GRAS Notice for Sodium Salts of Lactylates of Lauric and Myristic Acids (C12/C14 Lactylates)

Corbion (Purac Biochem bv)
Arkelsedijk 46
4206 AC Gorinchem
Netherlands

GRAS Notice for Sodium Salts of Lactylates of Lauric and Myristic Acids (C12/C14 Lactylates)

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NOMENCLATURE

The following nomenclature is used within the GRAS Notice to refer to the raw materials, the GRAS substance and the marketed formulation for use in animal feed. A declaration confirming ALOAPUR® and ALOAPUR® PM are identical is provided in Appendix 001.

Raw Materials to Sodium Salts of Lactylates of Fatty Acids	
Lauric acid	C12
Myristic acid	C14
Lactic acid	LA
Sodium Salts of Lactylates of Fatty Acids (GRAS Substance)	
Sodium salts of lactylates of lauric and myristic acids	C12/C14 lactylates
	Puramix 100
Market Formulation (for Addition to Animal Feed)	
Sodium salts of lactylates of lauric acid and myristic acid on a diatomaceous earth carrier	ALOAPUR® PM (Trade name), previously known as ALOAPUR®: 30 to 40% (b) (4) C12/C14 lactylates and 60 to 70% diatomaceous earth (b) (4)
Other Formulations Tested in Animal Studies	
Sodium salts of lactylates of lauric acid and myristic acid on solid and liquid carriers (developmental products prior to final formulation of ALOAPUR® PM)	Puramix 30S: 38 to 42% C12/C14 lactylates and 58 to 62% diatomaceous earth (b) (4) Puramix 30L: 38 to 42% C12/C14 lactylates and 58 to 62% monopropylene glycol (MPG)

GRAS Notice for Sodium Salts of Lactylates of Lauric and Myristic Acids (C12/C14 Lactylates)

PART 1. §570.225. SIGNED STATEMENTS AND CERTIFICATION

In accordance with 21 CFR §570 Subpart E consisting of §570.203 to 280, Corbion (Purac Biochem bv) (hereafter referred to as "Corbion"), hereby informs the United States (U.S.) Food and Drug Administration (FDA) that they are submitting a Generally Recognized As Safe (GRAS) notice for the sodium salts of lactylates of lauric and myristic acids.

1.1 NAME AND ADDRESS OF ORGANIZATION

Purac Biochem B.V. doing business as Corbion
Arkelsedijk 46
4206 AC Gorinchem
Netherlands

1.2 NAME OF THE NOTIFIED SUBSTANCE

The notified substance is sodium salts of lactylates of lauric and myristic acids (C12/C14 lactylates).

1.3 INTENDED CONDITIONS OF USE

Sodium salts of lactylates of lauric and myristic acids (C12/C14 lactylates) is intended for use as a nutritional ingredient (source of lauric and myristic acid) in the food of all categories and species of animals in the U.S.

C12/14 lactylates will be marketed as a formulation on an inert diatomaceous earth carrier under the trade name "ALOAPUR® PM". The carrier complies with the specifications laid down in Title 21 of the Code of Federal Regulations (21 CFR) Part 573.340 for diatomaceous earth (U.S. FDA, 2021) and is intended to aid mixing and handling of C12/C14 lactylates during finished feed manufacture by providing the fatty acid-containing ingredient in the form of a free flowing powder.

The practical use level of C12/C14 lactylates as a source of lauric and myristic acid in animal food is anticipated to be in the range of 0.7 g/kg complete feed for all categories and species of animal, equivalent to 2 g ALOAPUR® PM/kg complete feed. The use level will not exceed 1.8 g C12/C14 lactylates/kg complete feed as-fed, equivalent to 5 g ALOAPUR® PM/kg complete feed as-fed.

1.4 STATUTORY BASIS FOR THE CONCLUSION OF GRAS STATUS

Pursuant to 21 CFR §570.30(a) and (b), sodium salts of lactylates of lauric and myristic acids, has been concluded to have GRAS status for use as a nutritional ingredient in the food of all categories and species of animal under the conditions described in Part 1.3, on the basis of scientific procedures.

1.5 PREMARKET EXCEPTION STATUS

Corbion hereby informs the U.S. FDA of the view that sodium salts of lactylates of lauric and myristic acids is not subject to the premarket approval requirements of the Federal Food, Drug and Cosmetic Act (FFDCA) based on Corbion's conclusion that the notified substance is GRAS under the conditions of intended use as described in Part 1.3 above.

1.6 AVAILABILITY OF INFORMATION

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

Purac Biochem B.V. doing business as Corbion
Arkelsedijk 46
4206 AC Gorinchem
Netherlands

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

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

In Corbion's view, with the exception of Sections 2.2.2 and 2.2.3, and Appendices 002 to 007 (all subparts) and 009 to 016, all data and information presented in Parts 2 through 7 of this notice do not contain any trade secret, commercial or financial information that is privileged or confidential, and therefore, all data and information presented herein are not exempt from the Freedom of Information Act, 5 U.S.C. Section 552.

1.8 CERTIFICATION

Maurits van Kolck hereby certifies that to the best of his knowledge, all data and information presented in this notice constitutes a complete, representative and balanced submission, which includes all unfavorable as well as favorable information known to Corbion and pertinent to the evaluation of the safety and GRAS status of sodium salts of lactylates of lauric and myristic acids.

Signed,

ValidSigned by Maurits Kolck, van
on 23-Dec-2022

Maurits van Kolck

23-12-2022

Date

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

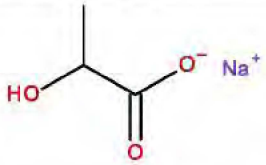
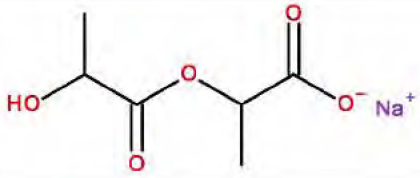
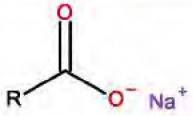
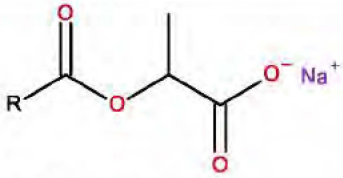
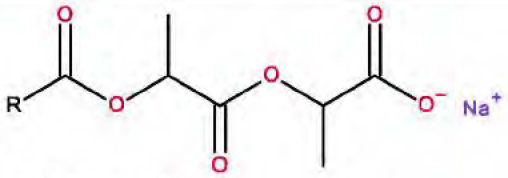
2.1 IDENTITY

2.1.1 Common or Usual Names

The common or usual name for the ingredient is the sodium salts of lactylates of lauric and myristic acids, which can be abbreviated to C12/C14 lactylates. Compositionally, the ingredient is a mixture of substances of which sodium lactylate of lauric acid and sodium lactylate of myristic acid are the primary components (see Section 2.1.2).

2.1.2 Description and Identity

C12/C14 lactylates is the product of esterification of lauric (C12) and myristic (C14) fatty acids with lactic acid in the presence of sodium hydroxide. The esterification reaction yields an equilibrium mixture of the sodium salts of the raw materials with the chemical structures and identity of each of the components of the ingredient provided in Table 2.1.

Table 2.1: Identity of the Components of C12/C14 Lactylates	
Chemical Structure	**Identification**
	**Sodium L-lactate (*ca*. 98%)** CAS No. 79-33-4 MW 112 **Sodium D-lactate (*ca*. 2%)** CAS No. 867-56-1 MW 112
	**Sodium lactoyl lactate** CAS No. None assigned MW 201
 R = C12 fatty acid  R = C14 fatty acid 	**Sodium laurate** CAS No. 629-25-4 MW 222 **Sodium myristate** CAS No. 822-12-8 MW 250
 R = C12 fatty acid  R = C14 fatty acid 	**Sodium lauroyl-1-lactylate** CAS No. 42415-70-3 MW 294 **Sodium myristoyl-1-lactylate** CAS No. None assigned MW 322
 R = C12 fatty acid  R = C14 fatty acid 	**Sodium lauroyl-2-lactylate** CAS No. 13557-75-0 MW 366 **Sodium myristoyl-2-lactylate** CAS No. None assigned MW 394

Abbreviations: CAS No. = Chemical Abstracts Service Registry Number; MW = molecular weight.

2.1.3 Market Formulation and Trade Name

C12/C14 Lactylates (30 to 40% by weight) are mixed with an insert diatomaceous earth carrier (60 to 70% by weight) to produce a formulation which is marketed under the trade name ALOAPUR® PM for incorporation into animal feeds. The composition of the market formulation (ALOAPUR® PM) is provided in Table 2.2.

Table 2.2: List of Formulation Components (ALOAPUR® PM)	
Composition	**Typical Content (% w/w)**
C12/C14 Lactylates	30-40
Diatomaceous earth	60-70

2.2 METHOD OF MANUFACTURE

2.2.1 Raw Materials and Processing Aids

The raw materials used in the production of C12/C14 lactylates are provided in Table 2.3. All of the raw materials are considered safe and suitable for feed production and have a history of use as ingredients for direct use in animal feed. Specifications and production control documentation for each of the raw materials are provided in Appendices 002A to I (CONFIDENTIAL).

Table 2.3: Raw Materials and Processing Aids Used in the Manufacture of C12/C14 Lactylates			
Material	**Function**	**Regulatory Status**	**Quality**
Lactic acid (*ca.* 98% L-lactic acid; *ca.* 2% D-lactic acid)	(b) (4)	GRAS for use as general purpose food additives in accordance with good manufacturing practice (21 CFR §582.1061; U.S. FDA, 2021)	(b) (4) (Manufactured by Corbion for use as a feed additive also) [Appendix 002A; CONFIDENTIAL]
Lauric acid (C12)		AAFCO OP Ingredient Definition 33.2 Vegetable fat, or oil; and 33.3 Hydrolyzed __ fat, or oil, feed grade (AAFCO, 2022)	(b) (4) Food grade (b) (4)) [Appendices 002B, C, F and G; CONFIDENTIAL]
Myristic acid (C14)		AAFCO OP: Ingredient definition 33.2 Vegetable fat, or oil and 33.3 Hydrolyzed __ fat, or oil, feed grade (AAFCO, 2022)	(b) (4) Food grade (b) (4) [Appendices 002D, E, F and G; CONFIDENTIAL]
Sodium hydroxide		GRAS for use as general purpose food additives in accordance with good manufacturing practice (21 CFR §582.1763; U.S. FDA, 2021)	(b) (4) [Appendix 002H and I; CONFIDENTIAL]

C12/C14 lactylates is marketed as a formulation under the trade name ALOAPUR® PM by mixing with a diatomaceous earth carrier. The raw materials used in the production of the market formulation (ALOAPUR® PM) are provided in Table 2.4. Both the processing aid and carrier are considered safe and

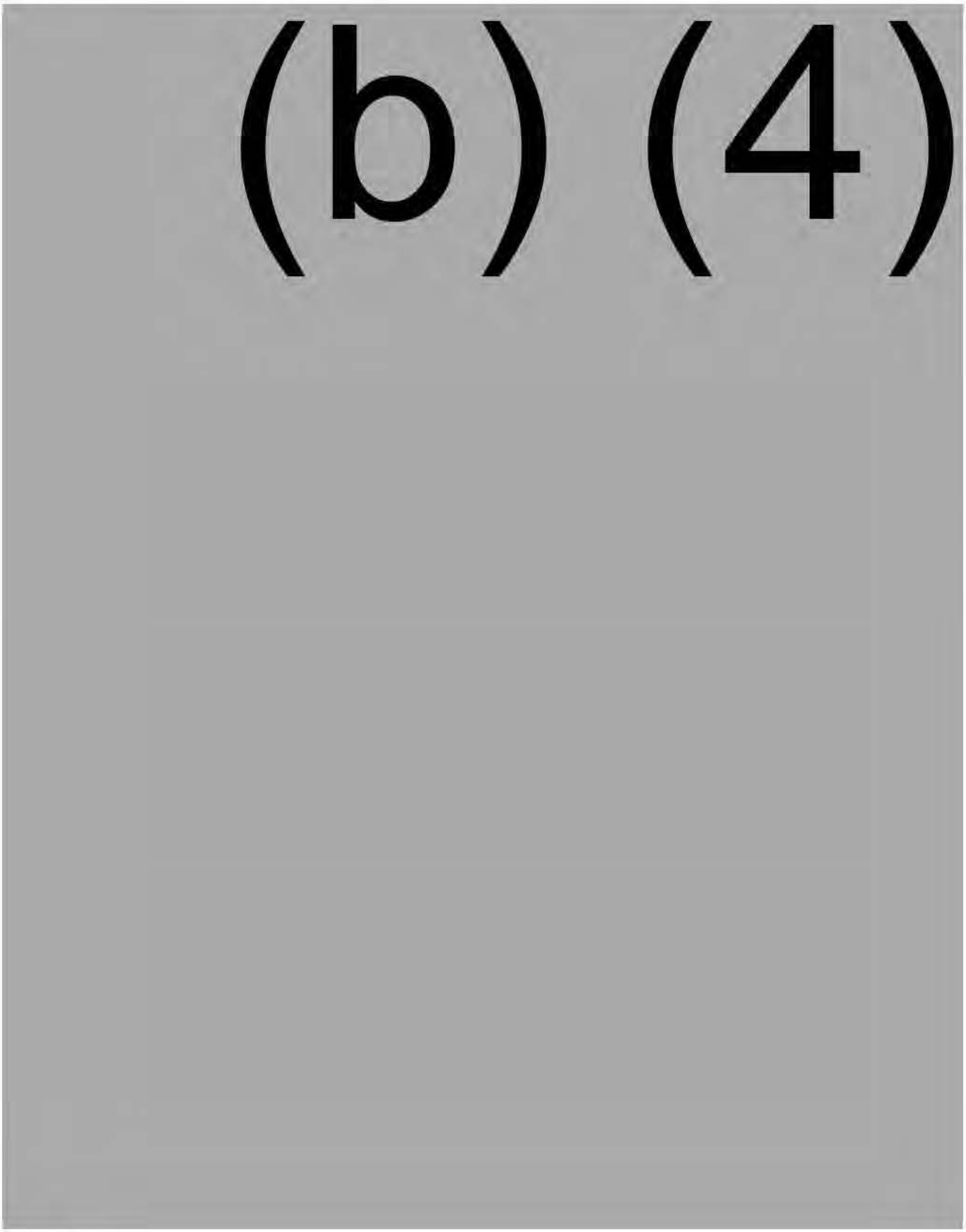
suitable for use in the production of feed and have a history of use as direct ingredients for use in animal feed. Specifications for the raw materials are provided in Appendices 003A and B (CONFIDENTIAL).

