(initial body weight 20.85 ± 0.48 g) and separated into fifteen 300 L tanks (n = 30/tank, 3 replicates per treatment). Trout were fed diets with 0.25%, 0.5%, 0.75%, and 1% XOS inclusion, four times a day, for 56 days. Final body weights were significantly higher in the 0.75% and 1% XOS inclusion groups. Weight gain rate increased only in the 1% XOS inclusion group. The 0.75% and 1% XOS inclusion groups also had increased specific growth rates when compared to control. Amylase activity was significantly higher in the 0.5%, 0.75%, and 1% XOS inclusion groups when compared to control, with lipase activity being higher in the 0.75% inclusion when compared to control. Villus height increased in the 0.75% and 1% XOS inclusion groups. Crypt depth showed an increasing trend with increased amounts of XOS inclusion. Muscular thickness increased in the 0.5%, 0.75%, and 1% XOS inclusion groups.

13. Van Doan et al. (2018) conducted a study to determine the effects of XOS inclusion on Nile tilapia fingerlings (*Oreochromis niloticus*). Fish were acclimated for 2 weeks prior to study (initial body weights 20.72 ± 0.02g) and separated into sixteen 150 L tanks (n = 20/tank, with four replicates per treatment). Tilapia were fed diets with 0.5%, 1%, and 2% XOS inclusion, twice a day, for 8 weeks. Skin mucus peroxidase, lysozyme, and alternative complement activities were increased in all XOS inclusion groups. There were increases in final body weight, weight gain, growth rate, and feed conversion ratio in all XOS inclusion groups as compared to the control group.

14. Poolsawat et al. (2021) investigated the effects of XOS inclusion on tilapia (*Oreochromis niloticus* x *Oreochromis aureus*). Fish were acclimated for 2 weeks prior to study (initial body weight 5 g) and separated into three indoor pools with 5 cages in each pool (n = 25/tank, with three replicates per treatment). Tilapia were fed diets with 0.05%, 0.1%, 0.2%, and 0.4% XOS, three times a day, inclusion for 8 weeks. Final body weights, weight gain, and specific growth rate increased in the XOS inclusion groups when compared to controls. Feed conversion was improved in all XOS inclusion groups when compared to control. Hepatosomatic index increased in the 0.4% XOS inclusion groups. Viscerosomatic index increased in all XOS inclusion groups when compared to control. Protein and lipid retention was increased in the XOS inclusion groups when compared to controls. There was no effect on the apparent digestibility coefficient of crude protein or crude lipid. The apparent digestibility coefficient of dry matter was increased in the 0.2% XOS inclusion group. The 0.1%, 0.2%, and 0.4% XOS inclusion groups had significantly higher alkaline phosphate levels when compared to control. Acid phosphatase levels were also significantly higher in all but the 0.4% XOS inclusion group when compared to control. Nitric oxide levels were similar between all test groups, with lysozyme being similar as well except for the 0.4% inclusion group which was significantly higher. Superoxide dismutase activity increased in XOS inclusion groups when compared to control, with no effect on malondialdehyde activity. Catalase activity was increased in the 0.2% and 0.4% XOS inclusion groups when compared to control. Amylase activity increased in all XOS inclusion groups while protease activity was increased only in the 0.05%, 0.1%, and 0.2% XOS groups. Villus height was greater in the 0.1%, 0.2%, and 0.4% XOS inclusion groups when compared to controls, and all XOS inclusion groups had a decrease in

villus width when compared to control. The 0.4% XOS inclusion group had an increase in muscular thickness.

15. Chen et al. (2022) investigated the effects of XOS inclusion on the diets of blunt snout bream (*Megalobrama amblycephala*). Fish were acclimated for 2 weeks prior to study (initial body weights 44.69 $\pm$ 0.11 g) and separated into twenty-four floating net cages (n = 15/cage, with four replicates per treatment). Bream were fed diets with 0.5%, 1%, 1.5%, and 2% XOS inclusion, three times daily, for 12 weeks. Final body weights, weight gain, intraperitoneal fat ratio, survival rates, feed intake, protein efficiency, hepatosomatic index, and visceralsomatic index showed no significant differences among the inclusion groups when compared to control. Nitrogen and energy retention percent were significantly greater in XOS inclusion groups when compared to control. Liver glycogen levels showed an increase at 1.5% and 2% XOS inclusion. Liver lipid contents were increased in the 1.5% and 2% XOS inclusion group and muscle lipid content levels increased in the 1%, 1.5%, and 2% XOS inclusion groups. Plasma glucose showed no significant difference amongst treatment groups when compared to controls. Plasma triglycerides, total cholesterol, and glycated serum protein all decreased linearly with increasing levels of XOS inclusion.

Safety of Residual Xylooligosaccharides

Published data were found on the exposure to and consumption of XOS by humans. These data have been extracted from *Notice to US Food and Drug Administration of the Conclusion that the Intended Use of Prenexus Xylooligosaccharides (XOS), Derived from Sugarcane (XOS95®) is Generally Recognized as Safe.* This document was submitted to the Division of Biotechnology and GRAS Notice Review, Office of Food Additive Safety (HFS-200), Center for Food Safety and Applied Nutrition, Food and Drug Administration, on September 27, 2018.

As part of an animal feed ingredient approval, potential human exposure through consumption of meat, milk, and eggs produced by animals consuming the proposed new ingredient must be assessed. Data are available from the European Food Safety Authority (EFSA) and FDA GRAS Notices relative to direct consumption of XOS in human foods. However, as a feed additive for poultry, swine, and fish, XOS will be fermented by the gut flora, and the fermentation products will be metabolized by the fed animal, negating any exposure via consumed meat or eggs.

Safety in Other Animals

Published safety and toxicity data in relevant species (e.g., monogastric species) were identified in an EFSA review (2018) and are also summarized in several FDA GRAS notifications (FDA, 2013; 2019).

ADME (absorption, distribution, metabolism, excretion)

A diet delivering 2.4 g XOS/kg bw/day (Xylooligo 95®, obtained from birch wood hydrolyzed by *Trichoderma*-derived xylanase, consisting predominantly of xylobiose, xylotriose, xylotetraose) and 5% cellulose was fed to 10 male Sprague–Dawley rats for 14 days (Campbell et al., 1997, as cited by FDA, 2013; EFSA, 2018). XOS treatment increased cecal and fecal total anerobic bacteria, bifidobacteria, and short-chain fatty acids compared to controls.

Genotoxicity

Two bacterial reverse mutation studies (Ames test) with *Salmonella typhimurium* have been reported with XOS at concentrations up to 5,000 ug/plate. Both studies were negative with and without metabolic activation; however, neither met the OECD TG 471 because each study was missing one or more strains of *S. typhimurium* (Oh et al., 1999 as cited by FDA, 2013; Fu et al., 2012 as cited by FDA, 2013; 2019; EFSA, 2018).

A bone marrow micronucleus test was reported by Fu et al. (2012, as cited by FDA, 2013; 2019; EFSA, 2018). Mice (5/sex/group) received 2.5, 5.0, and 10.0 g XOS/kg bw via gavage twice in 24 hours. There were no differences in the rate of micronucleated polychromatic erythrocytes between XOS and control groups.

Acute Toxicity

Acute toxicity studies were done with XOS in rats and mice. The oral LD_{50} was determined to be greater than 10,000 mg XOS/kg bw in rats (Park et al., 1999 as cited by FDA, 2013; 2019; EFSA, 2018) and greater than 32 g XOS/kg bw in mice (Gao et al., 2012, as cited by FDA, 2013; 2019; EFSA, 2018).

Repeat Dose Toxicity

Rats

Park et al. (1999 as cited by FDA, 2013; 2019; EFSA, 2018) administered 0, 333, 1000, or 3000 mg XOS/kg bw/day, orally, to Sprague-Dawley rats (n=10/sex/group) for 13 weeks. XOS treatment did not cause mortality, adverse clinical effects, changes in body weight, food or water consumption, clinical pathology, gross pathology, absolute or relative organ weights, or histopathology compared to controls. The no-observed-adverse-effect-level (NOAEL) was determined to be 3000 mg XOS/kg bw/day.

Gao et al. (2012 as cited by FDA, 2013; 2019; EFSA, 2018) fed diets containing up to 10% XOS to rats (n=10/sex/group) for 13 weeks (0, 1.1, 3.6, 9.8, and 11.5 g XOS/kg bw per day for females and 0, 1.4, 4.7, 13.8, and 15 g XOS /kg bw per day for males). There was no mortality, ophthalmological changes, or adverse clinical signs observed. XOS treatment had no adverse effect on body weight, food consumption, clinical pathology, absolute or relative organ weights, or histopathology compared to controls. The NOAEL was determined to be the highest dose, which corresponded to 11.5 and 15 g/kg bw per day in females and males, respectively.

Dogs

A repeat dose study was reported in Beagle dogs (Gao et al., 2017, as cited by EFSA, 2018; FDA, 2019). Dogs (7–9 months of age; bw range: 8.1–10.0 kg in females and 8.0–11.0 kg in males; n=4/sex/group) received XOS orally at doses of 0, 1.25, 2.5, and 5 g/kg bw, for up to 26 weeks. One dog/sex/group was killed at 13-weeks, 2/sex/group were killed at 26-weeks, and 1/sex/group was killed following a 28-day recovery period. There was no mortality in the study. Diarrhea and vomiting occurred with all the high dose animals throughout the treatment phase of the study and diarrhea occurred in the first two weeks of treatment for mid-dose animals. There were no XOS effects on body temperature, food consumption, EKG results, or in the ophthalmological exams. There were no dose-related, significant changes in hematological and clinical biochemistry parameters or in relative organ weights at weeks 13 and 26 and at the end of the 4-week recovery period. There were no XOS-related histopathological changes. Authors determined the NOAEL to be 2.5 g XOS/kg bw/day. This level is equivalent to approximately 12.5% XOS dietary inclusion assuming 10 kg bw and 200 g feed intake per day (2% of body weight).

Chronic Toxicity/Carcinogenicity

A 2-year rat carcinogenicity study was reported with D-xylose (Kuroiwa et al., 2005, as cited by FDA, 2013; 2019; EFSA, 2018). Rats (n=55/sex/group) were fed 0, 2.5, or 5% (equivalent to 2214 mg/kg bw/day for males and 2513 mg/kg bw/day for females) D-xylose in the diet. There were no differences in survival, no adverse effects, and no carcinogenicity that could be attributed to treatment. Some changes in organ weight (brain, kidney) were observed but there were no corresponding changes in the histopathological exam of those organs.

Reproductive/Developmental Toxicity

No data were reported.

Safety Conclusions

The intended use of XOS is as a feed ingredient to provide a source of xylooligosaccharides for normal intestinal microbiota. The intended inclusion level is up to 1% in poultry, swine, and fish feed.

Monogastric animals such as poultry, swine, and finfish do not produce the enzymes required for enzymatic hydrolysis of XOS which allows it to serve as a prebiotic that is fermented by the microbial community in the lower gut. The use of prebiotics can improve intestinal microflora communities and subsequent intestinal health across poultry, swine, and fish species that represent feeding habits ranging from carnivorous through omnivorous to herbivorous. The microbial communities use the XOS for microbial growth with a concomitant production of short-chain fatty acids such as acetate, propionate, and butyrate that can be used as an energy source by the host target species and reduce the pH in the lumen of the gut thereby promoting growth of known beneficial microbes.

A total of eighteen studies were identified with XOS used in broiler feed. Levels of XOS in feed ranged from 0.0002% (42 days) to 2% (59 days), and study duration ranged from 12 to 59 days. In all studies, broilers fed an XOS-supplemented feed had normal growth performance, and mortality was not different than controls. A few studies reported an increase in intestinal villus height, a decrease in crypt depth, and greater short-chain fatty acid concentrations with XOS supplementation that were considered indicative of positive intestinal health. Biochemical parameters and antibody titers were measured in several studies and, in general, were either not different from untreated controls or improved.

Four studies were identified with XOS added to the feed of layers. Levels of XOS in diet ranged from 0.0005% (56 days) to 0.6% (70 days), and study durations ranged from 8 to 12 weeks. In all studies, animals fed an XOS-supplemented feed had normal egg production and egg quality parameters and normal to improved biochemical parameters. Villus height and villus-height:crypt-depth ratio were also increased in a few studies. When measured, immunoglobulin levels were increased with XOS. Two studies showed positive shifts in intestinal bacterial communities with increased presence of *Bifidobacteria* and *Lactobacillus* and decreased presence of *Escherichia coli*, *Bacteroides*, and *Campylobacter*. Use levels of up to 1% of the diet were justified based on the documented lack of any adverse effect in broilers and the similar metabolic fate of XOS in broilers and layers.

There were 12 studies with XOS use in swine feed. Levels of XOS in feed ranged from 0.01% (up to 56 days) to 3% (28 days), and study duration ranged from 28 – 84 days. In all studies, animals fed an XOS-supplemented feed had normal growth performance parameters and mortality was not different than controls. A few studies noted decreased diarrhea occurrence, improved intestinal morphology, and greater short-chain fatty acid concentrations with XOS supplementation. When evaluated, hematological and biochemical parameters and immunoglobin levels were somewhat improved or not different from untreated controls. XOS supplementation was also associated with positive shifts in intestinal bacterial communities with increased presence *Bifidobacteria* and *Lactobacillus* and decreased presence of *Escherichia coli* and *Clostridium*.

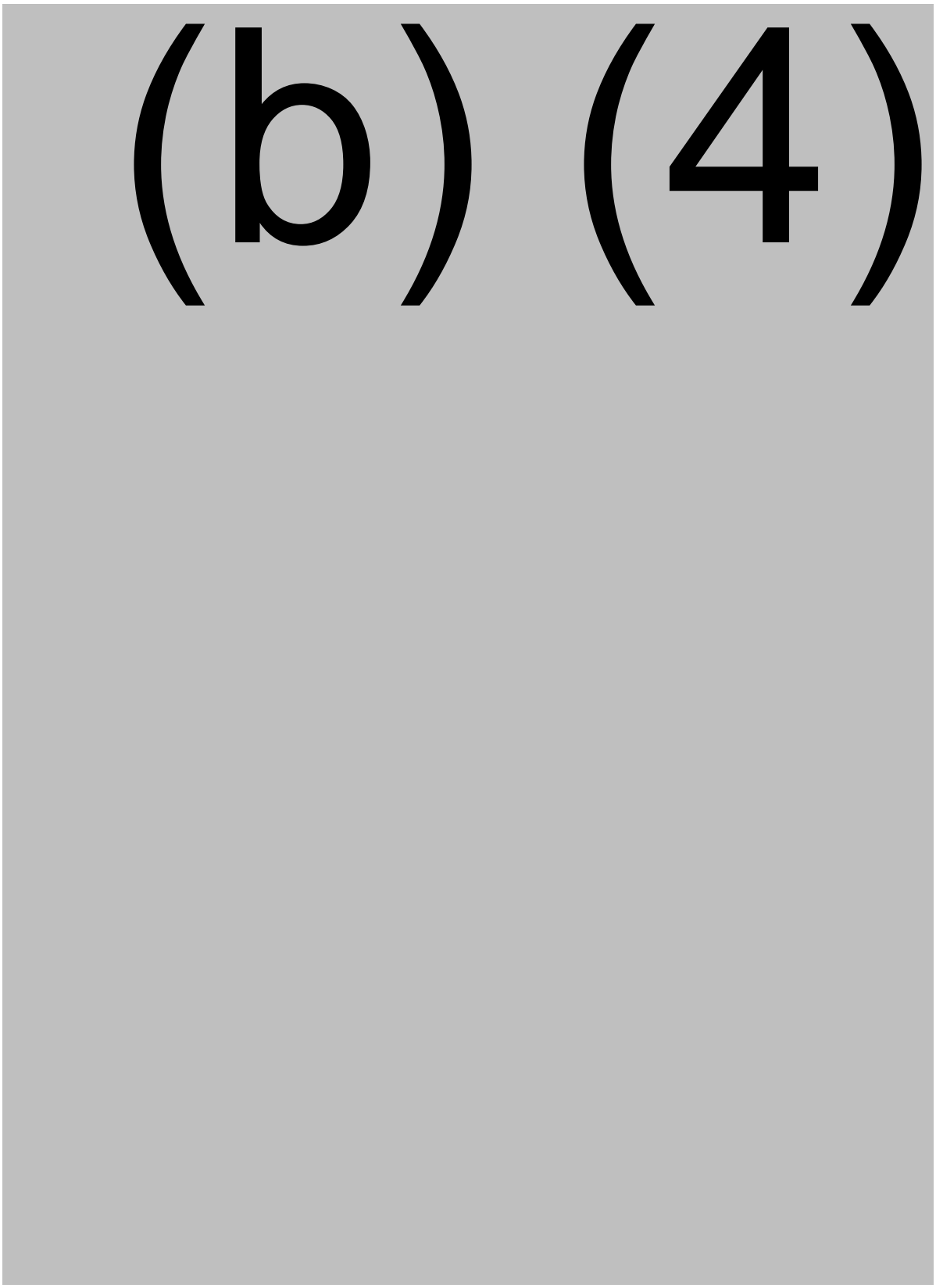
Fifteen published studies were found examining the effects of XOS feed supplementation in fish. Species studied included carp, sea bass, blunt snout bream, seabream, Caspian white fish, tilapia, rainbow trout, white shrimp, and grouper which represent a variety of feeding behaviors including herbivores, omnivores, carnivores, and detrivores. Feed supplementation ranged from 0.005% for 45 days to 3% XOS for 56 days. In all studies, animals fed an XOS-supplemented feed had normal to improved growth performance parameters. Survival rates were also similar to controls. Intestinal morphology was improved with XOS in feed.

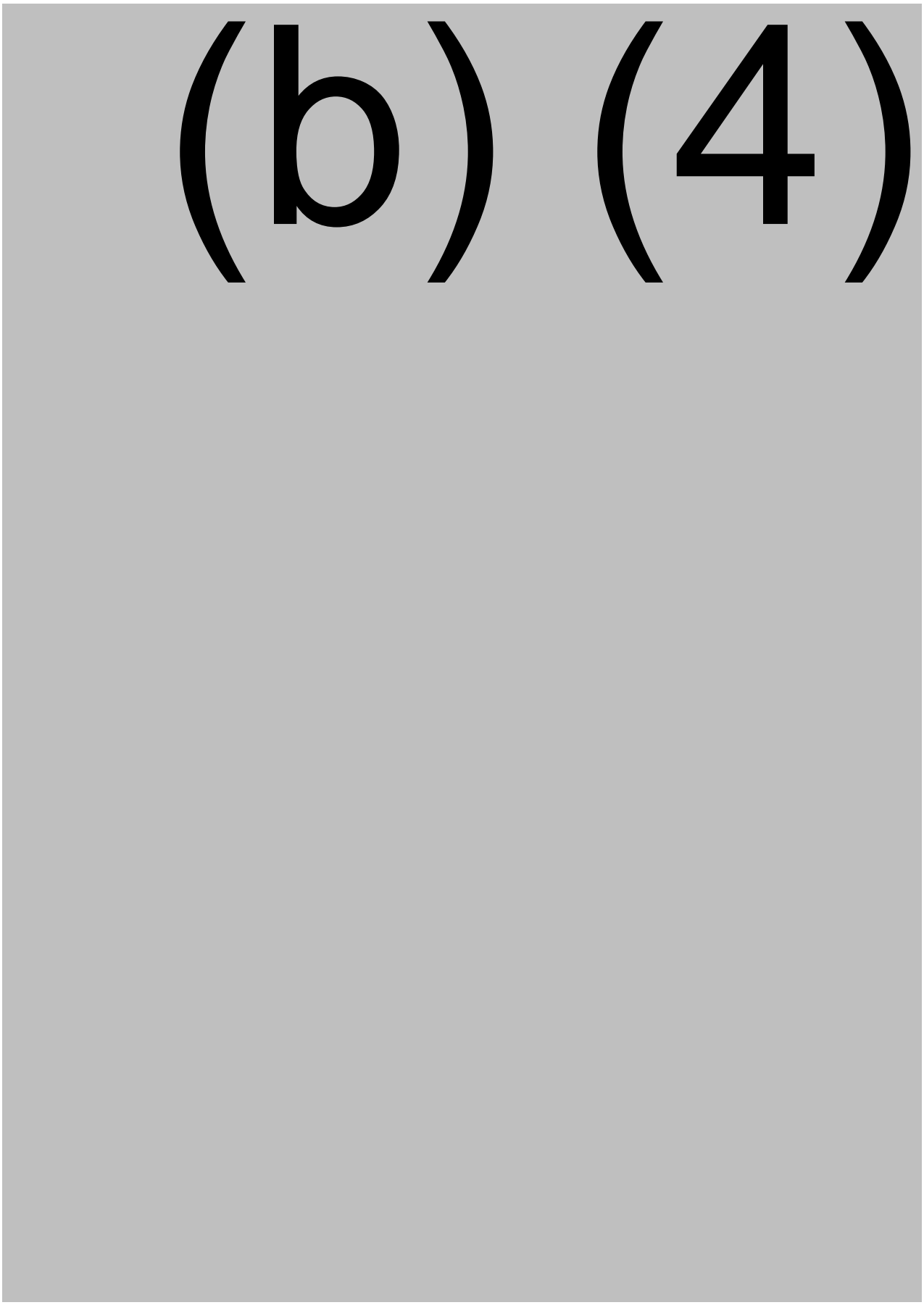
The studies conducted in the target species demonstrated maximum inclusion levels of XOS fed to broiler chickens (2% for up to 59 days), layers (0.6% for up to 70 days), swine (3% for up to 28 days), and finfish (3% for up to 56 days) had no adverse effects and potential positive impacts. Overall, there were no changes in growth performance or adverse effects in the supplemented groups compared to unsupplemented controls. Intestinal morphology and health showed improvements in XOS supplementation when compared to controls. Biochemical parameters were either not different or slightly improved when compared to controls.

When combining the data from poultry, swine, and finfish while considering the similarities and differences of the target species and age groups within the species including their diversity of feed habits and known ability of prebiotics to improve intestinal microbial ecology and subsequent intestinal health across these species, an inclusion level of 1% XOS would be supported for poultry, swine, and finfish.

A margin of safety for the proposed 1% use level was not deemed necessary as XOS is considered to have nutrient value, has a known microbial metabolism in the lower gastrointestinal tract, has no demonstrated adverse effects at dietary levels up to 3% in the target species, and is supported by published NOAELs that are 3-4 times the proposed level in toxicity studies conducted in rats and dogs.

General Recognition of Safety

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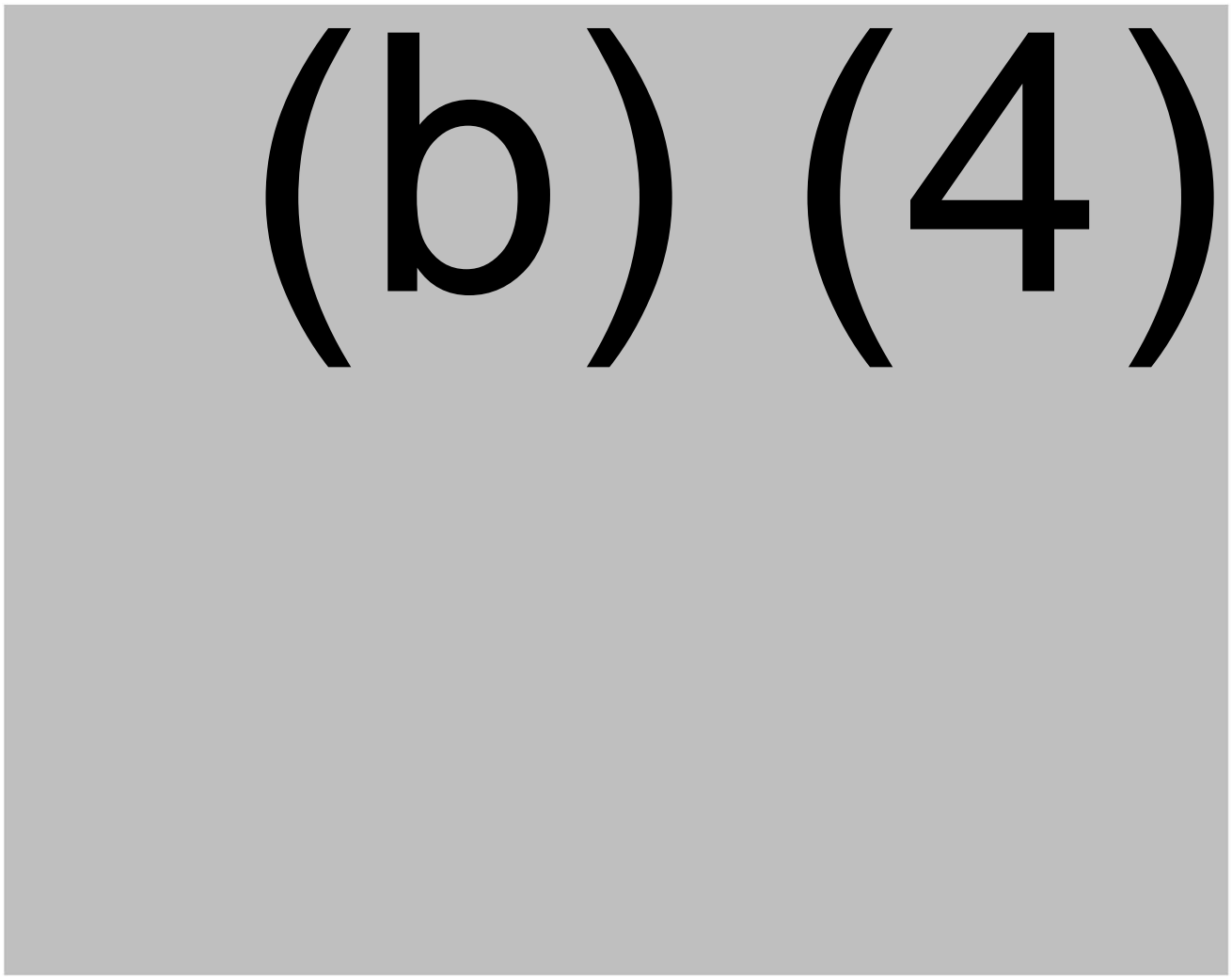
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§ 570.255 Part 7, Supporting Data and Information

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APPENDIX A

Certificates of Analysis and Other Analytical Data

Performance Fibers

Marketing and Research

Certificate of Analysis

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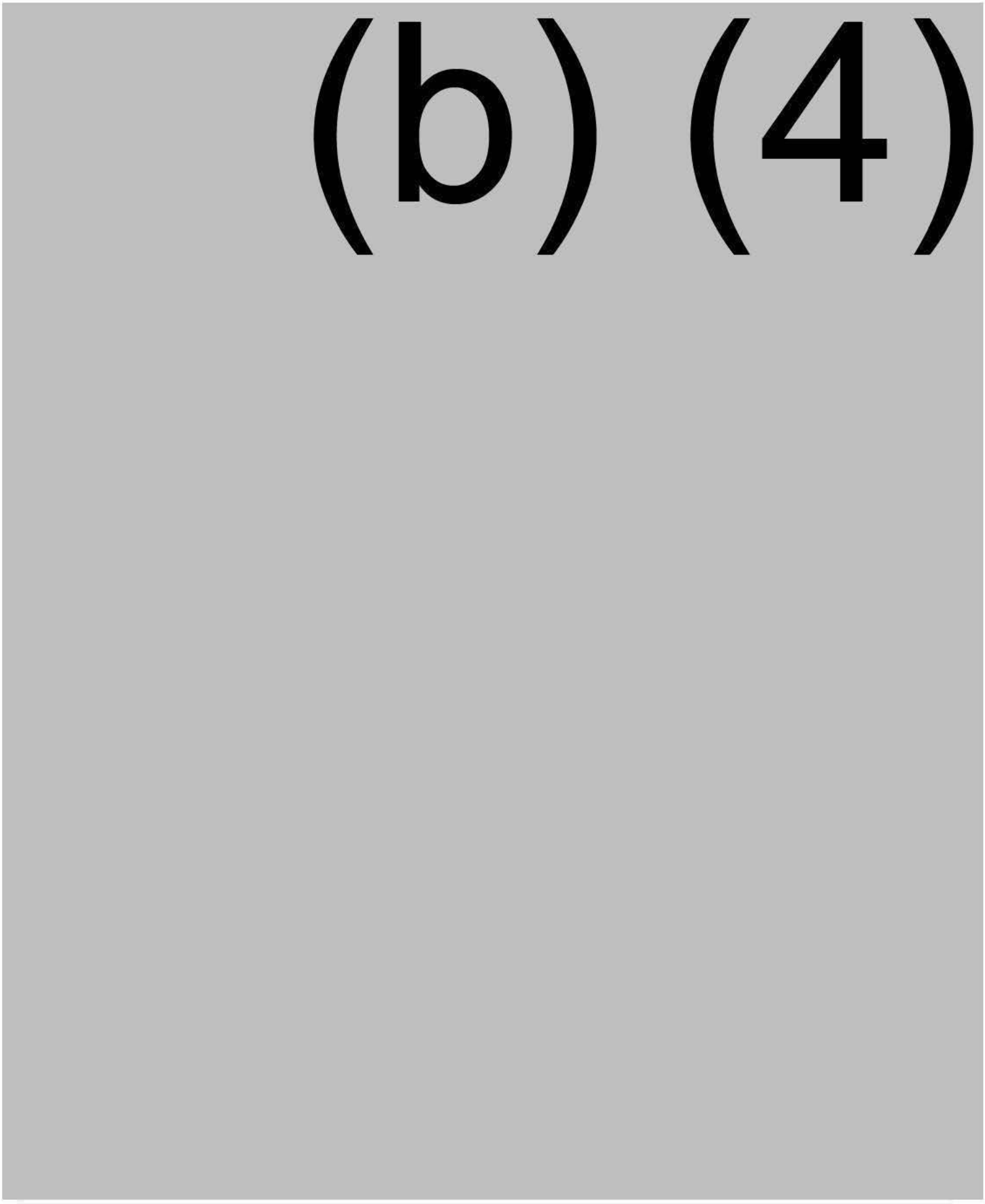
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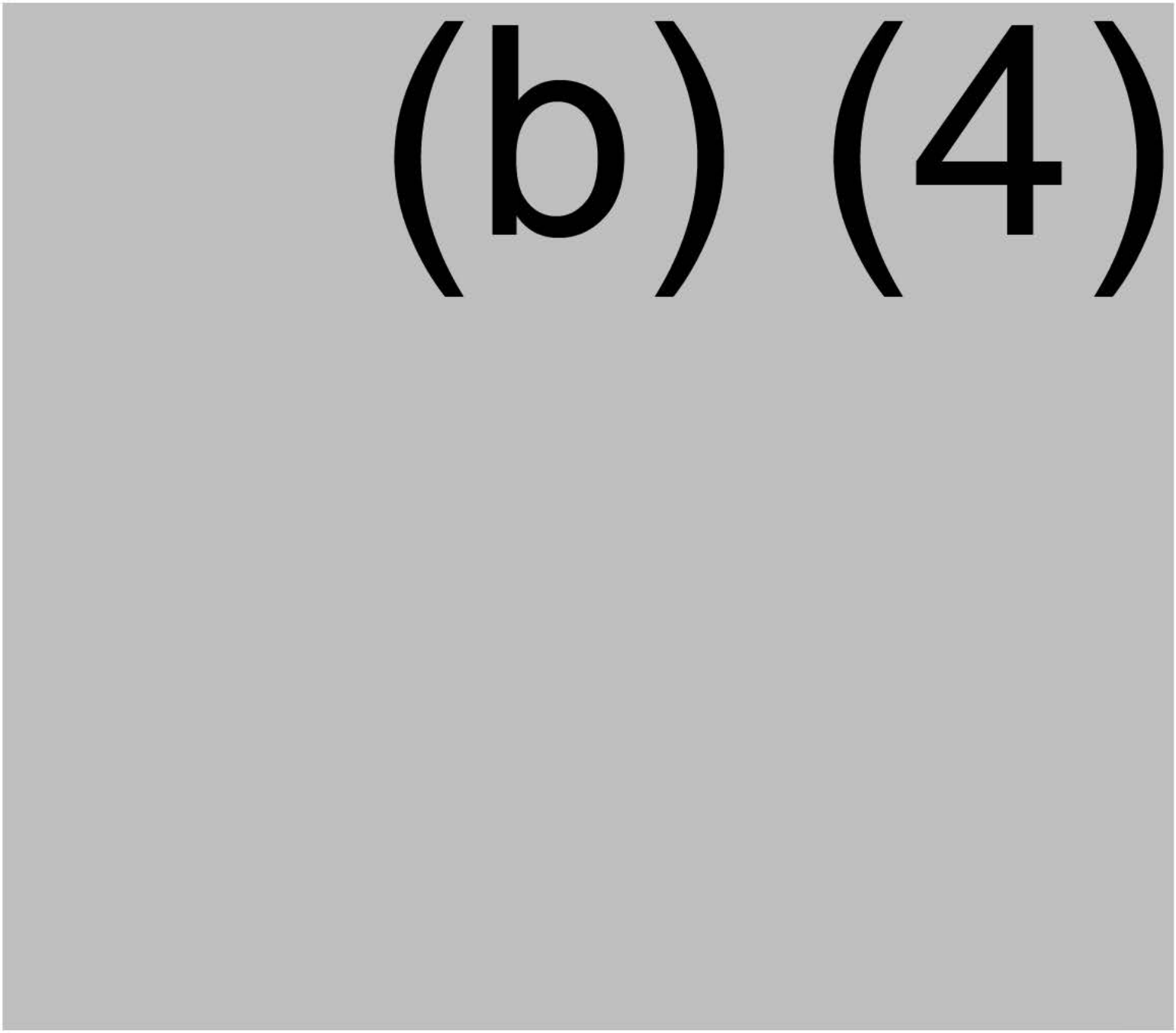
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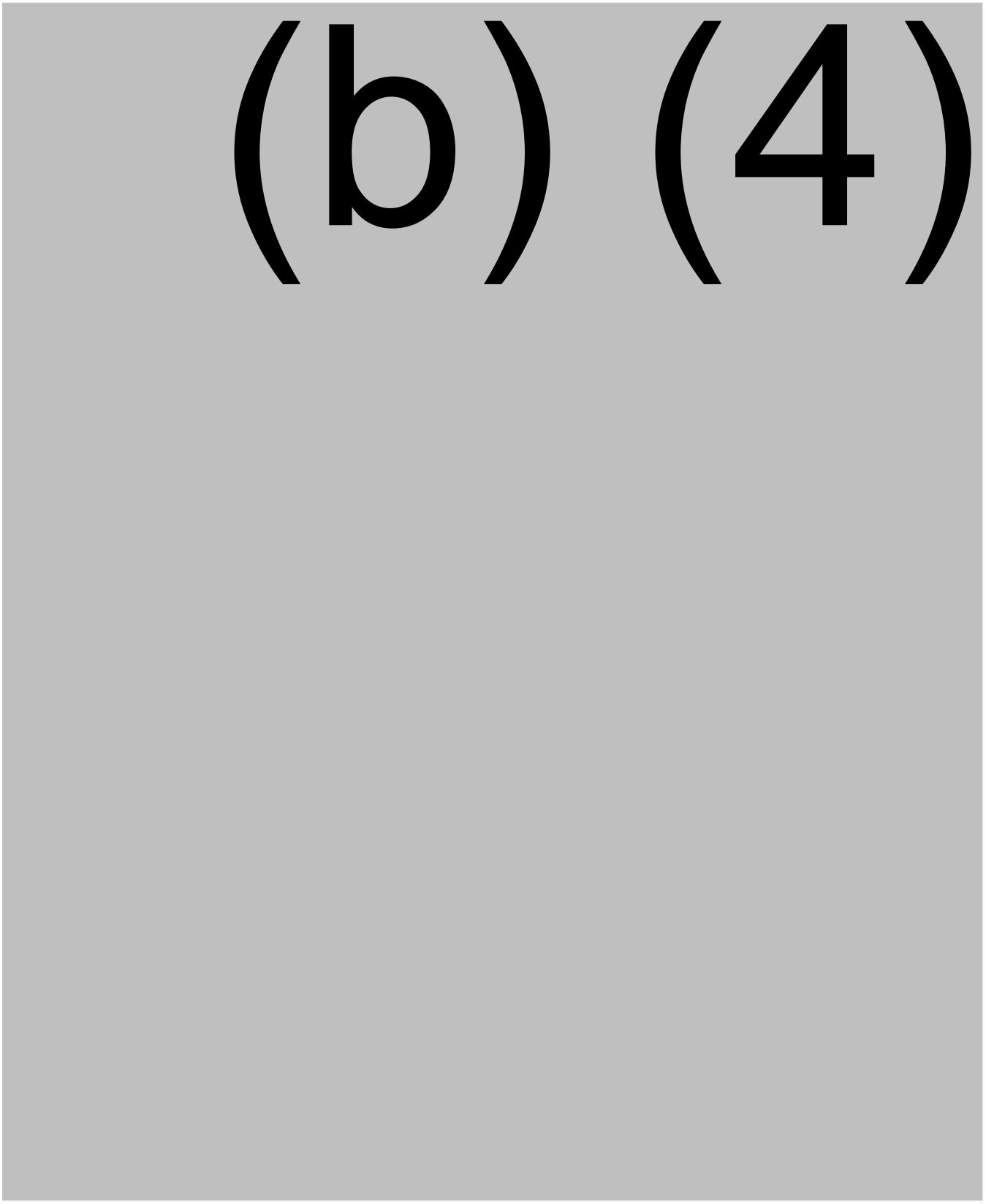
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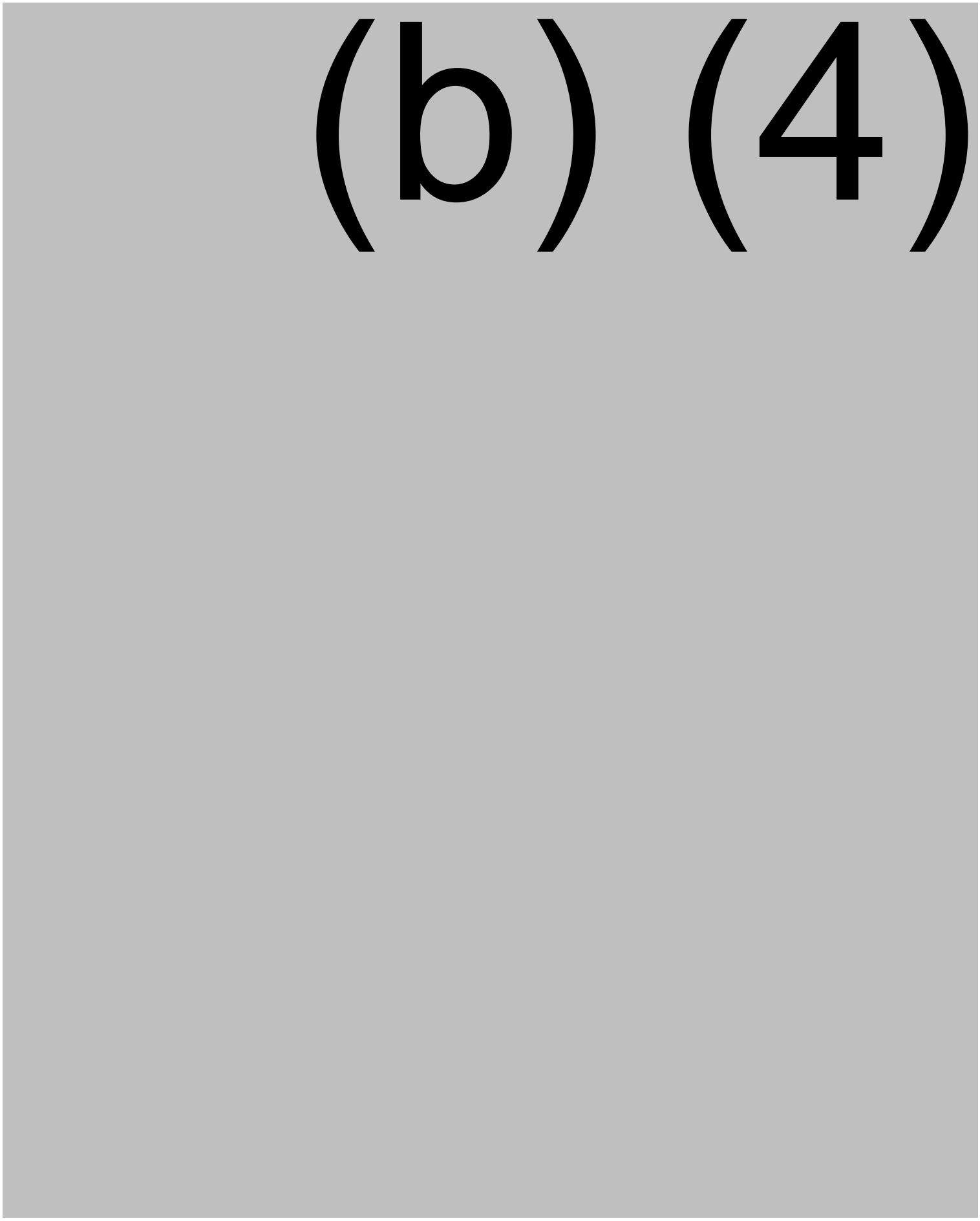
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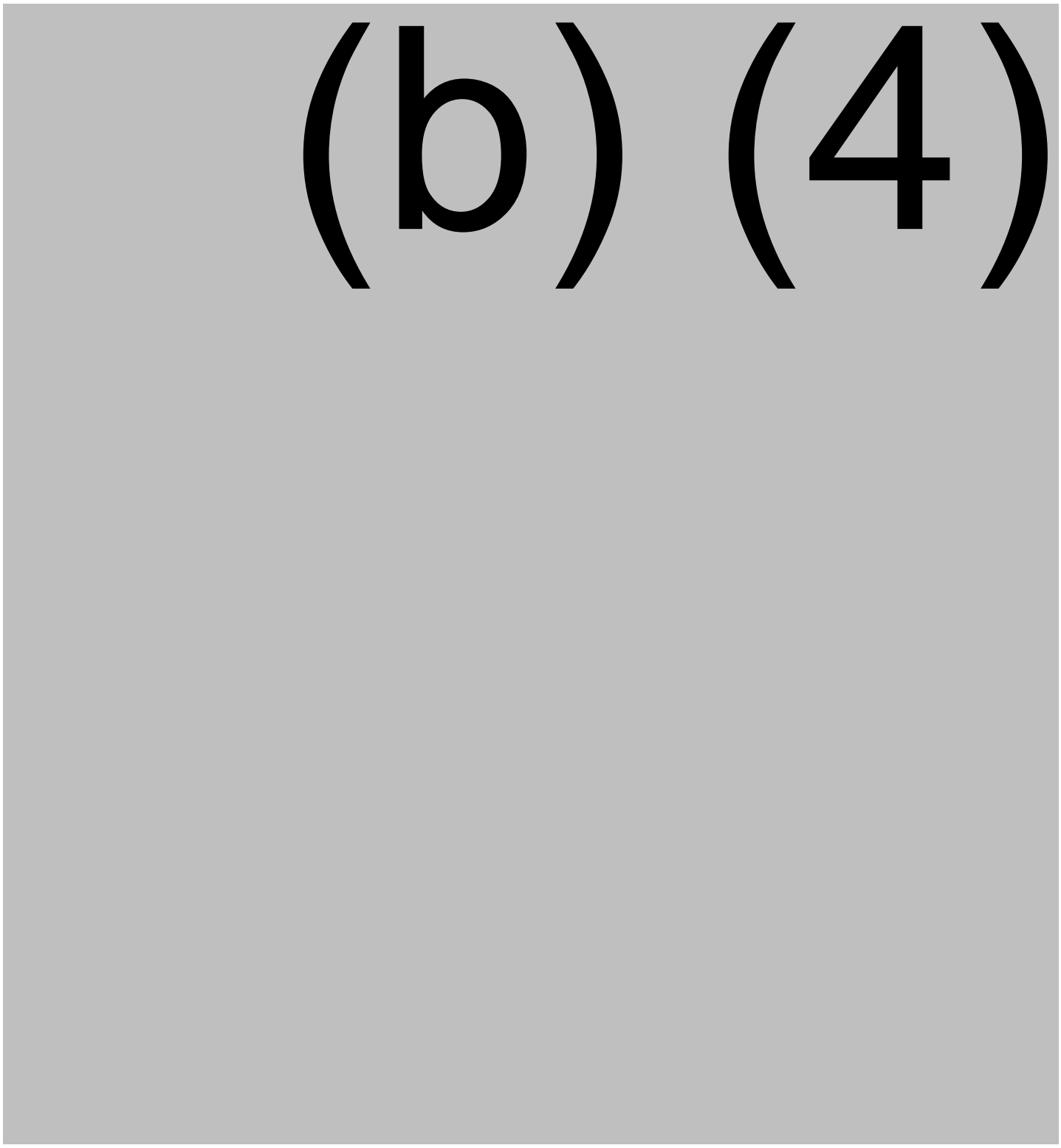
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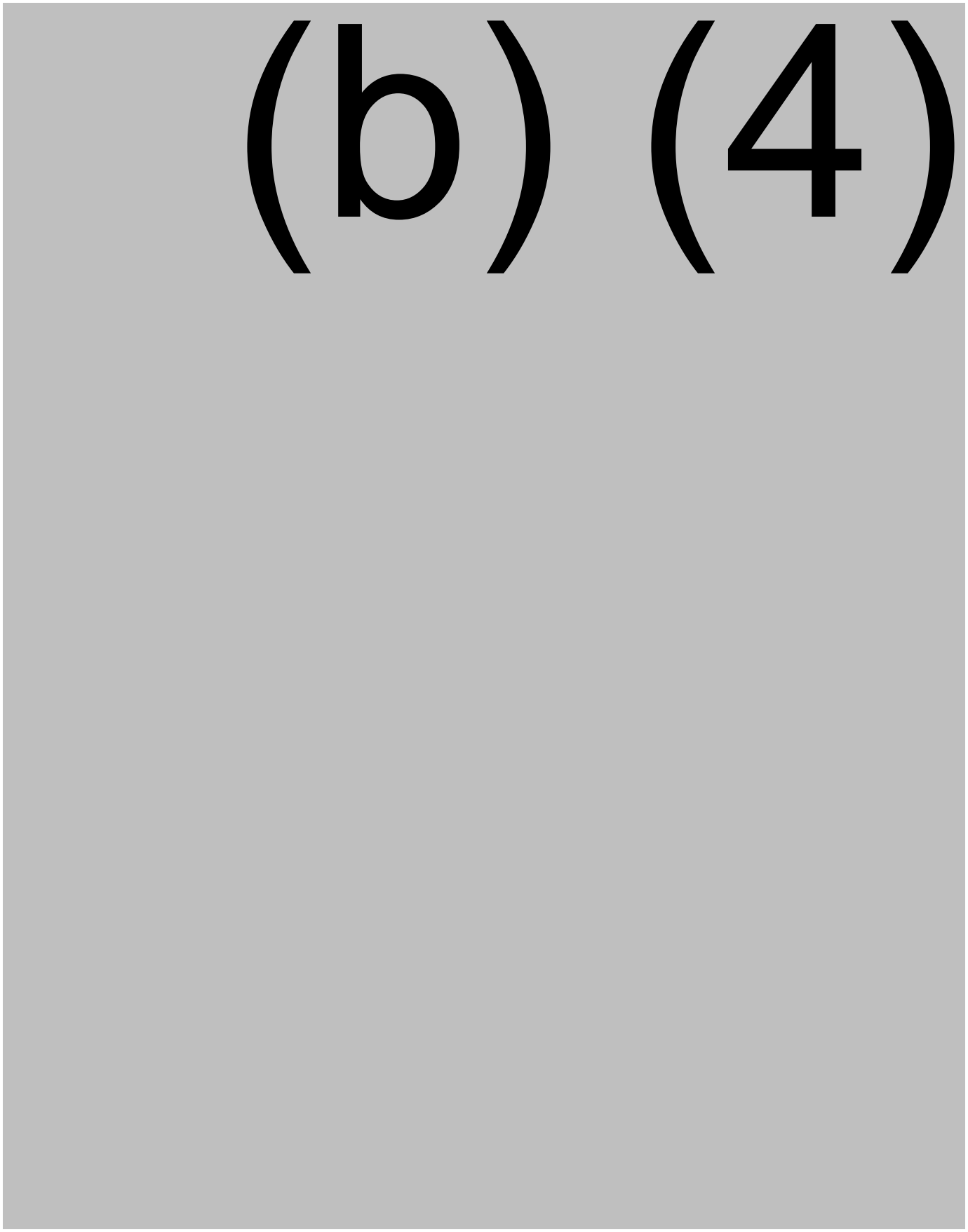
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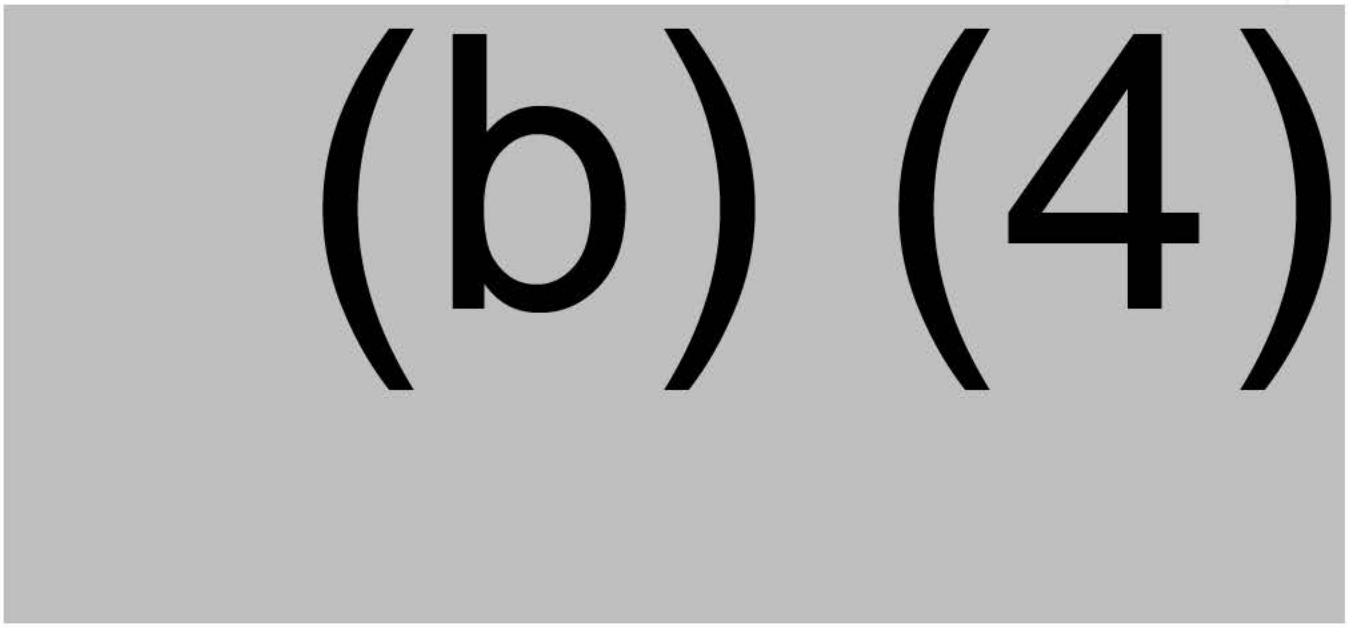
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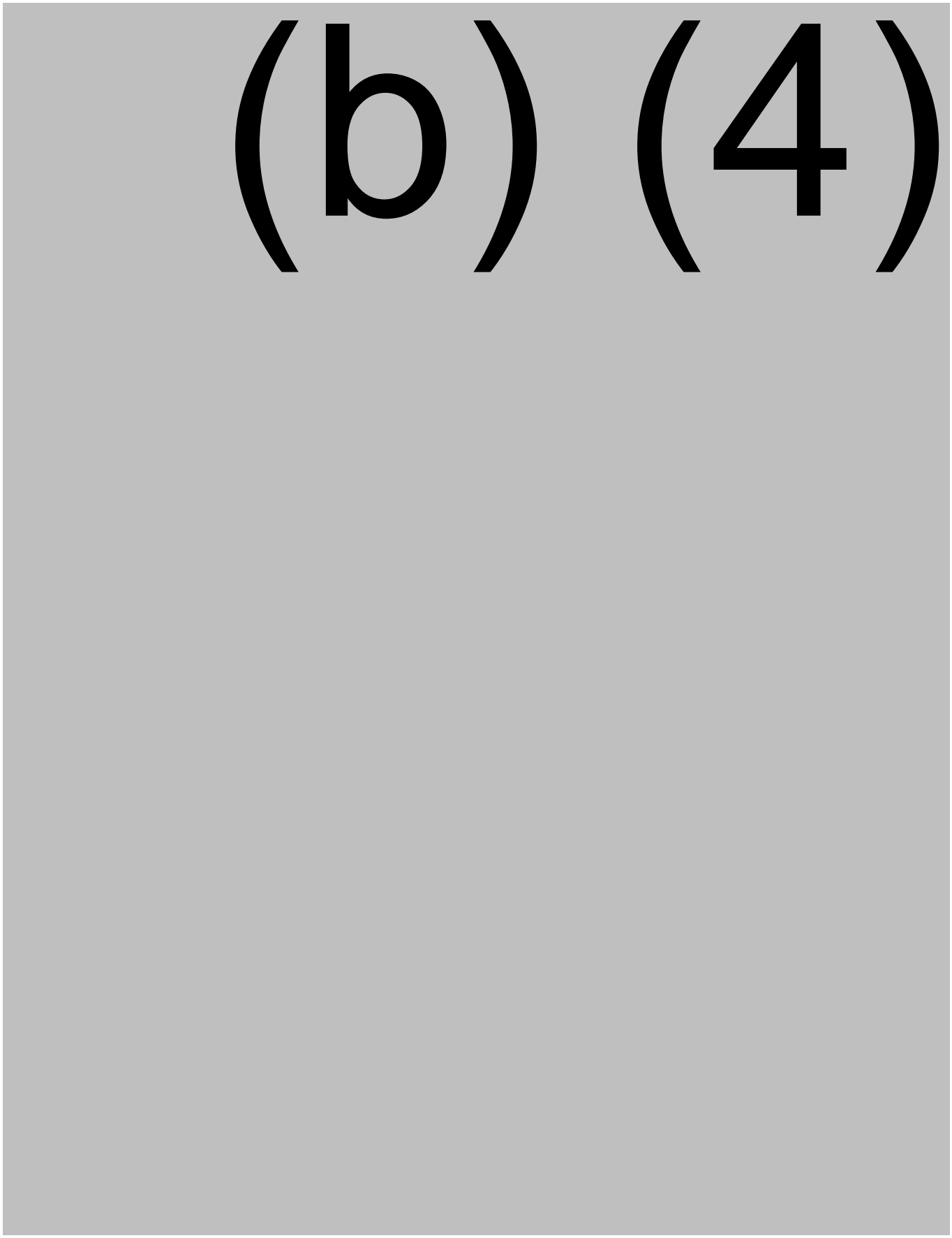