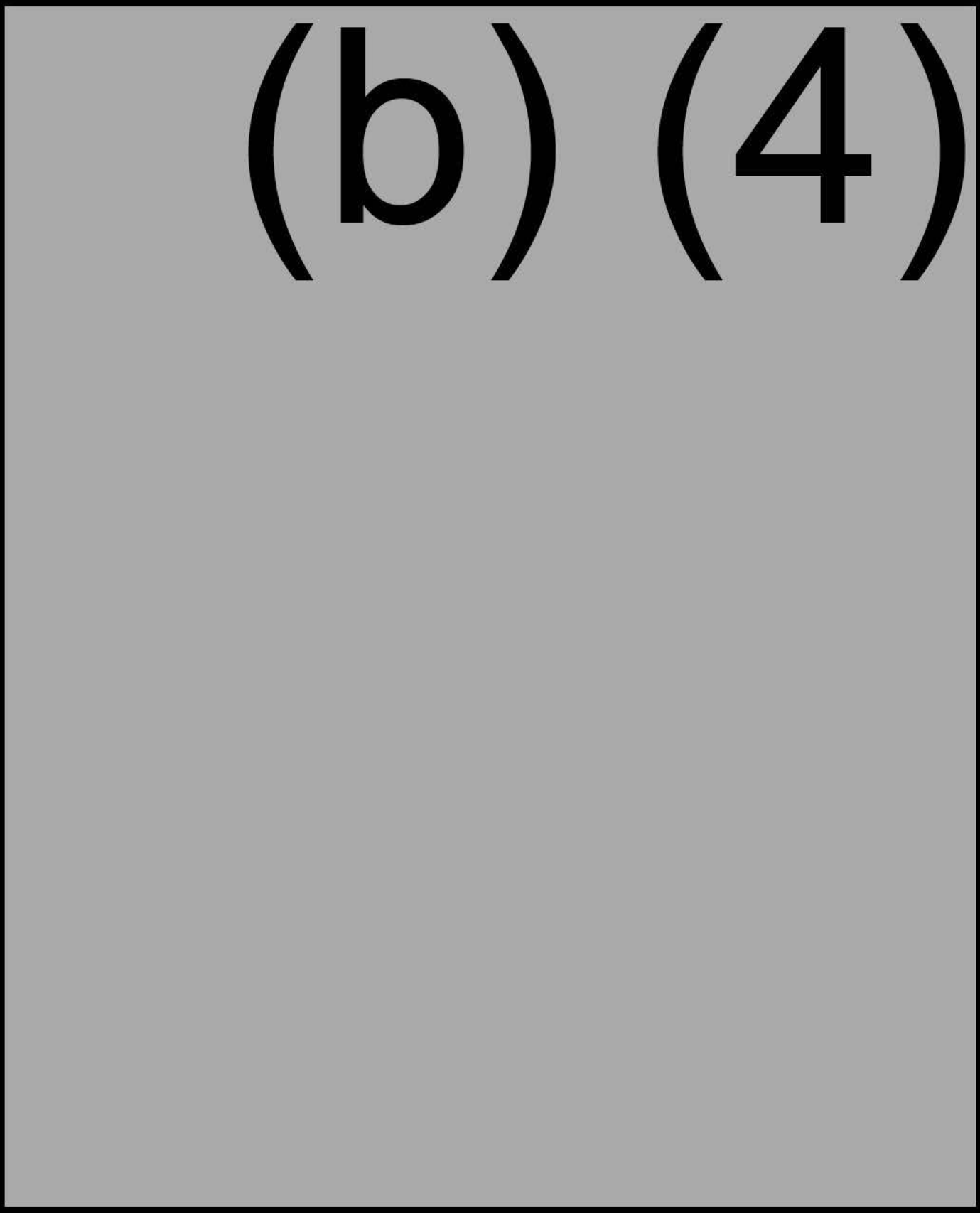
Table 2.4: Raw Materials and Processing Aids Used in the Manufacture of ALOAPUR® PM

Material	Function	Regulatory Status	Quality
C12/C14 Lactylates	(b) (4)	Not applicable	Not applicable
Sodium sulfate		AAFCO Ingredient Definition 57.109 for use as a mineral product (AAFCO, 2022)	(b) (4)
Diatomaceous earth		Food additive permitted in feed and drinking water of animals (21 CFR §573.340; U.S. FDA, 2021)	

2.2.2 Manufacture of C12/C14 Lactylates

(b) (4)


(b) (4)


(b) (4)

The formulated product is manufactured in accordance with cGMP and a HACCP system in place. The production is conducted in compliance with GMP+ requirements and a valid Process Certificate is provided in Appendix 006 (CONFIDENTIAL). The manufacturer will comply with the requirements for importing feed into the U.S. laid down by the Food Safety Modernization Act (FSMA) and the Bioterrorism Act (2002).

2.3 PRODUCT SPECIFICATIONS AND ANALYTICAL DATA FOR C12/C14 LACTYLATES

2.3.1 Product Specification for C12/C14 Lactylates

Appropriate compositional feed grade specifications have been established for C12/C14 lactylates and are presented in Table 2.7. Copies of the methods of analyses are provided in Appendices 007D, E and G (CONFIDENTIAL). All of the methods are based on internationally recognized procedures [e.g., European standard (EN) methods].

(b) (4)

(CONFIDENTIAL). As mentioned in Section 2.2.2, C12/C14 lactylates comprises an equilibrium mixture of raw materials and esterification products, and the extent of the reaction is monitored by following the AV and EV, which can be summed to give the SV. Thus, specific AV, EV and SV of (b) (4) KOH/g are targeted for C12/C14 lactylates with acceptable ranges of 50 to 70, 130 to 160 and 180 to 230 mg KOH/g, respectively set by the product specifications to ensure the composition is consistent (i.e., equilibrium mixture). The sodium content (6.0 to 8.0% w/w) provides further validation of the esterification reaction and quality of C12/C14 lactylates.

The levels of contaminants in C12/C14 lactylates are primarily controlled by ensuring that food grade raw materials (lactic acid, lauric acid, myristic acid and sodium hydroxide) are used in the manufacturing process (see Table 2.3). Maximum limits for arsenic, lead, mercury and cadmium of 10, 10, 0.2 and 1 mg/kg are proposed as part of the product specifications. Maximum tolerable limits (MTLs) for arsenic, lead, mercury and cadmium in the feed have been established for animals by the National Research Council (NRC, 2005). The MTLs vary by animal, with a level of 30 mg/kg DM feed established for arsenic for all animals except fish, for which a level of 5 mg/kg DM feed is set. A MTL of 10 mg/kg DM feed is set for lead for all animals except cattle and ruminants for which the level is 100 mg/kg DM feed. For inorganic and organic mercury, the MTL is 0.2 and 2 mg/kg DM feed, respectively for sheep, cattle and swine, and 0.2 and 1 mg/kg DM feed, respectively for poultry and fish. The MTL for cadmium is 10 mg/kg DM feed for all animal species. As mentioned in Section 3.1, C12/C14 lactylates is intended for use as a source of lauric and myristic acids in the diets of animals at levels not exceeding 1.0 g/kg complete feed as-fed, equivalent to 2.0 g/kg DM feed assuming a 12% moisture content. Under these conditions of use, the contribution by C12/C14 lactylates to the arsenic, lead, mercury and cadmium contents of the complete feed will not exceed 0.02, 0.01, 0.0004 and 0.002 mg/kg as-fed from C12/C14 lactylates. These levels fall well below (<1%) the MTLs established by the NRC for these heavy metals in the feed of animals and are not expected to pose a safety concern.

Table 2.7: Proposed Product Specifications for C12/C14 Lactylates		
Parameter	**Specification**	**Method of Analysis**
Appearance and Composition		
Color	(b) (4)	Visual inspection
Acid value (AV)		(b) (4)
Ester value (EV)		
Saponification value (SV)		
Sodium		
Heavy Metals		
Arsenic		
Lead		
Mercury		
Cadmium		

Abbreviations: AAS = atomic absorption spectrometry; ICP-OES = inductively coupled plasma-optical emission spectrometry.

2.3.2 Conformance of C12/C14 Lactylates with Product Specifications

Analytical data for 5 independently produced commercial lots of C12/C14 lactylates are summarized in Table 2.8. The Certificates of Analysis are provided in Appendices 007A, H to L, and N to R (CONFIDENTIAL). The analytical lot results demonstrate that C12/C14 lactylates can be manufactured in conformance with the proposed product specifications and exhibits acceptable lot to lot variation.

The AV and EV are relatively consistent across the 5 lots, varying from (b) (4) KOH/g and (b) (4) to (b) (4) mg KOH/g, respectively. Likewise, the SV is similar for the 5 lots with levels varying from (b) (4) mg KOH/g. Further validation of the consistency of the production process is provided by the sodium content which varies from (b) (4) across the 5 lots. No heavy metals were identified above detection limits for any of the lots tested.

Table 2.8: Analytical Data for 5 Production Lots of C12/C14 Lactylates							
Parameter	**Unit**	**Specification**	**Analytical Data[1]**				
Appearance and Composition							
Color	-						
Acid value (AV)	mg KOH/g						
Ester value (EV)	mg KOH/g						
Saponification value (SV)	mg KOH/g						
Sodium	% w/w						
Heavy Metals							
Arsenic	mg/kg	≤10	<4.8	<4.8	<4.8	<4.8	<4.8
Lead	mg/kg	≤5	<1.2	<1.2	<1.2	<1.2	<1.2
Mercury	mg/kg	≤0.2	<0.1	<0.1	<0.1	<0.1	<0.1
Cadmium	mg/kg	≤1	<0.1	<0.1	<0.1	<0.1	<0.1

(b) (4)

(b) (4)

Abbreviations: w/w = on a weight basis;

[1]Results of analysis for AV, EV and SV as reported by the manufacturer of the lots of C12/C14 lactylates – the results of analysis on the same lots conducted by Corbion are also provided in Appendices 007A, H to L, and N to R (CONFIDENTIAL) and are consistent with those of the manufacturer.

2.3.3 Composition of C12/C14 Lactylates

The composition of 5 independent commercial lots of C12/C14 lactylates was analyzed by gas chromatography (GC) and the results are summarized in Table 2.9. The study report is provided in Appendix 007A (CONFIDENTIAL) and a copy of the method in Appendix 007C (CONFIDENTIAL).

C12/C14 lactylates typically comprises a mixture of sodium lauroyl-1-lactylate (*ca.* (b) (4) sodium myristoyl-1-lactylate (*ca.* (b) (4) sodium laurate (C12 fatty acid; *ca.* (b) (4) sodium myristate (*ca.* (b) (4) sodium lactate (*ca.* (b) (4) sodium lauroyl-2-lactylate (*ca.* (b) (4) sodium myristoyl-2-lactylate (*ca.* (b) (4) sodium lactoyl lactate (*ca.* (b) (4) and minor amounts of oligomers of lactate (not quantified). The composition of C12/C14 lactylates was consistent across the 5 lots analyzed, displaying less than 2% variation in any individual component.

Table 2.9: Composition of 5 Production Lots of C12/C14 Lactylates						
Composition	**Analytical Data (% w/w)[1]**					**Mean Content (% w/w)[2]**
Sodium lactate	(b) (4)					9.0
Sodium lactoyl lactate						2.8
Sodium laurate (C12 fatty acid)						18.4
Sodium myristate (C14 fatty acid)						8.1
Sodium lauroyl-1-lactylate (monomer)						33.4
Sodium myristoyl-1-lactylate (monomer)						14.0
Sodium lauroyl-2-lactylate (dimer)						6.2
Sodium mristoyl-2-lactylate (dimer)						2.2
Total						***94.1***
Oligomers of lactate (minor components)	Minor amounts (not quantified)	Minor amounts (not quantified)	Minor amounts (not quantified)	Minor amounts (not quantified)	Minor amounts (not quantified)	Minor amounts (not quantified)

Abbreviations: w/w = on a weight basis; C12 = dodecanoyl/lauroyl moiety; C14 = tetradecanoyl/myristoyl moiety;
[1]Each lactate and lactylate component is expressed as the sodium salt;
[2]Expressed as the mean of the 5 representative lots of C12/C14 lactylates.

2.3.4 Dioxins and PCBs in C12/C14 Lactylates

The results of dioxin and PCB analysis for 3 independent commercial lots of C12/C14 lactylates are presented in Table 2.10. The Certificates of Analysis are provided in Appendices 007S to U (CONFIDENTIAL). The individual dioxins and PCBs analyzed and reported as sums of dioxins, dioxins and dioxin-like PCBs, and non-dioxin-like PCBs expressed in World Health Organization (WHO) Toxic Equivalency Factors (TEQs) were below the limits of quantification.

In the European Union (EU), specification limits are set under Directive 2002/32/EC (as amended; EC, 2002) for dioxins and dioxins and dioxin-like PCBs of not more than 0.75 ng WHO-PCDD/F-TEQ and 1.5 WHO-PCDD/F-PCB-TEQ/kg complete feed (12% moisture), respectively and for non-dioxin-like PCBs of not more than 10 µg/kg complete feed (12% moisture) in vegetable oils and their by-products intended

for use as feed materials. The analytical results for the 3 commercial lots of C12/C14 lactylates fall well below these specification limits.

Table 2.10: Results of Dioxins, Dioxin-like PCBs and Non-Dioxin-Like PCBs Analysis for 3 Production Lots of C12/C14 Lactylates

Parameter	Unit	EU Specification	Analytical Data[2]		
			(b) (4)		
Dioxins[1]	ng WHO-PCDD/F-TEQ/kg	≤0.75	0.156	0.156	0.156
Dioxins + dioxins-like PCBs[1]	ng WHO-PCDD/F-PCB-TEQ/kg	≤1.5	0.222	0.222	0.222
Non-dioxin-like PCBs[1]	µg/kg	≤10	3.0	3.0	3.0

Abbreviations: PCB = polychlorinated biphenyls; PCDD = polychlorinated dibenzo-p-dioxins; PCDF = polychlorinated dibenzofurans; TEQ = toxic equivalency; WHO = World Health Organization;
[1]Upper-bound concentrations are calculated on the assumption that all values of the different congeners below the limit of quantification are equal to the limit of quantification;
[2]Sums of dioxins, dioxin and dioxin-like PCBs and non-dioxin-like PCBs are identical on the basis that the values for the different congeners were below the limits of quantification.

2.4 PRODUCT SPECIFIATIONS AND ANALYTICAL DATA ON ALOAPUR® PM

2.4.1 Product Specifications for ALOAPUR® PM

Appropriate feed grade specifications have been established for the market formulation, ALOAPUR® PM and are presented in Table 2.11. Copies of the methods of analysis are provided in Appendices 007E and 008 (CONFIDENTIAL).

(b) (4)

There are no other compositional or chemical changes that occur on preparation of the market formulation (ALOAPUR® PM). The ester value is a measure of the lactic acid ester components (total) and is the most appropriate analytical means of confirming the composition and inclusion level of C12/C14 lactylates.

The EV and ash content are evaluated on every lot of ALOAPUR® PM prior to product release. The

(b) (4)

Table 2.11: Proposed Product Specifications for ALOAPUR® PM		
Parameter	**Specification**	**Method of Analysis**
Composition		
Ester value (EV)	≥45 mg KOH/g	(b) (4) (Titration) (Appendix 007E – CONFIDENTIAL)
Ash/residue on ignition	≤70%	Commission Regulation (EC) No 152/2009 (Appendix 008)

Appropriate specifications for heavy metals are set separately for C12/C14 lactylates (see Table 2.6) and diatomaceous earth (see Appendix 003B – CONFIDENTIAL) in order to control the levels of contaminants in ALOAPUR® PM. Dioxins and PCBs levels comply with the maximum limits set in the EU under Directive 2002/32/EC on undesirable substances in animal feed (as amended; EC, 2002).

2.4.2 Conformance of ALOAPUR® PM with Product Specifications

The results of analysis for EV and ash content for 5 independent commercial lots of ALOAPUR® PM are presented in Table 2.12. The Certificates of Analysis are provided in Appendices 009A to E (CONFIDENTIAL). The analytical lot results demonstrate that ALOAPUR® PM can be manufactured in conformance with the product specifications and exhibits acceptable lot to lot variation. Across the 5 lots, the ester value varied from (b) (4) mg KOH/g and the ash content (b) (4)%.

Table 2.12: Analytical Data for 5 Production Lots of ALOAPUR® PM			
Parameter	**Unit**	**Specification**	**Analytical Data**
Ester value	mg KOH/g		(b) (4)
Ash/residue on ignition	% w/w		(b) (4)

Abbreviations: w/w = on a weight basis.

2.4.3 Composition of ALOAPUR® PM

As mentioned in Section 2.3.3, C12/C14 lactylates comprises an equilibrium mixture of sodium lauroyl-1-lactylate (*ca.* 33%), sodium myristoyl-1-lactylate (*ca.* 14%), sodium laurate (C12 fatty acid; *ca.* 18%), sodium myristate (*ca.* 8%), sodium lactate (*ca.* 9%), sodium lauroyl-2-lactylate (*ca.* 6%), sodium myristoyl-2-lactylate (*ca.* 2%), sodium lactoyl lactate (*ca.* 3%) and minor amounts of oligomers of lactate (not quantified). C12/C14 lactylates (*ca.* (b) (4) range 30 to 40%) is mixed with diatomaceous earth (*ca.* (b) (4) range 60 to 70%) to form the market formulation, ALOAPUR® PM. The typical amount of each component of the C12/C14 lactylates ingredient in the market formulation (ALOAPUR® PM) for direct use in animal feed is summarized in Table 2.13.

Table 2.13: Composition of the Formulation (ALOAPUR® PM)	
Composition	**Typical Content (w/w %)**
Sodium salts of lactylates of lauric and myristic acids (C12/C14 lactylates), of which[1]:	(b) (4)
Sodium lactate	
Sodium lactoyl lactic acid	
Sodium laurate (C12 fatty acid)	
Sodium myristate (C14 fatty acid)	
Sodium lauroyl-1-lactylate (monomer)	
Sodium myristoyl-1-lactylate (monomer)	
Sodium lauroyl-2-lactylate (dimer)	
Sodium myristoyl-2-lactylate (dimer)	
Diatomaceous earth	
Total	***100***

(b) (4) (with rounding).

Analytical data for 3 independent commercial lots of ALOAPUR® PM are presented in Table 2.14. The composition of C12/C14 lactylates was consistent across the 3 lots analyzed, displaying no more than 2% variation in any individual component. The sum of the major C12/C14 lactylates components in the market formulation varied from (b) (4) (mean (b) (4)) and overall, the data are consistent with the calculated contents. The results are provided in Appendix 010 (CONFIDENTIAL) and these lots were also used in the stability study described in Section 2.5.