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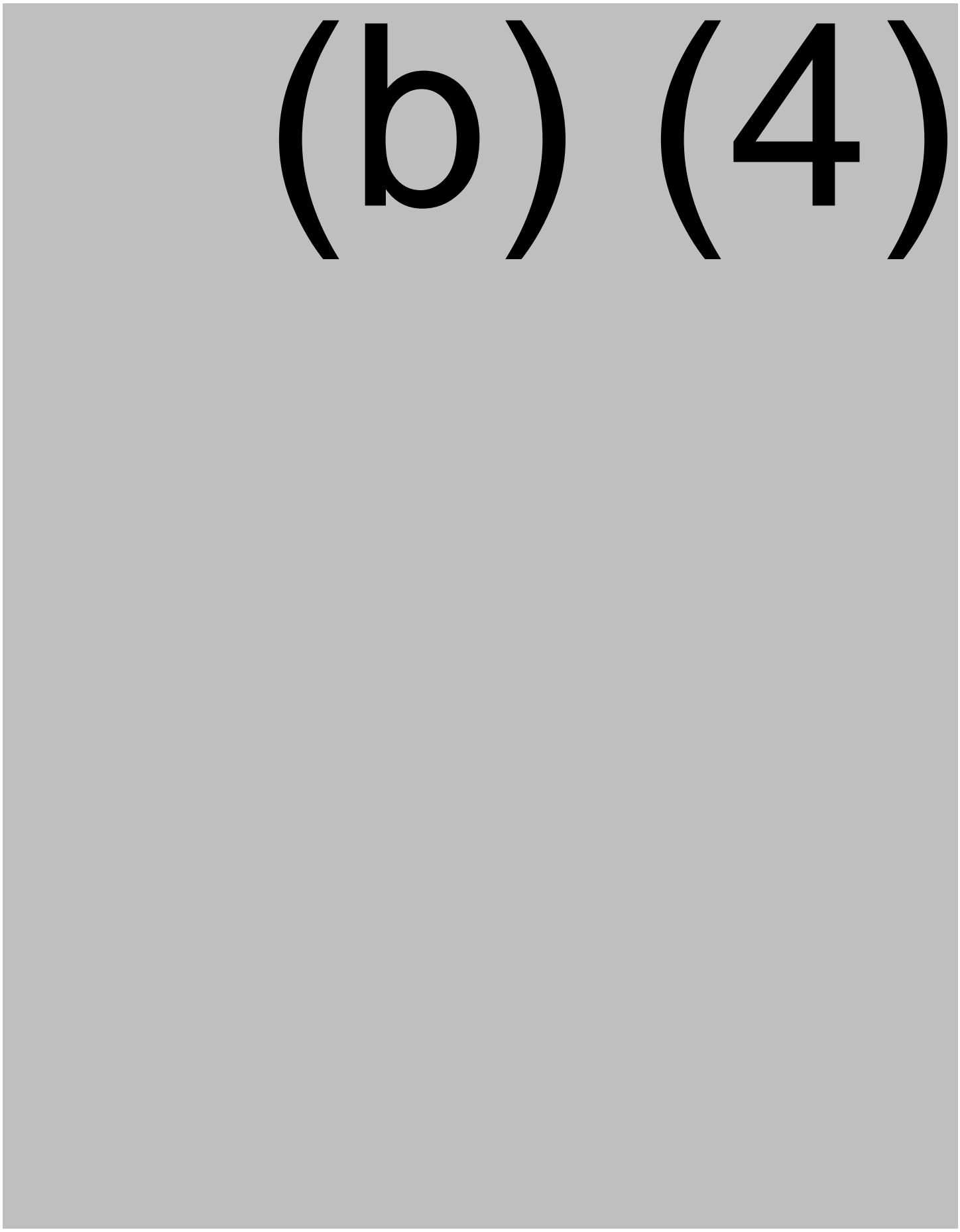
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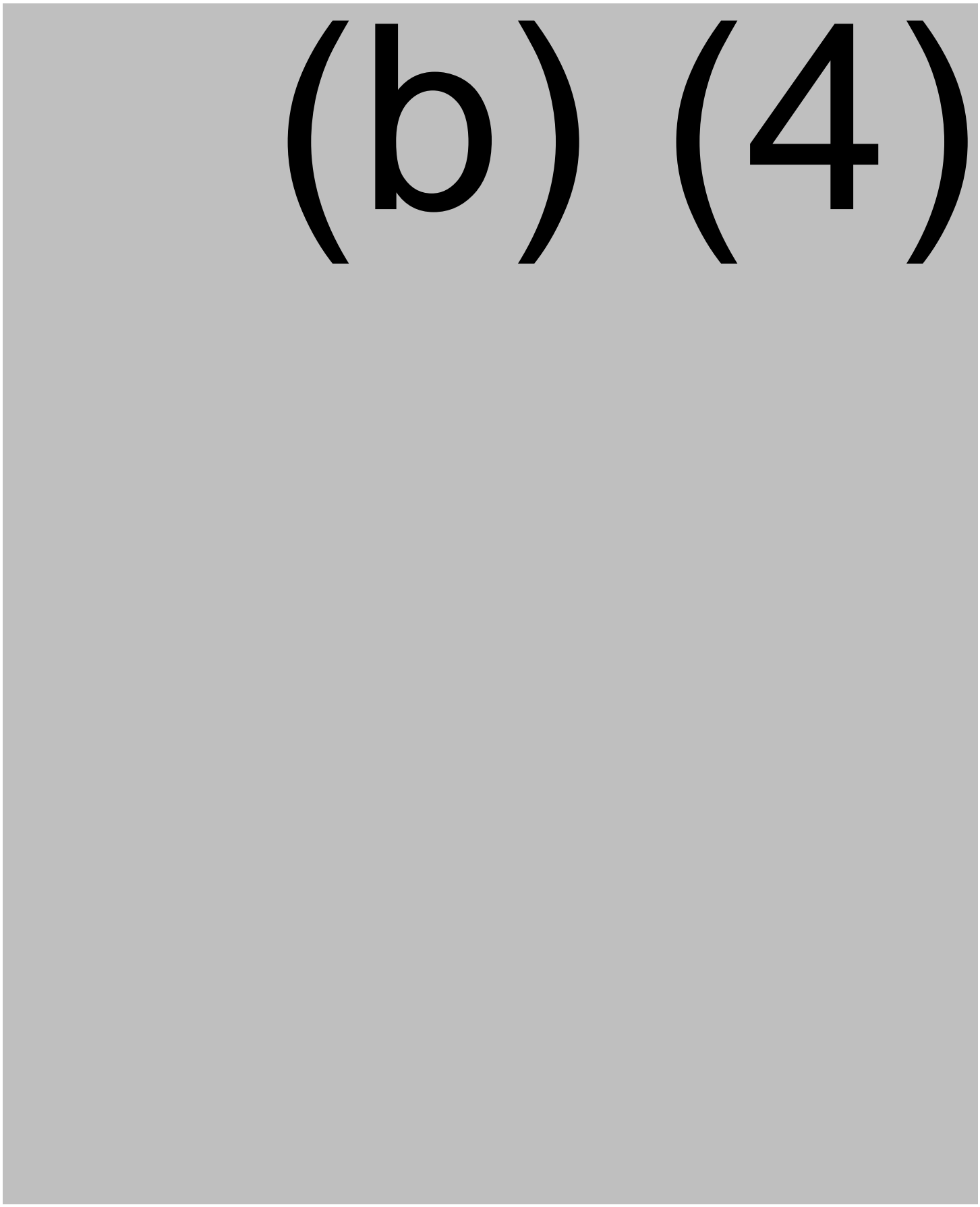
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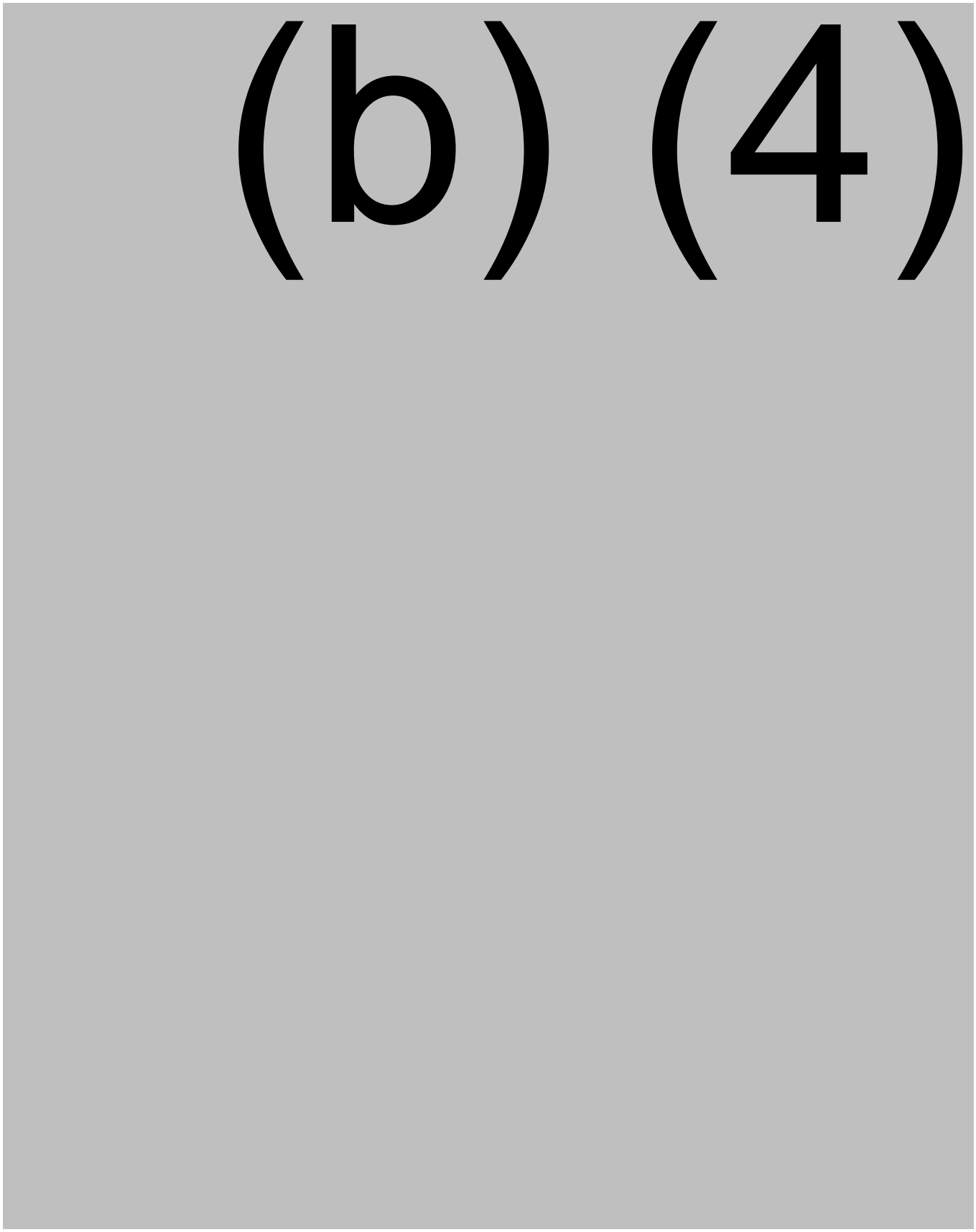
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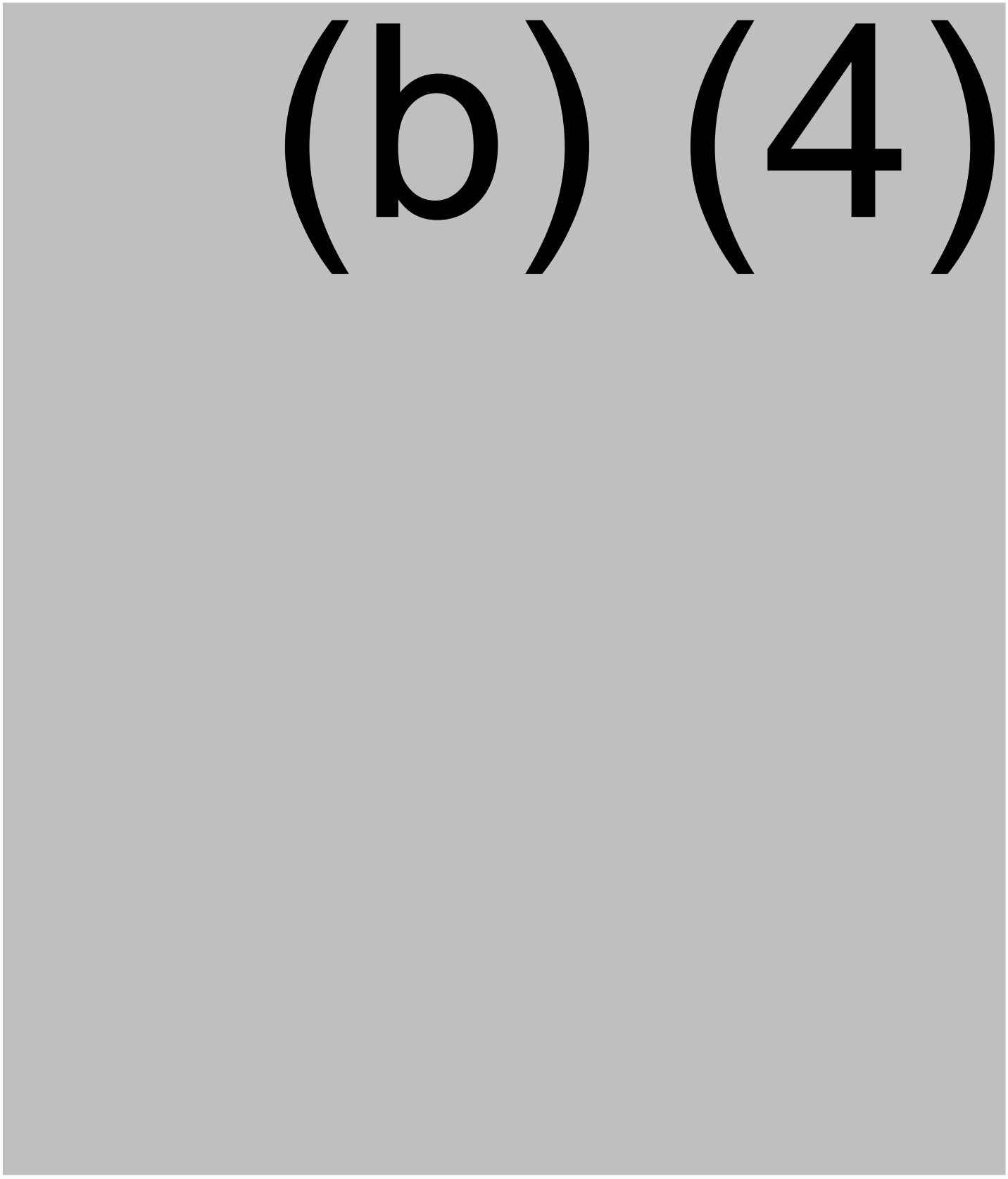
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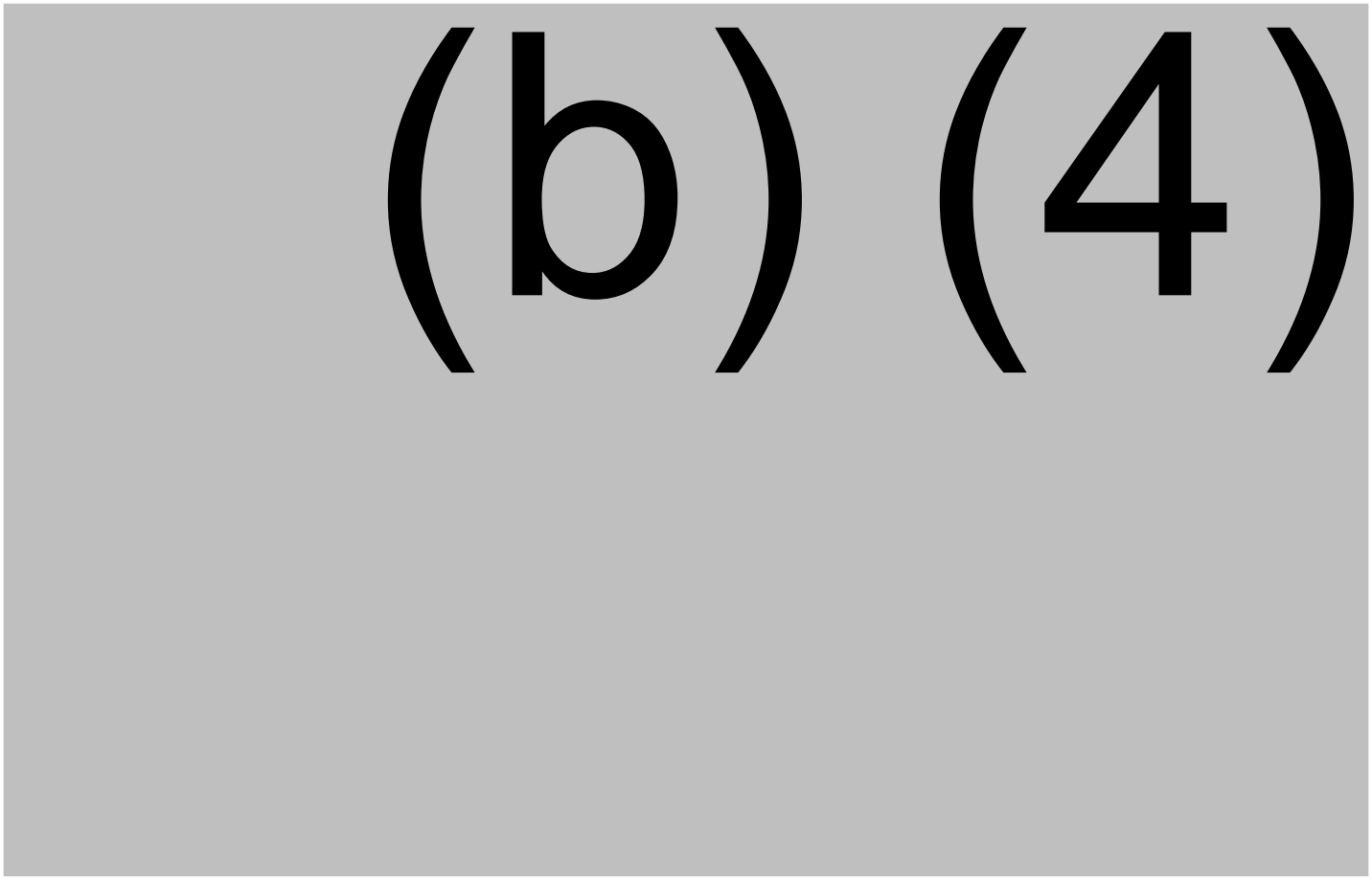
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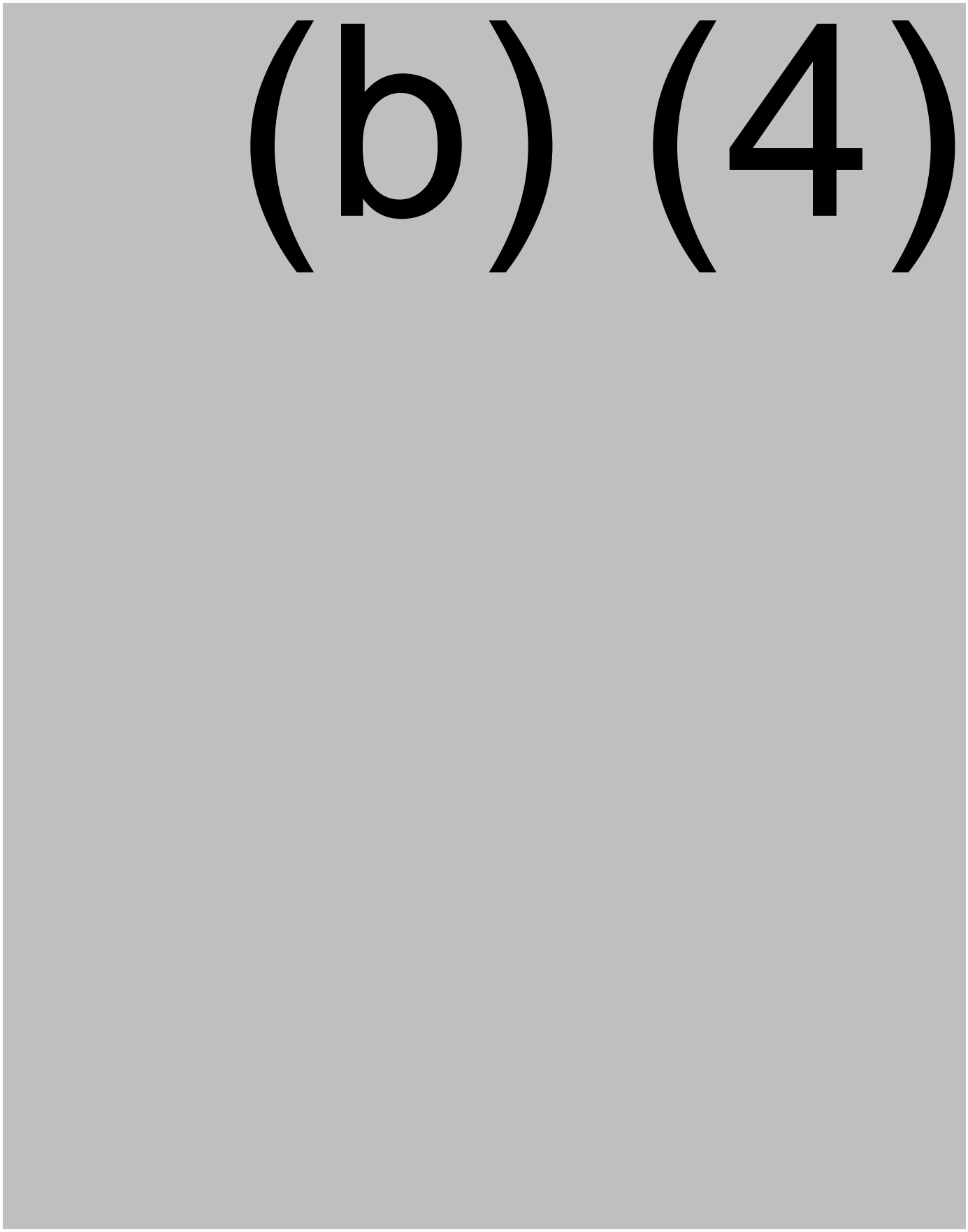
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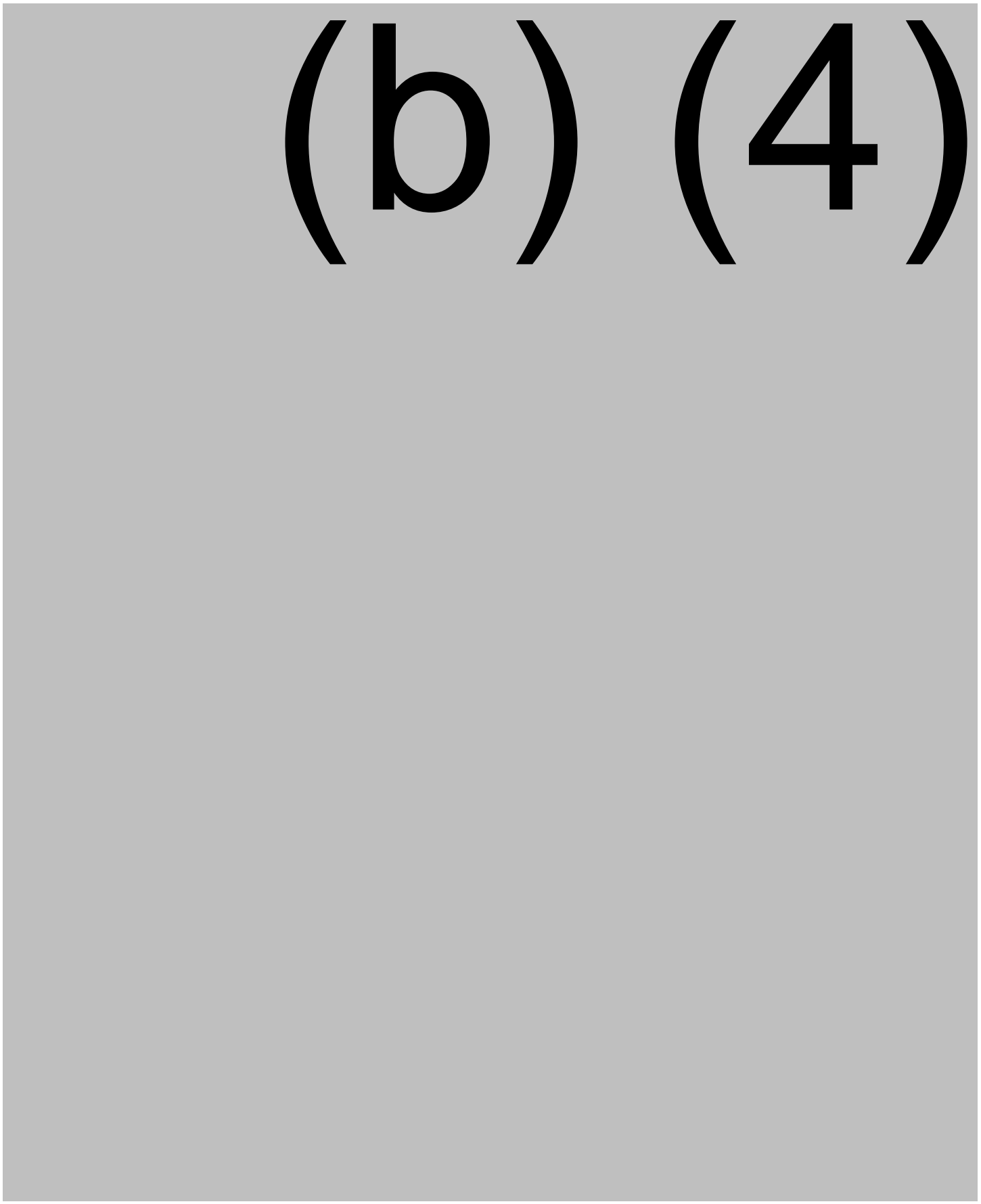
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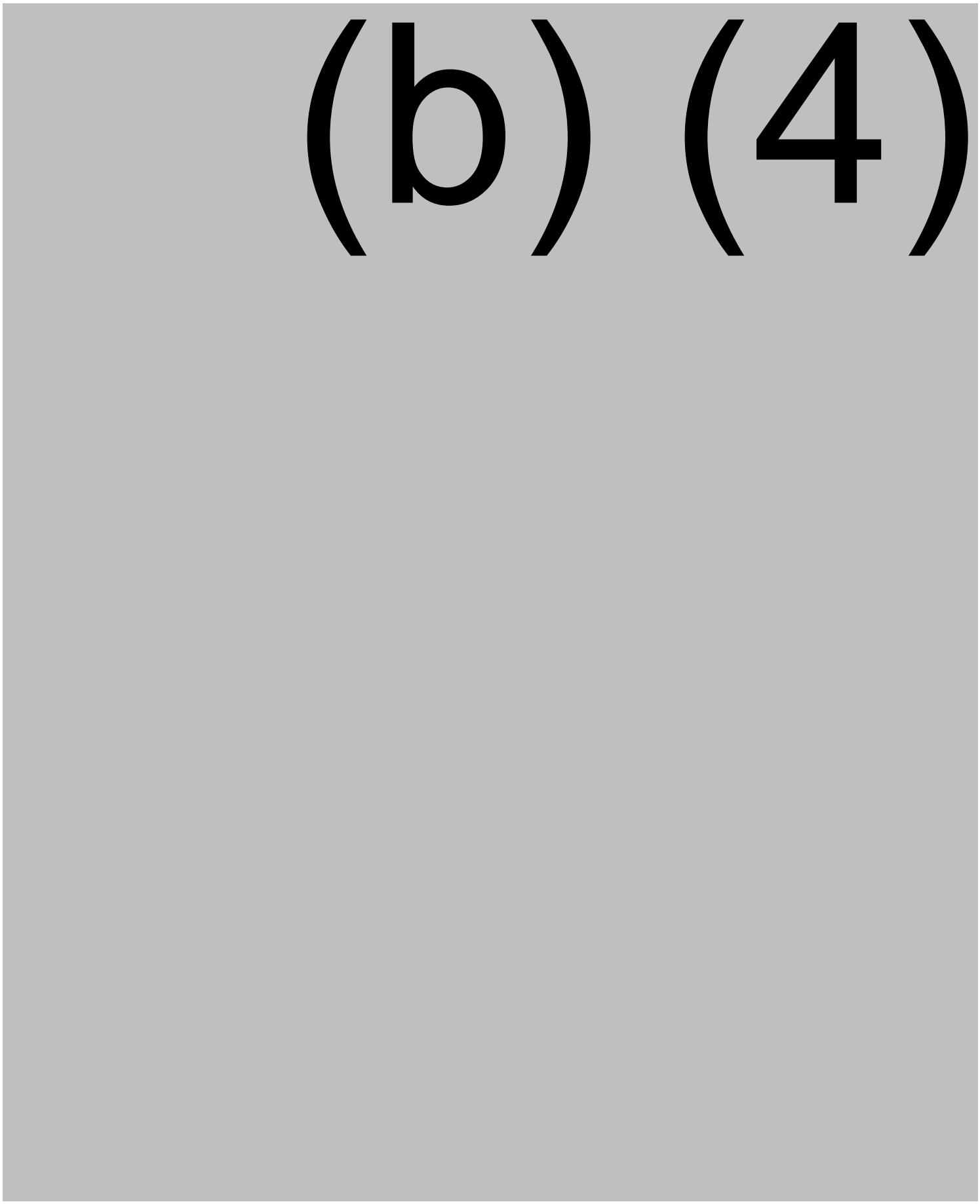
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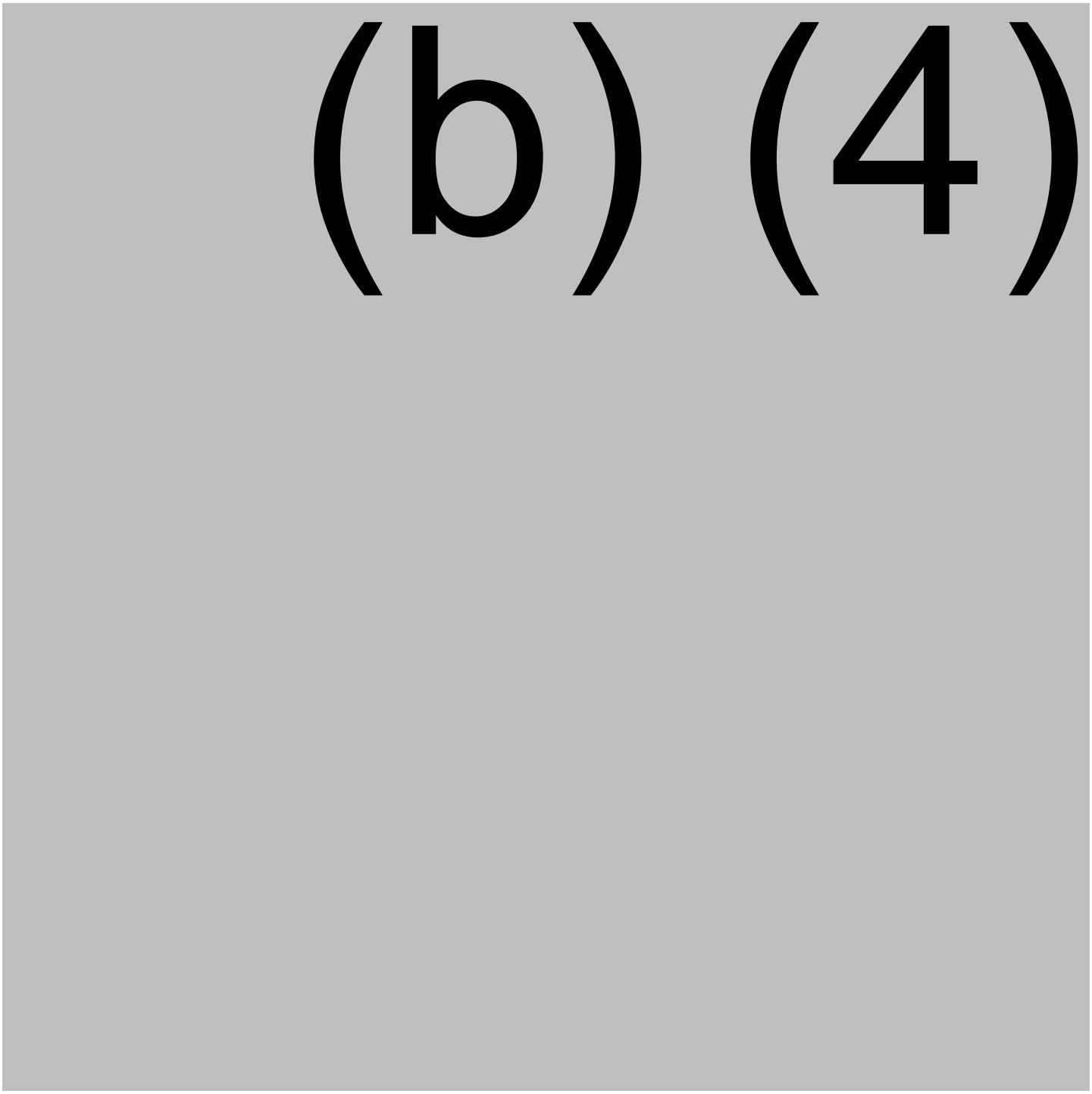
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RYAM STANDARD TEST INSTRUCTION

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This RYAM Standard Test Instruction (RSTI) is the Master Instruction for all RYAM. Additional instructions may be used in individual laboratories to accommodate the physical layout of the laboratory, equipment details, sampling schedules, etc.

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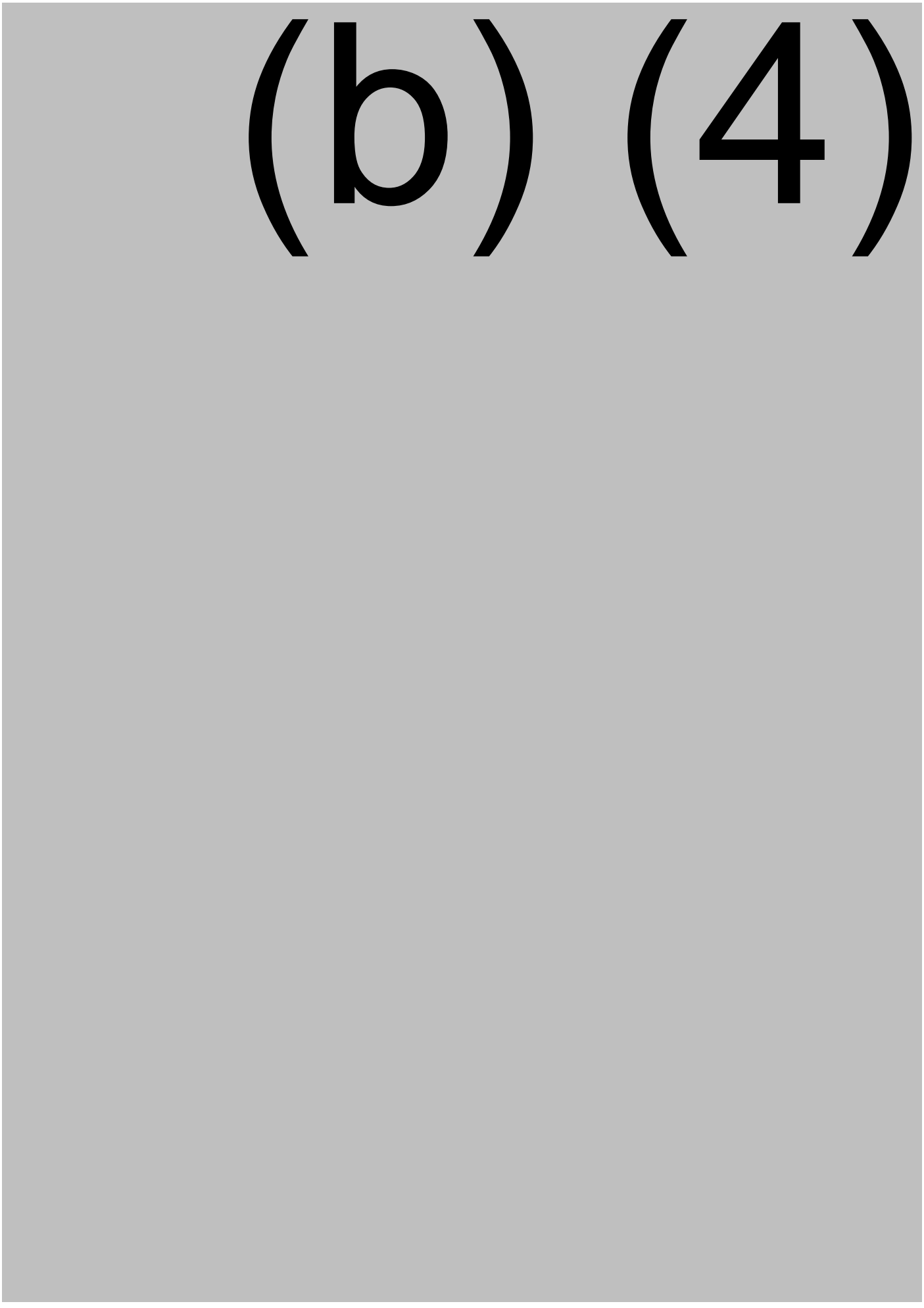
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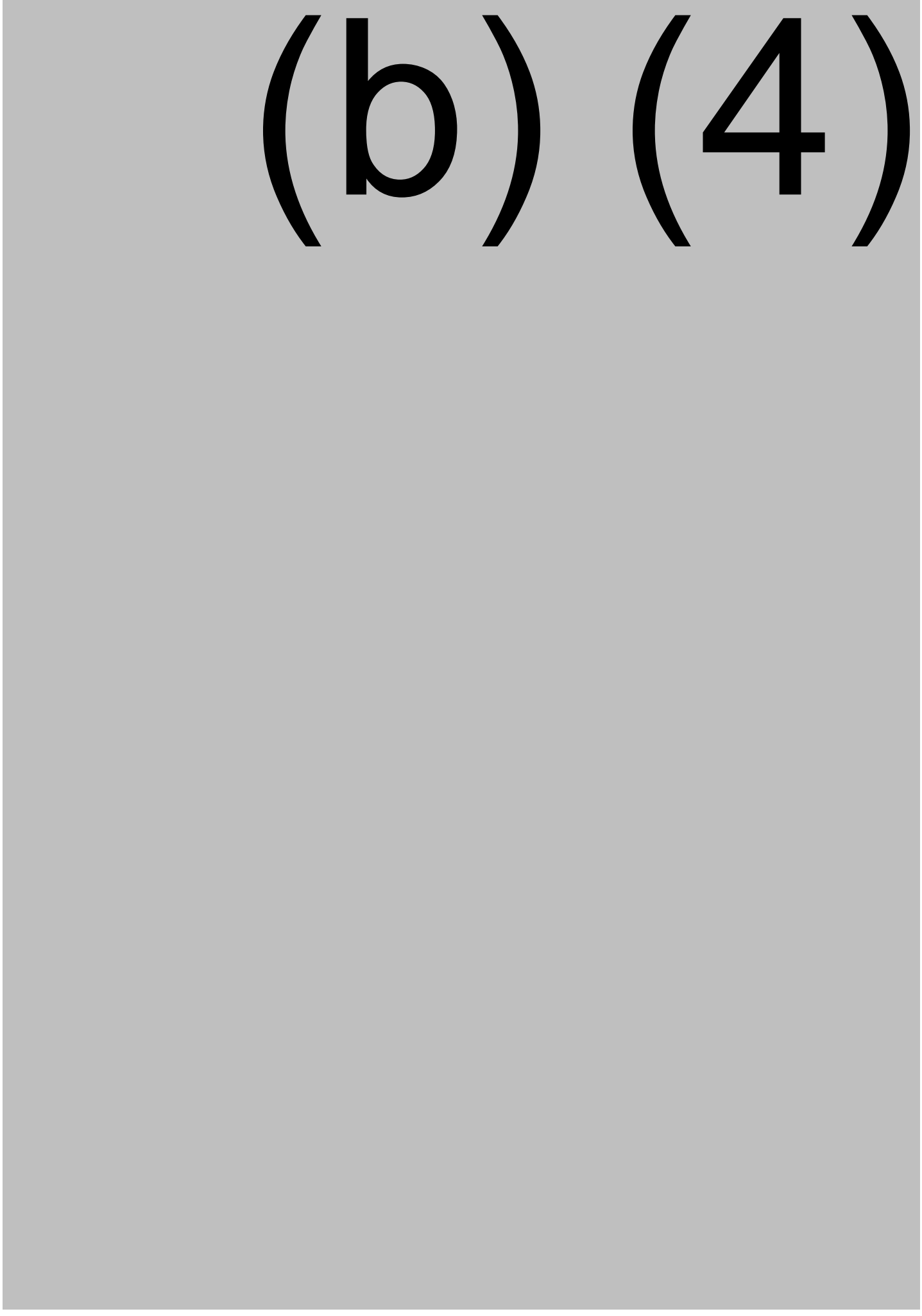
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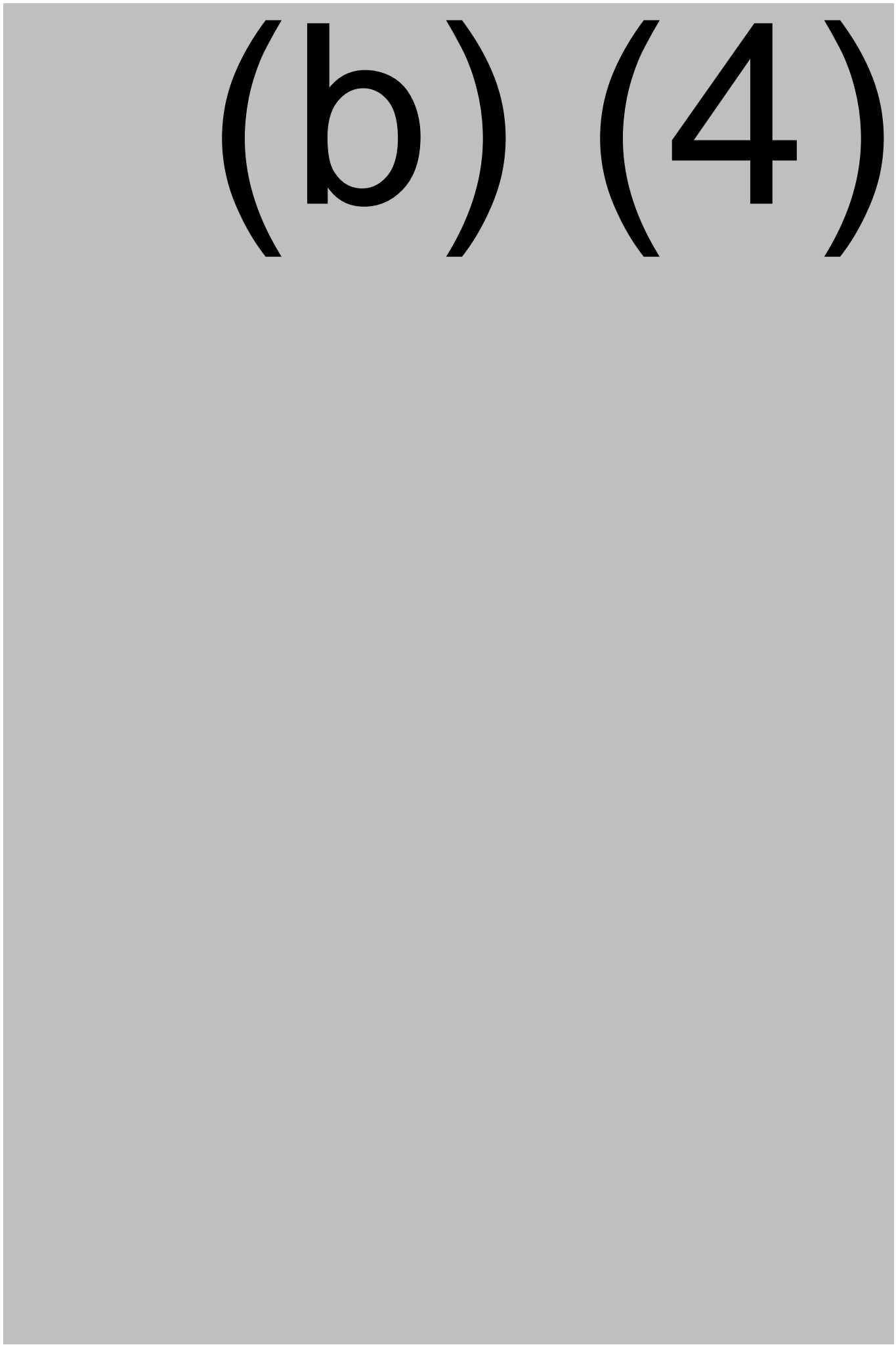
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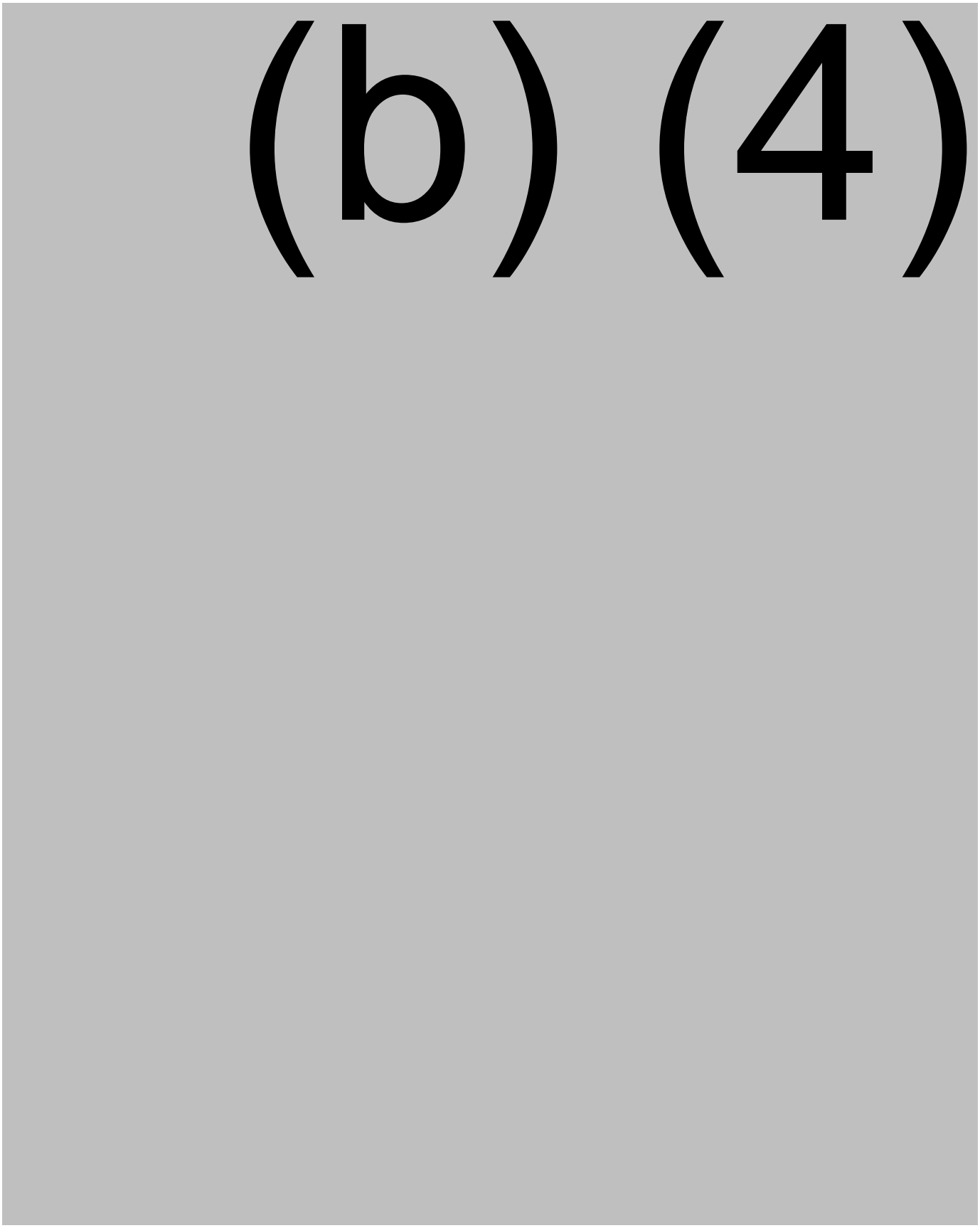
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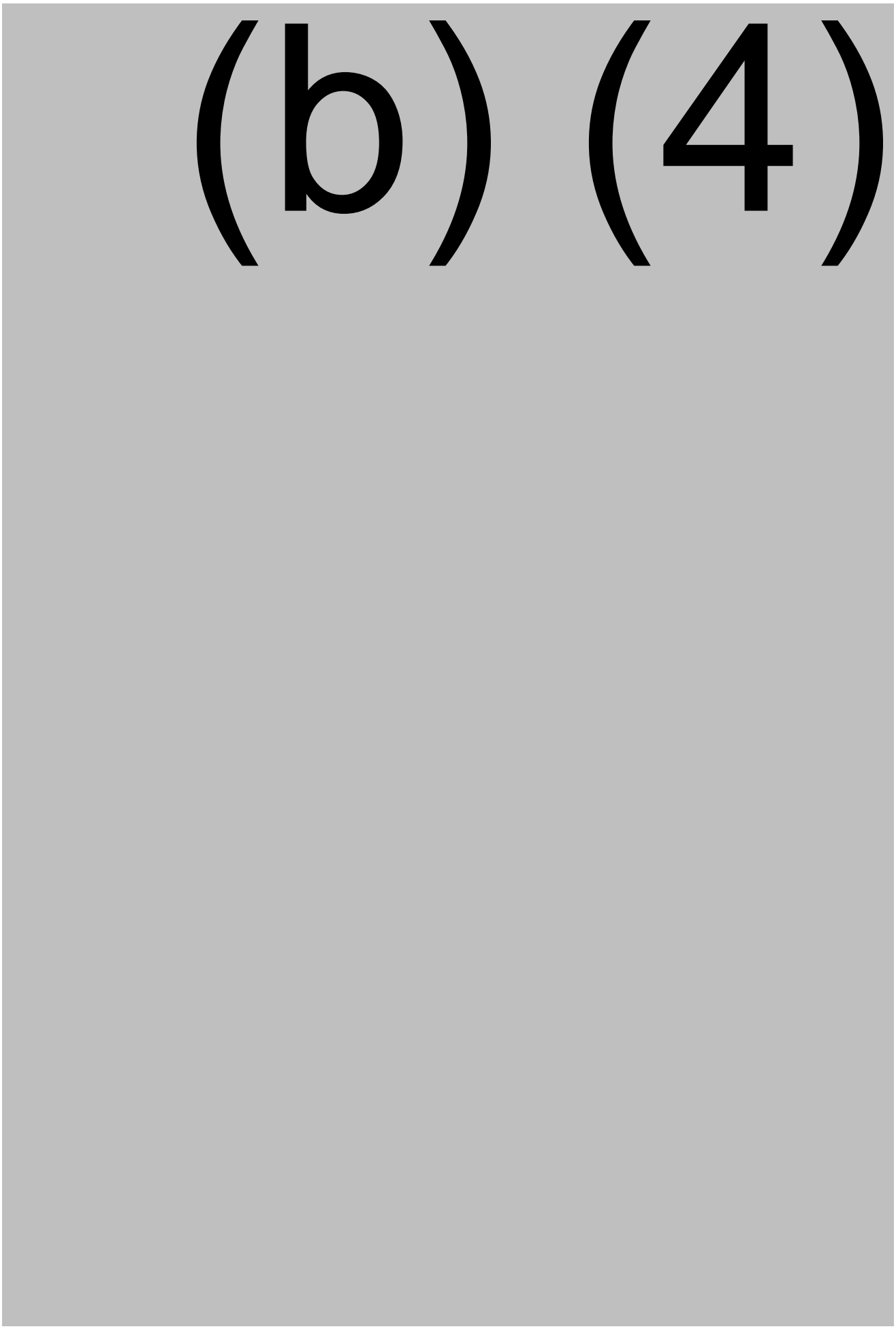
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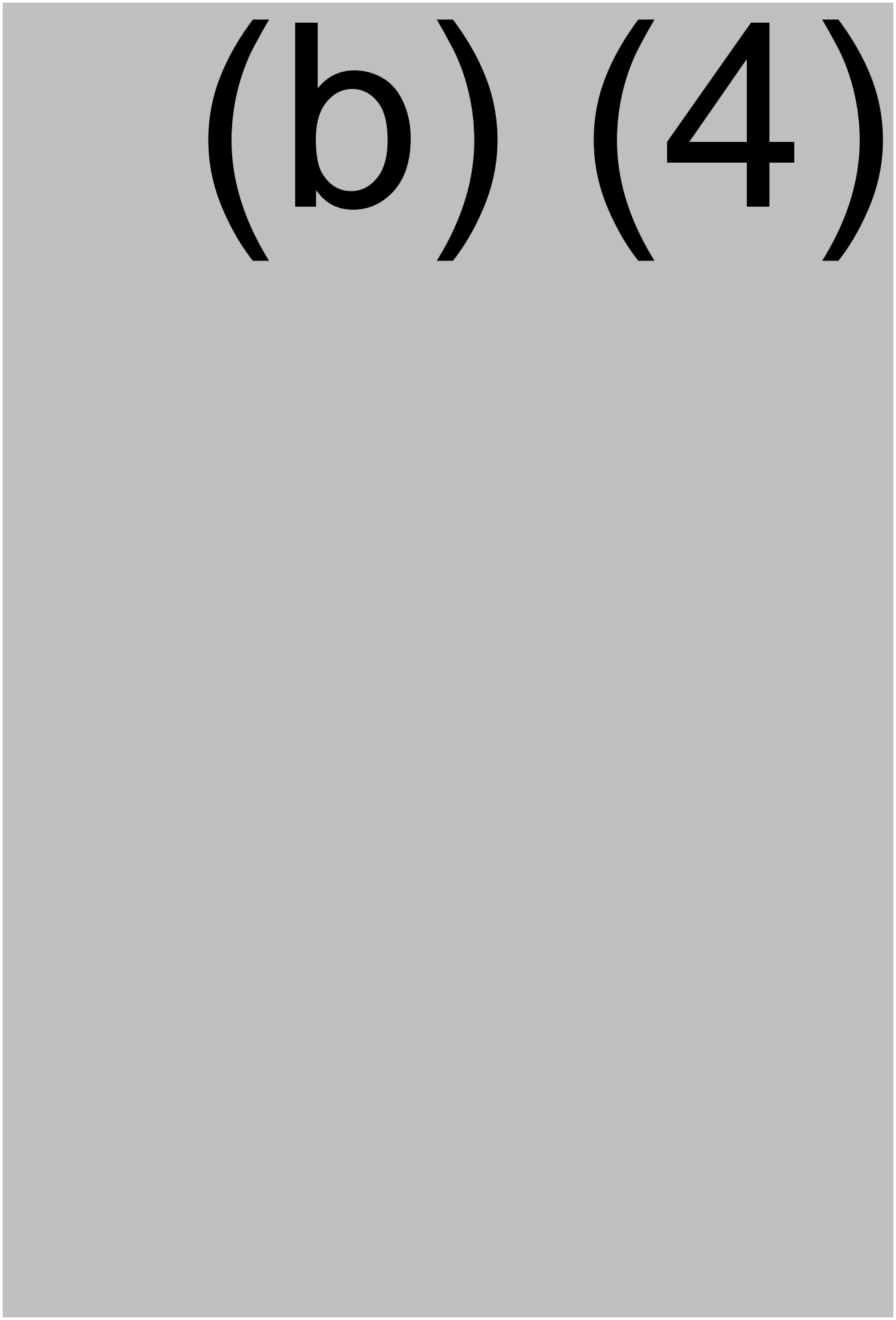
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RYAM STANDARD TEST INSTRUCTION

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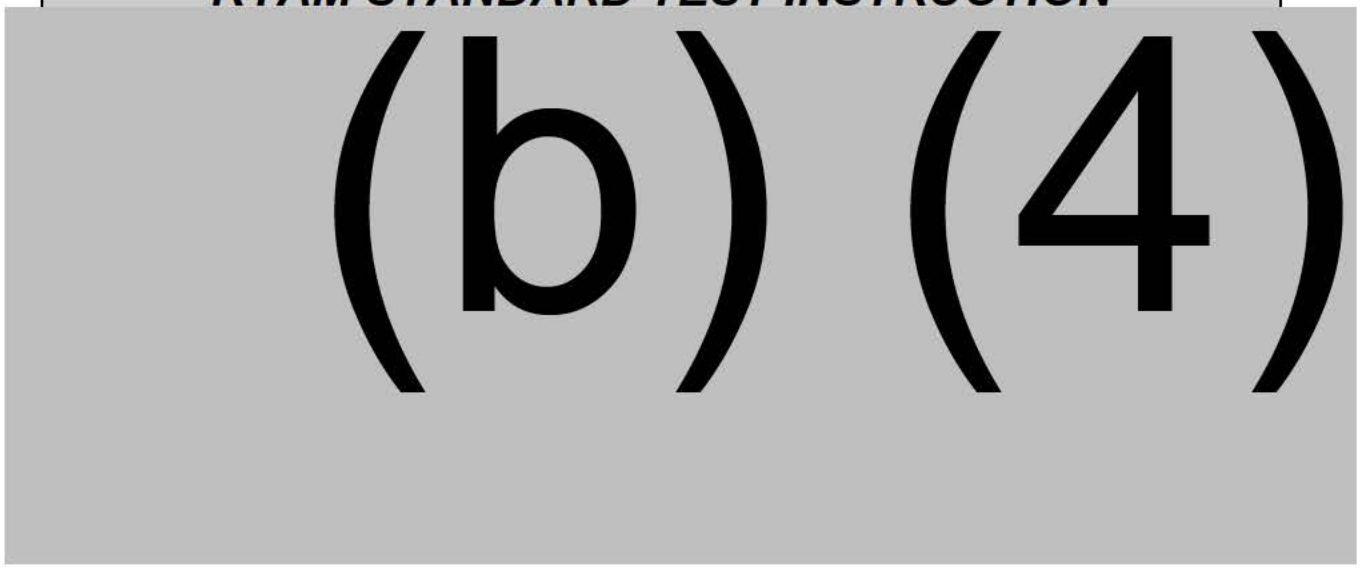
RYAM STANDARD TEST INSTRUCTION



RAYONIER ADVANCED MATERIALS STANDARD TEST INSTRUCTION

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EXHIBIT 1

Report of the Expert Panel

Opinion of an Expert Panel on the Safety and Generally Recognized as Safe (GRAS) Status of Xylooligosaccharides for Use in Poultry, Swine, and Fish Feed

Introduction

An independent panel of experts (GRAS Panel), qualified by scientific training and experience to evaluate the safety of animal food ingredients, was specially convened on behalf of Rayonier Advanced Materials Inc (RYAM) by ToxStrategies LLC to conduct a critical and comprehensive evaluation of the available pertinent data and information, and determine whether, under the conditions of intended use of xylooligosaccharides (XOS) as a feed supplement to provide a source of XOS for swine, poultry, and fish, is safe and "Generally Recognized as Safe" ("GRAS") based on scientific procedures in accordance with 21 CFR § 570.30(a) and (b).

The proposed intended use of the XOS is for swine, poultry, and fish feeds at a dietary inclusion rate of up to 3%.

The proposed use of XOS as a source of fermentable fiber will provide energy to and support normal intestinal function and microbiota populations. XOS will be fermented by the gut flora in the intestinal tract to produce short-chain fatty acids. The fermentation products will be metabolized by the animal and their microbiota for energy. Metabolization of XOS will negate human exposure via consumed meat or eggs.

A detailed review based on the existing scientific literature (through November 2023) on the safety of XOS was conducted for RYAM and is summarized in the attached dossier.

The GRAS Expert Panel included the following individuals: Michael Carakostas, DVM, Ph.D., MC Scientific Consulting LLC; George C. Fahey, Jr., Ph.D., University of Illinois; and Delbert M. Gatlin III, Ph.D., Texas A&M University. These individuals are qualified by scientific training and experience to evaluate the safety of substances intended to be added to animal foods. They have critically reviewed and evaluated the publicly available information summarized in this document and have individually and collectively concluded via teleconference, on April 9, 2024, that XOS, produced in a manner consistent with GMP and meeting the specifications described herein, is safe under its intended conditions of use. The Panel further unanimously concludes that the intended use of XOS is GRAS, based on scientific procedures, and that other experts qualified to assess the safety of animal foods and food additives would concur with these conclusions. A summary of the basis for the GRAS Panel's conclusion is provided below.

Summary and Basis for GRAS Determination

The name of the substance that is the subject of this GRAS determination is XOS.

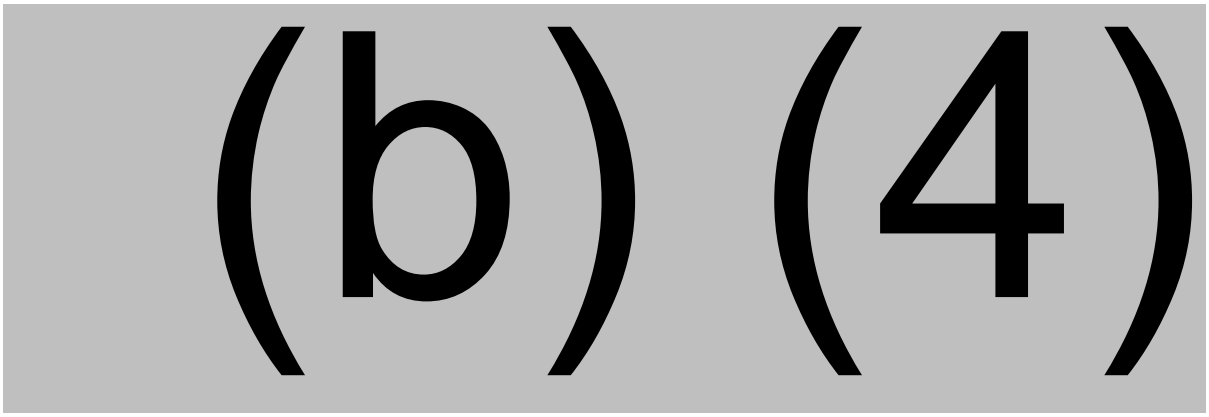
The intended use of the XOS is for poultry, swine, and fish feeds at a dietary inclusion rate

of up to 3% as a source of fermentable fiber to provide energy to and support normal intestinal function and microbiota populations.

As a feed additive for poultry, swine, and fish, the XOS will be fermented by the gut flora in the intestinal tract to produce short-chain fatty acids. The fermentation products will be metabolized by the animal and their microbiota for energy, negating any exposure via consumed meat or eggs. Similarly, the sodium chloride and sodium caseinate will be fully digested by the animal, absorbed, and metabolized, negating any exposure via consumed meat or eggs.

Xylooligosaccharides are a recognized ingredient by the European Food Safety Authority (EFSA, 2018) and FDA GRAS Notices (GRN 343; GRN 458; GRN 816) relative to direct consumption of XOS in human foods. These authorities allow inclusion of XOS in a wide variety of foods up to 2.4 grams/serving. In addition, sodium chloride (21 CFR 182.1(a)) and sodium caseinate (21 CFR 182.1748) are GRAS for use in human foods when used in accordance with good manufacturing practice.

Manufacturing Process



Technical Effect

The XOS will provide a source of fermentable fiber.

Intake Assessment

The intended use of the XOS is for poultry, swine, and fish feeds at a dietary inclusion rate of up to 3% as a source of fermentable fiber to provide energy to and support normal intestinal function and microbiota populations. However, as a feed additive for poultry, swine, and fish, the XOS will be fermented by the gut flora in the intestinal tract to produce short-chain fatty acids. The fermentation products will be metabolized by the animal and their microbiota for energy, negating any exposure via consumed meat or eggs. Similarly, the sodium chloride and sodium caseinate will be fully digested by the animal, absorbed, and metabolized, negating any exposure via consumed meat or eggs.

Human Exposure

Data are available from the European Food Safety Authority (EFSA, 2018) and FDA GRAS Notices (GRN 343; GRN 458; GRN 816) relative to direct consumption of XOS in human foods. These authorities allow inclusion of XOS in a wide variety of foods up to 2.4 grams/serving. In addition, sodium chloride (21 CFR 182.1(a)) and sodium caseinate (21 CFR 182.1748) are generally recognized as safe for use in human foods when used in accordance with good manufacturing practice.

As part of an animal feed ingredient approval, potential human exposure through consumption of meat, milk, and eggs produced by animals consuming the proposed new ingredient must be assessed. However, as a feed additive for poultry, swine, and fish, the XOS will be fermented by the gut flora in the intestinal tract to produce short-chain fatty acids. The fermentation products will be metabolized by the animal and their microbiota for energy, negating any exposure via consumed meat or eggs. Similarly, the sodium chloride and sodium caseinate will be fully digested by the animal, absorbed, and metabolized, negating any exposure via consumed meat or eggs.

However, XOS will be used as a source of fermentable fiber in feed for poultry, swine, and fish. As a result, there will be no additional human exposure to XOS from the use of XOS in feed after gut flora fermentation to produce short-chain fatty acids from XOS. The sodium chloride and sodium caseinate portion of XOS will be metabolized by poultry, swine, and fish as an energy source with no exposure directly to humans.

Safety Data

The intended use of XOS is as a feed ingredient to provide a source of fermentable fiber for normal intestinal microbiota. The intended inclusion level is up to 3% in poultry, swine, and fish feed.

Monogastric animals such as poultry, swine, and finfish do not produce the enzymes required for enzymatic hydrolysis of XOS which allows it to serve as a prebiotic that is fermented by the microbial community in the lower gut. The use of prebiotics can improve intestinal microflora communities and subsequent intestinal health across poultry, swine, and fish species that represent feeding habits ranging from carnivorous through omnivorous to herbivorous. The microbial communities use the XOS for microbial growth with a concomitant production of short-chain fatty acids such as acetate, propionate, and butyrate that can be used as an energy source by the host target species and reduce the pH in the lumen of the gut thereby promoting growth of known beneficial microbes.

A total of eighteen studies were identified with XOS used in broiler feed. Levels of XOS in feed ranged from 0.0002% (42 days) to 2% (59 days), and study duration ranged from 12 to 59 days. In all studies, broilers fed an XOS-supplemented feed had normal growth performance, and mortality was not different than controls. A few studies reported an increase in intestinal villus height, a decrease in crypt depth, and greater short-chain fatty acid concentrations with XOS supplementation that were considered indicative of positive intestinal health. Biochemical parameters and antibody titers were measured in several

studies and, in general, were either not different from untreated controls or improved. Use levels of up to 3% of the diet were justified based on the documented lack of any adverse effects at the 2% level in a published study, the known metabolism of XOS, and the known metabolic fate and lack of toxicity of the short-chain fatty acid metabolites.

Four studies were identified with XOS added to the feed of layers. Levels of XOS in diet ranged from 0.0005% (56 days) to 0.6% (70 days), and study durations ranged from 8 to 12 weeks. In all studies, animals fed an XOS-supplemented feed had normal egg production and egg quality parameters and normal to improved biochemical parameters. Villus height and villus-height:crypt-depth ratio were also increased in a few studies. When measured, immunoglobulin levels were increased with XOS. Two studies showed positive shifts in intestinal bacterial communities with increased presence of *Bifidobacteria* and *Lactobacillus* and decreased presence of *Escherichia coli*, *Bacteroides*, and *Campylobacter*. Use levels of up to 3% of the diet were justified based on the documented lack of any adverse effect in broilers and the similar metabolic fate of XOS in broilers and layers.

There were 12 studies with XOS use in swine feed. Levels of XOS in feed ranged from 0.01% (up to 56 days) to 3% (28 days), and study duration ranged from 28 – 84 days. In all studies, animals fed an XOS-supplemented feed had normal growth performance parameters and mortality was not different than controls. A few studies noted decreased diarrhea occurrence, improved intestinal morphology, and greater short-chain fatty acid concentrations with XOS supplementation. When evaluated, hematological and biochemical parameters and immunoglobin levels were somewhat improved or not different from untreated controls. XOS supplementation was also associated with positive shifts in intestinal bacterial communities with increased presence of *Bifidobacteria* and *Lactobacillus* and decreased presence of *Escherichia coli* and *Clostridium*.

Fifteen published studies were found examining the effects of XOS feed supplementation in fish. Species studied included carp, sea bass, blunt snout bream, seabream, Caspian white fish, tilapia, rainbow trout, white shrimp, and grouper which represent a variety of feeding behaviors including herbivores, omnivores, carnivores, and detrivores. Feed supplementation ranged from 0.005% for 45 days to 3% XOS for 56 days. In all studies, animals fed an XOS-supplemented feed had normal to improved growth performance parameters. Survival rates were also similar to controls. Intestinal morphology was improved with XOS in feed.

The studies conducted in the target species demonstrated maximum inclusion levels of XOS fed to broiler chickens (2% for up to 59 days), layers (0.6% for up to 70 days), swine (3% for up to 28 days), and finfish (3% for up to 56 days) had no adverse effects and potential positive impacts. Overall, there were no changes in growth performance or adverse effects in the supplemented groups compared to unsupplemented controls. Intestinal morphology and health showed improvements in XOS supplementation when compared to controls. Biochemical parameters were either not different or slightly improved when compared to controls.

When combining the data from poultry, swine, and finfish while considering the similarities and differences of the target species and age groups within the species including

their diversity of feed habits and known ability of prebiotics to improve intestinal microbial ecology and subsequent intestinal health across these species, an inclusion level of 3% XOS would be supported for poultry, swine, and finfish.

A margin of safety for the proposed 3% use level was not deemed necessary as XOS is considered to have nutrient value, has a known metabolism in the lower gastrointestinal tract, has no demonstrated adverse effects at dietary levels up to 3% in the target species, and is supported by published NOAELs that are 3-4 times the proposed level in toxicity studies conducted in rats and dogs.