Table 2.14: Composition of 3 Lots of ALOAPUR® PM					
Parameter	**Calculated Content (% w/w)[1]**	**Analytical Results (% w/w)[2]**			**Mean Content (% w/w)[3]**
		Lot	Lot	Lot	
Sodium lactate		(b) (4)			2.9
Sodium lactoyl lactate					0.9
Sodium laurate (C12 fatty acid)					6.9
Sodium myristate (C14 fatty acid)					3.0
Sodium lauroyl-1-lactylate (monomer)					12.7
Sodium myristoyl-1-lactylate (monomer)					5.3
Sodium lauroyl-2-lactylate (dimer)					2.1
Sodium myristoyl-2-lactylate (dimer)					0.9
Total					***34.7***

Abbreviations: w/w = on myristoyl moiety;
[1]As reported in Table 2.13;
[2]Each lactate and lactylate component is expressed as the sodium salt.

2.4.4 Sulfur and Sulfate Content of ALOAPUR® PM

The sulfur and sulfate content of 3 independent commercial lots of ALOAPUR® PM were analyzed and the results are presented in Table 2.15. Across the 3 lots tested, the mean sulfur and sulfate contents were 1,667 and 4,967 mg/kg, respectively. The level of sulfate is notably higher than the amount added in the form of sodium sulfate (b) (4) mg/kg of sodium sulfate, corresponding to <(b) (4) mg/kg of sulfate) which will be due to the additional contribution of the diatomaceous carrier. The full study report is provided in Appendix 011 (CONFIDENTIAL). A safety assessment of the levels of sulfate in ALOAPUR® PM for the target animal is conducted in Section 6.1.6.

Table 2.15: Results of Sulfur and Sulfate Analysis for 3 Production Lots of ALOAPUR® PM

Parameter	Unit	Analytical Data	Mean Content
Sulfur	mg/kg	(b) (4)	1,667
Sulfate	mg/kg		4,967

2.4.5 Heavy Metals in ALOAPUR® PM

The results of heavy metal analysis for 3 independently produced commercial lots of ALOAPUR® PM are summarized in Table 2.16. The Certificates of Analysis are provided in Appendices 012A to C (CONFIDENTIAL).

The MTLs for heavy metals set by the NRC (2005) in feed vary by animal, with a level of 30 mg/kg DM feed established for arsenic for all animals except fish, for which a level of 5 mg/kg DM feed is set. A MTL of 10 mg/kg DM feed is set for lead for all animals except cattle and ruminants for which the level is 100 mg/kg DM feed. For inorganic and organic mercury, the MTL is 0.2 and 2 mg/kg DM feed, respectively for sheep, cattle and swine, and 0.2 and 1 mg/kg DM feed, respectively for poultry and fish. The MTL for cadmium is 10 mg/kg DM feed for all animal species. As mentioned in Section 3.1, ALOAPUR® PM is incorporated into the diets of animals at levels not exceeding 5 g/kg complete feed as-fed, equivalent to 5.7 g/kg DM feed assuming a 12% moisture content. Under these conditions of use, the analytical data indicate that the contribution by ALOAPUR® PM to the arsenic, lead, cadmium and mercury levels in the feed will be no more than 0.07, 0.005, 0.0005 and 0.03 mg/kg as-fed, respectively. These levels fall well below the MTLs (<2%) established by the NRC for these heavy metals in the feed of animals and are not expected to pose a safety concern.

Table 2.16: Results of Heavy Metals Analysis for 3 Production Lots of ALOAPUR® PM

Parameter	Unit	Analytical Data (b) (4)		
Arsenic	mg/kg	13.7	13.8	11.8
Cadmium	mg/kg	1.040	0.850	0.620
Mercury	mg/kg	<0.10	<0.10	<0.10
Lead	mg/kg	6.02	6.01	6.17

2.4.6 Dioxins and PCBs in ALOAPUR® PM

The results of analysis for dioxins and PCBs for 3 independent commercial lots of ALOAPUR® PM representative of the commercial material is presented in Table 2.17. The Certificates of Analysis are provided in Appendix 012A to C (CONFIDENTIAL).

In the EU, specification limits are set under Directive 2002/32/EC (as amended; EC, 2002) for dioxins and dioxins and dioxin-like PCBs of not more than 0.75 ng WHO-PCDD/F-TEQ and 1.5 WHO-PCDD/F-PCB-TEQ/kg complete feed (12% moisture), respectively and for non-dioxin-like PCBs of not more than 10 µg/kg complete feed (12% moisture) in compound (mixed) feed intended for use as feed materials. The analytical results for the individual congeners in the 3 commercial lots of C12/C14 lactylates were all below detection limits and therefore, conform to the maximum limits set in the EU.

Table 2.17: Results of Dioxins and Dioxin-like PCBs Analysis for 3 Lots of ALOAPUR® PM

Parameter	Unit	EU Specification	Analytical Data[2]		
			Lot (b) (4)	Lot	Lot
Dioxins[1]	ng WHO-PCDD/F-TEQ/kg	0.75	0.222	0.222	0.222
Dioxins + dioxins-like PCBs[1]	ng/kg WHO-PCDD/F-PCB-TEQ/kg	1.5	0.156	0.156	0.156
Non-dioxin-like PCBs[1]	µg/kg	≤10	3.0	3.0	3.0

Abbreviations: PCB = polychlorinated biphenyls; PCDD = polychlorinated dibenzo-p-dioxins; PCDF = polychlorinated dibenzofurans; TEQ = toxic equivalency; WHO = World Health Organization;
[1]Upper-bound concentrations are calculated on the assumption that all values of the different congeners below the limit of quantification are equal to the limit of quantification;
[2]Sums of dioxins, dioxin and dioxin-like PCBs and non-dioxin-like PCBs are identical on the basis that the values for the different congeners were below the limits of quantification.

2.5 SHELF-LIFE AND STABILITY DATA

2.5.1 Evaluation of Potential Degradation Pathways

As mentioned, C12/C14 lactylates comprises the lactic acid esters of lauric (C12) and myristic (C14) fatty acids. The chemical structures and identity of each of the components are provided in Table 2.1 (above). ALOAPUR® PM is specifically formulated to generate an easily handled, and relatively stable, form of lauric and myristic acids for animals. Lauric and myristic acids are widely distributed naturally occurring fatty acids, with sources of lauric acid including coconut and palm kernel oils, as well as milk fats, and sources of myristic acid, coconut oil, nutmeg butter, palm seed oils and milk fats (CIR, 1987).

In terms of degradation, saturated fatty acids such as lauric acid and myristic acid are less susceptible to oxidation than unsaturated fatty acids, but under extreme processing conditions, polymerization and dehydration may occur, as well as reduction to aldehydes or alcohols (CIR, 1987). However, under the conditions of storage of C12/C14 lactylates and its use in animal feed, these degradation processes will not occur.

Furthermore, the background diet of animals contains significant levels of saturated and unsaturated fatty acids, and thermal processing, handling and storage conditions of feed are designed to reduce the potential significant fatty acid degradation, in order to maintain the nutritional value and organoleptic properties of the complete feed.

The long and established history of use of lauric and myristic acids in food manufacture, cooking and cosmetic products also provides corroborative evidence of the relative stability of these saturated fatty acids (CIR, 1987).

Hydrolysis of the ester bonds may occur but typically only under aqueous acidic or basic conditions, or in the presence of hydrolytic enzymes. Only low levels of hydrolysis are anticipated in C12/C14 lactylates under the conditions of manufacture, handling and storage of the market formulation or feeds.

On this basis, the shelf-life studies were primarily focused on ensuring conformance with the product specifications (see Section 2.5.2) whereas the in-feed stability studies followed the stability of the primary esters

Taking into account the primary anticipated degradation pathways, shelf-life studies using ALOAPUR® PM were primarily designed to demonstrate conformance with the product specifications (see Section 2.5.2) over the storage period. In-feed studies by contrast, primarily considered the potential for hydrolysis of the ester bonds and therefore, monitored the effect of storage on the primarily esters lauryl-1-lactylate and myristoyl-1-lactylate (see Section 2.5.3).

2.5.2 Shelf-Life and Stability

A shelf-life of 24 months is proposed for C12/C14 lactylates in the form of ALOAPUR® PM (market formulation) when stored in the original, unopened packaging under cool (<20°C) and dry in the absence of light or excessive humidity. Details of the packaging materials are provided in Section 2.2.3 above.

A real-time stability study was conducted by Corbion on one representative lot of ALOAPUR® PM stored in a climate-controlled chamber at (b) (4)% relative humidity (RH) in commercial packaging for 24 months. The results of the stability study are summarized in Table 2.18 and the full report is provided in Appendix 013A (CONFIDENTIAL). The methods of analysis used to measure EV, ash and sodium contents are those used by Corbion to demonstrate compliance with the product specifications and copies are provided in Appendices 007D, 007E and 008 (CONFIDENTIAL). Over the 24-month period, the EVs and ash contents remained within specification limits of no less than 40 mg KOH/g and not more than 67% (by weight), respectively. Although not part of the product specifications, the sodium content remained within the anticipated limit of not more than 3%. The bulk density and particle size were not significantly altered over the 24-month storage period but there was a slight initial reduction in bulk density during the first month (b) (4) kg/m^3). Overall, the results of the stability study indicate that the composition of ALOAPUR® PM remains compliant with the product specifications and there is no significant change in physical form over the proposed 24-month shelf-life for the product under the recommended storage conditions.

Table 2.18: Stability Study Results for 1 Lot of ALOAPUR® PM (25°C; 60% RH)								
Parameter	**Unit**	**Spec.[1]**	**Analytical Results (Months); Lot 1603003760**					
			t=0	**t=3**	**t=6**	**t=9**	**t=12**	**t=18**	**t=24**
Ester value	mg KOH/g	≥40	(b) (4)						
Ash, residue on ignition	%	≤67							
Sodium	%	≤3.0							
Bulk density	kg/m³	567-771							
Particle size >1000 µm	%	≤2							
Particle size <125 µm	%	≤2							

Abbreviations: RH = relative humidity; Spec. = specification; t = time;
[1]Note that specification limits were set for parameters evaluated in the stability study which are not part of the compositional product specifications and are not routinely evaluated.

Additionally, samples of 2 commercial lots of ALOAPUR® PM retained at Corbion's warehouse under ambient conditions in the original packaging for between 5 and 6 years, were re-tested for conformance to product specifications. A copy of the study report is provided in Appendix 013B (CONFIDENTIAL). The results of the re-testing are presented in Table 2.19 and confirm that after 5 to 6 years of storage, the ALOAPUR® PM lots, stored under representative warehouse conditions, continue to conform to the compositional and physical specifications. Although, the original analyses are not available for the date of manufacture, Corbion can confirm that they met the product specifications and were released for use commercially by quality control. Overall, the findings support the proposed shelf-life of ALOAPUR® PM of 24 months under ambient conditions.

Table 2.19: Stability Study Results for 2 Lots of ALOAPUR® PM (Ambient Warehouse Conditions)			
Parameter	**Unit**	**Spec.[1]**	**Analytical Results (5-6 Years)**
Ester value	mg KOH/g	≥40	(b) (4)
Ash, residue on ignition	%	≤67	
Sodium	%	≤3.0	
Bulk density	kg/m³	567-771	
Particle size >1,000 µm	%	≤2	
Particle size <125 µm	%	≤2	

Abbreviations: RH = relative humidity; Spec. = specification; T = time;
[1]Note that specification limits were set for parameters evaluated in the stability study which are not part of the compositional product specifications and are not routinely evaluated.

An accelerated shelf-life study was also conducted in which 3 representative lots of ALOAPUR® PM were stored in a climate-controlled chamber for 6 months at (b) (4) RH in commercial packaging. The methods of analysis used to measure EV and lactylates components are those used by Corbion to demonstrate conformance with the product specifications and to characterize the composition of its lactylates products and copies are provided in Appendices 007C and E (CONFIDENTIAL). The results of

the stability study are summarized in Table 2.20, and the protocol and study report are provided in Appendix 014A and B (CONFIDENTIAL), respectively.

Table 2.20: Stability Study Results for 3 Lots of ALOAPUR® PM ((b) (4) RH)									
Parameter	**Unit**	**Analytical Results (Months)**								
		(b) (4)								
		t=0	**t=3**	**t=6**	**t=0**	**t=3**	**t=6**	**t=0**	**t=3**	**t=6**
Ester value	mg KOH/g	(b) (4)								
Caking	-									
Moisture	% w/w									
Sodium lactate	% w/w									
Sodium lactoyl lactate	% w/w									
Sodium laurate (C12 fatty acid)	% w/w									
Sodium myristate (C14 fatty acid)	% w/w									
Sodium lauroyl-1-lactylate (monomer)	% w/w									
Sodium myristoyl-1-lactylate (monomer)	% w/w									
Sodium lauroyl-2-lactylate (dimer)	% w/w									
Sodium myristoyl-2-lactylate (dimer)	% w/w									

Abbreviations: t = time; w/w = by weight.

Across the 3 lots tested, the compositional changes observed over the 6-month storage period are summarized in Table 2.21. Over the 6-month storage period, an increase in sodium lactate, sodium laurate (C12 fatty acid) and sodium myristate (C14 fatty acid) were observed. A concomitant decrease in sodium lactoyl lactate, sodium lauroyl-1-lactylate, sodium myristoyl-1-lactylate, sodium lauroyl-2-lactylate and sodium myristoyl-2-lactylate was observed with time. These findings are consistent with hydrolysis of the lactic acid esters over time to release lactic and the free fatty acids and are further supported by the decrease in ester value over the 6-month period. These effects are not considered biologically significant or to impact the nutritional value of C12/C14 lactylates on the basis that the esters are enzymatically hydrolyzed to the same species in the gastrointestinal (GI) tract of the animal prior to being absorbed and metabolized (see Part 6).

Table 2.21: Summary of Compositional Changes Over the 6-Month Storage Period

Species	Analytical Results (%)	
	Range at t=0 Months	Range at t=6 Months
Ester value	(b) (4)	
Sodium lactate		
Sodium lactoyl lactate		
Sodium laurate (C12 fatty acid)		
Sodium myristate (C14 fatty acid)		
Sodium lauroyl-1-lactylate (monomer)		
Sodium myristoyl-1-lactylate (monomer)		
Sodium lauroyl-2-lactylate (dimer)		
Sodium myristoyl-2-lactylate (dimer)		

Abbreviations: t = time.

2.5.3 In-Feed Stability Study

Homogeneity and Pelleting Stability Studies

A study was conducted to evaluate (a) the ability of C12/C14 lactylates in the form of PURAMIX 30S[1] to homogeneously distribute in wheat- or corn-based mash and pelleted feeds; and (b) the stability of C12/C14 lactylates to withstand the conditions of the pelleting process. Considering the compositional similarities between PURAMIX 30S and ALOAPUR® PM, including the use of a diatomaceous earth carrier, the findings of the homogeneity and pellet stability studies can reasonably be extrapolated to support ALOAPUR® PM (market formulation).

PURAMIX 30S was added to mash feeds at a level of 5 g/kg complete feed and (b) (4) subsamples were taken from each sample. The contents of sodium lauroyl-1-lactylate and sodium myristoyl-1-lactylate were evaluated in the samples as an indicator of the distribution and levels of C12/C14 lactylates in the feed. Additionally, the mash samples were subject to pelleting at different temperatures ((b) (4) and (b) (4) °C).

[1] PURAMIX 30S comprises (b) (4)% C12/C14 lactylates and (b) (4) diatomaceous earth vs. ALOAPUR® PM which contains (b) (4)% C12/C14 lactylates and (b) (4)% diatomaceous earth.

The results of the homogeneity and pelleting testing are summarized in Table 2.22 and the study report is provided in Appendix 015A (CONFIDENTIAL) with a translation of the feed composition in English available in Appendix 015B (CONFIDENTIAL). The coefficients of variation (CVs) for the sodium lauroyl-1-lactylate and sodium myristoyl-1-lactylate contents in wheat-based feed samples in mash and pellet form varied from 4 to 11%. Similarly, the sodium lauroyl-1-lactylate and myristoyl-1-lactylate contents in corn-based feed samples in mash and pellet form varied from 5 to 12%. Taking into account that the method of analysis also has a reported variability of 75 to 125%, the CVs were considered acceptable in both the wheat- and corn-based diets indicating that C12/C14 lactylates in the form of PURAMIX 30S can be homogeneously distributed in animal feed based on either wheat or corn.

In wheat-based feed, under the conditions of pelleting (b) (4)), the content of sodium lauroyl-1-lactylate (measured as the lactylate ion) was observed to range from (b) (4) mg/kg mash feed to (b) (4) mg/kg in pelleted feed subjected to a temperature of (b) (4) °C. The content of sodium myristoyl-1-lactylate (measured as the lactylate ion) was observed to vary from (b) (4) mg/kg in mash feed to (b) (4) mg/kg in pelleted feed subject to a temperature of (b) (4) °C. A linear trend was displayed with the sodium lauroyl-1-lactylate and sodium myristoyl-1-lactylate contents reduced as the pellet processing temperature was increased.

The same trend was displayed in corn-based feeds with the sodium lauroyl-1-lactylate and sodium myristoyl-1-lactylate contents decreasing with increased pelleting temperature. The content of sodium lauroyl-1-lactylate (measured as the lactylate ion) varied from (b) (4) mg/kg in mash feed to (b) (4) and (b) (4) mg/kg in feed pelleted at (b) (4) °C, respectively. Similarly, the content of sodium myristoyl-1-lactylate (measured as the lactylate ion) varied from (b) (4) mg/kg in mash feed to (b) (4) mg/kg in feed pelleted at 85°C.

Overall, relative to the starting sodium lauroyl-1-lactylate and sodium myristoyl-1-lactylate contents in the mash feed, the recovery of these fatty acid derivatives after pelleting was at least 80% for all pelleting conditions. There were no observed differences in the stability of sodium lauroryl-1-lactylate and sodium myristoyl-1-lactylate to the pelleting process when incorporated into wheat- or corn-based feeds. Thus, it may be concluded that C12/C14 lactylates in the form of PURAMIX 30S exhibits acceptable stability in feed under typical pelleting conditions.

In these experiments, C12/C14 lactylates was added to the feed at around 2 g/kg in the form of 5 g of PURAMIX 30S/kg complete feed which is in the same range as that provided by ALOAPUR® PM at 1.8 g/kg when included at the same inclusion level (the recommended use level; see Section 2.6). Thus, equivalent behavior is expected when ALOAPUR® PM is formulated into corn- and wheat-based feeds.

Table 2.22: Results of Homogeneity and Pelleting Stability Studies in Wheat- and Corn-Feeds Containing PURAMIX 30S					
Sample	No. Subsamples	Mean Content (mg/kg)		CV (%)	
		Lauroyl-1-lactylate	Myristoyl-1-lactate	Lauroyl-1-lactylate	Myristoyl-1-lactate
Wheat (blank)	4	(b) (4)		-	-
Wheat (mash) + PURAMIX 30S	11			11	11
Wheat (pellets) + PURAMIX 30S, 65°C	11			5	5
Wheat (pellets) + PURAMIX 30S, 75°C	11			4	6
Wheat (pellets) + PURAMIX 30S, 85°C	11			6	10
Corn (blank)	4			-	-
Corn (mash) + PURAMIX 30S	11			9	10
Corn (pellets) + PURAMIX 30S, 65°C	11			6	6
Corn (pellets) + PURAMIX 30S, 75°C	11			10	12
Corn (pellets) + PURAMIX 30S, 85°C	11			5	6

Abbreviations: CV = coefficients of variation.

In-Feed Stability Study

The stability of C12/C14 lactylates in the form of ALOAPUR® PM in feed on storage for a period of up to 3 months under ambient (warehouse) conditions was evaluated in wheat- and corn-based broiler diets. PURAMIX 30S was added to wheat- and corn-based mash feeds at 5 g/kg equivalent to 2 g C12/C14 lactylates/kg and samples stored for the 3 month period in mash form or after subsequent pelleting at 65°C. The sodium lauroyl-1-lactylate and sodium myristoyl-1-lactylate contents were analyzed on preparation (time = 0 days) and again after 29, 57 and 92 days and the recoveries relative to the content at day 0 calculated. The results are summarized in Table 2.23 and the study report is provided in Appendix 015A (CONFIDENTIAL). (b) (4) (CONFIDENTIAL).

Over the 3-month storage period, the recovery of sodium lauroyl-1-lactylate (measured as the lactylate ion) from wheat- and corn-based mash and pelleted feed was at least 58%. Similarly, the recovery of sodium myristoyl-1-lactylate (measured as the lactylate ion) from wheat- and corn-based mash and pelleted feed was at least 74%. With the exception of myristoyl-1-lactylate in corn pellets containing C12/C14 lactylates in the form of PURAMIX 30S, the amount of lauryl-1-lactylate or myristoyl-1-lactyate extracted from the feed is reduced over the 92-day storage period. Although, there may be some degradation of the C12/C14 lactylates, it is also expected that the fatty acid-derived products will bind within the feed matrix, reducing the amount of lauroyl-1-lactylate and myristyl-1-lactylate that can be recovered. Any degradation that does occur, is likely to be hydrolysis of the lactic acid esters to the free lauric and myristic acids as demonstrated in the accelerated shelf-life study for ALOAPUR® PM above. Considering that C12/C14 lactylates will also hydrolyze in the GI tract of the animal following ingestion

of ALOAPUR® PM to form the free fatty acids which are absorbed (see Part 6), any observed hydrolysis in the feed is not expected to impact the nutritional value of the ingredient.

Overall, it may be concluded that C12/C14 lactylates in the form of ALOAPUR® PM displays acceptable stability in broiler feed based on wheat or corn under typical storage conditions at the anticipated use level. Moreover, the stability of C12/C14 lactylates is not expected to be influenced to a significant degree by differences in the relative amounts of different cereal and other ingredients in the feed, and the results of the stability study in broiler feed may also be reasonably extrapolated to feeds for other animals.

Table 2.23: Results of Stability Studies in Wheat- and Corn-Feeds Containing PURAMIX 30S				
Sample	Stability (% Recovery from Content at Day 0)[1]		CV (%)	
	Lauroyl-1-lactylate	Myristoyl-1-lactate	Lauroyl-1-lactylate	Myristoyl-1-lactate
Wheat (mash) + PURAMIX 30S				
Day 0	100	100	11	11
Day 29	(b) (4)		13	13
Day 57			11	14
Day 85			4	6
Day 92			11	10
Wheat (pellets) + PURAMIX 30S, 65°C (pelleting temperature)				
Day 0	100	100	5	5
Day 29	(b) (4)		5	3
Day 57			9	7
Day 85			7	5
Day 92			7	9
Corn (mash) + PURAMIX 30S				
Day 0	100	100	9	10
Day 29	(b) (4)		15	13
Day 57			9	8
Day 85			9	5
Day 92			8	7
Corn (pellets) + PURAMIX 30S, 65°C (pelleting temperature)				
Day 0	100	100	6	6
Day 29	(b) (4)		9	6
Day 57			5	3
Day 85			2	4
Day 92			5	4

Abbreviations: CV = coefficients of variation;
[1]Mean recoveries and CV based on 4 subsamples per feed sample.

Method of Analysis for Identifying C12/C14 Lactylates Components in Feed

The method of analysis for determining the lauroyl-1-lactylate and myristoyl-1-lactylate contents of feed samples also is provided in Appendix 016 (CONFIDENTIAL).

2.6 INTENDED USE AND USE LEVEL IN ANIMAL FOOD

C12/C14 lactylates is intended for use as a source of lauric acid and myristic acid in the diet of animals in the U.S. The target animals are all major and minor food-producing animals as well as companion animals. The fatty acid-containing ingredient will be marketed as a formulation on a diatomaceous earth carrier comprising *ca.* (b) (4) C12/C14 lactylates and *ca.* (b) (4) carrier under the trade name ALOAPUR® PM. The use of the diatomaceous carrier allows C12/C14 lactylates to be supplied for use in animal feed in a homogenous powdered form which is readily mixed with finished feeds. The finished feeds may be provided to the animal in mash form or pelleted. Stability studies conducted on ALOAPUR® PM demonstrate that two of the primary components of C12/C14 lactylates, lauroyl-1-lactylate and myristoyl-1-lactylate are stable under commercial pelleting conditions (see Section 2.5.3).

C12/C14 lactylates comprises an equilibrium mixture of sodium lauroyl-1-lactylate (*ca*. 33%), sodium myristoyl-1-lactylate (*ca*. 14%), sodium laurate (C12 fatty acid; *ca*. 18%), sodium myristate (*ca*. 8%), sodium lactate (*ca*. 9%), sodium lauroyl-2-lactylate (*ca*. 6%), sodium myristoyl-2-lactylate (*ca*. 2%), sodium lactoyl lactate (*ca*. 3%) and minor amounts of oligomers of lactate (not quantified) (see Table 2.8). In this respect, animals will be provided with lauric (C12) and myristic (C14) acids as well as lactic acid in the free and esterified forms. Based on this typical composition, C12/C14 lactylates is calculated to comprise around 42% lauric acid, 19% myristic acid and 28% lactic acid[2]. The total fatty acid content is therefore, in the region of 61% (i.e., lauric acid + myristic acid). By extrapolation, the formulated product (ALOAPUR® PM) will comprise around 15% lauric acid, 7% myristic acid, 22% total fatty acids (lauric acid + myristic acid) and 10% lactic acid. A copy of the calculations performed is provided in Appendix 017.

The practical use level of C12/C14 lactylates as a source of lauric and myristic acid in feed is anticipated to be in the range of 0.7 g/kg complete feed, equivalent to 2 g ALOAPUR® PM/kg complete feed and will not exceed 1.8 g/kg complete feed as-fed, equivalent to 5 g ALOAPUR® PM/kg complete feed as-fed (see Part 3). At the maximum use level of 5 g ALOAPUR® PM/kg complete feed, corresponding to 1.8 g C12/C14 lactylates/kg complete feed, animals are estimated to consume 0.8 g lauric acid, 0.3 g myristic acid and 0.5 g lactic acid/kg complete feed. The combined lauric acid and myristic acid intake from C12/C14 lactylates is estimated to be around 1.1 g/kg complete feed.

2.7 Technical Effect of C12/C14 Lactylates in Animal Food

The requirements for the identity, method of manufacture, specifications and physical or technical effect part of a GRAS notice for a feed substance are laid down in 21 CFR §570.230 Subpart E (U.S. FDA, 2020). In accordance with 21 CFR §570.230(d) relevant data and information bearing on the physical or other technical effect the notified substance is intended to produce, including the quantity of the notified substance required to produce such an effect, must be included only when necessary to demonstrate safety.

As mentioned above, C12/C14 lactylates is intended for use as a source of lauric and myristic acids in the diet of animals and will be marketed as a formulation on a diatomaceous earth carrier comprising *ca*. 40% C12/C14 lactylates and *ca*. 60% carrier under the trade name ALOAPUR® PM. The target animals are all major and minor food-producing animals as well as companion animals. C12/C14 lactylates comprises an equilibrium mixture of sodium lauroyl-1-lactylate (*ca*. 33%), sodium myristoyl-1-lactylate (*ca*. 14%), sodium laurate (C12 fatty acid; *ca*. 18%), sodium myristate (*ca*. 8%), sodium lactate (*ca*. 9%), sodium lauroyl-2-lactylate (*ca*. 6%), sodium myristoyl-2-lactylate (*ca*. 2%), sodium lactoyl lactate (*ca*. 3%) and minor amounts of oligomers of lactate (not quantified). Based on this typical composition, C12/C14 lactylates comprises around 42% lauric acid, 19% myristic acid and 28% lactic acid in total in the free fatty acid and esterified forms. The combined content of lauric and myristic acids is in the region of

[2] Calculated from the mean contents from the 5 lots analyzed (see Table 2.9) and the molecular weight (MW) of each component as the sodium salt. The amounts are reported in the acid form (approximately equal to the anion) for the purposes of the safety assessment but represent the lactic acid, lauric acid and myristic acid contents as the sum available in the free (sodium salt) or ester (lactic acid ester) form.

61%. By extrapolation, the formulated product (ALOAPUR® PM) will comprise around 15% lauric acid, 7% myristic acid, 22% total fatty acids (lauric acid + myristic acid) and 10% lactic acid.

Lauric and myristic acids also can be present in the background diet of animals at different levels depending on the ingredients used to formulate the feed as described in Section 6.1.3 below. Coconut and palm kernel-derived products such as coconut oil (CO), coconut meal (CM), palm kernel oil (PKO) and palm kernel meal (PKM) are rich in lauric acid. Myristic acid occurs widely in ingredients of vegetable and animal origin, but coconut and palm kernel by-products are the most significant sources in feed. C12/C14 lactylates is intended only for use in feeds which do not contain any significant sources of lauric or myristic acids from other sources and would not be included in combination with coconut or palm kernel-derived ingredients.

Medium- and long-chain saturated fatty acids are recognized nutritional ingredients in the feed of animals, although unlike polyunsaturated fatty acids (PUFAs) such as linoleic acid (C18:2, n-6), eicosapentaenoic acid (EPA; C20:5, n-3) and docosahexaenoic acid (DHA; 22:6n-3) there are no specific requirements set by the NRC or other bodies (MSD Veterinary Manual, 2022). Therefore, C12/C14 lactylates is intended as a supplemental source of lauric and myristic acids in the diet contributing generally to the overall profile of fatty acids consumed by the animal. In this respect, C12/C14 lactylates will be included in the diet alongside other common fats and fat-containing nutritional ingredients. At the maximum intended use level of 1.8 g C12/C14 lactylates/kg complete feed (or 5 g ALOAPUR® PM/kg complete feed), animals will be provided with around 1.1 g lauric and myristic acids combined[3]. By comparison, linoleic acid requirements of broilers, as an example of a major category of food-producing animal, are established by the NRC to be 1.0% in the diet (NRC, 1994). Similarly, the NRC has recommended a minimum of 0.5 to 1.0% EPA and DHA (combined) are included in the diet of salmonids (NRC, 2011). These levels of individual fatty acid incorporation in the diet of animals are in the range of that proposed for lauric and myristic acids.

Notably, at the maximum intended use level of 1.8 g C12/C14 lactylates/kg complete feed (or 5 g ALOAPUR® PM/kg complete feed), equivalent to around 1.1 g lauric and myristic acids (total; free fatty acid and esterified form), animals are supplied approximately 10 calories. Compared to the daily metabolizable energy requirements of animals from the feed of, for example, 3,200 kcal/kg complete feed, C12/C14 lactylates will have a minimal impact on the daily energy intakes under the intended conditions of use. Therefore, the energy requirements of the animal will be met primarily by other nutritional ingredients, such as grains, oilseed meals and animal or vegetable fats.

Considering that C12/C14 lactylates will provide a source of lauric and myristic acids and will be used alongside, rather than as a substitute for, other fat sources in the diet, there are no anticipated nutritional disadvantages associated with the intended use in feed at levels not exceeding 1.8 g/kg complete feed as-fed. On this basis, beyond its compositional value as a source of fatty acids, the technical effect of C12/C14 lactylates on the feed does not have a bearing on safety and no further evaluation of utility is required.

[3] Calculated as the amount of lauric and myristic acids present in the form of sodium salts or lactylates (esters) based on the equilibrium mixture of species obtained.

PART 3. §570.235 – TARGET ANIMAL AND HUMAN EXPOSURES

3.1 EXPOSURE BY ANIMALS

3.1.1 Use Levels and Anticipated Exposure by Target Animals

As mentioned in Part 2 of the GRAS Notice (Section 2.6), C12/C14 lactylates is intended for use as a supplementary source of lauric and myristic acids in the diet of animals, providing these fatty acids in the free fatty acid (or sodium salt) and lactic acid ester forms. The level of C12/C14 lactylates in the form of ALOAPUR® PM added to the diet will depend on the nutritional needs of the animal as well as the composition of the basal feed. Considering that C12/C14 lactylates contains only lauric and myristic acids, it is anticipated that the ingredient will act as a supplemental source of specific fatty acids and will not replace other fat products in the diet. In this respect, C12/C14 lactylates will contribute to the total intake of fatty acids from the diet and be included alongside conventional sources of fat such as fish oil (FO), soy oil (SO) and palm oil (PO). The practical use level of C12/C14 lactylates as a source of lauric and myristic acid in feed is anticipated to be in the range of 0.7 g/kg complete feed, equivalent to 2 g ALOAPUR® PM/kg complete feed and will not exceed 1.8 g/kg complete feed as-fed, equivalent to 5 g ALOAPUR® PM/kg complete feed as-fed.

As mentioned above, C12/C14 lactylates is calculated to comprise around 42% lauric acid, 19% myristic acid and 28% lactic acid in different forms (i.e., free fatty acid/organic acid or ester form). The estimated exposure by animals to C12/C14 lactylates and to the individual lauric acid, myristic acid and lactic acid components under the practical conditions of use of 0.7 g/kg complete feed are presented in Table 3.1. At these levels of inclusion of C12/C14 lactylates in the diet, animals will be exposed to around 0.3 g lauric acid, 0.1 g myristic acid and 0.2 g lactic acid/kg complete feed. The combined lauric and myristic acid intake from C12/C14 lactylates is around 0.4 g/kg complete feed. Similarly, the estimated exposure by animals to C12/C14 lactylates to the individual components at the highest anticipated use level of 1.8 g/kg complete feed are presented in Table 3.1. At these levels of inclusion of C12/C14 lactylates in the diet, animals are estimated to consume 0.8 g lauric acid, 0.3 g myristic acid and 0.5 g lactic acid/kg complete feed. The combined lauric acid and myristic acid intake from C12/C14 lactylates is estimated to be around 1.1 g/kg complete feed.