General Recognition of Safety

The intended use of XOS has been determined to be safe through the scientific procedures set forth in 21 CFR § 570.3(b), thus satisfying the so-called "technical" element of the GRAS determination, based on the following:

- The XOS ingredient that is the subject of this GRAS determination is produced during production of highly purified cellulose that contains xylan-rich hemicelluloses. This XOS solution is removed from the cellulose stream by filtration and neutralized to produce high purity XOS.
- This ingredient is considered to be within specifications at greater than 70% XOS.
- The proposed intended use of XOS is for swine, poultry, and fish feeds, at a dietary inclusion rate of up to 3% to provide a source of fermentable fiber.
- There are no published studies specifically addressing potential exposure of humans to XOS from edible animal tissues. Data are available from the European Food Safety Authority (EFSA) and FDA GRAS Notices relative to direct consumption of XOS in human foods. However, as an ingredient for poultry, swine, and fish, XOS will be fermented by the gut flora, and the fermentation products will be metabolized by the fed animal, negating any exposure via consumed meat or eggs.
- Peer-reviewed studies in broilers indicated no adverse effects on growth performance when they were exposed to XOS feed supplementation ranging from 0.0002% (42 days) to 2% (59 days), and study duration ranging from 12 to 59 days. When evaluated, studies reported normal to improved intestinal morphology. Biochemical parameters and antibody titers were measured in several studies and, in general, were either no different from untreated controls or improved. Use levels of up to 3% of the diet were justified based on the documented lack of any adverse effects at the 2% level in a published study, the known metabolism of XOS, and the known metabolic fate and lack of toxicity of the short-chain fatty acid metabolites.
- Peer-reviewed studies in layers found no adverse effects on egg production and egg quality parameters with XOS added to the feed. Levels of XOS in diet ranged from 0.0005% (56 days) to 0.6% (70 days), and study durations ranged from 8 to 12 weeks. In all studies, animals fed an XOS-supplemented feed had normal or improved biochemical parameters, intestinal morphology, and immune function parameters. Two studies showed positive shifts in intestinal bacterial communities

with increased presence of *Bifidobacteria* and *Lactobacillus* and decreased presence of *Escherichia coli*, *Bacteroides*, and *Campylobacter*. Use levels of up to 3% of the diet were justified based on the documented lack of any adverse effect in broilers and the similar metabolic fate of XOS in broilers and layers.

- Peer-reviewed studies in swine found no adverse effects on growth performance in weanling pigs exposed to levels of XOS in feed ranging from 0.01% (up to 56 days) to 3% (28 days), and study duration ranged from 28 – 84 days. A few studies noted decreased diarrhea occurrence, improved intestinal morphology and greater short-chain fatty acid concentrations with XOS supplementation. When evaluated, hematological and biochemical parameters and immunoglobin levels were not different from untreated controls or somewhat improved. XOS supplementation was also associated with positive shifts in intestinal bacterial communities with increased presence of *Bifidobacteria* and *Lactobacillus* and decreased presence of *Escherichia coli* and *Clostridium*.

- Peer-reviewed studies in multiple fish species (carp, sea bass, blunt snout bream, seabream, Caspian white fish, tilapia, rainbow trout, white shrimp, and grouper) with a diversity of feeding behaviors indicated that XOS feed supplementation (0.005% for 45 days to 3% XOS for 56 days) led to normal to improved growth performance parameters. Survival rates were also similar to controls. Intestinal morphology was improved with XOS in feed.

- When combining the data from poultry, swine, and finfish while considering the similarities and differences of the target species and age groups within the species including their diversity of feed habits and known ability of prebiotics to improve intestinal microbial ecology and subsequent intestinal health across these species (Baker et al., 2021), an inclusion level of 3% XOS would be supported for poultry, swine, and finfish.

- A margin of safety for the proposed 3% use level was not deemed necessary as XOS is considered to have nutrient value, has a known metabolism in the lower gastrointestinal tract, has no demonstrated adverse effects at dietary levels up to 3% in the target species, and is supported by published NOAELs that are 3-4 times the proposed level in toxicity studies conducted in rats and dogs.

- In summary, peer-reviewed studies in poultry, swine, and fish support the conclusion that XOS is safe when used in poultry, swine, and fish feed not to exceed 3% XOS to provide energy and support normal intestinal health and microbiota populations in poultry, swine, and fish feed.

Because this safety evaluation was based on generally available and widely accepted data and information, it also satisfies the "common knowledge" element of a GRAS determination.

Conclusions of the GRAS Panel

We, the undersigned independent, qualified members of the GRAS Panel, have individually and collectively critically reviewed the published and ancillary information pertinent to the identification, use, and safety of RYAM's XOS ingredient for use in poultry, swine, and fish feed. We unanimously conclude that the intended use of RYAM's XOS produced consistent with current Good Manufacturing Practice (cGMP) and meeting the appropriate animal food-grade specifications, as presented in the supporting dossier is safe.

We, the members of the GRAS Panel, further unanimously conclude that the intended uses and use levels of RYAM's XOS ingredient for use in poultry, swine, and fish feed, produced consistent with cGMP and meeting the appropriate animal food-grade specifications, as presented in the supporting dossier, is Generally Recognized as Safe (GRAS) based on scientific procedures under the conditions of intended use in foods as described herein.

It is our professional opinion that other qualified experts critically evaluating the same information would concur with this conclusion.

(b) (6) 15 May 2024

Michael Carakostas, DVM, Ph.D. Date
Consultant
MC Scientific Consulting LLC

George C. Fahey, Jr., Ph.D. Date
Professor Emeritus of Animal Sciences
University of Illinois at Urbana-Champaign

Delbert M. Gatlin III, Ph.D. Date
Regents Professor
Department of Ecology and Conservation Biology

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Michael Carakostas, DVM, Ph.D. Date

Consultant

MC Scientific Consulting LLC

(b) (6) May 15, 2024

George C. Fahey, Jr., Ph.D. Date

Professor Emeritus of Animal Sciences

University of Illinois at Urbana-Champaign

Delbert M. Gatlin III, Ph.D. Date

Regents Professor

Department of Ecology and Conservation Biology

Texas A&M University

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Michael Carakostas, DVM, Ph.D. Date

Consultant

MC Scientific Consulting LLC

George C. Fahey, Jr., Ph.D. Date

Professor Emeritus of Animal Sciences

University of Illinois at Urbana-Champaign

(b) (6) May 15, 2024

Delbert M. Gatlin III, Ph.D. Date

Regents Professor

Department of Ecology and Conservation Biology

Texas A&M University

RYAM™

Ms. Wasima Wahid
Division of Animal Feeds (HFV- 220)
Center for Veterinary Medicine
Food and Drug Administration
7519 Standish Pl.
Rockville, MD 20855

September 23, 2024

Subject: GRAS Notice for Xylooligosaccharides

Notifier: Rayonier Advanced Materials Inc (RYAM)
1301 Riverplace Blvd #2300,
Jacksonville, FL 32207

Dear Ms. Wahid,

On September 20, 2024, you requested clarification on the utility of the substance as related to safety. We appreciate the teleconference between you and Dr. Jennifer van de Ligt on September 23, 2024, to further explain the request for clarification.

The intended use of the xylooligosaccharides is as a source of fermentable fiber at a dietary inclusion rate of up to 3% to provide energy and support normal intestinal health and microbiota populations in poultry, swine, and fish. This use has been demonstrated by a thorough review of published well-controlled studies evaluating various levels of xylooligosaccharides in feed for poultry, swine, and fish. However, under the regulations covering GRAS notification, the intended use is not related to safety beyond the compositional value as a supplementary source of fermentable fiber. As such, demonstration of utility of the GRAS substance is not required (21 CFR 570.230(d)).

Sincerely,

(b) (6)

Larissa S. Fenn, PhD, MBA
Director, New Products Research and Development

Marketing and Research
Center, Jesup, GA US

912.588.8261 office
(b) (6) mobile

larissa.fenn@rayonieram.com
www.RYAMglobal.com

ToxStrategies

Innovative solutions
Sound science

May 15, 2025

Attn: Ms. Wasima Wahid, M.S.
Biologist
Center for Veterinary Medicine
Office of Surveillance and Compliance
Division of Animal Food Ingredients (DAFI)

SUBJECT: GRAS Notice Submission
Xylooligosaccharide
AGRN 73

SUBMITTER: Rayonier Advanced Materials Inc
1301 Riverplace Blvd. #2300
Jacksonville, FL 32207

Ms. Wahid,

Please find Rayonier's response (and accompanying references) to the question posed in the GRAS Notice Submission AGRN 73 email dated May 9, 2025 below in Appendix A.

Thank you for the opportunity to clarify this question. Please let us know if any further clarifications are needed.

Sincerely,

(b) (6)

Jennifer Hinerman, Ph.D.
Senior Scientist, ToxStrategies
Consultant to Rayonier Advanced Materials Inc.

cc: Larissa Fenn, Rayonier Advanced Materials Inc.

Appendix A

Rayonier's response to question posed in May 9, 2025 regarding GRAS Notice Submission AGRN 73 and supporting literature

May 15, 2025

Attn: Ms. Wasima Wahid, M.S.
Biologist
Center for Veterinary Medicine
Office of Surveillance and Compliance
Division of Animal Food Ingredients (DAFI)

SUBJECT: GRAS Notice Submission
Xylooligosaccharide
AGRN 73

SUBMITTER: Rayonier Advanced Materials Inc
1301 RiverPlace Blvd. #2300
Jacksonville, FL 32207

Ms. Wahid,

Please find our responses to each question posed in the GRAS Notice Submission AGRN 73 email dated May 9, 2025. The original question is displayed in italics and the answers are provided in plain text.

Question: *In our January 29, 2025 email, we indicated that "As a source of fermentable fiber, we expect utility to be demonstrated as compared with an appropriate control in studies utilizing healthy animals (e.g., no challenge studies). The notifier should provide data and information that support the utility of the notified substance as a fermentable fiber in the target animal species." Alternatively, we offered that "the notifier may consider changing the intended use of the notified substance from "a source of fermentable fiber" to "a source of xylooligosaccharides. If pursuing this option, the notifier should clarify in its amendment that the utility of the notified substance as a source of XOS does not impact safety of the target animal when the substance is used at the proposed use rate."*

The notifier's February 12, 2025 response indicated "Rayonier is amendable to decreasing the proposed inclusion rate to up to 1 % XOS, consistent with its demonstrated, utility as a fermentable fiber." We have interpreted this response to indicate that the intended use should remain "as a source of fermentable fiber". However, additional data and information to support utility of this intended use were not provided as part of the amendment.

If it is the Notifier's intention to maintain the assertion made in the original cover letter that utility does not affect the safety of the notified substance, then the Notice should be amended to reflect an intended use "as a source of xylooligosaccharides." Under this scenario information supporting utility of XOS would not be necessary; however, the

amended Notice would need to include an explanation of why the utility the intended use as a source of XOS would not impact the safety of target animals consuming diets containing the notified substance. Additionally, the Notice would need to be amended to remove any reference to the utility of the substance and to affirm that utility does not impact safety of the target animal.

Response: We are slightly confused by the request to choose between a utility statement of "as a source of fermentable fiber" or "as a source of xylooligosaccharides." Fiber is defined as non-digestible soluble and insoluble carbohydrates and is well established as a dietary nutrient with a wide range inclusion levels across and among animal species. Xylooligosaccharides are carbohydrates that are non-digestible because the target animal species do not produce enzymes capable of digesting them. As a result, xylooligosaccharides meet the definition of fiber. In addition, xylooligosaccharides, like most oligosaccharides and many other fibers, can be fermented in the intestine of the target animal species thereby meeting the definition of fermentable fiber.

Although xylooligosaccharides are intended to be used in animal food, the principle of dietary fiber is the same in other animal species including humans. For example, the FDA established the definition of dietary fiber with respect to human food labeling (FDA-2016-D-3401, FDA-2018-D-1323). This definition of dietary fiber includes "certain naturally occurring fibers that are "intrinsic and intact" in plants and added isolated or synthetic non-digestible soluble and insoluble carbohydrates that have beneficial physiological effects to health." Examples of fiber in the human food labeling guidance include long-chain fibers such as cellulose and arabinoxylans, and short-chain fibers like addition, McClearly (2023) discusses oligosaccharide analysis, including xylooligosaccharides, by the Total Dietary Fiber (TDF) method.

The physiological and nutritive effects of fiber occur primarily through two mechanisms: 1) physiological effects such as improved laxation or stool bulking when insoluble or non-fermentable fibers are consumed, and 2) nutritive effects when soluble or fermentable fibers are consumed. In the target animal species, nutritive effects of fermentable fiber include increased growth due to energy release from fermentation of the non-digestible carbohydrates, shifting microbiota to species affiliated with gut development, release of volatile fatty acids that are used by the intestinal enterocytes, improved intestinal morphology, and others (Singh and Kim, 2021). The theme of the nutritive effect of fermentable fibers is improved intestinal health due to the interaction between microbiota, volatile fatty acids, and the intestinal cells.

Because many feedstuffs include very long chain non-digestible and poorly fermentable fibers as part of their cell walls, the inclusion of enzymes, such as

additions to feed (Singh and Kim, 2021). The action of xylanase is to break the long-chain xylans in these feedstuffs within the lumen of the gut to produce fermentable xylooligosaccharides that provide the nutritive effect of fermentable fiber. Singh and Kim (2021) state that common fermentable dietary fibers in feeds are "small fragments of carbohydrates which are oligosaccharides of fructose, xylose, mannose, or galactose, etc." As a result, the inclusion of xylooligosaccharides directly to feed provides the fermentable fiber nutritive effect without the use of dietary xylanases.

The utility, or nutritive effect, 'as a source of fermentable fiber' or 'as a source of xylooligosaccharides' can be demonstrated from the same data provided to demonstrate safety. The following are a selection of data for each target animal species that was included in the AGRN. These selections are not intended to be comprehensive.

Yadav et al. (2024) demonstrated a linear increase toward more *Biidobacterium* with increasing XOS and a trend for increased villi height: crypt depth in broilers receiving 0.05 and 0.2% XOS supplementation groups compared to both the control and the 0.1% XOS group. These findings are indicative improved intestinal development, a known nutritive effect of fiber consumption. Lin et al. (2023) observed increased cecal total short chain fatty acids, acetate, and butyrate in the XOS-supplemented, unchallenged group when compared to unchallenged control. Yuan et al. (2018) found that cecal acetate and butyrate were increased with XOS supplementation. Yuan et al. (2018) further stated that butyrate is a primary energy source for colonic cells and has other effects considered positive for intestinal health. The short chain fatty acid results clearly demonstrate that XOS is a fermentable fiber and provides nutritive effects. Zhenping et al. (2013) demonstrated improved body weight gain and feed efficiency for broilers consuming 1% XOS. De Maesschalck et al. (2015) found increased villi height and increased butyrate-producing bacteria and lactobacilli in broiler ceca with XOS supplementation.

Ding et al. (2018) and Zhou et al. (2021) demonstrated increased villi height: crypt depth ratio as with XOS supplementation in layers. Ding et al. (2018) also observed increased cecal short chain fatty acids, acetate, and butyrate in XOS-fed layers.

Liu et al. (2018) and Hou et al. (2020) observed increased growth metrics in weanling pigs fed XOS. Liu et al. (2018) and Chen et al. (2021a) observed increased villi height: crypt depth ratio for weanling pigs fed XOS. Chen et al. (2021a) observed increased total short-chain fatty acids, propionate, and butyrate concentrations in the cecum and Ding et al. (2021) observed increased butyrate in the ileum in XOS-fed weanling pigs.

Abasubong et al. (2022) observed improved body mass index in common carp fed XOS. Guerreiro et al. (2015) observed improved final body weight and body weight gain in sea bass

fed XOS. Sun et al. (2019) demonstrated improved feed efficiency, survival rate, and intestinal villi height and area in shrimp fed XOS.

Not all studies with XOS result in demonstrable nutritive effects from inclusion of low levels of fermentable fiber. It is well-known in animal nutrition that studies demonstrating nutritive effects of fermentable fibers often have non-significant differences in some or all measures depending upon husbandry practices, dietary formulation, nutrient concentration, environmental and disease pressure, etc. However, the majority of the studies included in the dossier to demonstrate safety of XOS also demonstrate at least one nutritive effect of fermentable fiber observed with the addition of xylooligosaccharides to the feed.

Xylooligosaccharides are, by definition, a fermentable fiber as demonstrated by numerous controlled studies in healthy animals. Rayonier is amenable to using any of the following utility statements - "as a source of fiber" or "as a source of fermentable fiber" or "as a source of xylooligosaccharides" - because the statements are synonymous with more specificity provided in each subsequent statement by declaring the type of fiber (i.e., fermentable) in the second option and the chemistry of the fiber (i.e., xylooligosaccharides) in the third option. The updated intended use should be considered in all discussion of intended use throughout the notice. Whether the utility is considered "as a source of fiber" or "as a source of fermentable fiber" or "as a source of

References (those not included in original submission)

Singh, A. K., & Kim, W. K. (2021). Effects of Dietary Fiber on Nutrients Utilization and Gut Health of Poultry: A Review of Challenges and Opportunities. *Animals*, *11*(1), 181. https://doi.org/10.3390/ani11010181

McCleary B. V. (2023). Measurement of Dietary Fiber: Which AOAC Official Method of AnalysisSM to Use. *Journal of AOAC International*, 106(4), 917-930. https://doi.org/10.1093/jaoacint/qsad051

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Review Article

Human Nutrient Methods

Measurement of Dietary Fiber: Which AOAC *Official Method of Analysis*[SM] to Use

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Abstract

A broad range of AOAC *Official Methods of Analysis*[SM] (OMA) have been developed and approved for the measurement of dietary fiber (DF) and DF components since the adoption of the Prosky method (OMA 985.29). OMA 985.29 and other OMA were developed to support the Trowell definition of DF. However, these methods do not measure DF as defined by the "new," physiologically relevant, Codex Alimentarius definition. Methodology to support the Codex definition has been developed and updated in recent years. In this article, the relevance of each OMA in supporting the Codex definition of DF is described and suggestions are presented on the most appropriate method, together with proposals for changes in title and application statements for the "historic" OMA methods.