Table 3.1: Practical Intended Use Levels of C12/C14 Lactylates in Animal Feed

Use Level of C12/C14 Lactylates in the Diet (g/kg As-Fed)	Exposure to the Components of C12/C14 Lactylates (g/kg As-Fed)[1]			
	Lauric Acid	Myristic Acid	Total Lauric + Myristic Acids	Lactic Acid
0.7 (practical use level)	0.3	0.1	0.4	0.2
1.8 (maximum use level)	0.8	0.3	1.1	0.5

[1]Based on C12/C14 lactylates comprising a total of 41% lauric acid, 18% myristic acid and 27% lactic acid in the free fatty acid/organic acid and ester forms.

3.1.2 Regulatory Status in Animal Feed

3.1.2.1 Animal Feed Use in the U.S.

C12/C14 lactylates was previously marketed under the Association of American Feed Control Officials (AAFCO) ingredient definition for "Fat Product, Feed Grade" (Ingredient Definition 33.5) until it was

removed from the OP in 2016. Currently, there is no AAFCO ingredient definition under the Section for Fats and Oils (Section 33) which captures C12/C14 lactylates within its scope.

3.1.2.2 Animal Feed Use in the EU

Corbion currently markets C12/C14 lactylates in the form of ALOAPUR® PM for use as a feed material in the EU. Salts of lactylates of fatty acids are currently defined in the EU Catalogue of Feed Materials (Entry 13.6.13) for use as a fat source in animal feed. The current entry listed in Commission Regulation (EU) 2017/1017 on the Catalogue of Feed Materials is summarized in Table 3.2 (EC, 2017).

Table 3.2: Feed Material Catalogue for Salts of Lactylates of Fatty Acids

Name	Description	Compulsory Label Declarations
Salt of lactylates of fatty acids	Non-glyceride ester of fatty acids. The product can be a calcium, magnesium, sodium or potassium salt of fatty acids esterified with lactic acid. It may contain the salts of free fatty acids and lactic acid.	Crude fat Moisture (if >1%) Nickel (if >20 mg/kg) Ca, Na, K or Mg (as appropriate)

3.2 ESTIMATED HUMAN EXPOSURE

3.2.1 Potential for Deposition of C12/C14 Lactylates or Its Metabolites in Edible Tissues

C12/C14 lactylates is intended for use as a source of lauric and myristic acids in feed for all animals at levels not to exceed 1.8 g/kg complete feed (5 g ALOAPUR® PM/kg complete feed), equivalent to 0.8 g lauric acid, 0.3 g myristic acid and 0.5 g lactic acid/kg complete feed. As demonstrated in animal studies summarized in Part 6, on ingestion, C12/C14 lactylates will be hydrolyzed by gastric lipases to release lauric acid, myristic acid and lactic acid. These components will be metabolized by established fatty acid and tricarboxylic acid pathways in animals to ultimately form carbon dioxide, which is excreted by exhalation. Lauric and myristic acids are normal constituents of biological fat and as such are consumed by animals and humans as part of the background diet. No differences are anticipated in the metabolism of lauric and myristic acids as components of C12/C14 lactylates and as constituents of biological fats forming part of the normal diet. Moreover, under the conditions of intended use, the exposure by animals to lauric and myristic acids from C12/C14 lactylates will not exceed that from other dietary sources such as coconut-derived products (see Section 6.1.3).

Similarly, lactic acid is an endogenous intermediate of carbohydrate and amino acid metabolism in animals and humans. It has a long and established history of consumption by animals as a normal constituent of the diet and as a preservative in feed at levels exceeding that from the intended use of C12/C14 lactylates. The lactic acid component of C12/C14 lactylates is expected to be rapidly absorbed from the GI tract of animals and ultimately metabolized to carbon dioxide which will be exhaled (see Section 6.1.4).

The use of C12/C14 lactylates as a source of lauric acid and myristic acid in the diet of animals should not therefore, lead to the deposition of substances not normally present, or at levels not usually observed, in the edible tissues of animals.

3.2.2 Human Food Use in the U.S.

Salts of lactylates of fatty acids have an established history of use as emulsifiers, conditioning agents and stabilizers in foods and in food production in the U.S., especially by the bakery industry. The current regulatory status of these ingredients for food use are summarized in Table 3.3.

Table 3.3: Regulatory Status of Salts of Lactylates of Fatty Acids for Use in Human Food in the U.S.

Additive	Description	Status
Lactylic esters of fatty acids 21 CFR §172.848 (U.S. FDA, 2022)	Prepared from lactic acid and fatty acids meeting the requirements of 21 CFR 172.860(b) and/or oleic acid derived from tall oil fatty acids meeting the requirements of 21 CFR 172.862	Emulsifiers, plasticizers, or surface-active agents in bakery mixes, baked products, cake icings, filling and toppings, dehydrated fruits and vegetables, dehydrated fruit and vegetable juices, edible vegetable fat-water emulsions, frozen desserts, liquid shortening, pancake mixes, pre-cooked instant rice and pudding mixes Permitted for use in an amount not greater than that required to produce the intended physical or technical effect, and they may be used with shortening and edible fats and oils when required
Calcium stearoyl-2-lactylate 21 CFR §172.844 (U.S. FDA, 2022)	Mixture of calcium salts of stearoyl lactylic acid and minor proportions of other calcium salts of related acids, it is manufactured by the reaction of stearic acid and lactic acid and conversion to the calcium salts	Dough conditioner in yeast-leavened bakery products and prepared mixes for yeast-leavened bakery products in an amount not to exceed 0.5 parts for each 100 parts by weight of flour used As a whipping agent in: – Liquid and frozen egg white at a level not to exceed 0.05% – Dried egg white at a level not to exceed 0.5% – Whipped vegetable oil topping at a level not to exceed 0.3% of the weight of the finished whipped vegetable oil topping As a conditioning agent in dehydrated potatoes in an amount not to exceed 0.5% by weight
Sodium stearoyl lactylate 21 CFR §172.846 (U.S. FDA, 2022)	Mixture of sodium salts of stearoyl lactylic acids and minor proportions of sodium salts of related acids, it is manufactured by the reaction of stearic acid and lactic acid and the conversion to the sodium salts Meets the specifications of the FCC	In prepared mixes for the below listed foods following the conditions specified in each of these categories: – Dough strengthener, emulsifier, or processing aid in bakery products, pancakes and waffles in an amount not to exceed 0.5 part for each 100 parts by weight of flour used – As a surface-active agent, emulsifier or stabilizer in icings, fillings, puddings and toppings at a level not to exceed 0.2% by weight of the finished food – As an emulsifier or stabilizer in liquid and solid edible fat-water emulsions intended for use as substitutes for milk or cream in beverage coffee, at a level not to exceed 0.3% by weight of the finished edible fat-water emulsion – As a formulation aid, processing aid or surface active agent in dehydrated potatoes, in an amount not to exceed 0.5% of the dry weight food

Table 3.3: Regulatory Status of Salts of Lactylates of Fatty Acids for Use in Human Food in the U.S.		
Additive	**Description**	**Status**
		– As an emulsifier, stabilizer or texturizer in snack dips, at a level not to exceed 0.2% by weight of the finished product – As an emulsifier, stabilizer, or texturizer in cheese substitutes and imitations and cheese product substitutes and imitations, at a level not to exceed 0.2% by weight of the finished food – As an emulsifier, stabilizer, or texturizer in sauces or gravies, and the products containing the same, in an amount not to exceed 0.25% by weight of the finished food As an emulsifier, stabilizer or texturizer in cream liqueur drinks, at a level not to exceed 0.2% by weight of the finished product

Abbreviations: CFR = Code of Federal Regulations; FCC = Food Chemicals Codex.

3.2.3 Human Food Use in the EU

Sodium stearoyl-2-lactylate (E 481) and calcium stearoyl-2-lactylate (E 482) are permitted for use as food additives in accordance with Commission Regulation (EU) No 1129/2011 (EC, 2011) in the EU. Sodium stearoyl-2-lactylate has a history of use as a dough conditioner/emulsifier in high fat, yeast leavened baked goods, as an aerating agent in both dairy and non-dairy whipped toppings and desserts, and as a surfactant and complexing agent in coffee creamer. Similarly, calcium stearoyl-2-lactylate is used in the production of yeast-leavened bakery products where it acts in combination with gluten in dough to provide greater tolerance to processing conditions, and as an egg-white whipping aid. Both sodium and calcium stearoyl-2-lactylate may be used as fat replacers, where the sodium salt has a history of use in whitening products and the calcium salt in fine bakery wares. They are also both used in fat spreads, dairy fat spreads and blended spreads. The maximum permitted levels of sodium and calcium stearoyl-2-lactylates in food varies from 2,000 to 10,000 mg/kg depending on the application. Examples of the maximum permitted levels include, but are not limited to, 5,000 mg/L in flavored fermented milk products included heat-treated products, 2,000 mg/kg in fruit and vegetable preparations excluding compote, 5,000 mg/kg in non-fruit based decorations, coatings and fillings, 5,000 mg/kg in breakfast cereals, 5,000 mg/kg in bread rolls and 4,000 mg/kg in heat-treated processed meat (minced, diced or canned).

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

The use of C12/C14 lactylates will be self-limiting on the basis that there are detrimental nutritional effects associated with excessive consumption of fat.

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

Not applicable.

PART 6. §570.250. NARRATIVE

6.1 INFORMATION TO ESTABLISH SAFETY FOR THE TARGET ANIMALS

6.1.1 Introduction

C12/C14 lactylates is the product of esterification of lauric (C12) and myristic (C14) acids with lactic acid in the presence of sodium hydroxide. The fatty acid raw materials are obtained from food grade PKO and food-grade lactic acid. The resultant product comprises an equilibrium mixture of sodium lauroyl-1-lactylate (*ca*. 33%), sodium myristoyl-1-lactylate (*ca*. 14%), sodium laurate (C12 fatty acid; *ca*. 18%), sodium myristate (*ca*. 8%), sodium lactate (*ca*. 9%), sodium lauroyl-2-lactylate (*ca*. 6%), sodium myristoyl-2-lactylate (*ca*. 2%), sodium lactoyl lactate (*ca*. 3%) and minor amounts of oligomers of lactate (not quantified). Based on this typical composition, C12/C14 lactylates comprises around 42% lauric acid, 19% myristic acid and 28% lactic acid in the free fatty acid and esterified form. The combined content of lauric and myristic acids is in the region of 61%.

The lactic acid esters of lauric and myristic acid will be enzymatically hydrolyzed to lauric acid, myristic acid and lactic acid in the GI tract of animals. Evidence for the hydrolysis of C12/C14 lactylates by gastric lipases is provided by the findings of published (Phillips *et al*., 1981) and unpublished (Hodge, 1961) *in vitro* studies using a structurally similar lactylate, calcium stearoyl-lactylate. The available details of these studies are provided in Section 6.1.2.2. Lactic acid esters of lauric acid, myristic acid and stearic acid are expected to be hydrolyzed in an analogous manner in animals and the findings of the studies using calcium stearoyl-lactylate may be extrapolated to C12/C14 lactylates. Consistent with hydrolysis of lactylates to their component parts, the metabolism of a mixture of stearic acid and lactic acid, and calcium stearoyl-lactylate was demonstrated to be comparable in an unpublished study by Hodge (1955) summarized in Section 6.1.2.2. Corroborating evidence for the absorption or digestion of the fatty acid components of C12/C14 lactylates by animals is provided by the findings of an unpublished study conducted by Corbion at the University of Wageningen (Jansman and van Wikselaar, 2013). Details of the study are provided in Section 6.1.2.1. Likewise, in Section 6.1.2.2, summaries of a series of ^{14}C-labeling experiments in mice and guinea pigs following the metabolism of the structurally related lactylate, calcium stearoyl (C18) lactylate also are provided (Phillips *et al*., 1981).

Lauric acid, myristic acid and lactic acid derived from hydrolysis of C12/C14 lactylates will be rapidly absorbed from the GI tract of animals and metabolized via established fatty acid and tricarboxylic acid pathways to ultimately form carbon dioxide, which is excreted by exhalation. Therefore, the toxicological assessment of C12/C14 lactylates may be based on the individual components.

Medium- and long-chain saturated fatty acids such as lauric acid and myristic acid are normal components of vegetable and animal fats. They also have a history of use as additives in human food and as such have been the subject of evaluations by authoritative bodies as described in Section 6.1.3.1. Lauric and myristic acid from C12/C14 lactylates are expected to be metabolized by established fatty acid pathways in animals as outlined in Section 6.1.3.2. The levels of lauric and myristic acid in common fats used in the diet of animals, and the potential exposure by animals to these fatty acids from the daily ration are considered in Sections 6.1.3.3 to 6.1.3.5. There are a number of studies in the published literature in which animals were fed diets containing lauric or myristic acids as the free fatty acids.

These are briefly considered in Section 6.1.3.6. Additionally, a limited toxicological data set is available which is summarized in Section 6.1.3.7.

Lactic acid and calcium lactate have a long and established history of use as a technological additive (preservative) in feed and food in the U.S., the EU and elsewhere. A summary of the scientific evaluations by authoritative bodies to support the use as additives in feed and food is provided in Section 6.1.4.1. Lactic acid is rapidly absorbed by animals and ultimately metabolized to carbon dioxide and water as outlined in Section 6.1.4.2 using publicly available information. Animals will be exposed to lactic acid from the normal diet, particularly through the use of Fermented Liquid feeds (FLF) for pigs and ensiled forages for ruminants. Background exposure by animals to lactic acid from the diet is estimated in Section 6.1.4.4. On the basis that exposure by animals to lactic acid from the background diet is lower for poultry, swine and ruminants, a summary of the available published literature in which animals were fed diets containing lactic acid are summarized in Section 6.1.4.5. These data provide supporting evidence of the safety of this component for the target animals. Additionally, a limited set of toxicological data are available and outlined in Section 6.1.4.7.

It is recognized that C12/C14 lactylates will also provide a source of sodium. The sodium content of C12/C14 lactylates is limited to not more than 8% by the product specifications for the ingredient (see Table 2.7). The impact of the sodium content of C12/C14 lactylates on the nutritional status of animals is considered in Section 6.1.5.

As mentioned in Part 2, sodium sulfate is added to the market formulation, ALOAPUR® PM as a processing aid at a level of less than 0.1% by weight. The safety of the sulfate component of C12/C14 lactylates for animals under the conditions of intended use is evaluated in Section 6.1.6.

The data summarized in Part 6 may be extrapolated to support the safety of all categories and species of animal on the basis that (a) the metabolic fate of C12/C14 lactylates is common in all animals; (b) lauric acid, myristic acid and lactic acid are metabolized by well-established pathways; and (c) there is a long and established history of safe consumption of these components from the background diet as well as from additive use (lactic acid).

6.1.2 Absorption, Distribution, Metabolism and Excretion (ADME) of Lactylates

An unpublished study has been conducted by Corbion to evaluate the digestion and kinetics of disappearance of C12/C14 lactylates in broilers. Additionally, the metabolic fate of calcium stearoyl-lactylate has been examined in a series of animal and *in vitro* studies which were evaluated by the Joint Food and Agricultural Organization (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) and European Food Safety Authority (EFSA) to establish the safety of sodium and calcium stearoyl-lactylate as food additives (JECFA, 1974a; EFSA, 2013). The structural similarities of calcium stearoyl-lactylate to C12/C14 lactylates allows extrapolation of the findings of these studies to support the assessment of the ADME of Corbion's ingredient. The available data are outlined below and together provide evidence for the appropriateness of basing the safety assessment on the individual components of C12/C14 lactylates.

6.1.2.1 Study in Broilers using C12/C14 Lactylates

An unpublished study was conducted by (b) (4) in which the digestion and kinetics of disappearance of sodium lauroyl (C12)-lactylate, sodium myristoyl (C14)-lactylate and palmitoyl (C16)-lactylate was investigated following administration in the diet of broiler chickens. The C12/C14 lactylates test articles used in the study are liquid (Puramix 30L) and solid (Puramix 30S) formulations prepared by combining 38 to 42% C12/C14 lactylates with either monopropylene glycol or (b) (4) (b) (4) (natural clay/sediment from diatoms) as the diluent/carrier. The treatment levels in the study were 7.5 g/kg complete feed of Puramix 30L or 30S. A summary of the study is provided in Appendix 018.