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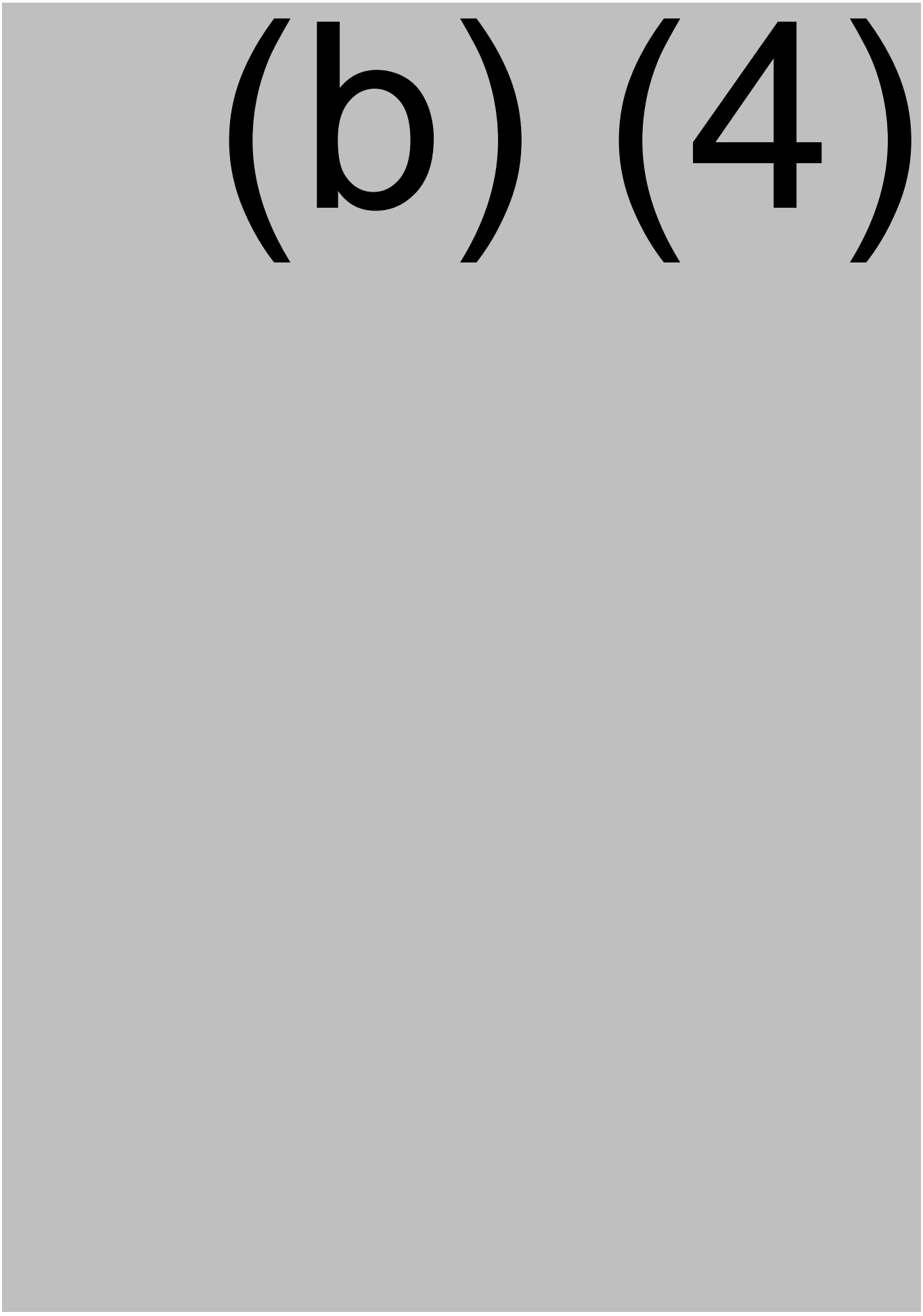
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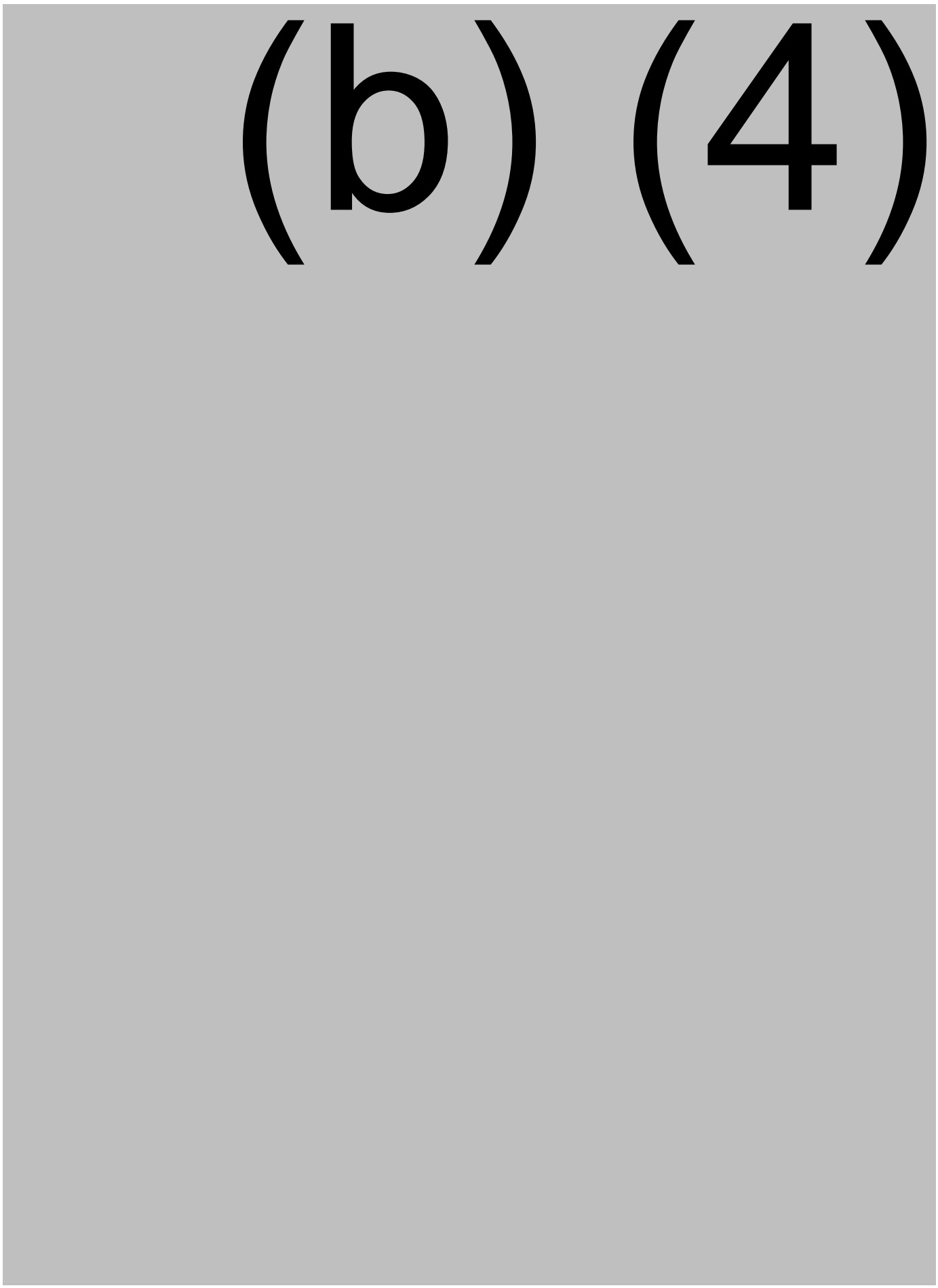
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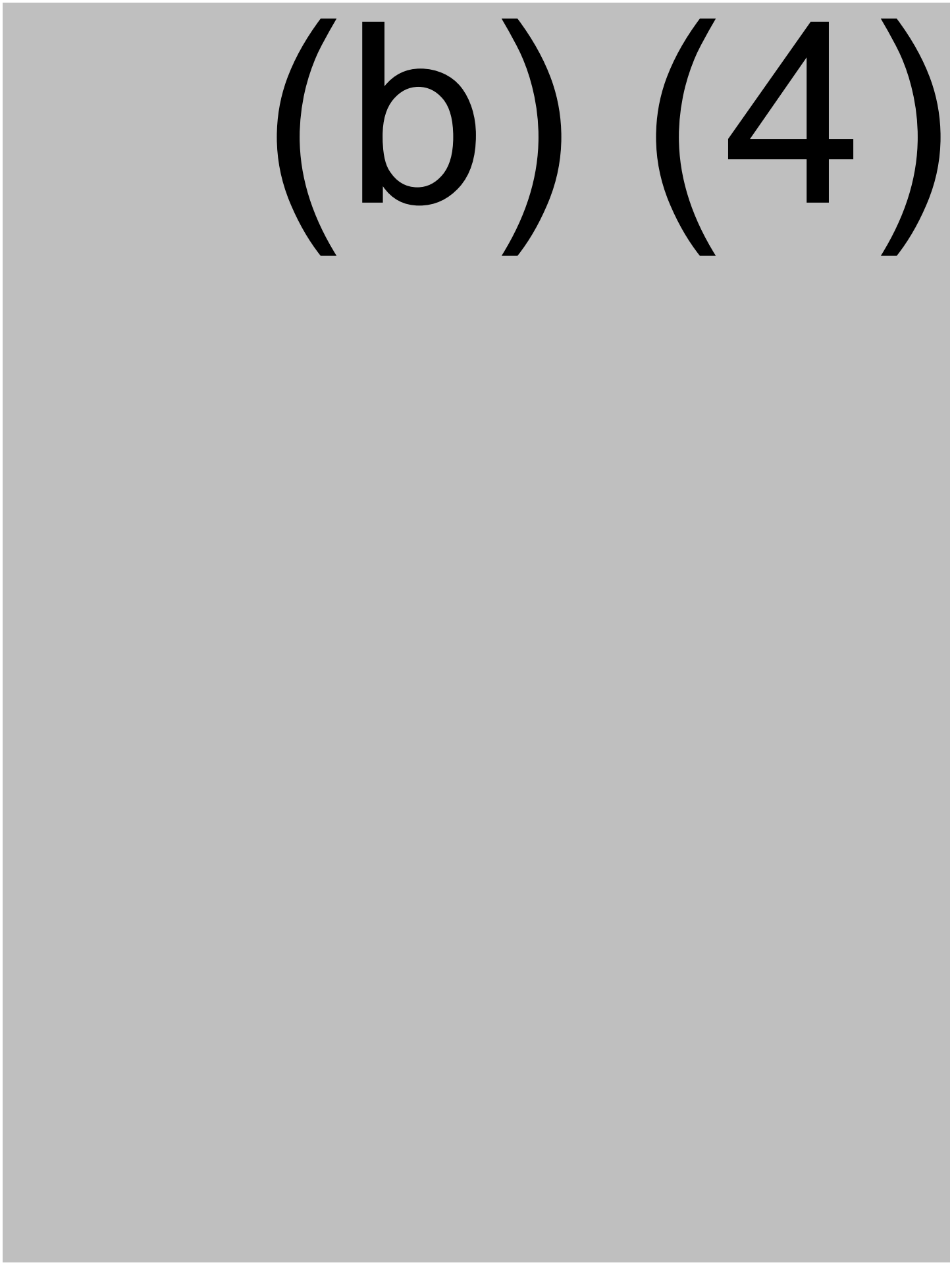
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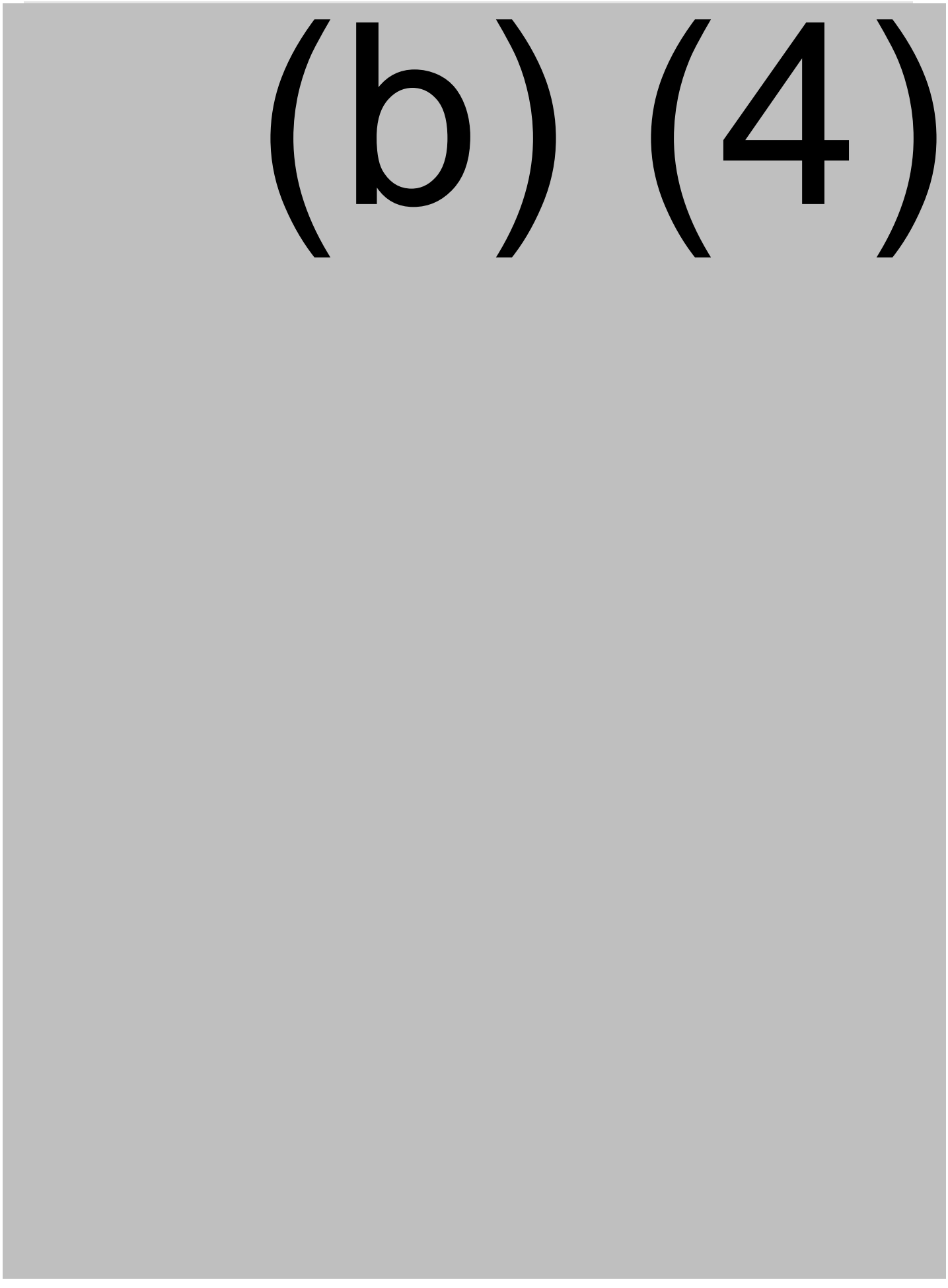
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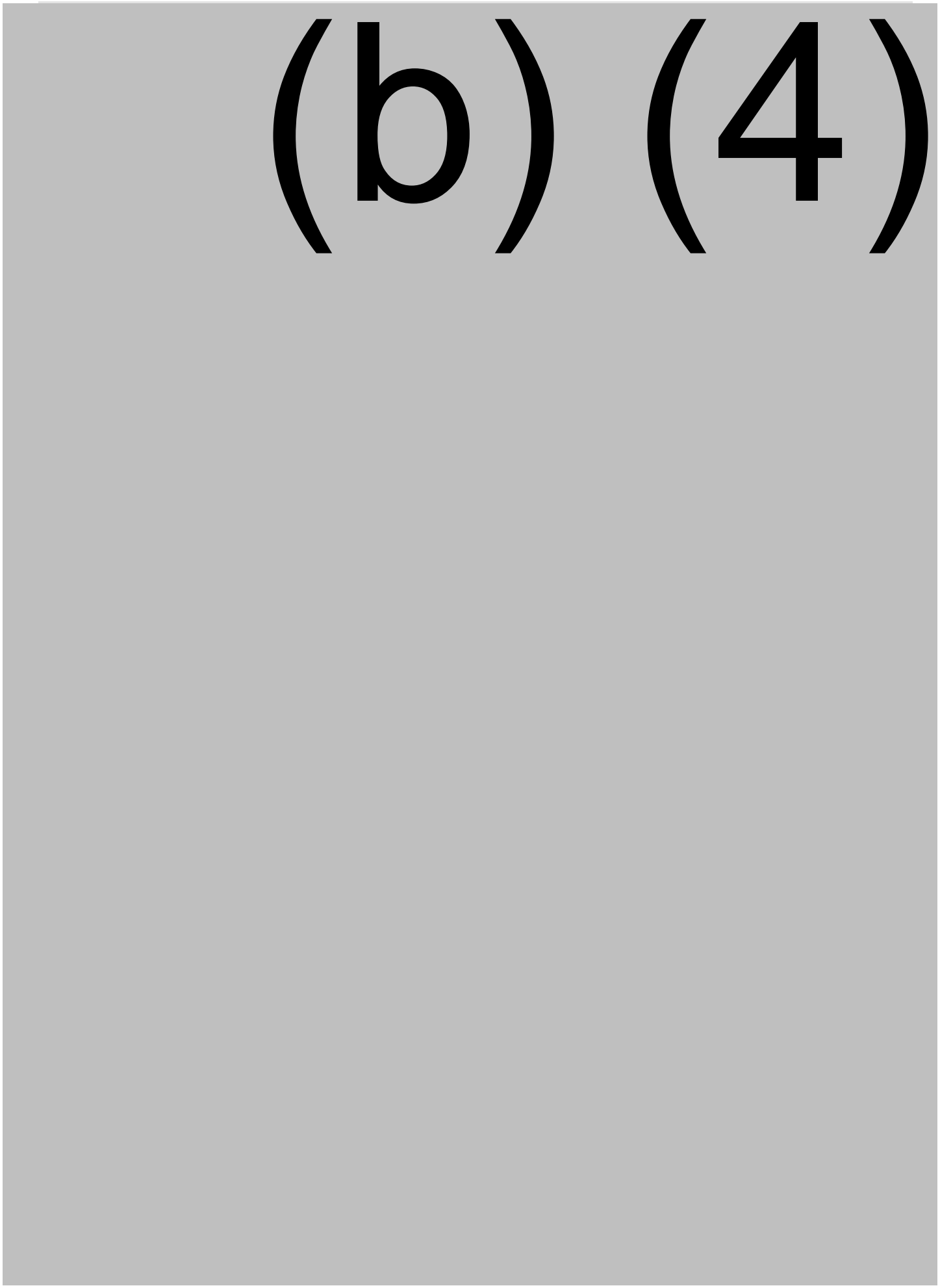
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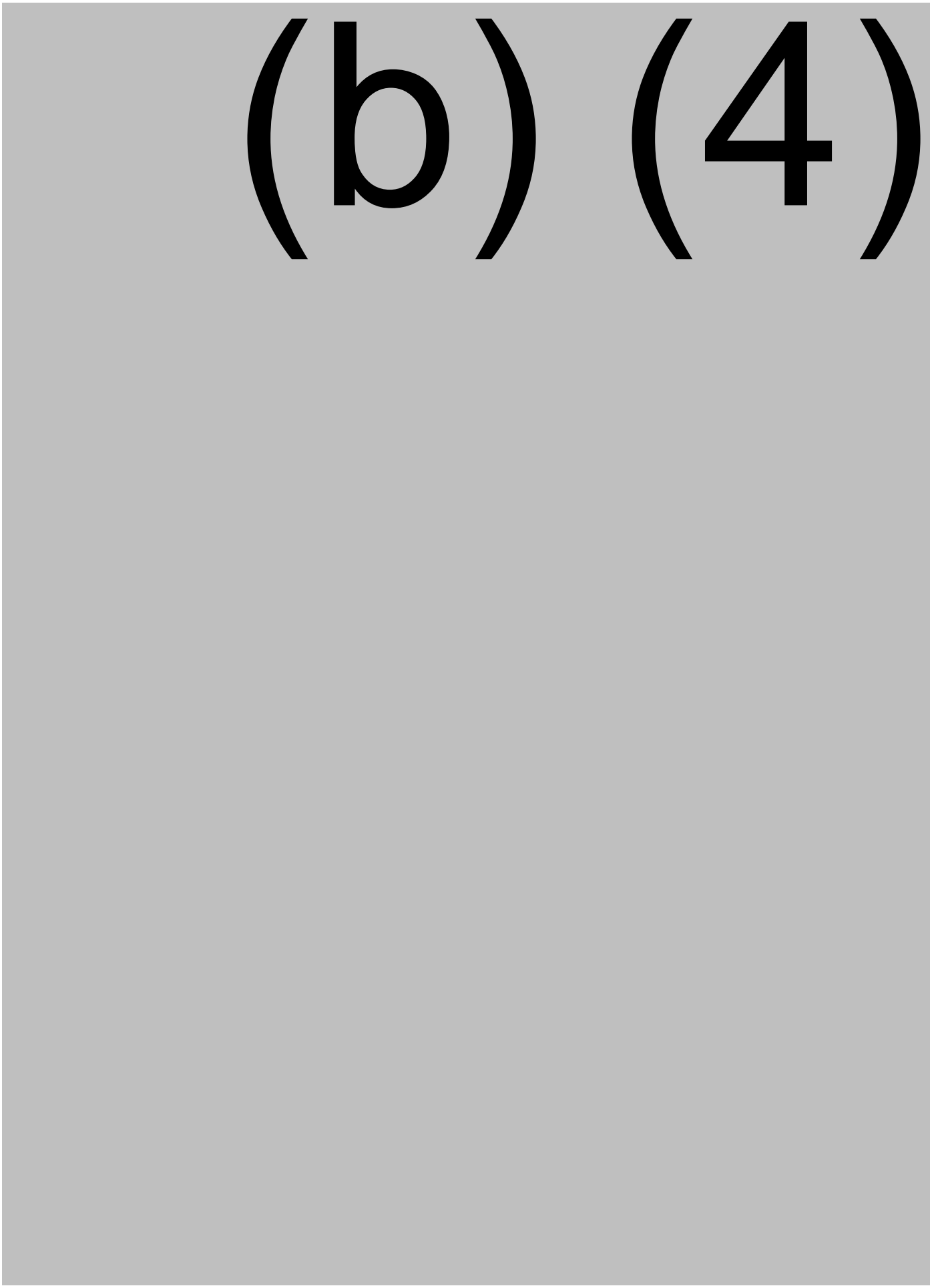
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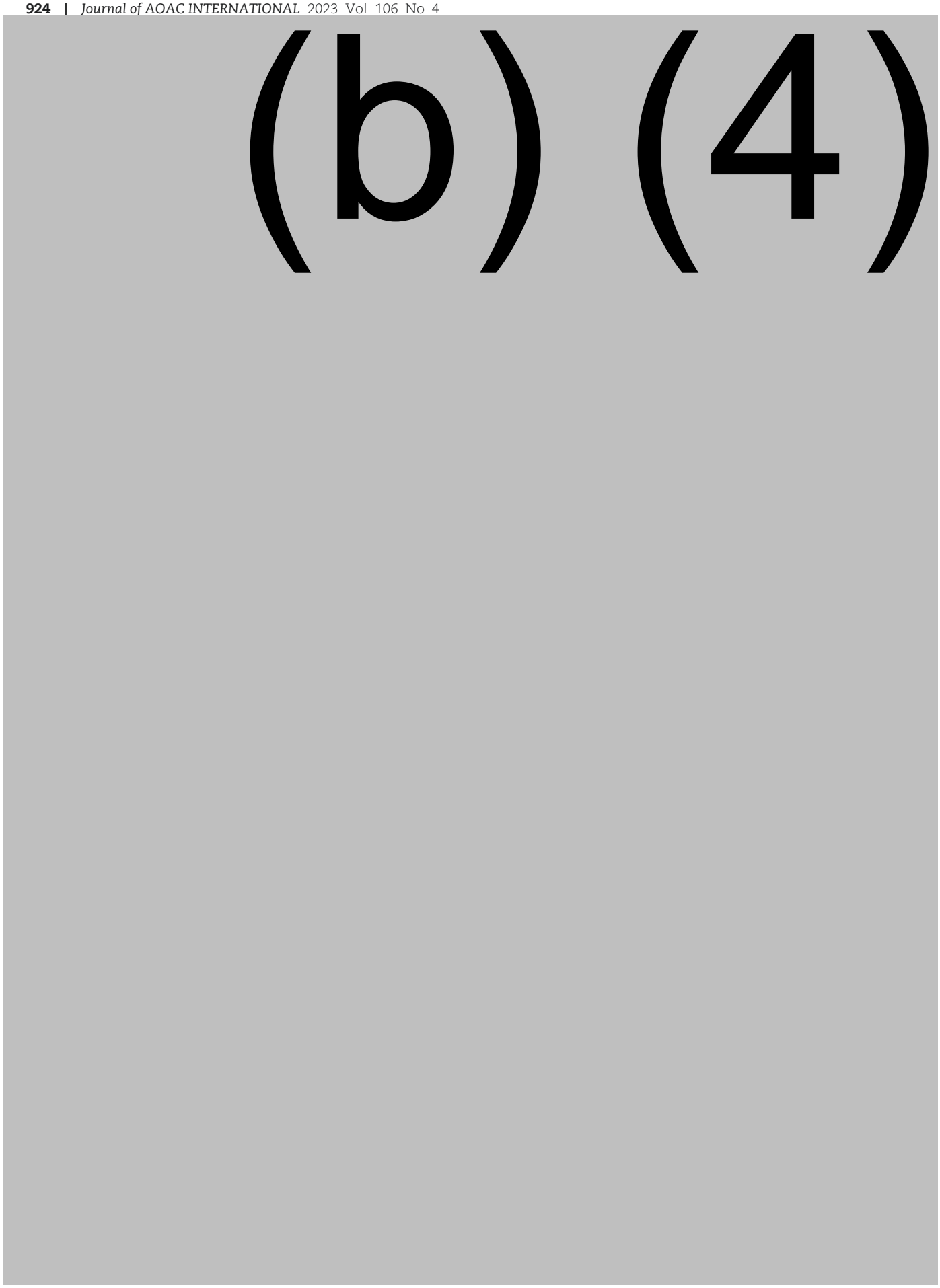
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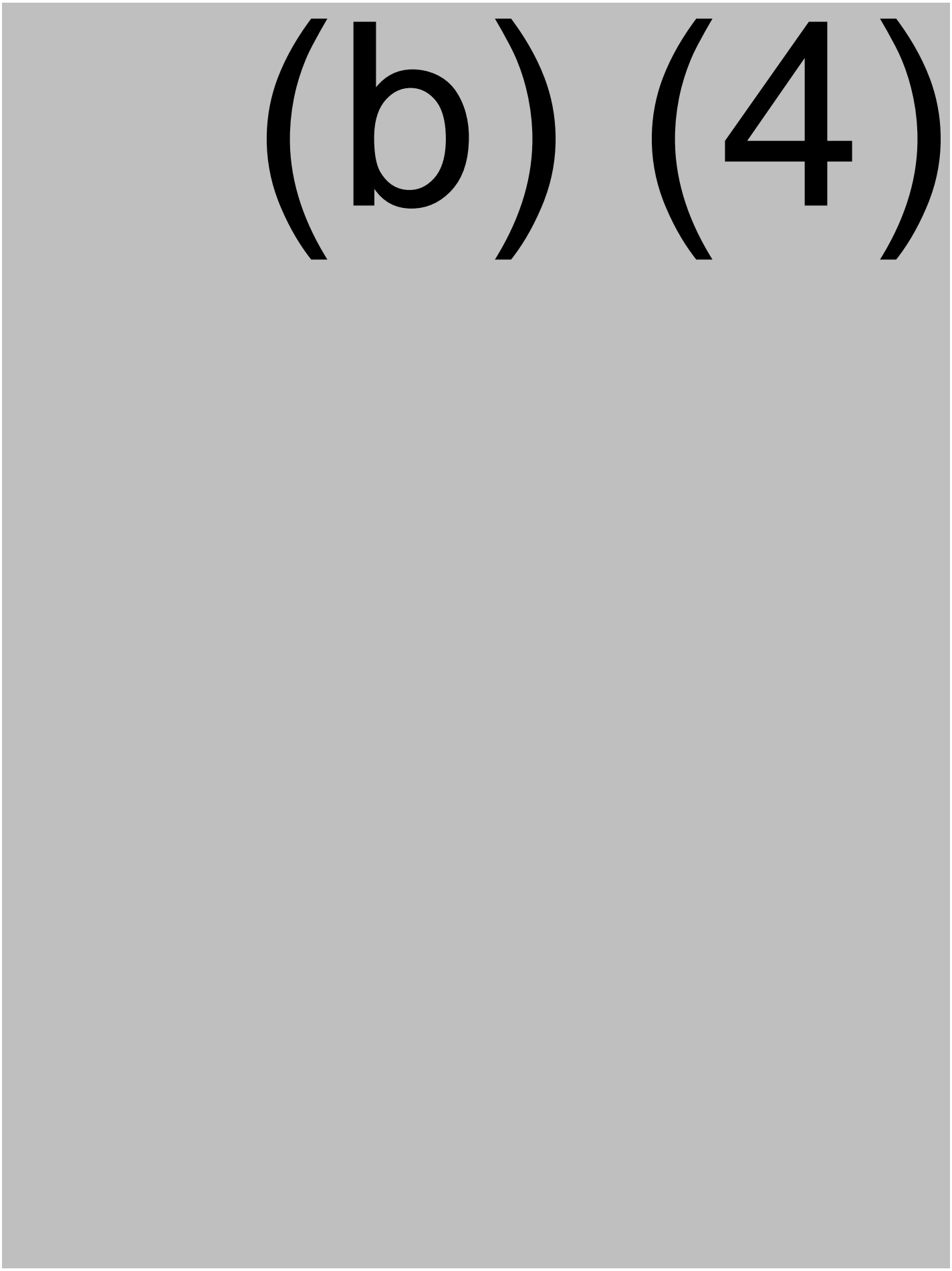
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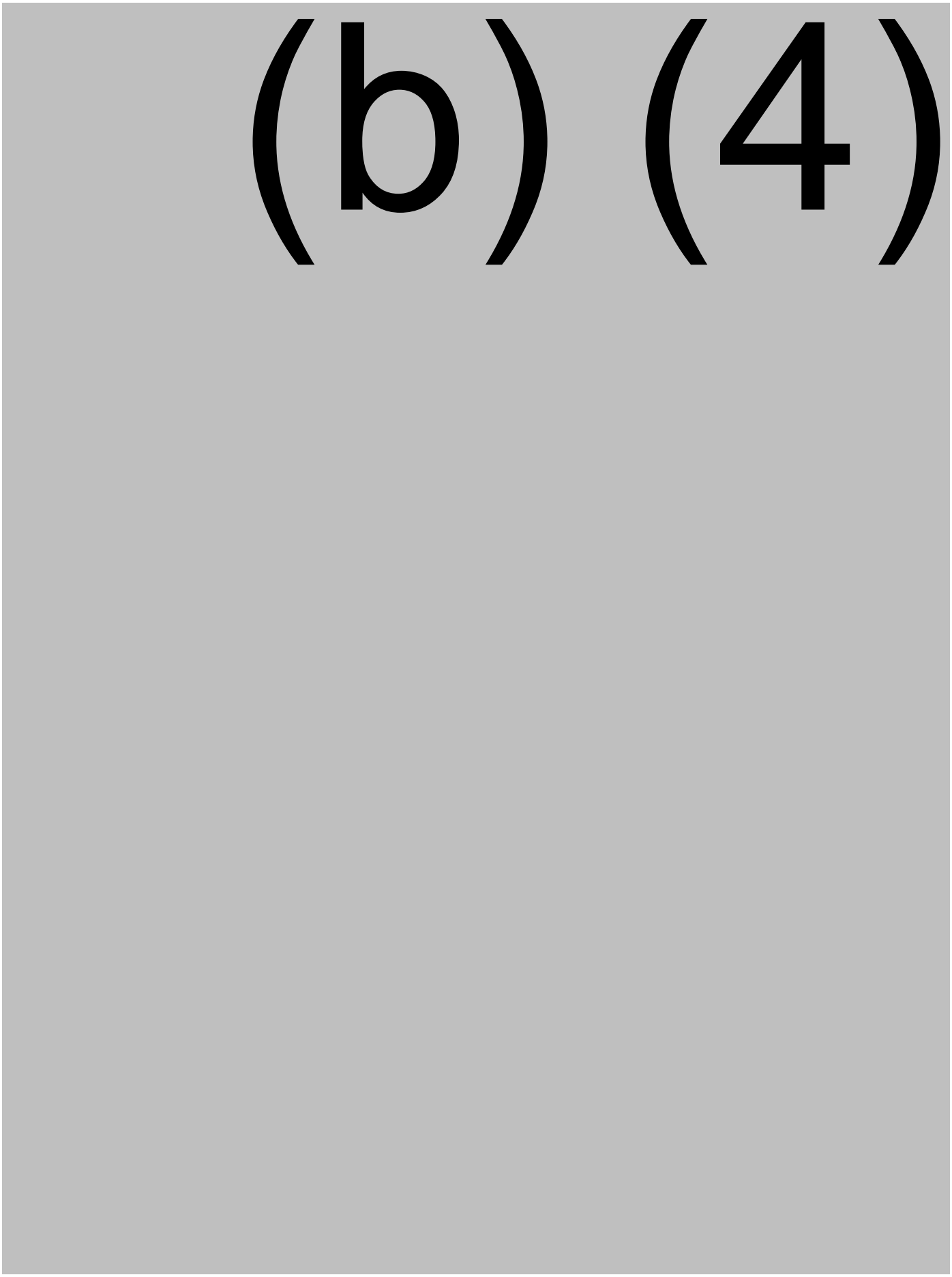
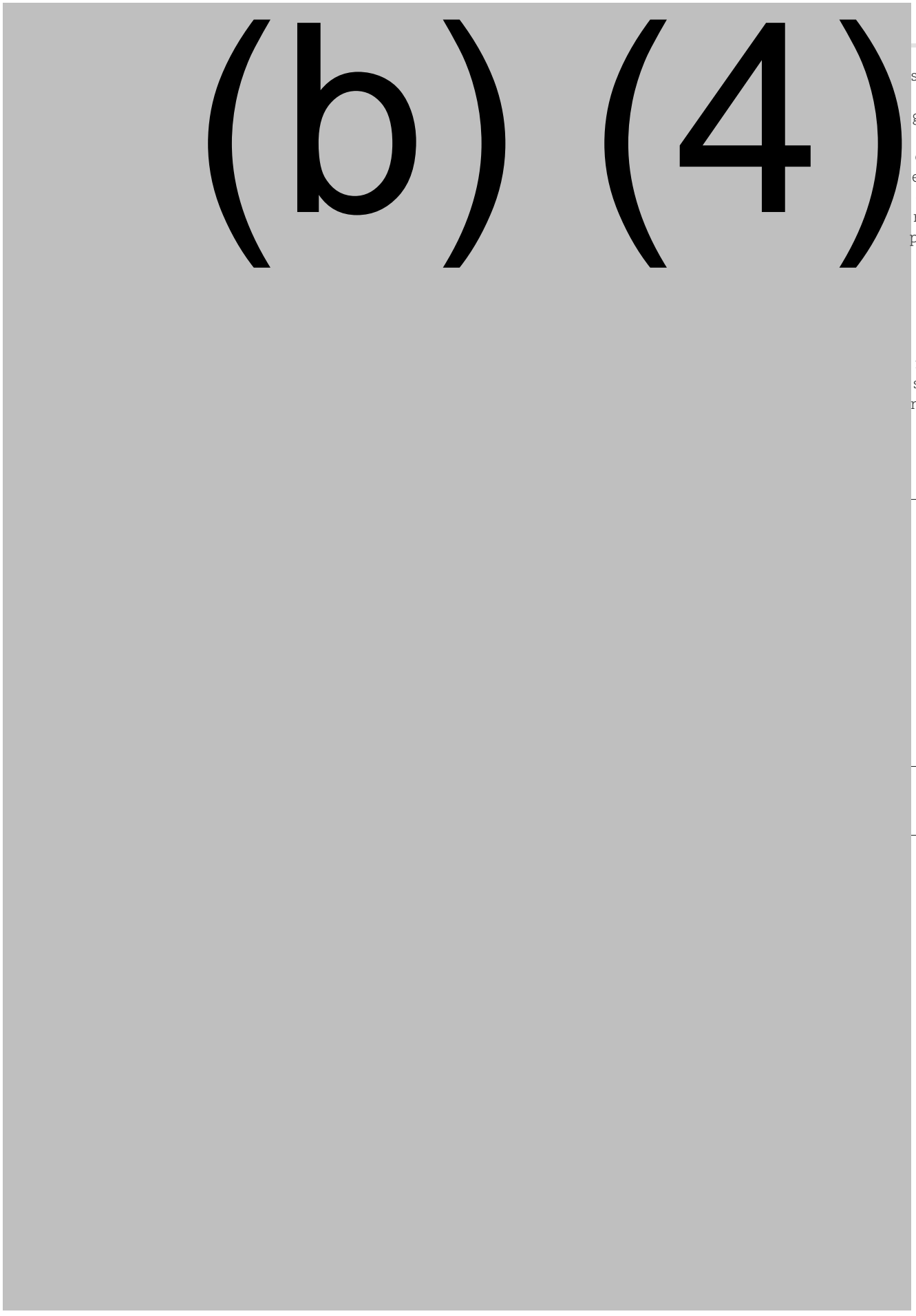


[a] Reprinted from McCleary, B.V, Sloane, N. & Draga, A. (2015) *Starch*, **67**, 860 863.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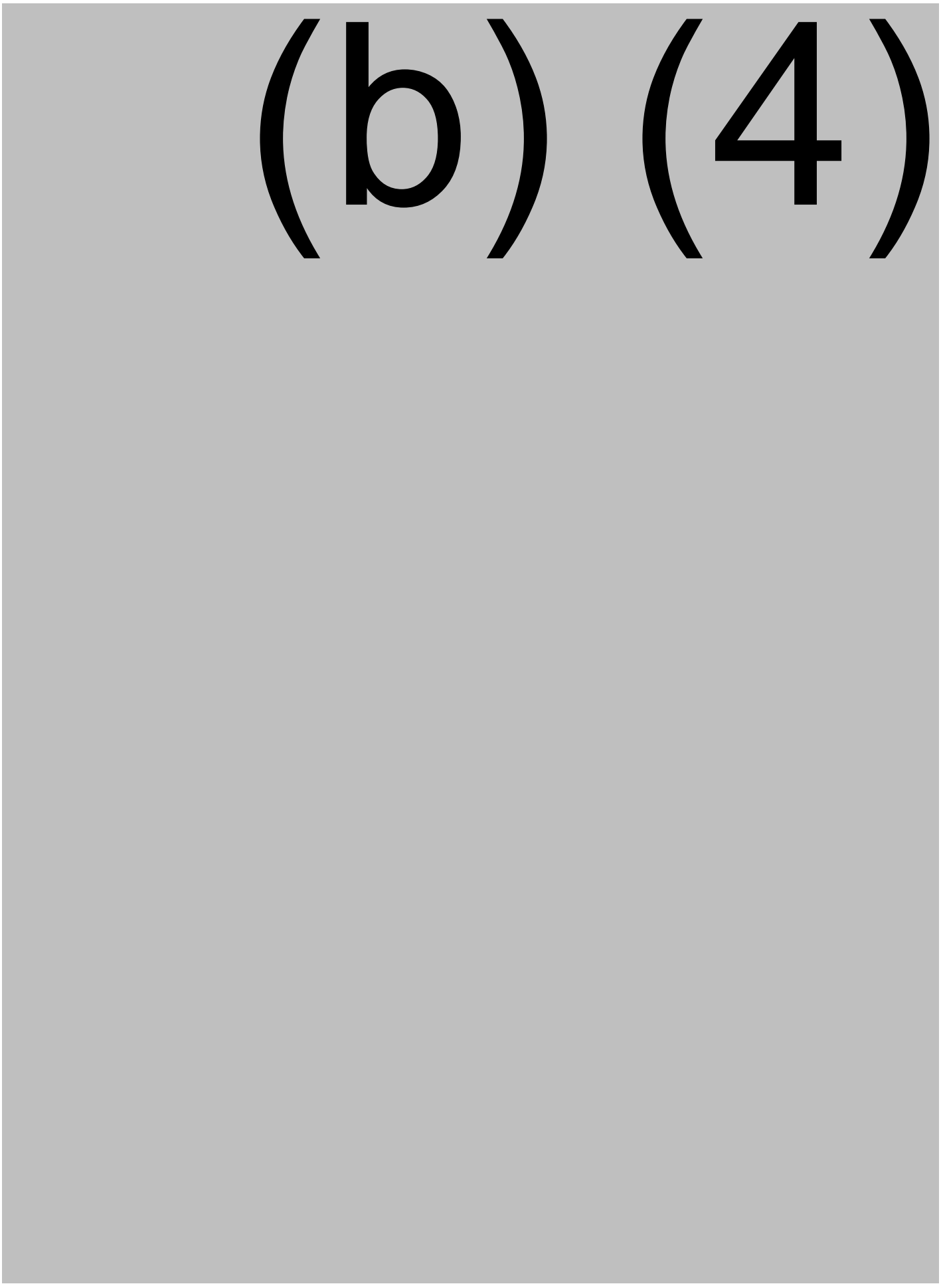
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Review

Effects of Dietary Fiber on Nutrients Utilization and Gut Health of Poultry: A Review of Challenges and Opportunities

Amit Kumar Singh and Woo Kyun Kim *

Department of Poultry Science, University of Georgia, Athens, GA 30602, USA; aksingh@uga.edu
* Correspondence: wkkim@uga.edu

Simple Summary: The inclusion of agricultural co-products has been increased to utilize the nutrients in these products available at low cost, but inherently, it adds a high dietary fiber content in the poultry diets. The use of exogenous feed enzymes along with advancements in feed milling, feed formulation, and processing of these non-conventional ingredients to improve their digestibility and utilization have played an emphatic role in boosting their use globally. Despite such developments, the presence of a high level of dietary fibers (DF) acting in an anti-nutritive manner still poses challenges in poultry feeding. Various isolated forms of fiber or feed enzymes to break DF into fermentable substrates are being used extensively to provide potential prebiotics to support beneficial gut microbiota or probiotics to improve the gut health of poultry raised without antibiotic growth promoters (AGP). This review reports and discusses the existing challenges in feeding high-DF feed ingredients to poultry and the opportunities that are available to improve the nutritive value of such non-conventional feed ingredients by adopting various technologies.

Abstract: Many fibrous ingredients incorporated in poultry feed to reduce production costs have low digestibility and cause poor growth in poultry. However, all plant-based fibers are not equal, and thus exert variable physiological effects on the birds, including but not limited to, digestibility, growth performance, and microbial fermentation. Several types of fibers, especially oligosaccharides, when supplemented in poultry diets in isolated form, exhibit prebiotic effects by enhancing beneficial gut microbiota, modulating gut immunity, boosting intestinal mucosal health, and increasing the production of short-chain fatty acids (SCFA) in the gut. Recently, poultry producers are also facing the challenge of limiting the use of antibiotic growth promoters (AGP) in poultry feed. In addition to other alternatives in use, exogenous non-starch polysaccharides digesting enzymes (NSPase) and prebiotics are being used to provide substrates to support the gut microbiome. We also conducted a meta-analysis of different studies conducted in similar experimental conditions to evaluate the variability and conclusiveness in effects of NSPase on growth performance of broilers fed fibrous ingredients. This review presents a holistic approach in discussing the existing challenges of incorporating high-fiber ingredients in poultry feed, as well as strategies to fully utilize the potential of such ingredients in improving feed efficiency and gut health of poultry.