Two hundred and twenty, 1-day old healthy male Ross 308 broilers were randomly assigned to one of 20 cages containing 11 birds per cage. There were 4 cages per treatment. The birds were fed diets containing no lactylates (control; treatment 1), 7.5 g C12/C14 lactylates in liquid form (Puramix 30L[4]; treatment 2), 7.5 g C12/C14 lactylates in solid form (Puramix 30S[5]; treatment 3), 7.5 g C14 lactylate in solid form (Puramix 14S; treatment 4) or 7.5 g C16 lactylate in solid form (Puramix 16S; treatment 5) per kg complete feed for 27 days. On the basis that treatments 4 and 5 were not part of the digestion and kinetics assessment in the study, no further information on these groups is provided herein. Birds received a starter diet from day 0 to day 7, and a grower diet from day 7 to day 27. Chromium oxide was included in the feed as a digestibility marker. Feed and water were available *ad libitum* for the duration of the study. The calculated and analyzed concentrations of the C12, C14 and C16 components in the treatment diets are provided in Table 6.1.

Table 6.1: Calculated and Analyzed Concentrations of Lactylates in the Treatment Diets

Component	Analyzed Concentrations[1]				
	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
	Control (0 g/kg Complete Feed)	7.5 g Puramix 30L/kg Complete Feed (C12/C14 Lactylates)	7.5 g Puramix 30S/kg Complete Feed (C12/C14 Lactylates)	7.5 g Puramix 14S/kg Complete Feed (C14 Lactylates)	7.5 g Puramix 16S/kg Complete Feed (C16 Lactylates)
C12 lactylate (mg/kg complete feed)	<0.5	584	792	24	2
	(0)	(900)	(900)	(0)	(0)
C14 lactylate (mg/kg complete feed)	<0.5	276	321	992	49
	(0)	(375)	(375)	(1,275)	(0)
C16 lactylate (mg/kg complete feed)	<0.5	<0.5	<0.5	<0.5	1,073
	(0)	(0)	(0)	(0)	(1,313)

[1]Values in brackets () are calculated values from feed preparation.

The animals were housed in metabolic cages in an environmentally controlled room for the duration of the study. Behavior and physical condition of the birds was inspected at least once per day over the 27-

[4] Puramix 30L: (38 to 42%; mean 40%) C12/C14 lactylates diluted using monopropylene glycol as a liquid (58 to 62%)

[5] Puramix 30S: (38 to 42%; mean 40%) C12/C14 lactylates on a solid carrier (58 to 62%) (natural clay/sediment from diatoms) – (b) (4)

day feeding period. Mortality was recorded as well as the body weight of dead birds. Individual body weights of birds were measured at day 0, 7 and 27. Feed consumption was measured per pen for the periods 0 to 7, 7 to 14 and 21 to 27 days. Blood samples were taken for analysis of C12, C14 and C16 component concentrations from 12 birds per treatment (3 birds/cage) at day 27. The same birds were euthanized by lethal injection on day 27 for necropsy. Upon necropsy, samples of liver, skin and breast meat tissues were taken as well as digesta samples from the crop, gizzard, duodenum, proximal and distal small intestine, ileum and colon. For treatments 1 to 3 only, the concentrations of C12, C14 and C16 components in blood plasma and the digesta from the various compartments of the GI tract were determined. The apparent absorption coefficient (AC) of lactylates from the different compartments of the digestive tract was calculated for treatments 1 to 3 in order to evaluate the apparent quantitative absorption/metabolism of C12/C14 lactylates from the feed.

During the study, 7 birds died or were removed from the study due to adverse health conditions. None of the mortalities were associated with treatment although, the reason of death for 5 of the birds was unclear.

Feed intake, body weight gain (BWG) and feed conversion ratio (FCR) of the birds over the duration of the study (0 to 27 days) is reported in Table 6.2. There was no effect of treatment on feed intake, BWG or FCR among treatments (P>0.05).

Table 6.2: Effect of Lactylates Supplementation on Performance of Broilers

Parameter	Performance[1]					P-value	LSD
	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5		
	Control (0 g/kg Complete Feed)	7.5 g Puramix 30L/kg Complete Feed (C12/C14 Lactylates)	7.5 g Puramix 30S/kg Complete Feed (C12/C14 Lactylates)	7.5 g Puramix 14S/kg Complete Feed (C14 Lactylates)	7.5 g Puramix 16S/kg Complete Feed (C16 Lactylates)		
FI (g/day)	87.1	88.1	84.6	87.7	88.1	0.22	3.9
BWG (g/day)	1,704	1,752	1,661	1,706	1,742	0.51	119
FCR	1.381	1.369	1.375	1.391	1.367	0.82	0.04

Abbreviations: BWG = body weight gain; FCR = feed conversion ratio; FI = feed intake; LSD = least significant difference;
[1]Significance determined at P<0.05.

The concentrations of the C12, C14 and C16 lactylates components in the plasma of birds in treatments 1 to 3 on day 27 of the study are reported in Table 6.3. The plasma concentrations of the C12 lactylates component was lower (P<0.05) in birds fed diets containing 7.5 g/kg complete feed of Puramix 30L compared to Puramix 30S (treatments 2 and 3; 308 vs. 389 ng/mL). The plasma concentration of C14 lactylates component was comparable (P>0.05) between birds fed diets containing 7.5 g/kg complete feed of Puramix 30S and 30L (treatments 2 and 3; 80 vs. 83 ng/mL).

Table 6.3: Effect of Lactylates Supplementation on Plasma Fatty Acids of Broilers					
Lactylate Component	**Plasma Fatty Acid Levels**			**P-value**	**LSD**
	Treatments 1	**Treatment 2**	**Treatment 3**		
	Control (0 g/kg Complete Feed)	**7.5 g Puramix 30L/kg Complete Feed (C12/C14 Lactylates)**	**7.5 g Puramix 30S/kg Complete Feed (C12/C14 Lactylates)**		
C12 lactylate (ng/mL)	<50[a]	308[b]	389[c]	<0.001	71
C14 lactylate (ng/mL)	<50[a]	80[b]	83[b]	<0.001	14
C16 lactylate (ng/mL)	<50[a]	<50	<50	-	-

Abbreviations: LSD = least significant difference;

[abc] Values with different superscripts within a row differ significantly at P<0.05.

The concentrations of C12, C14 and C16 lactylate components in the digesta of different compartments of the digestive tract are presented in Table 6.4 on an as-is basis. In digesta samples from treatments 2 and 3, the concentrations of C12 and C14 lactylate components were reported to be highest in the crop at 111 and 161 mg/kg for C12 lactylate, respectively and 56 and 71 mg/kg for C14 lactylate, respectively. The concentrations of C12 and C14 lactylates components decreased progressively through the compartments of the digestive tract and were ≤3 mg/kg in the ileum and colon for birds from treatments 2 and 3. The digesta concentrations are reported on an as-is basis and will have been influenced by the digestion and/or absorption of the C12 and C14 lactylate components, as well as changes in DM content.

Table 6.4: Concentration of Lactylates in the Digesta of Broilers on Day 27						
Lactylate Component	Compartment	Digesta Concentrations			P-value[1]	LSD[1]
		Treatment 1	Treatment 2	Treatment 3		
		Control (0 g/kg Complete Feed)	7.5 g Puramix 30L/kg Complete Feed (C12/C14 Lactylates)	7.5 g Puramix 30S/kg Complete Feed (C12/C14 Lactylates)		
C12 lactylate (mg/kg as-is)	Crop	<0.1[a]	111[b]	161[b]	<0.01	63
	Gizzard	<0.1[a]	98[b]	152[c]	<0.001	44
	Duodenum	<0.1[a]	29[b]	43[b]	<0.05	25
	Proximate SI	<0.1[a]	24[b]	29[b]	<0.05	27
	Distal SI	<0.5[a]	3[b]	2[b]	<0.05	1.9
	Ileum	<0.5[a]	1[b]	2[b]	<0.05	1.4
	Colon	<0.5[a]	3[c]	1[b]	<0.01	1.2
C14 lactylate (mg/kg as-is)	Crop	<0.2[a]	56[b]	71[b]	<0.01	28
	Gizzard	<0.2[a]	45[b]	62[b]	<0.001	19
	Duodenum	<0.5[a]	14[b]	17[b]	<0.05	11
	Proximate SI	<0.5[a]	12[c]	7[b]	<0.01	4.5
	Distal SI	<0.5[a]	4[b]	3[b]	<0.05	2.4
	Ileum	<0.5[a]	3[b]	2[b]	<0.05	1.9
	Colon	<0.5[a]	3[b]	2[b]	<0.05	1.8
C16 lactylate (mg/kg as-is)	Crop	1.0[b]	0.1[a]	0.3[a]	<0.01	0.2
	Gizzard	0.7[b]	0.2[a]	0.3[a]	<0.05	0.3
	Duodenum	0.1	<0.15	<0.15	0.42	0.1
	Proximate SI	<0.15	<0.15	<0.15	-	-
	Distal SI	<0.15	<0.15	<0.15	-	-
	Ileum	<0.15	<0.15	<0.15	-	-
	Colon	<0.15	<0.15	<0.15	-	-

Abbreviations: LSD = least significant difference; SI = small intestine;
[abc] Values with different superscripts within a row differ significantly at P<0.05;
[2]P-Value and LSD for the overall effect of experimental treatment; in the statistical analysis of data values < lowest level of quantification (LLOQ) were set at 50% of the LLOQ value.

The dry matter (DM) contents of the digesta in the crop ranged from 292 to 356 g/kg among treatments (not shown). Lower DM contents ranging from 123 to 215 g/kg were reported for the gizzard, duodenum, proximal and distal small intestine, ileum and colon. Within compartments of the GI tract, there were no differences in DM content between treatments.

The apparent digestibility of DM and ACs of C12 and C14 lactylates components from the digesta of different compartments of the digestive tract in birds fed treatment diets 1 to 3 are reported in Table 6.5. The ACs for C12 lactylate component were lowest in the gizzard (7 and -5%, respectively for treatments 2 and 3) and increased to around 100% in the distal small intestine, ileum and colon for treatments 2 and 3. The calculated ACs for C12 lactylate component were higher in the crop (35 and 47%, respectively for treatments 2 and 3) compared to the gizzard (7 and -5%, respectively for treatments 2 and 3). Likewise, the ACs for C14 lactylate component were lowest in the gizzard (13 and -4%, respectively for treatments 2 and 3) and increased to around 100% in the distal small intestine, ileum and colon for treatments 2 and 3. The calculated ACs for C14 lactylate component were higher in

the crop (32 and 43%, respectively for treatments 2 and 3) compared to the gizzard (13 and -4%, respectively for treatments 2 and 3). These findings indicate complete apparent absorption of C12 and C14 lactylates components from the GI tract. The study investigators suggested that the higher observed ACs in the crop compared to the gizzard may be due to non-synchronized passage of C12/C14 lactylates and the digestibility marker (chromium oxide) through the crop, resulting in unrealistic ACs for this compartment. There were no significant differences in ACs for C12 or C14 lactylates components within compartments of the GI tract between treatments 2 and 3.

Table 6.5: Apparent Digestibility of Dry Matter and Apparent Absorption Coefficient (AC) of Lactylates from the Digesta of Broilers on Day 27

Lactylate Component	Compartment	Apparent Digestibility of DM and AC[1]			P-value	LSD
		Treatment 1	Treatment 2	Treatment 3		
		Control (0 g/kg Complete Feed)	7.5 g Puramix 30L/kg Complete Feed (C12/C14 Lactylates)	7.5 g Puramix 30S/kg Complete Feed (C12/C14 Lactylates)		
Dry Matter (Apparent digestibility, %)	Crop	7	0	2	0.30	11
	Gizzard	-12	-14	-16	0.94	25
	Duodenum	-13	-19	-1	0.17	40
	Proximate SI	53	51	52	0.53	4.5
	Distal SI	70	67	70	0.45	4.9
	Ileum	71	72	68	0.35	6.1
	Colon	70	69	67	0.43	7.2
C12 (Apparent absorption coefficient, %)	Crop	-	35	47	0.41	39
	Gizzard	-	7	-5	0.34	35
	Duodenum	-	(80)[2]	(33)[2]	-	-
	Proximate SI	-	90	91	0.80	8.7
	Distal SI	-	99	100	0.14	0.8
	Ileum	-	100	100	0.44	0.5
	Colon	-	99	101	0.27	1.7
C14 (Apparent absorption coefficient, %)	Crop	-	32	43	0.53	48
	Gizzard	-	13	-4	0.27	40
	Duodenum	-	(54)[2]	(65)[2]	-	-
	Proximate SI	-	90	95	0.05	4.4
	Distal SI	-	98	99	0.26	2.3
	Ileum	-	99	99	0.76	1.3
	Colon	-	98	97	0.74	4.4

Abbreviations: AC = absorption coefficient; LSD = least significant difference; SI = small intestine;
[1]The apparent absorption of C16 lactylates could not be determined because of low concentrations of C16 lactylates in the experimental diets;
[2]Estimates with a low accuracy as only based on n=2, as some pooled digesta samples were too small in size to perform analysis of the content of chromium oxide as digestibility marker.

Overall, there was no effect of dietary inclusion of C12/C14 lactylates, C14 lactylate or C16 lactylate on performance. However, the study was not designed as a performance trial and the animals were housed in cages rather than in floor pens.

Analyses of blood samples of the birds at day 27 revealed elevated concentrations of C12 lactylate and C14 lactylate components from both formulations (Puramix 30L and Puramix 30S) when compared to birds receiving the control diet. The authors of the study used data obtained from the birds in treatments 2 and 3 (Puramix 30L and Puramix 30S) to evaluate the pool size of C12 lactylate and C14 lactylates components in blood plasma. These calculations indicated that the pool size of C12 and C14 lactylates components was less than 0.05% of the mean daily dietary intake of these components.

The concentrations of C12 lactylate and C14 lactylate components were observed to decrease from the proximal to the distal part of the GI tract in treatments in which C12/C14 lactylates were added to the diet as Puramix 30L or Puramix 30S at 7.5 g/kg complete feed. The decrease in concentration of these lactylates in various parts of the GI tract is related to both the intestinal absorption and to changes in the DM intake, endogenous water secretion and intestinal water absorption. The lactylates appear to be absorbed or digested only to a limited extent prior to the small intestine. A significant portion of C12/C14 lactylates appears to be metabolized in the duodenum and proximal part of the small intestine, with the mean apparent ACs for the C12 lactylate component determined to be 90 and 95% for the liquid (Puramix 30L) and solid (Puramix 30S) formulations. These values increased further in the distal parts of the GI tract. Comparable results were obtained for the C14 lactylate component.

Taken together, the authors of the study concluded that C12/C14 lactylates included in the diet at dietary levels of 7.5 g Puramix 30L or Puramix 30S/kg complete feed, equivalent to 3 g C12/C14 lactylates/kg complete feed are extensively and readily metabolized (absorbed and/or digested) from the proximal part of the small intestine. Only a small portion of C12/C14 lactylates consumed in the diet was recovered intact in the blood plasma of broilers.

C12/C14 lactylates are intended for use in animal feed at levels typically in the range of 0.7 g/kg complete feed and not exceeding 1.8 g/kg complete feed. The amount of C12/C14 lactylates added to the feed of the broiler study (3 g C12/C14 lactylates/kg complete feed) exceeds the maximum anticipated use in practice. Thus, the findings with respect to the fate of the lauric and myristic acid components of C12/C14 lactylates have relevance to commercial conditions of use. Moreover, it is reasonable to assume that on the basis of the possession of gastric lipases, animals will process lactic acid esters of fatty acids by similar mechanisms and that the findings in broilers can be extrapolated to all target species.

6.1.2.2 Studies using Calcium Stearoyl-Lactylates

Published and unpublished studies have been conducted to evaluate the metabolic fate of calcium stearoyl-lactylate. These studies have been reviewed by both JECFA (1974a) and EFSA (2013) as part of the safety evaluation of calcium stearoyl-lactylate for use as a food additive. On the basis that the lactic acid ester of stearic acid should exhibit a comparable metabolic fate to the lactic acid esters of lauric and myristic acids in terms of enzymatic hydrolysis of the ester bond in the GI tract of animals, information on the ADME of this structurally related compound was considered relevant to C12/C14 lactylates.

The ADME of calcium stearoyl-lactylate (CSL) was compared with that of sodium DL-lactate in a radiolabeled study conducted in mice and Guinea pigs by Phillips *et al*. (1981). Animals were placed within specialized metabolic cages for the evaluation of expired gases. A single oral dose of ^{14}C-CSL (aqueous solution) was administered by gavage at a concentration of either 90 or 900 mg/kg body

weight to groups of 4 male Tuck TO mice or groups of 4 male Dunkin-Hartley guinea pigs. An additional group of 3 mice or 3 guinea pigs received DL-^{14}C-sodium lactate (^{14}C-lactate) by gavage at a concentration of 325 mg/kg body weight, providing the equivalent lactate concentration as 900 mg CSL/kg body weight. Radioactivity was determined in exhaled air, urine, feces, liver, kidneys, heart, lungs, spleen, testes and the GI tract for up to 48 hours after administration of ^{14}C-CSL.