Keywords: antinutrient; enzyme; fermentation; fiber; gut health; microbiota; meta-analysis; poultry; prebiotic

Citation: Singh, A.K.; Kim, W.K. Effects of Dietary Fiber on Nutrients Utilization and Gut Health of Poultry: A Review of Challenges and Opportunities. *Animals* **2021**, *11*, 181. https://doi.org/10.3390/ani11010181

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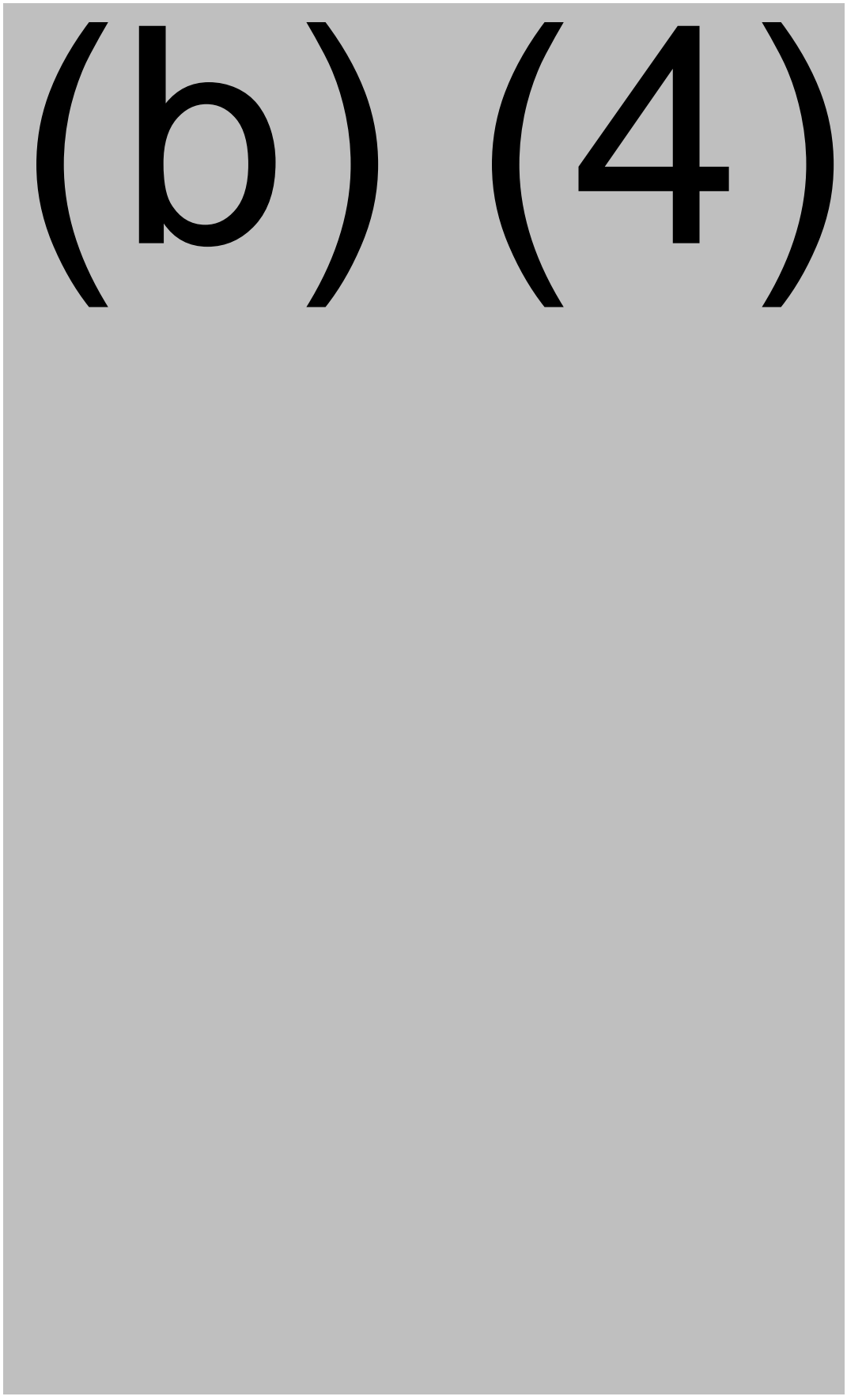
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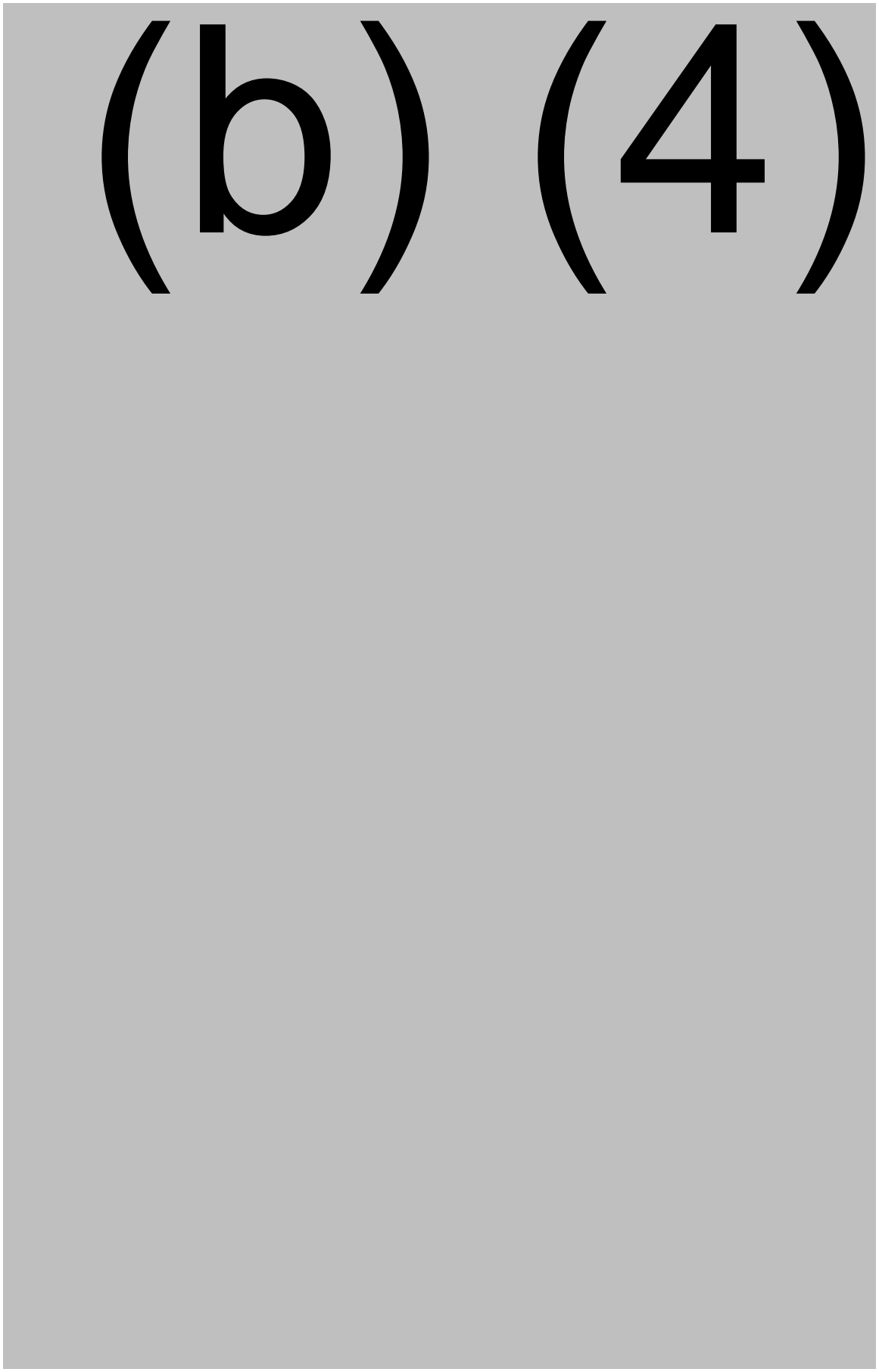
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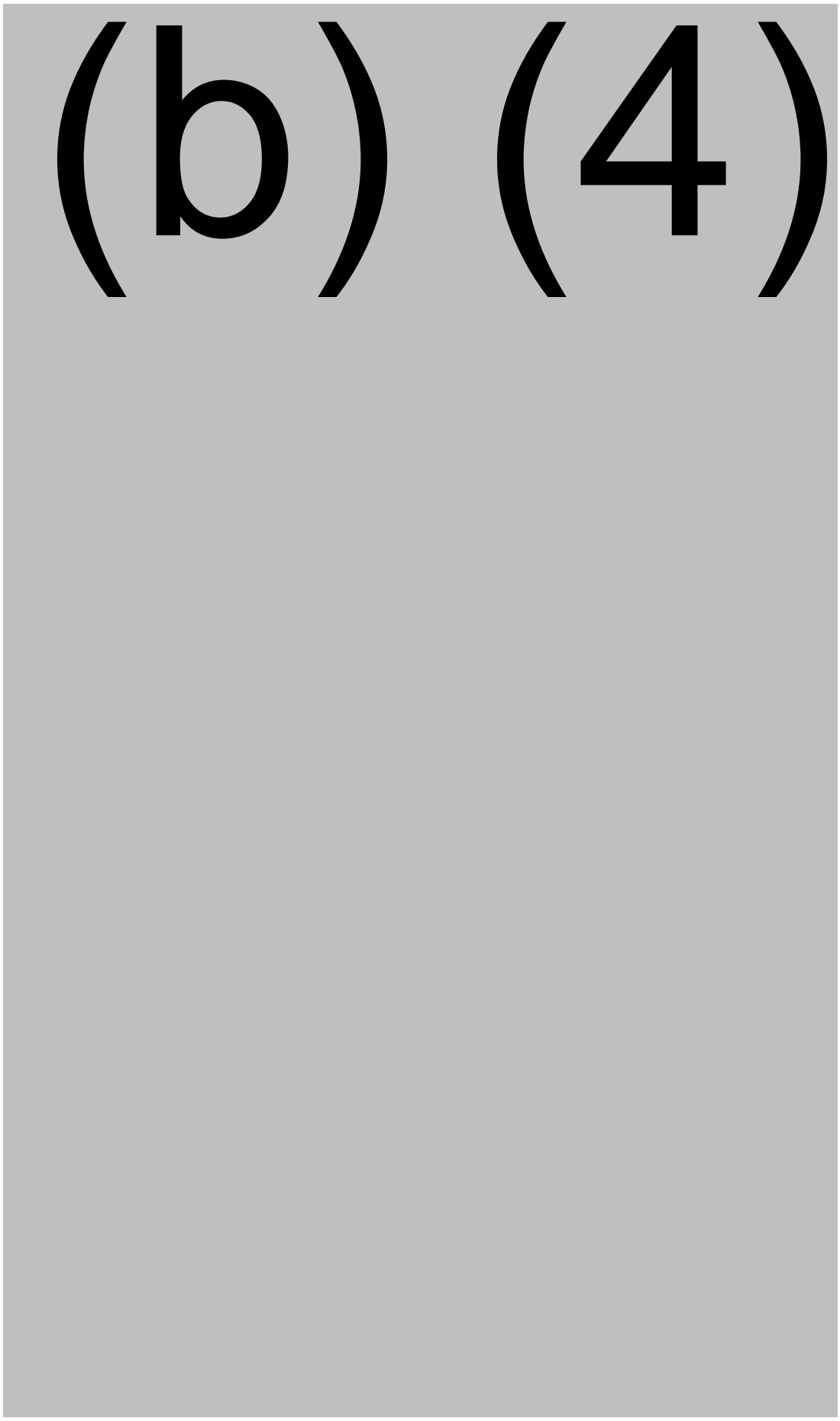
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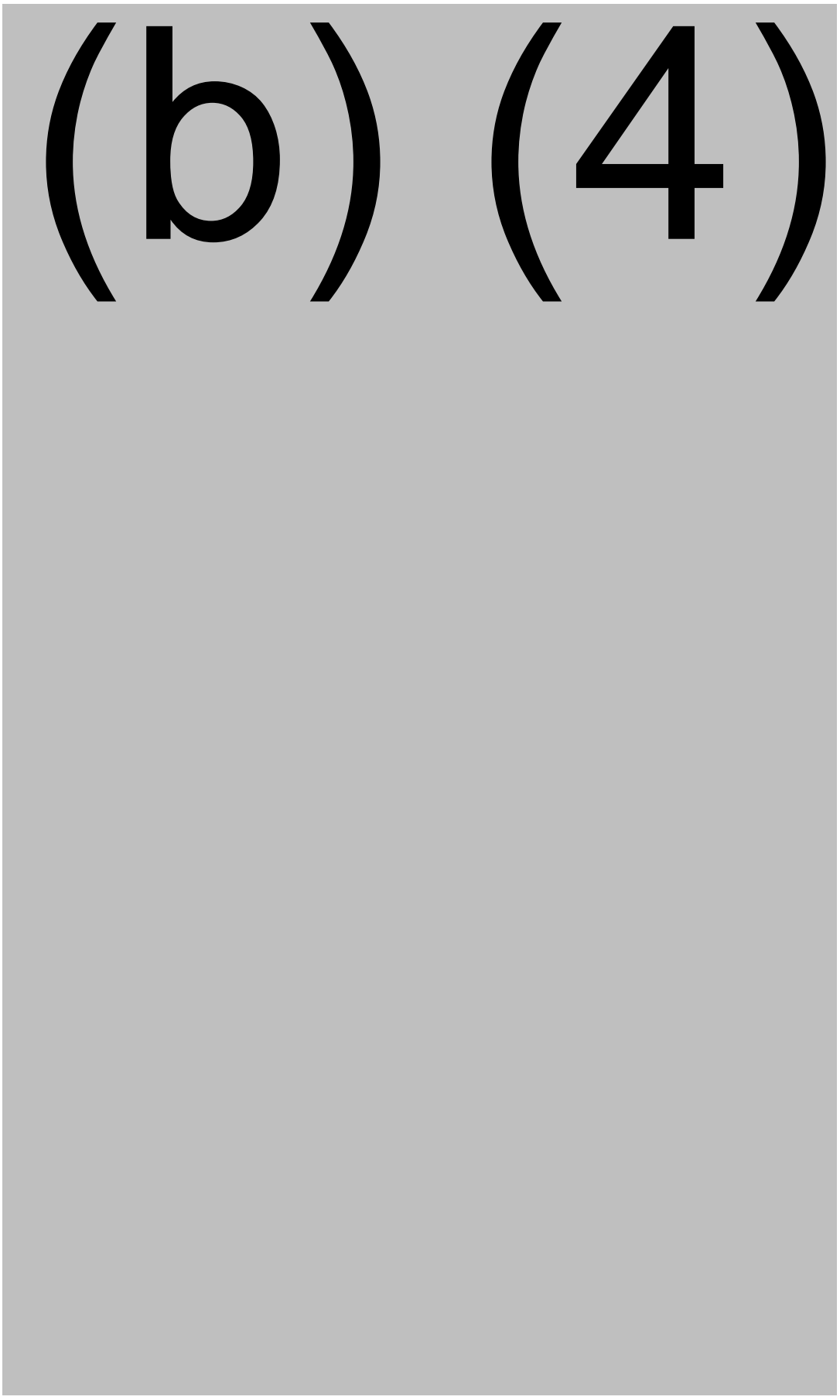
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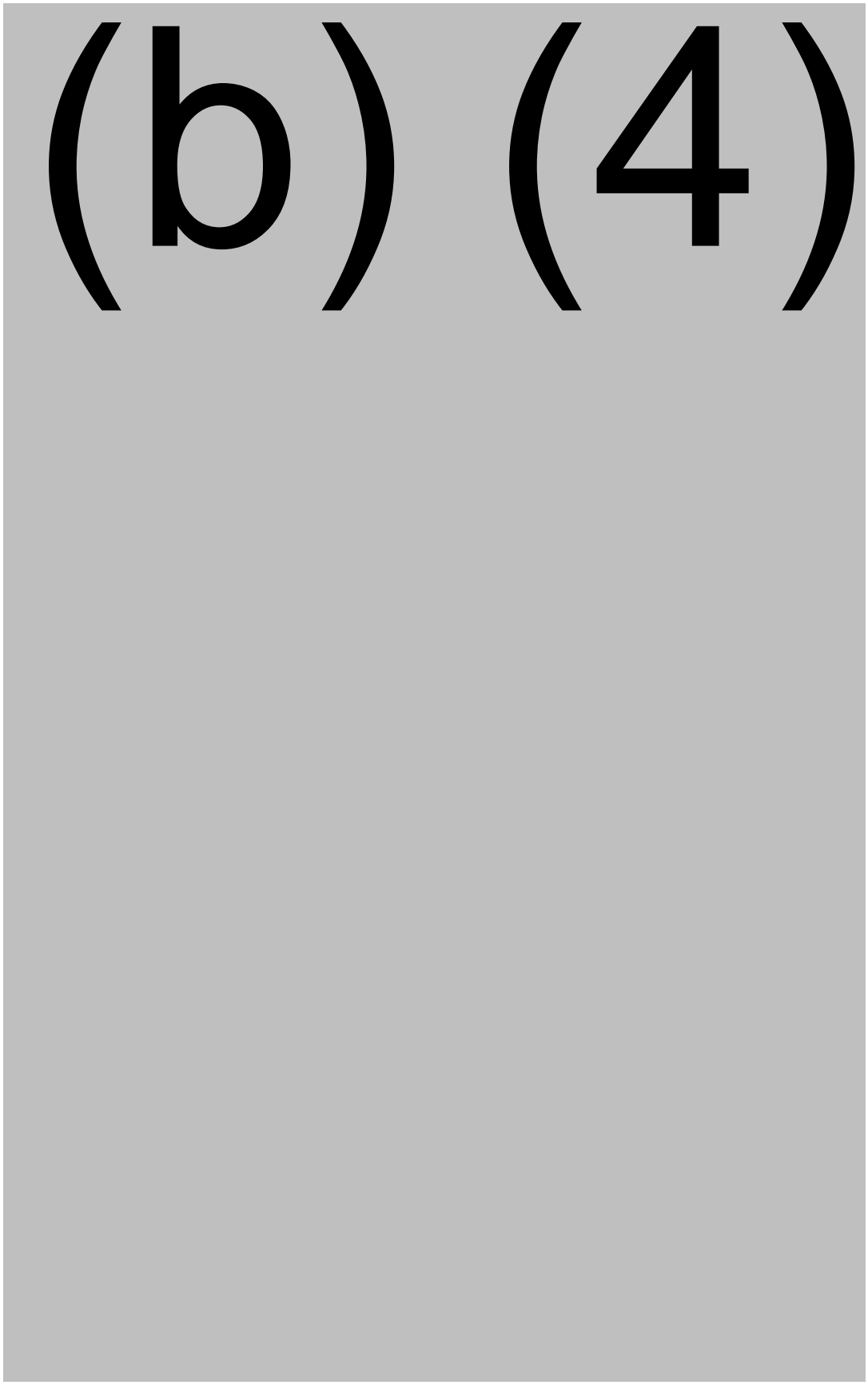
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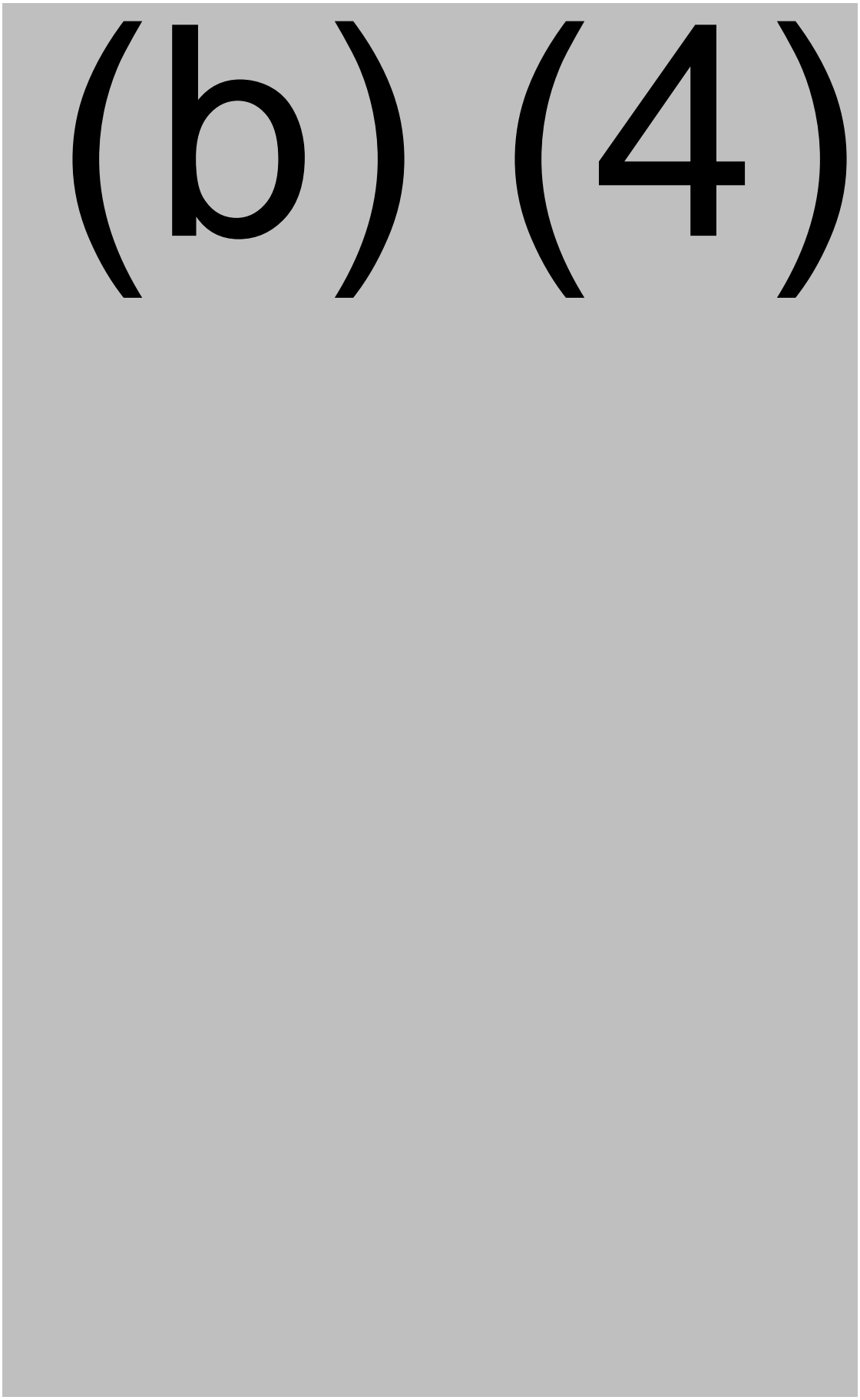
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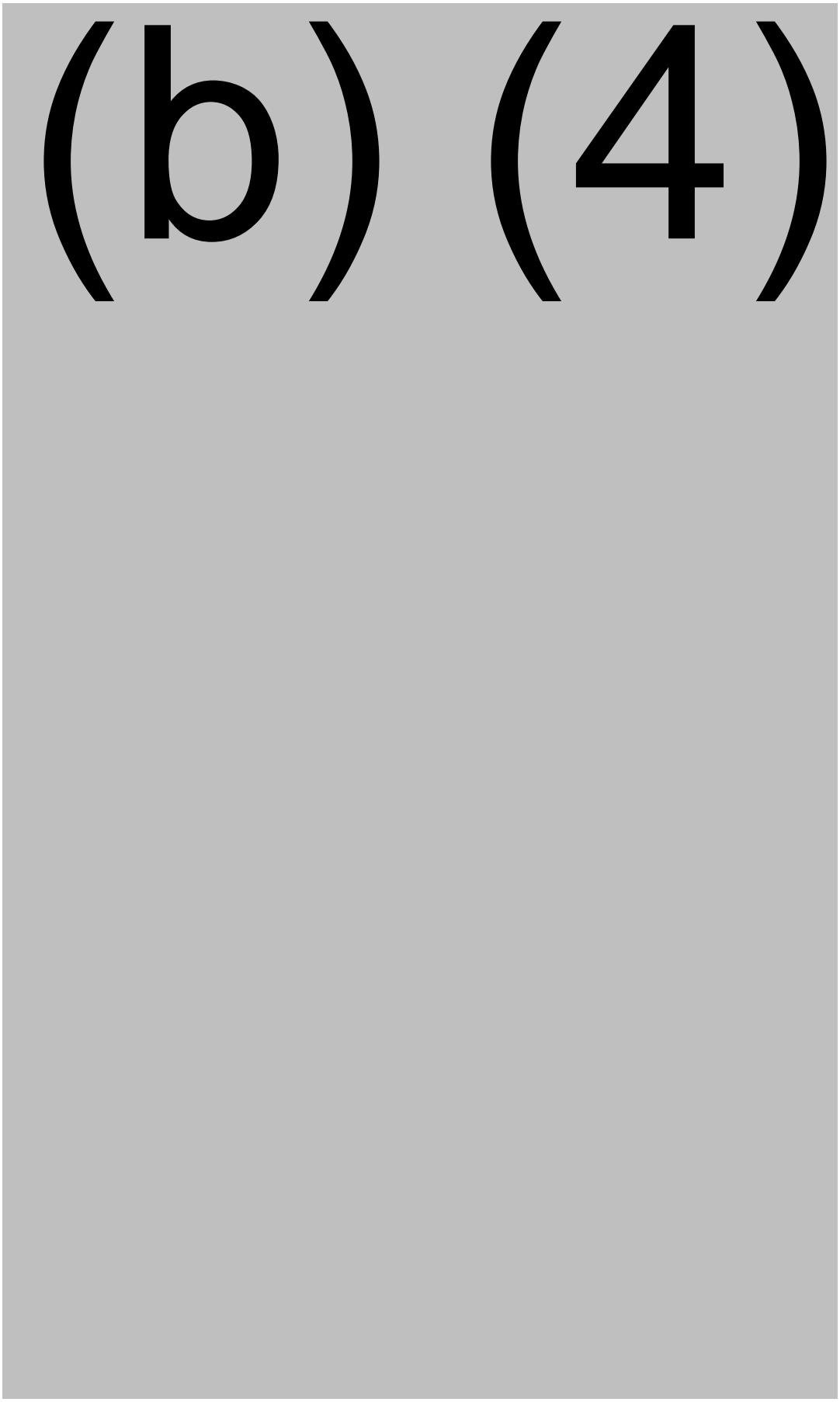
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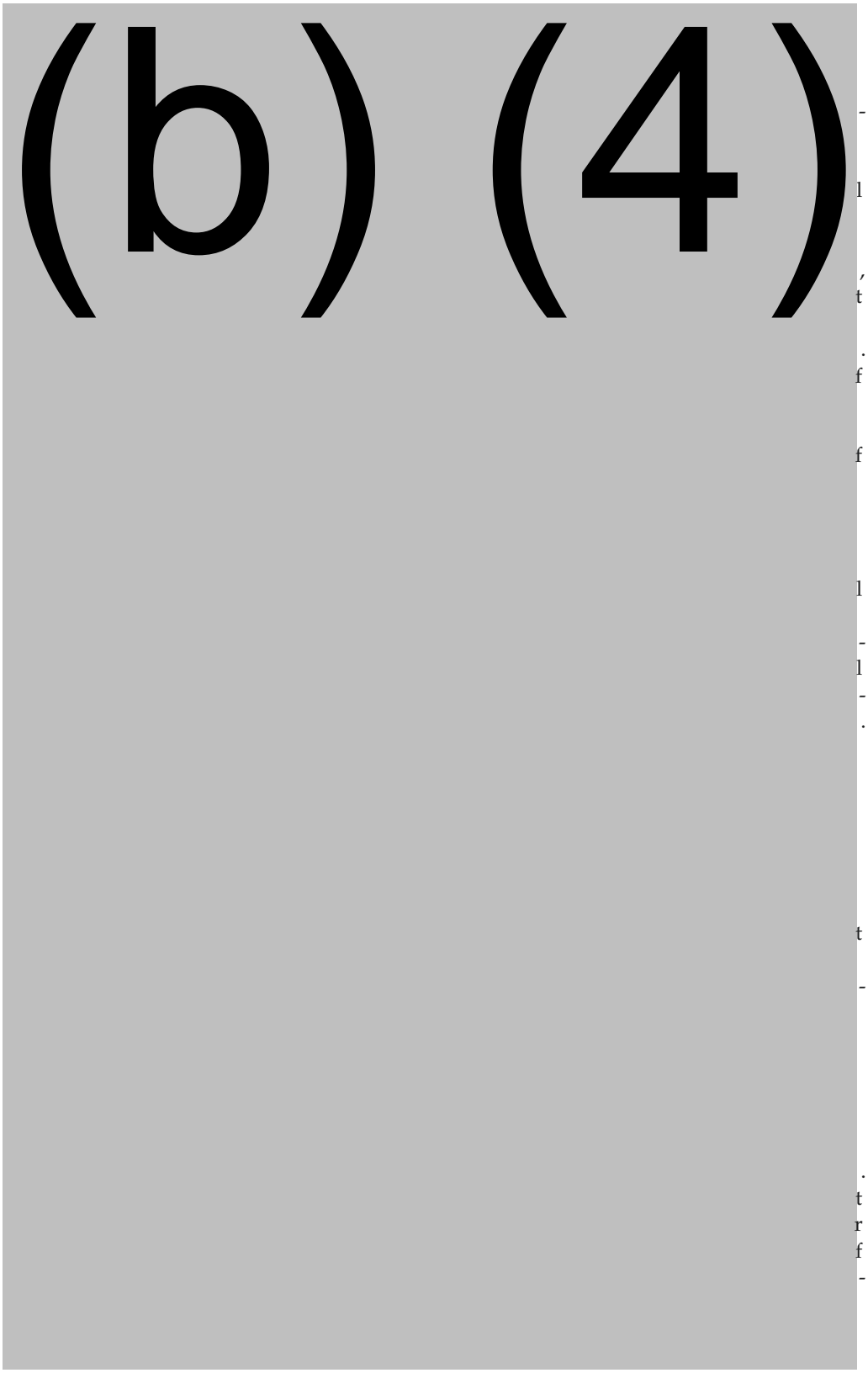
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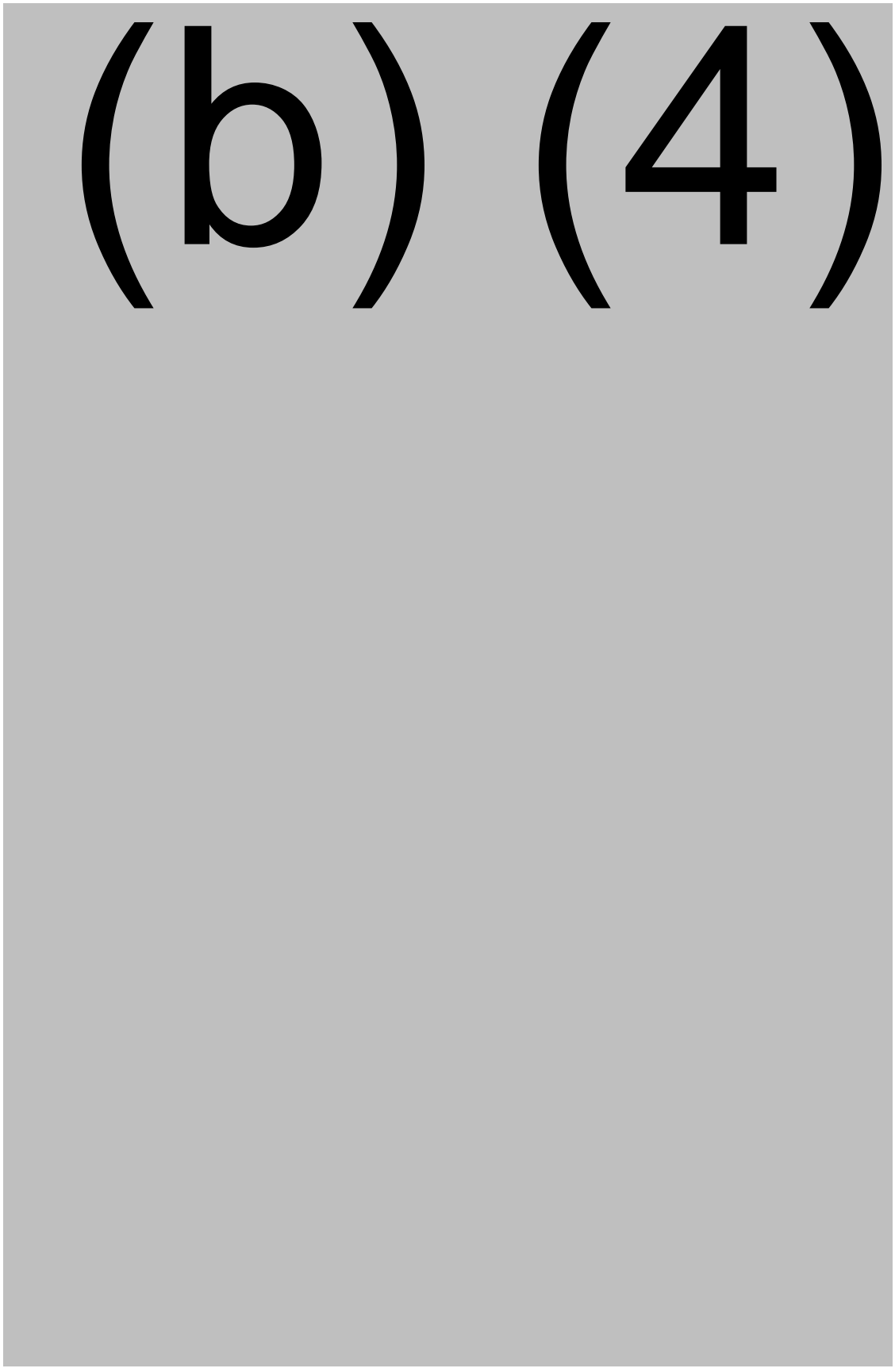
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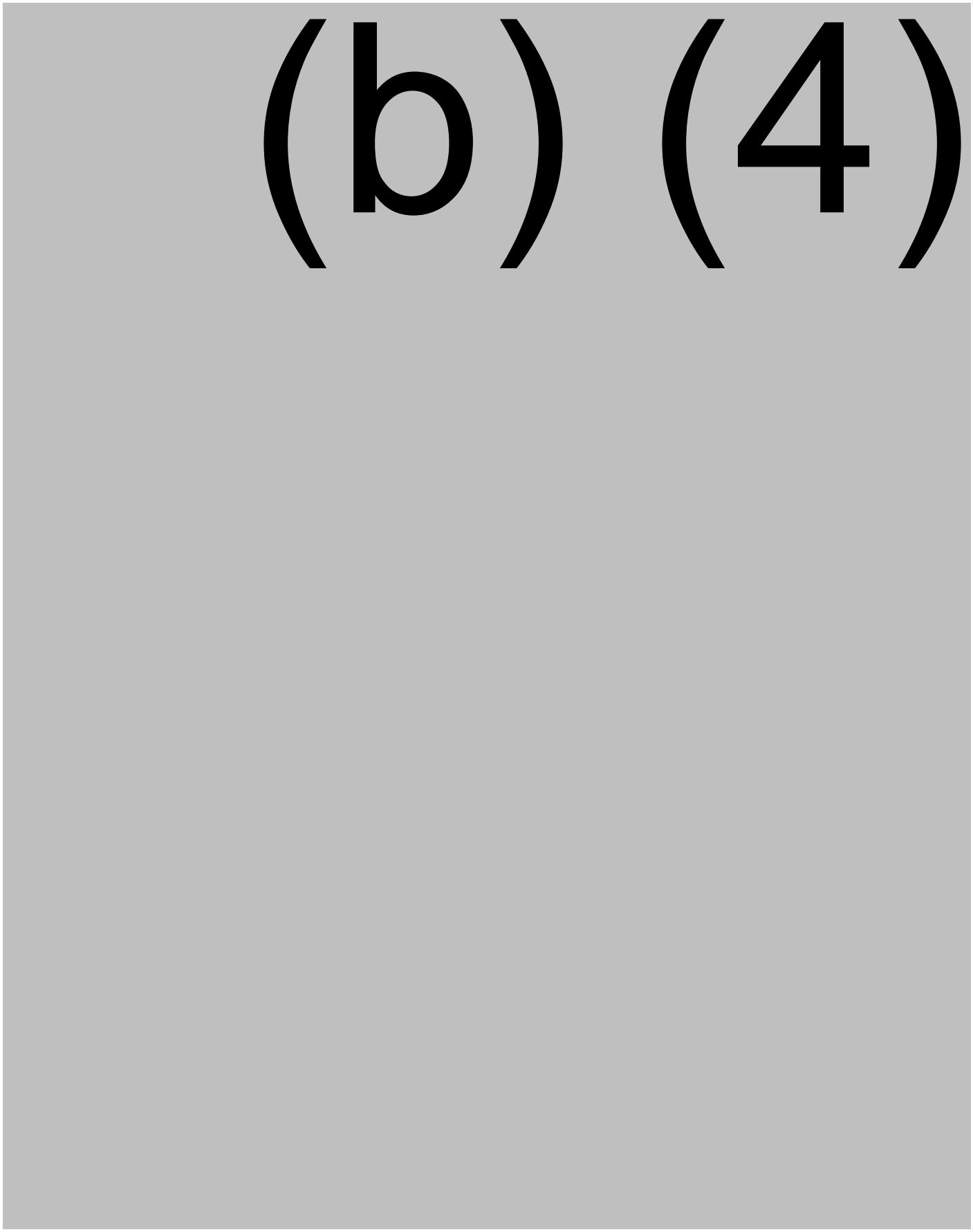
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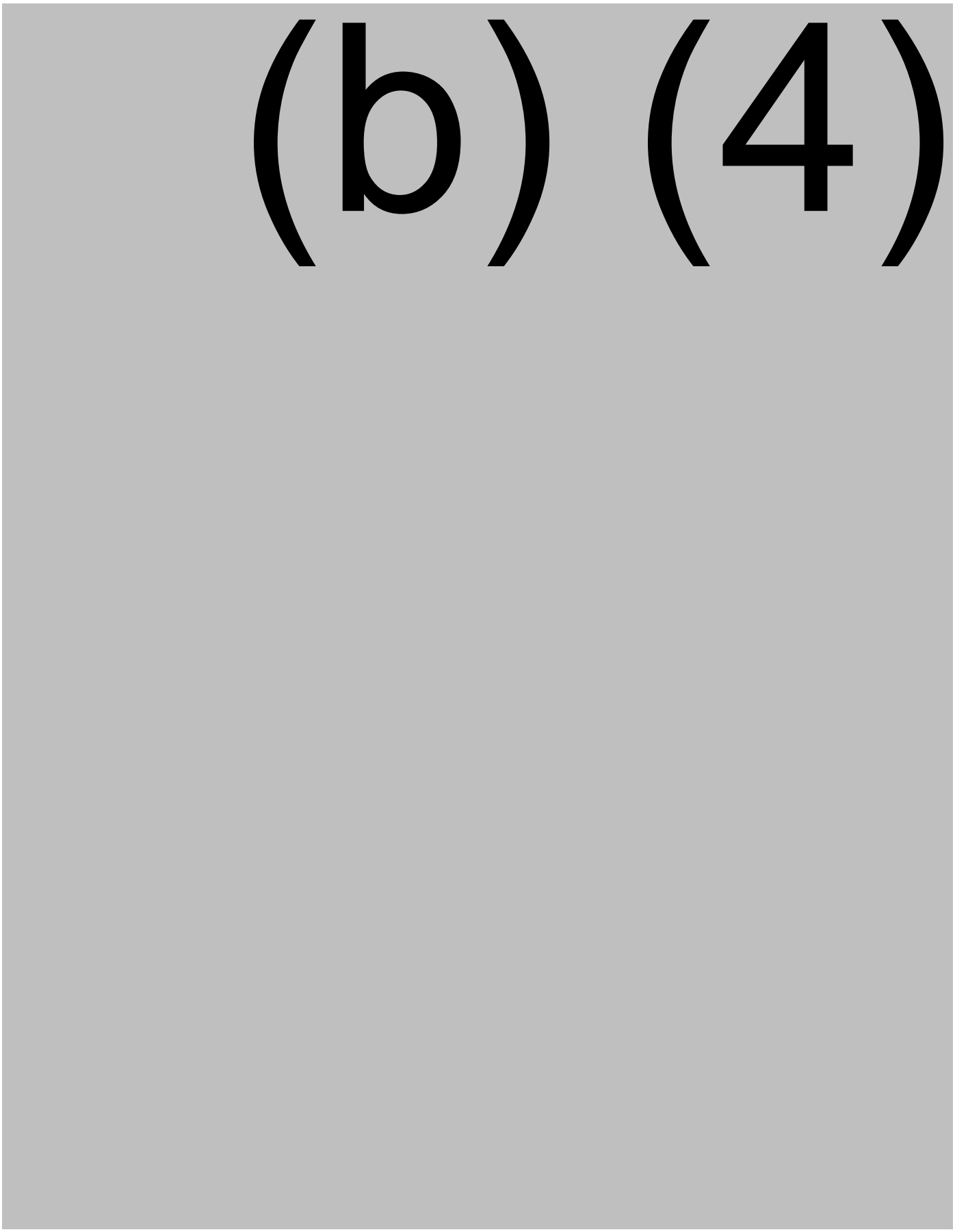
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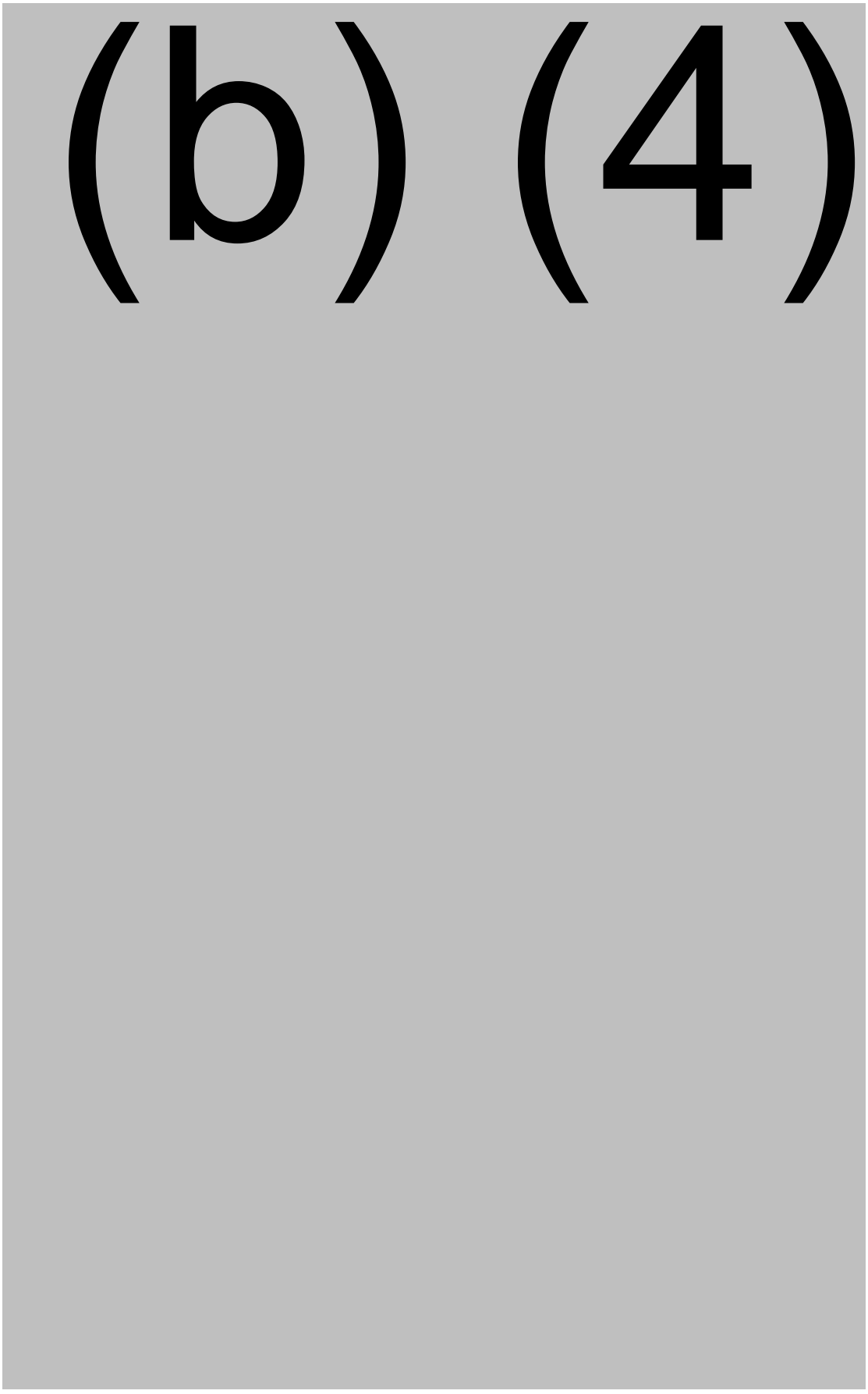
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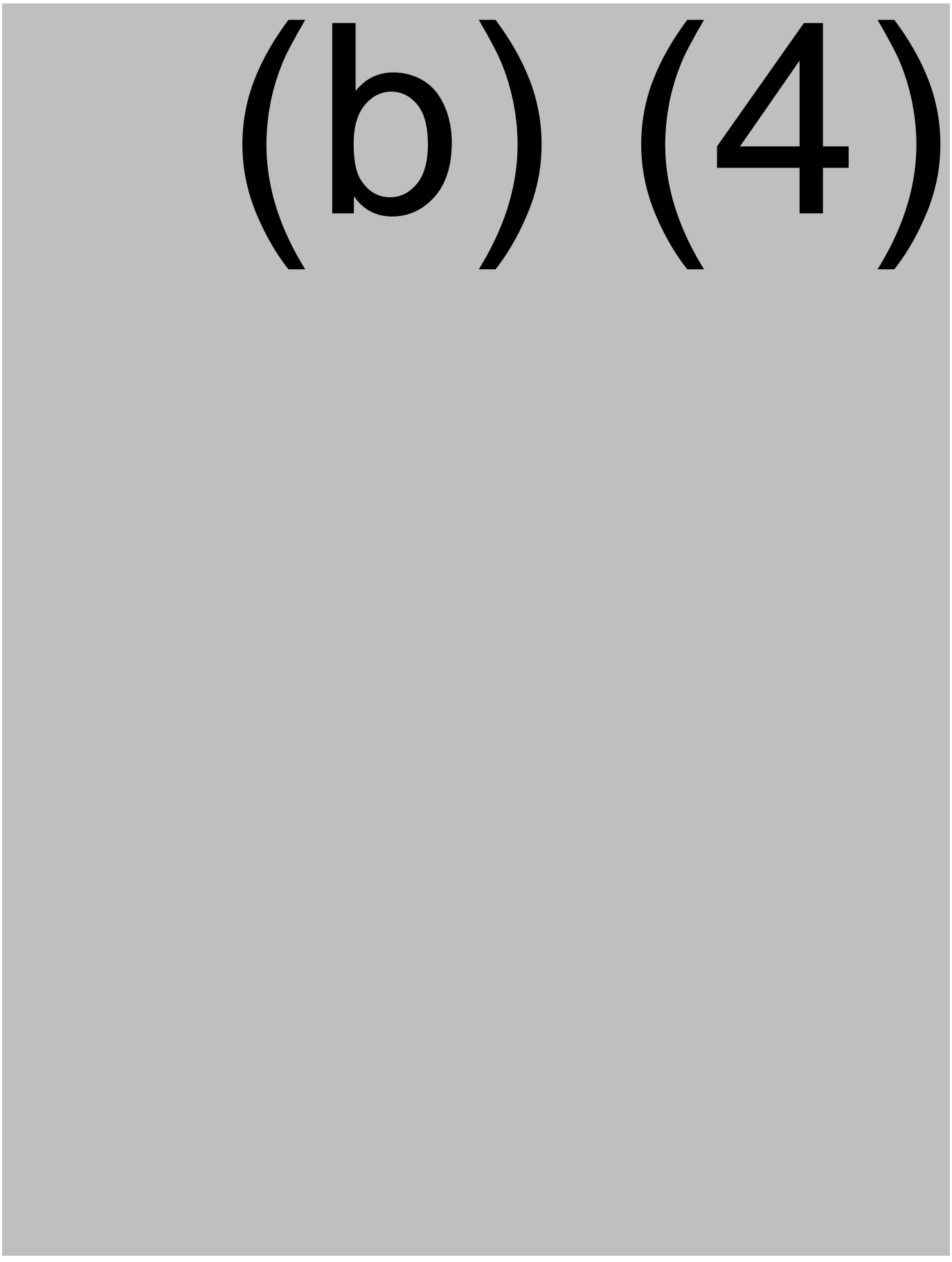
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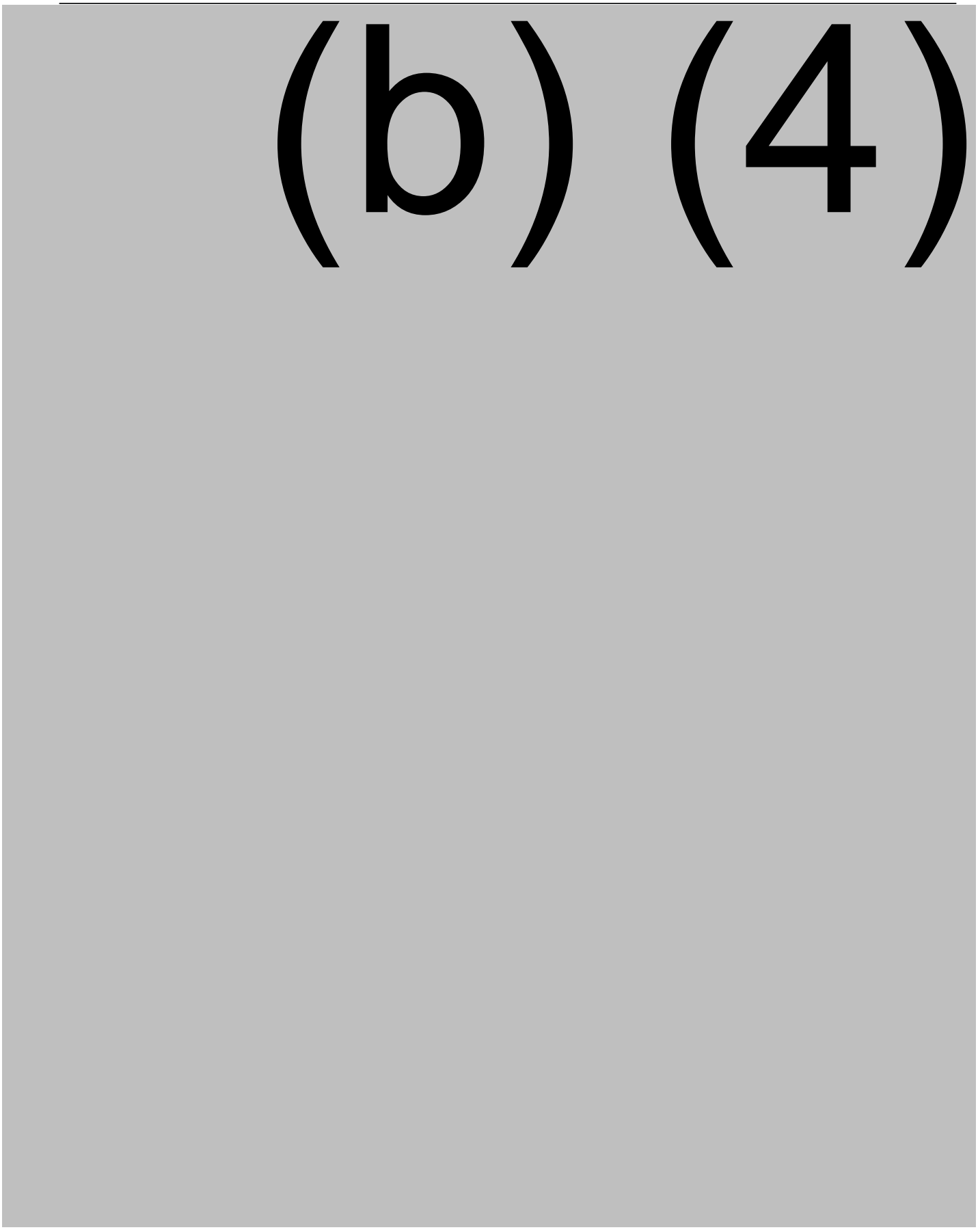