The excretion of radioactivity from the mice and guinea pigs during the 48 hours following administration of the single oral dose of ^{14}C-CSL or ^{14}C-lactate is summarized in Table 6.6. Following administration of ^{14}C-CSL at 90 mg/kg body weight to mice and guinea pigs, more than 97% of the radioactivity was eliminated within 48 hours with the majority (*ca*. 77%) excreted as ^{14}C-carbon dioxide within 24 hours. The remaining radioactivity was mostly excreted in the urine within 24 hours (14.8% of the administered dose), with only low levels measured in the feces (2.7% of the administered dose) after 48 hours and urine (0.7% of the administered dose) in the 24-to-48-hour period. At the higher dose of administration of ^{14}C-CSL of 900 mg/kg body weight, the level of radioactivity at 7 hours excreted as ^{14}C-carbon dioxide was less than in animals administered the lower dose of 90 mg/kg body weight (57.5 vs. 69.7% of the administered dose) but the total excreted after 48 hours was comparable (82.6 vs. 80.2% of the administered dose). The percentage of radioactivity excreted in the urine and feces within 48 hours was also comparable (16.2 vs. 15.5% of the administered dose in urine and 2.1 vs. 2.7% in feces). The majority of the radioactivity following administration of 325 mg ^{14}C-lactate acid/kg body weight (equivalent to the high dose of ^{14}C-CSL) to mice was also excreted as ^{14}C-carbon dioxide within 24 hours (92.2% of the administered dose). Less of the radioactivity was excreted in the urine and feces (4.0 and 1.1% of the administered dose, respectively after 48 hours).

The excretion of radioactivity by guinea pigs was similar to that observed in mice. The rate and extent of conversion of ^{14}C-CSL to ^{14}C-carbon dioxide was comparable in guinea pigs and mice administered 90 or 900 mg/kg body weight (78.8 and 81.9% of the administered dose, respectively after 48 hours). However, the percentage of the dose excreted in the urine (10.0 and 9.1% of the administered dose, respectively for the 2 dose groups), and by all three routes was less over the 48-hour period.

In both mice and guinea pigs, the total residual activity and distribution of radioactivity in tissues was similar after administration of 90 or 900 mg/kg body weight of ^{14}C-CSL, or 325 mg/kg body weight of ^{14}C-lactate. The total radioactivity sampled in the tissues of guinea pigs (6.1, 6.7 and 10.2% of the administered doses, respectively for each dose group) was higher than in mice (1.8, 2.1 and 2.1% of the administered dose, respectively for each dose group) at 48 hours. In both mice and guinea pigs, the radioactivity in tissues was mainly in the liver and GI tract. Lactic acid was identified by thin layer chromatography (TLC) to be the metabolite of ^{14}C-CSL and ^{14}C-lactate in the urine of both mice and guinea pigs.

Table 6.6: Excretion of Radioactivity by Male Mice and Guinea-Pigs Given a Single Oral Dose of ^{14}C-labelled Calcium Stearoyl-Lactylate or DL-[U-^{14}C]Lactate (Phillips *et al.*, 1981)				
Route of Excretion	**Time from Dosing (Hours)**	**Radioactivity Excreted (% of Dose) after Single Oral Administration**		
		^{14}C-CSL		**^{14}C-Lactate**
		90 mg/kg bw n=4	**900 mg/kg bw n=4**	**325 mg/kg bw n=3**
Mice				
Exhaled CO_2	0-24	76.8	79.8	89.3
	0-48	80.2	82.6	92.2
Urine	0-24	14.8	14.1	3.4
	0-48	15.5	16.2	4.0
Feces	0-24	2.4	1.8	0.9
	0-48	2.7	2.1	1.1
Organs[1]	At 48	1.8	2.1	2.1
Total recovery		100.2	103.0	99.4
Guinea Pigs				
Exhaled CO_2	0-24	75.3	78.6	81.6
	0-48	78.8	81.9	84.1
Urine	0-24	9.2	8.1	3.3
	0-48	10.0	9.1	3.7
Feces	0-24	3.0	2.3	1.6
	0-48	3.8	2.9	2.1
Organs[1]	At 48	6.1	6.7	10.2
Total recovery		98.7	100.6	100.1

Abbreviations: bw = body weight; CSL = calcium stearoyl-lactylate;
[1]Sum of organs evaluated (liver, kidneys, heart, lungs, spleen, testes and GI tract).

In an unpublished study reviewed by JECFA (1974a), experiments were conducted comparing the metabolism of mixed stearic (C18) acid and ^{14}C-lactic acid with calcium stearoyl-lactylate (^{14}C-CSL; lactic acid labeled) (Hodge, 1955; no further details available). 58% of the administered dose of radioactivity was excreted from the mixture, and 60% from ^{14}C-CSL within 24 hours. No differences were reported in the distribution or excretion of the radioactivity between the two groups.

In another study cited by JECFA (1974a) in which rats were fed calcium stearoyl-lactylate, only traces of lactate were detected in the fecal fat, with good utilization of stearic acid and calcium by the animal (Hodge, 1961; no further details available).

The results of *in vitro* studies conducted using calcium or sodium stearoyl-lactylate were also summarized by JECFA (Hodge, 1961 – unpublished study; JECFA, 1974a). The hydrolysis of calcium stearoyl-lactylate by lipases was reported to occur readily to form stearic and lactic acid (no further details specified).

A series of *in vitro* studies were also conducted by Phillips *et al.* (1981) to investigate the hydrolysis of ^{14}C-CSL from liver and GI homogenates of the rat, mouse and guinea pig as well as GI homogenate from human duodenum. The appearance of lactate and disappearance of stearoyl-lactate during hydrolysis of ^{14}C-CSL were monitored. The liver homogenates from all 3 species readily hydrolyzed ^{14}C-CSL with the highest rate exhibited by guinea pigs (24.7 µmol/g liver/hour) and the least in the mouse (7.5 µmol/g

liver/hour). In liver homogenates 55% (rat liver), 45% (guinea pig liver), or 35% (mouse liver) of the initial radioactivity occurred in the form of ^{14}C-lactate within 60 minutes after initiation of incubation. The hydrolysis of ^{14}C-CSL by homogenates of the GI mucosa also occurred rapidly. The initial rate of hydrolysis by the rat and the guinea pig were significantly faster than that of mouse mucosa, with 40 % (guinea pig) and 35% (rat) of the initial radioactivity present in the form of lactate after 1 hour compared to 30% in the mouse. The rate of hydrolysis by human intestinal mucosa was slower in comparison to intestinal mucosa samples from experimental animals with 18% (human) of the initial activity appearing as lactate after an hour. Whole blood from rats and mice hydrolyzed the compound, but at a much slower rate than in the liver or intestinal mucosa homogenates. No significant hydrolysis of ^{14}C-CSL was detected using human blood. Direct evidence for the hydrolysis of the ester linkage in calcium stearoyl-lactylate by gastric lipases is provided by the *in vitro* studies conducted by Hodge (1961 – unpublished; limited summary available in JECFA, 1974a) and Phillips *et al*. (1981). Another unpublished study by Hodge (1955, unpublished; limited summary available in JECFA, 1974a) indicates that the metabolism of calcium stearoyl-lactylate is comparable to a mixture of stearic acid and lactic acid. The results of other *in vitro* and *in vivo* data indicate that calcium stearoyl-lactylate is rapidly absorbed and utilized by animals. On the basis that lipases are demonstrated to hydrolyze the ester linkage, it is assumed that the absorption and metabolism of the lauric acid, myristic acid and lactic acid components of calcium stearoyl-lactylates will follow established pathways for these free fatty acids and organic acid in animals (see Sections 6.1.3 and 6.1.4, respectively). Considering the structural similarities of stearoyl (C18)-lactylate and C12/C14 lactylates, it is reasonable to conclude that similar behavior will be displayed by C12/C14 lactylates under the intended conditions of use as a nutritional ingredient in feed at levels of up to 5 g/kg complete feed. Although the rate of hydrolysis of C12/C14 lactylates may differ between species, these differences are not anticipated to impact the metabolic fate in animals. Thus, as outlined in Section 6.1, it is reasonable to assess the safety C12/C14 lactylates in terms of the individual components, lauric acid, myristic acid and lactic acid.

6.1.3 Information to Establish the Safety of Lauric and Myristic Acids for the Target Animal

6.1.3.1 Natural Occurrence in the Diet and Overview of Previous Safety Evaluations for Use in Food

Lauric and myristic acids are fatty acids which are widely consumed as part of the normal background diet of animals and humans. Their natural occurrence in the diet and well-established metabolic fate can therefore, be used as a basis for the safety determination as described below. The validity of this approach has been demonstrated in previous evaluations of lauric and myristic acids as additives in food. Lauric and myristic acids along with other structurally-related saturated straight-chain fatty acids have a long and established history of use as food additives and flavorings. The safety of the fatty acids for these human food uses has been evaluated by EFSA and JECFA, and the most recent evaluations are summarized in Table 6.7.

Table 6.7: Summary of Previous Scientific Evaluations of Lauric and Myristic Acids		
Reference	**Scope of Evaluation**	**Summary**
EFSA, 2017a	Re-evaluation of fatty acids (E 570) as food additives [caprylic (C8), capric (C10), lauric (C12), myristic (C14), palmitic (C16), stearic (C18) and oleic (C18:1) acids]	No safety concern at current usage levels
JECFA, 1998	Safety evaluation of saturated aliphatic, acyclic linear primary alcohols, aldehydes and acids for use as flavoring substances in food	No safety concern at current level of usage as a flavoring agent

Abbreviations: EFSA = European Food Safety Authority; JECFA = Joint FAO/WHO Expert Committee on Food Additives.

The dietary exposure by humans to these saturated straight-chain fatty acids from their use as technological additives or flavorings is low compared to that from the normal diet. On this basis, safety for the use as a technological additive or flavoring in food was largely based on the low anticipated contribution to total intakes together with the known rapid and extensive metabolism via normally fatty acid and tricarboxylic acid pathways. Although toxicological data were not critical to the determination of safety, the body of available data were reviewed by EFSA and JECFA as part of their assessments.

A similar approach can be taken in evaluating the safety of the lauric and myristic acid components of C12/C14 lactylates for animals. The metabolic fate of lauric and myristic acid by animals is evaluated in Section 6.3.2. These fatty acids are present in feed products marketed in the U.S. under the definitions laid down in Section 33 of Chapter 6 of the AAFCO OP, and in particular, as the free fatty acids under the definition for Hydrolyzed Fat or Oil, Feed Grade (33.3; AAFCO, 2023). The levels of lauric and myristic acids in fats with a history of use in animal feed is considered in Section 6.1.3.3. The anticipated intakes of lauric and myristic acids from the diet are compared with those from C12/C14 lactylates under the conditions of intended use in Section 6.1.3.4. Taken together, these data demonstrate that the lauric and myristic acid components of C12/C14 lactylates will not pose a safety concern to animals under the intended conditions of use. Although studies in target animals are not considered necessary to establish the safety of lauric and myristic acids for animals, there are a number of studies in the published literature in which these fatty acids were fed to poultry and livestock. These studies are briefly described for completeness in Section 6.1.3.6. In addition, the body of toxicological information evaluated by EFSA and JECFA, together with a more recent short-term feeding study in rats using lauric acid is outlined in Section 6.1.3.7. These studies provide corroborative evidence of the safety of the lauric and myristic acid components of C12/C14 lactylates under the conditions of intended use.

6.1.3.2 ADME of Lauric and Myristic Acids

An overview of general fat digestion and absorption by mammals is provided below [e.g., as described in CIR (1987), Xenoulis and Steiner (2010), EFSA (2013 and 2017a) and Adeva-Andany *et al.* (2019) considering dogs and humans]. The stomach is the major site of fat emulsification, mechanically breaking down larger aggregates which are virtually insoluble in the aqueous environment into smaller fragments. Fatty acid esters will generally be hydrolyzed by lipases secreted by the dorsal surface of the tongue or gastric lipases secreted by the stomach. Gastric lipases hydrolyze short-, medium- and longer-chain fatty acids into free fatty acids initiating lipid absorption. Medium-chain fatty acids such as lauric acid are absorbed via the stomach wall into the portal vein for transport to the liver for hepatic metabolism. Longer-chain fatty acids such as myristic acid and any remaining esters are transported

into the duodenum as droplets and the latter further hydrolyzed by the action of pancreatic lipases. Smaller lipids droplets are exposed to bile in the duodenum where they combine to form micelles (4-8 nm in diameter). These micelles (water soluble aggregates) are comprised of mixed lipids and bile acids which allows for absorption in the proximal small intestine. Fatty acids can enter the enterocytes of the small intestine by simple diffusion across the epithelial cell membrane or via a transporter protein. In the intestinal cell, the fatty acids are transported as triglycerides in chylomicrons and very low-density lipoproteins via the lymphatic system and enter systemic circulation. Ultimately, the fatty acids are metabolized by normal fatty acid and tricarboxylic acid pathways in animals to form carbon dioxide which is excreted via exhalation.

The digestion of fat in ruminants is markedly different to that in non-ruminants (Harrison and Leat, 1972; Nafikov and Beitz, 2007; Brzozowska and Oprzadek, 2016). Whereas in non-ruminants, little digestion of fat will occur before the digesta reaches the small intestine where, as described above, lipid solubilization occurs by the action of pancreatic lipases and bile acids. By comparison in ruminants, dietary lipids are generally modified in the forestomach by the microbial population of the rumen, acting to hydrolyze esters and hydrogenate unsaturated fatty acids. Saturated free fatty acids pass into the small intestine where absorption occurs.

Studies using Lauric Acid

Fasted and refed rats were administered 0.5 mL rat serum containing 0.1 µeq 1-^{14}C-labeled lauric acid in combination with 0.1 µeq 9,10-^{3}H-labeled palmitic acid by intravenous injection (Göransson, 1965; cited in EFSA, 2017a). The disappearance of the two labels was measured 1 to 5 minutes after injection. In both fasted and refed rats, lauric acid was observed to disappear from blood at a faster rate than palmitic acid. Analysis of tissues indicated that lauric acid was more rapidly oxidized than palmitic acid.

In a study by Rioux *et al*. (2003), 1-^{14}C-labeled lauric acid was rapidly taken up by cultured rat hepatocytes and the initial radioactivity was cleared from the medium after 4 hours of incubation. Incorporation of the radioactivity into cellular lipids was low due to the high β-oxidation of lauric acid in hepatocytes and it was preferentially incorporated into triglycerides. It was also rapidly converted into palmitic acid by two successive elongations. Labeling studies with 11,12-^{3}H-lautic acid showed incorporation of radioactivity in several proteins as well as labeling of its elongation product, myristic acid.

The metabolic pathways of lauric acid and other fatty acids in liver microsomes of various mammalian species was also investigated by Adas *et al*. (1999). Cytochrome P450 2E1 was reported to catalyze the hydroxylation of lauric acid across all species.

Studies using Myristic Acid

Fasted and refed rats were administered 0.5 mL rat serum containing 0.1 µeq 1-^{14}C-labeled myristic acid in combination with 0.1 µeq 9,10-^{3}H-labeled palmitic acid by intravenous injection (Göransson, 1965). The disappearance of the two labels was measured 1 to 5 minutes after injection. In both fasted and refed rats, myristic acid was observed to disappear from blood at a faster rate than palmitic acid. Analysis of tissues indicated that myristic acid was more rapidly oxidized than palmitic acid.

The metabolism of myristic and palmitic acids was compared in cultured rat hepatocytes using 1-^{14}C-labeled fatty acids (Rioux *et al*., 2000). Myristic acid was reported to be taken up more rapidly than palmitic acid (87 vs. 68% after 4 hours) and incorporation into cellular lipids was around 33% of initial radioactivity. After 12 hours of incubation, the radioactivity of cellular triglycerides, cellular phospholipids and secreted triglycerides was significantly lower for myristic than palmitic acid. Greater oxidation of myristic acid occurred than of palmitic acid with 15% of the initial radioactivity incorporated in β-oxidation products after 4 hours. Myristic acid also was elongated to palmitic acid to a greater degree than palmitic acid to stearic acid (12% vs. 5% of the initial radioactivity). The combination of elongation and β-oxidation of myristic acid resulted in the more rapid disappearance of myristic acid in cultured hepatocytes than palmitic acid.

Other Biological Considerations

Free fatty acids are recognized to specifically bind to G-protein coupled receptors (GPCR) (FFAR1-4, GPR84) which are widely expressed on a variety of cells and regulate both metabolic and immunological processes (Alvarez-Curto and Milligan, 2016). In this respect, free fatty acids can be critical signaling molecules and an imbalance of fatty acids ingested may have consequences for the animal. As mentioned in Sections 2.6, 3.1 and 3.2, C12/C14 lactylates are intended for use as part of nutritionally balanced diets alongside other fat sources such as grains, oilseed meals, SO and FO. Thus, no adverse effects as a result of fatty acid imbalances are expected under the intended conditions of use of C12/C14 lactylates in animal feed at practical levels of 0.7 g/kg complete feed and not exceeding 1.8 g/kg complete feed.