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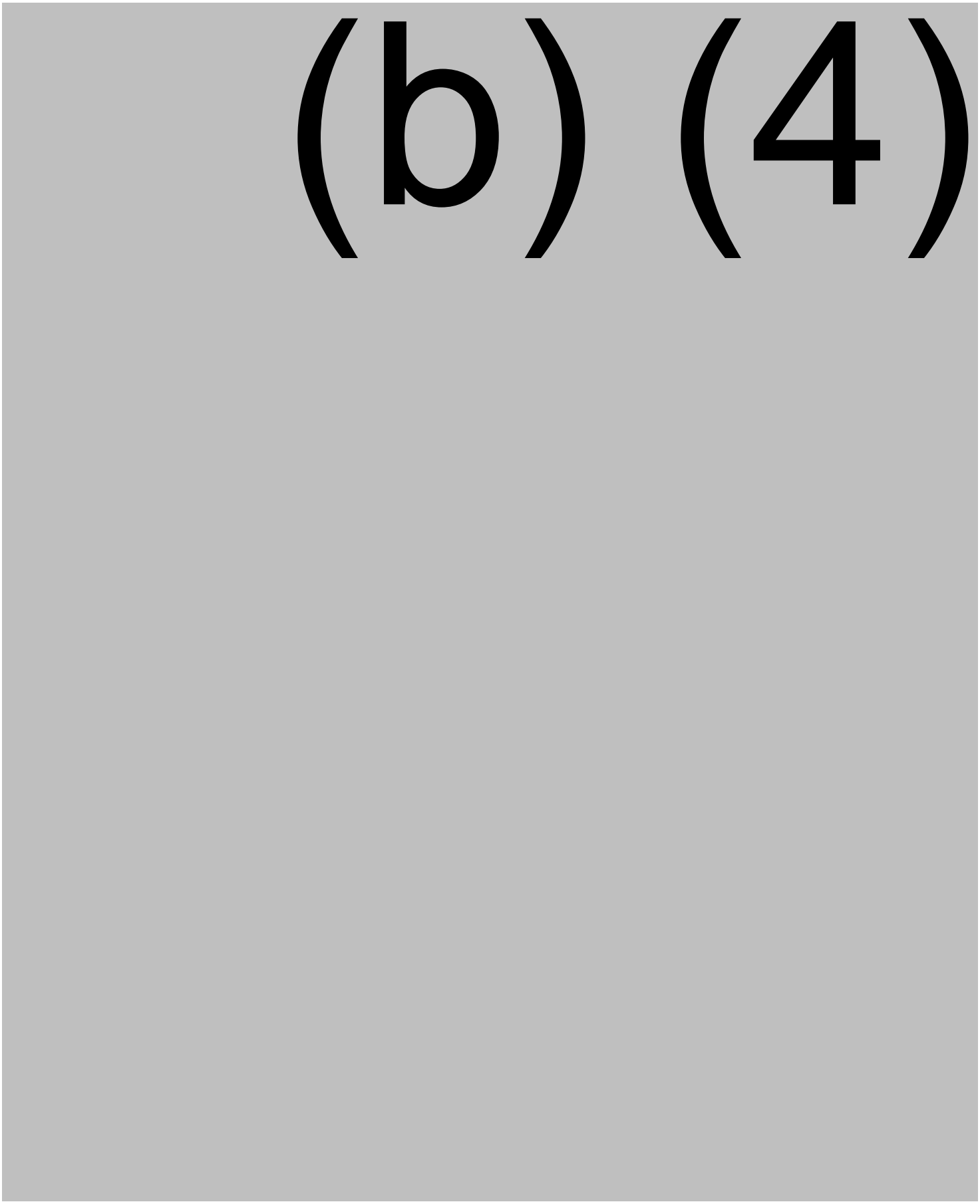
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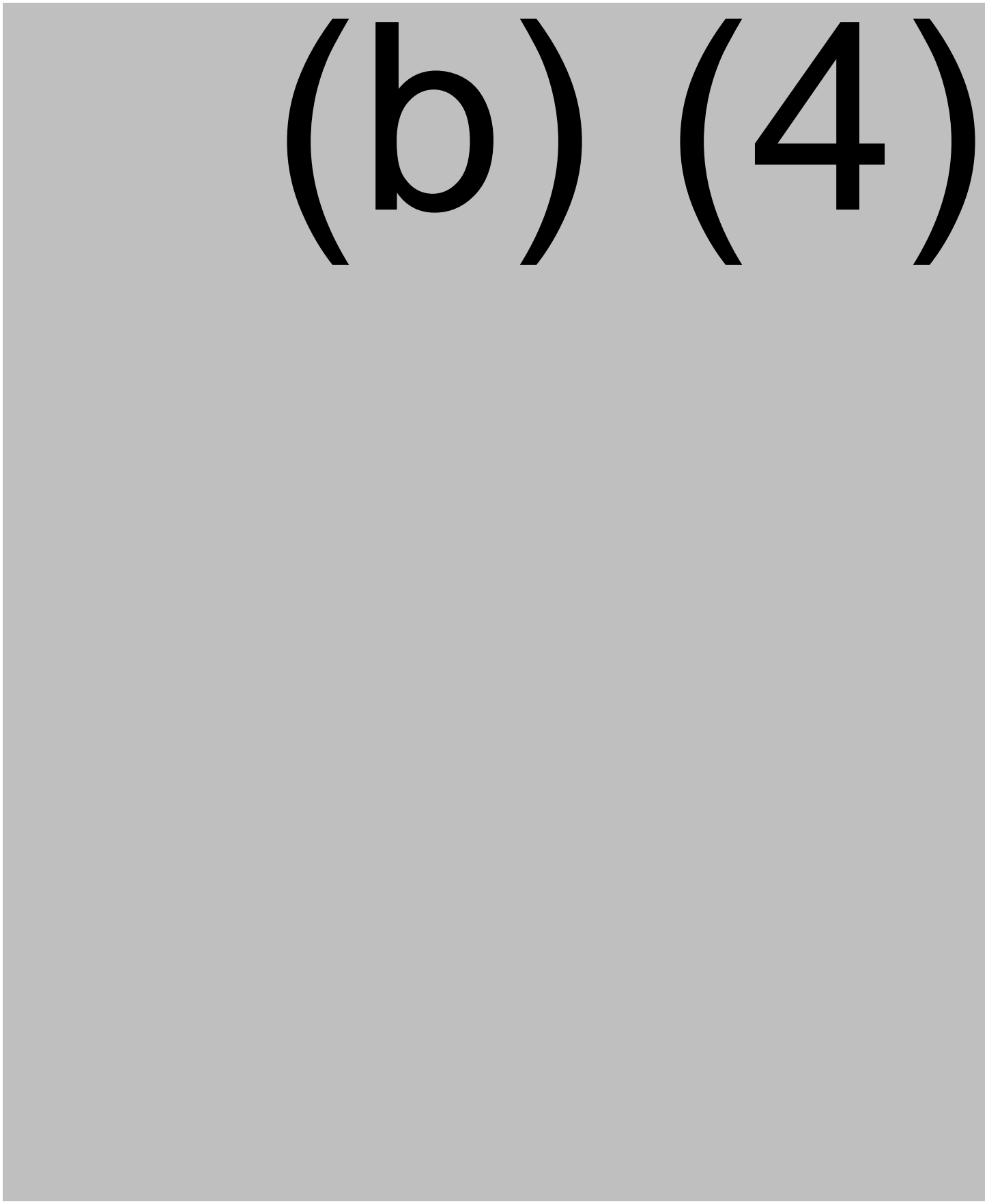
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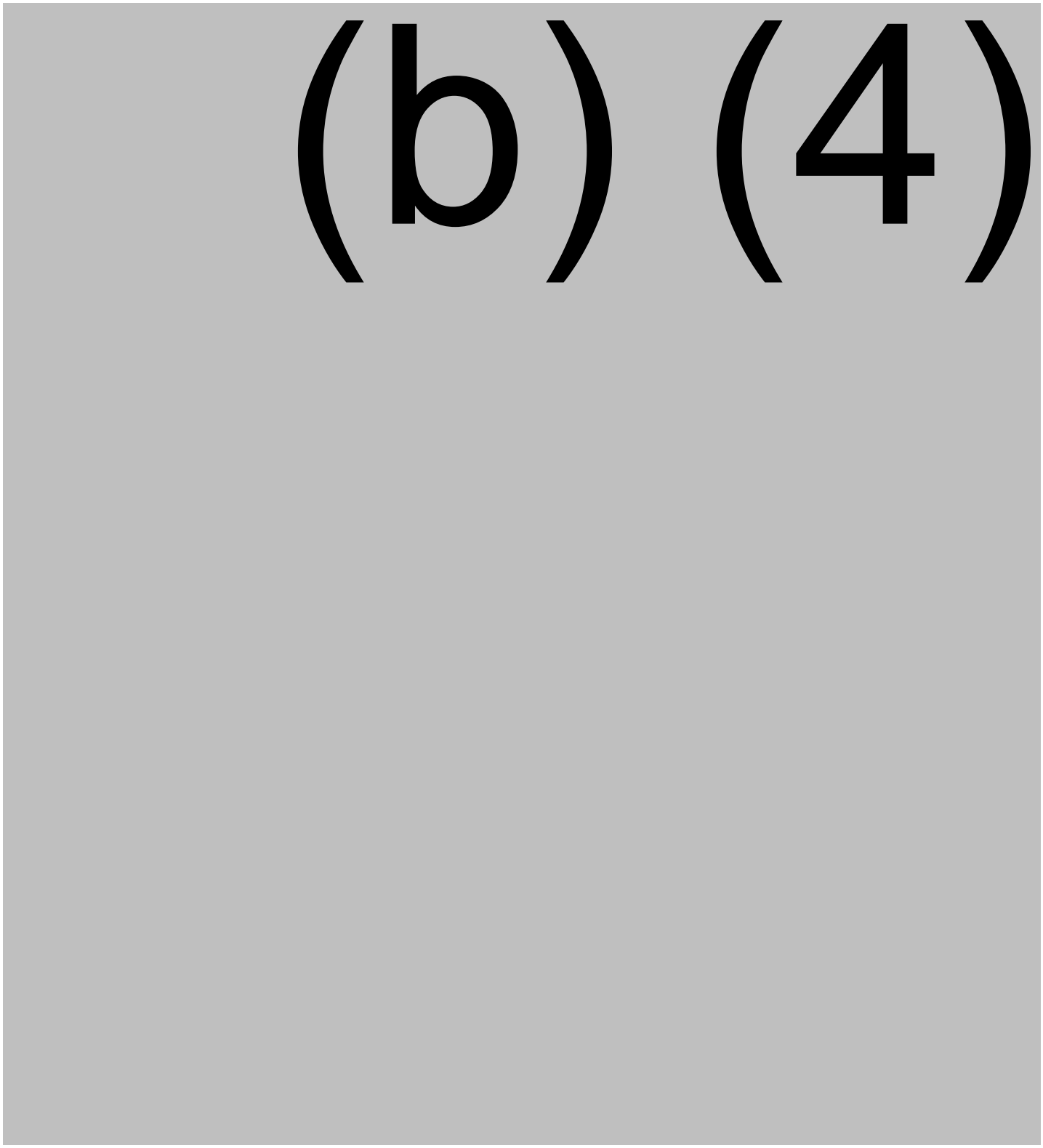
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RYAM™

Ms. Wasima Wahid
Division of Animal Feeds (HFV- 220)
Center for Veterinary Medicine
Food and Drug Administration
7519 Standish Pl.
Rockville, MD 20855

September 23, 2024

Subject: GRAS Notice for Xylooligosaccharides

Notifier: Rayonier Advanced Materials Inc (RYAM)
1301 Riverplace Blvd #2300,
Jacksonville, FL 32207

Dear Ms. Wahid,

On September 20, 2024, you requested clarification on the utility of the substance as related to safety. We appreciate the teleconference between you and Dr. Jennifer van de Ligt on September 23, 2024, to further explain the request for clarification.

The intended use of the xylooligosaccharides is as a source of fermentable fiber at a dietary inclusion rate of up to 3% to provide energy and support normal intestinal health and microbiota populations in poultry, swine, and fish. This use has been demonstrated by a thorough review of published well-controlled studies evaluating various levels of xylooligosaccharides in feed for poultry, swine, and fish. However, under the regulations covering GRAS notification, the intended use is not related to safety beyond the compositional value as a supplementary source of fermentable fiber. As such, demonstration of utility of the GRAS substance is not required (21 CFR 570.230(d)).

Sincerely,

(b) (6)

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