6.1.3.3 Comparison of the Composition of C12/C14 Lactylates and Other Sources of Dietary Fat

As mentioned above, C12/C14 lactylates comprises around 42% lauric acid and 19% myristic acid in the free fatty acid and lactic acid ester form. Animal fats, fish oil and vegetable oils are all commonly used as fat sources in practical animal diets in the U.S. Although, lauric acid is only found in vegetable fats, myristic acid occurs widely in vegetable and animal fats (Woodgate and van der Veen, 2014; Merriman *et al*., 2016). The fatty acid profiles of fish oil (FO), soy oil (SO), coconut (or copra) oil (CO), palm kernel oil (PKO)[6], palm oil (PO) and tallow which have a history of use by the U.S. feed industry are presented in Table 6.8. These fats were chosen for comparison to include those rich in lauric and myristic acids such as CO and PKO, but which have a limited history of use in the U.S., as well as those with a more established history of use such as SO, FO and tallow, but lower contents of lauric and myristic acids. Coconut oil and PKO contain *ca*. 41% and 49% lauric acid, respectively, which is similar to the level found in C12/C14 lactylates of *ca*. 42%. Fish oil, CO and PKO all contain significant levels of myristic acid at *ca*. 10, 17 and 16%, respectively, which are slightly lower than the levels reported in C12/C14 lactylates of *ca*. 19%. Consistent with animal fats generally, tallow is a source of myristic acid (*ca*. 3%) but contains only negligible levels of lauric acid (*ca*. 0.2%).

[6] Palm kernel oil is less widely used in the U.S. than other fats and oils listed in Table 6.8 but is available as a product of the palm oil industry and is included for completeness as a myristic acid-rich oil.

Table 6.8: Fatty Acid Profiles of Fats used in Animal Feed							
Fatty Acid	**Unit**	**Typical Values (% Fatty Acids)[1]**					
		CO	**FO**	**PKO**	**PO**	**SO**	**Tallow**
C6 to C10 fatty acids	%	15.5	-	8.2	-	-	-
Lauric acid (C12:0)	***%***	***40.7***	***0.15***	***48.7***	***0.16***	***<0.1***	***0.2***
Myristic acid (C14:0)	***%***	***17.1***	***9.9***	***15.6***	***0.95***	***0.2***	***3.2***
Palmitic acid (C16:0)	%	9.2	20.9	7.5	40.3	10.7	26.3
Palmitoleic acid (C16:1, n-7)	%	-	12.5	-	0.1	0.1	3.8
Stearic acid (C18:0)	%	2.9	3.4	1.8	4.1	3.6	21.2
Oleic acid (C18:1, n-9)	%	6.9	13.0	14.8	36.7	21.9	38.5
Linoleic acid (C18:2, n-6)	%	1.7	1.1	2.5	9.3	51.3	2.8
alpha-Linolenic acid (ALA; 18:3, n-3)	%	0.1	0.8	-	0.2	6.9	0.7
Arachidic acid (C20:0)	%	0.1	0.4	-	0.3	0.3	1.1
Eicosenoic acid (C20:1)	%	-	1.9	-	0.1	0.2	0.3
Arachidonic acid (C20:4)	%	-	0.6	-	-	-	-
Eicosapentaenoic acid (EPA; C20:5, n-3)	%	-	12.2	-	-	-	-
Behenic acid (C22:0)	%	-	0.2	-	-	0.4	0.1
Erucic (C22:1)	%	-	0.7	-	-	0.1	0.2
Docosapentanoic (C22:5)	%	-	1.7	-	-	-	-
Docosahexaenoic (DHA; C22:6, n-3)	%	-	7.9	-	-	-	-
Lignoceric acid (C24:0)	%	-	0.2	-	-	0.2	0.05

Abbreviations: CO = coconut (copra) oil; FO = fish oil; PKO = palm kernel oil; PO = palm oil; SO = soy oil;
[1]Fatty acid profiles taken from the INRAE-CIRAD-AFZ feed tables (2022) except for palm kernel oil which was taken from Pantzaris and Ahmad (2001).

6.1.3.4 Background Exposure to Lauric and Myristic Acid from Other Fats in the Diet

As mentioned in Section 6.1.3.3, CO in particular is a significant source of lauric and myristic acids containing in the region of 41 and 17% of these fatty acids, respectively, and has a history of use in animal feed as a partial replacement for other fats such as FO, SO and canola oil. The amount of CO included in the diet of animals will vary depending on the species but may range from 0.5 to 2% of the diet of poultry, swine, dairy cows or fish (e.g., FAO, 1983; Jørgensen *et al.*, 2000; Faciola and Broderick, 2014; Wang *et al.*, 2015; Braundmeier-Fleming *et al.*, 2020; Rolinec *et al.*, 2020). At these inclusion levels of CO in the feed of 5 or 20 g/kg complete feed as-fed, the lauric acid content will be in the region of 0.2 to 0.8% (2 or 8 g/kg complete feed), and the myristic acid content in the region of 0.09 to 0.3% (0.9 to 3 g/kg complete feed). By comparison, under the conditions of intended use of C12/C14 lactylates in feed of typically 0.7 g/kg as-fed, animals will be provided with 0.3 and 0.1 g/kg complete feed of lauric and myristic acids, respectively (see Table 3.1). At the maximum intended use level of 1.8 g C12/C14 lactylates/kg complete feed, the lauric and myristic acid contents will be 0.8 and 0.3 g/kg as-fed, respectively (see Table 3.1). Thus, background exposure to lauric and myristic acids from the use of CO as a fat at levels of 0.5 to 2% of the diet, are higher (2.5- to 10-fold for lauric acid; 3- to 10-fold for myristic acid) higher than from C12/C14 lactylates at the maximum intended use level.

Additionally, medium-chain fatty acids derived from coconut oil have a history of use in the pet food industry (Beynen, 2019; Petco Store, 2020). Coconut oil and its derivative, medium-chain triglycerides (MCT) oil comprising primarily C8 (caprylic) and C10 (capric) fatty acids, are considered to have potential nutritional benefits to dogs (e.g., Pan *et al.*, 2010; Hall and Jewell, 2012). Assuming a dog is provided

with a treat or supplement containing 2 g of CO, the animal will be exposed to around 0.8 g of lauric acid/day. By comparison, if a 15 kg dog consumes 250 g of a dry dog food containing 1.8 g C12/C14 lactylates/kg complete feed as-fed, daily exposure to lauric acid will be in the region of 0.2 g/day which is around 4 times lower than the estimated intake from 2 g of CO.

It is recognized that animal fats such as tallow or white grease are more commonly used in feed than CO. As mentioned in Section 6.1.3.3, tallow for example, contains around 3.2% myristic acid but only 0.2% lauric acid. Tallow can be incorporated into the diet of animals at higher levels in practice than CO, and may potentially represent around 8% of the feed (e.g., Reis de Souza *et al*., 1995; O'Neill *et al*., 1998; Tancharoenrat and Ravindran, 2014; Merriman *et al*., 2016). Incorporation of around 8% of tallow in the feed of animals will provide in the region of 0.02% lauric acid and 0.3% myristic acid (0.2 and 3.0 g/kg complete feed). Thus, the potential exposure to lauric acid from the use of tallow under typical conditions of inclusion in feed is lower than from the maximum intended use level of C12/C14 lactylates (0.2 vs. 0.8 g/kg complete feed) but exposure to myristic acid is higher (3.0 vs. 0.3 g/kg complete feed; 10-fold higher). Thus, the exposure by animals to myristic acid from the use of tallow in animal feed further corroborates the safety of this component of C12/C14 lactylates at the proposed inclusion levels. Black soldier fly larvae (BSFL) oil was also evaluated by the U.S. FDA in order to establish an AAFCO ingredient definition for use as a fat source in the diet of finfish, poultry, swine and adult dogs (AAFCO, 2023). The fatty acid profile of BSFL can be influenced to some extent by the larval feedstock but generally contains significant (*ca*. 30%) levels of lauric acid with lower amounts of myristic acid (*ca*. 5%) (Mai *et al*., 2019). Thus, BSFL oil represents another form of lauric and myristic acids which is not considered to pose a safety concern as a component of animal diets and has recently entered the U.S. market.

6.1.3.5 Background Exposure to Lauric and Myristic Acid from Oilseed Meals in the Diet

By-products of the CO and PO industries, in particular CM and PKM are also sources of lauric and myristic acids with a history of use as ingredients in animal feed, in particular in countries with extensive coconut and palm oil industries. The use of these oilseed meals is less common in the U.S., CM is a recognized feed ingredient and is defined in the AAFCO OP (see below; AAFCO, 2022) and is routinely included in U.S. relevant feed composition tables (e.g., United States-Canadian Tables of Feed Composition; NRC, 1982). Further details of the lauric and myristic acid contents of CM and PKM are provided below and the exposure by different species and categories of animals estimated under practical conditions of use. Although these ingredients have only a limited use in commercial feed in the U.S., these data are considered to provide corroborative evidence generally for the history of consumption of lauric and myristic acid-containing ingredients by a range of species as part of the normal diet.

Composition of CM and Scope of Use

Coconut meal is a by-product of the extraction of oil from dried coconut kernels (copra) and has a history of use as an ingredient in feed in the U.S. It is recognized as an economical feed which can partially replace other protein sources in the diet of animals (e.g., O'Doherty and McKeon., 2000; Kim *et al*., 2001; Stein *et al*., 2015) with AAFCO ingredient definitions established for coconut meal, mechanically extracted (71.60), and coconut meal, solvent extracted (71.61) (AAFCO, 2022). Coconut meal obtained by mechanical extraction for example, is reported to have a typical fat content of 9% of

which lauric and myristic acids represent 45 and 19%, respectively (INRAE-CIRAD-AFZ feed tables, 2022). On an ingredient basis, this equates to levels of lauric and myristic acid in CM of around 4 and 2%, respectively. The practical use of CM in animal feed includes poultry and swine feeds as detailed in the following section (Ravindran and Blair, 1992; Stein *et al*., 2015; Arbor Acres, 2017).

Estimated Exposure by Poultry and Swine to Lauric and Myristic Acids from CM

The use of CM as a nutrient source in poultry feed is limited by the amino acid profile and relatively high dietary fiber content although these effects can be overcome by dietary supplementation with lysine and methionine, and the use of enzymes (e.g., mannanases) to help degrade the fiber component (Khanongnuch *et al*., 2006; Sundu *et al*., 2008 and 2009; Diarra *et al*., 2014). However, use in layers, which have lower nutrient requirements than growing birds, is reported with optimal levels of use typically reported to be in the region of 10% (e.g., Moorthy and Viswanathan, 2006 and 2010; Sundu *et al*., 2009).

In a review of feeding studies evaluating the use of CM in swine feeds, Stein *et al*. (2015) concluded that on the basis of the available body of information, the optimum level of inclusion of CM is less than 15% in the diet of weaned piglets and less than 25% in the diets for grower-finisher pigs.

Assuming that CM is included in the diet of layers and pigs at a level of 10% which is within these recommended ranges of use of this ingredient in poultry and swine feeds, the complete feed will contain lauric and myristic acid at levels of 0.4 and 0.2%[7], respectively (or 4 and 2 g/kg complete feed as-fed). By comparison, under the maximum conditions of intended use of C12/C14 lactylates in feed of up to 1.8 g/kg as-fed, layers and swine will be provided with lauric and myristic acid at levels of 0.8 and 0.3 g/kg as-fed, respectively (see Table 3.1). Thus, the background exposure by layers and swine to lauric and myristic acids from the use of CM as a nutrient source at dietary levels of 10% is approximately 5- and 7-fold higher, respectively than from the maximum intended use of C12/C14 lactylates.

Composition of PKM and Scope of Use

Palm kernel meal is a by-product of the PO industry with an extensive history of use as a feed ingredient in Southeast Asian countries such as Malaysia, and also in parts of Africa (Perez *et al*., 2000; Alimon, 2004; FAO, 2012). PKM obtained by mechanical extraction only and referred to as PKM expeller, has a reported fat content of between 5 and 20% of which lauric acid represents around 48% and myristic acid around 16%. On an ingredient basis, PKM expeller containing 8% fat for example, will contain in the region of 4 and 1% lauric and myristic acids, respectively. It displays a chemical composition similar to that of coconut meal or corn gluten meal and on account of its fiber content (13-20% DM) and amino acid profile, is generally considered suitable for use in combination with other protein sources in ruminant diets (FAO, 2012; Stein *et al*., 2015). The practical use of PKM in cattle diets is considered below.

Estimated Exposure by Ruminants to Lauric and Myristic Acids from PKM

In Malaysia for example, PKM is widely used at levels of up to 80% of the total mixed ration (TMR) for feedlot cattle and buffaloes. Inclusion levels in dairy cattle rations are generally in the range of 30 to

[7] Calculation: 10 x [0.09 x 0.45 (C12)] or [0.09 x 0.19 (C14)] = %C12 or C14 fatty acid in the complete feed

40% and in sheep rations around 30% (Alimon, 2004; FAO, 2012). Assuming that feedlot cattle are provided a TMR containing 80% PKM expeller which has a fat content of 8%, the estimated dietary levels of lauric and myristic acids are 3 and 1%[8] (or 30 and 10 g/kg feed as-fed), respectively. Similarly, the lauric and myristic acid levels in a TMR for dairy cattle containing 35% PKM expeller of which 8% is fat, are estimated to be 1 and 0.4%[9] (or 10 and 4 g/kg feed as-fed), respectively. By comparison, under the maximum intended use level of C12/C14 lactylates in feed of 1.8 g/kg as-fed, cattle will be provided with lauric and myristic acid at levels of 0.8 and 0.3 g/kg as-fed, respectively (see Table 3.1). Background exposure to lauric and myristic acids from ingredient use of PKM expeller is approximately 38 and 33 times greater, respectively than from C12/C14 lactylates by feedlot cattle and approximately 12.5 and 13 times greater, respectively by dairy cattle at the maximum intended use level.

Estimated Exposure by Fish to Lauric and Myristic Acids from PKM

The amino acid contents and relatively high fiber contents of PKM and CM limit their use in fish feed. However, some use of PKM in fish is reported with levels typically not exceeding 10 to 20% (Ng *et al*., 2002; FAO, 2012; Park *et al*., 2016).

Assuming that fish are provided diets containing 10% PKM expeller, the estimated dietary levels of lauric and myristic acids are 0.4 and 0.1%[10], respectively (4 and 1 g/kg complete feed as-fed). By comparison, under the maximum intended use level of C12/C14 lactylates in feed of 1.8 g/kg as-fed, fish will be provided with lauric and myristic acid at levels of 0.8 and 0.3 g/kg as-fed, respectively (see Table 3.1). Background exposure to lauric and myristic acids from use of PKM expeller at 10% in the diet is 5- and 3-fold higher, respectively than from C12/C14 lactylates at the maximum intended use level in fish feed.

Summary and Conclusions

The exposure by poultry, swine, ruminants and aquaculture to lauric and myristic acids from the use of CO, CM and PKM as nutrient sources in feed under commercial feeding practices was estimated to be significantly greater than from C12/C14 lactylates under the intended conditions of use. CO and MCTs derived from CO were also reported to have a history of use in cat and dog food at levels higher than from the intended use of C12/C14 lactylates. Further evidence of the acceptability of lauric and/or myristic acid as components of the diet of animals is provided by the common use of tallow in feed in the U.S., as well as the development of an AAFCO ingredient definition for BSFL oil. Therefore, the safety of lauric and myristic acids in C12/C14 lactylates can be established from the higher background intakes of these fatty acids from the normal diet. As mentioned in Section 2.6, C12/C14 lactylates are not intended for use in feeds which contain other significant sources of lauric or myristic acids.

6.1.3.6 Studies in Target Animals using Lauric and Myristic Acids

Numerous studies are available in the published literature in which poultry, swine, ruminants or aquaculture were fed diets containing CO or PKO at levels comparable to, or higher than, the anticipated

[8] Calculation: 80 x [0.08 x 0.48 (C12)] or [0.08 x 0.16 (C14)] = %C12 or C14 fatty acid in the TMR
[9] Calculation: 35 x [0.08 x 0.48 (C12)] or [0.08 x 0.16 (C14)] = %C12 or C14 fatty acid in the TMR
[10] Calculation: 10 x [0.08 x 0.48 (C12)] or [0.08 x 0.16 (C14)] = %C12 or C14 fatty acid in the complete feed

exposure to lauric and myristic acids under the conditions of intended use of C12/C14 lactylates. These studies support the background intakes of these fatty acids from the normal diet as described above.

In addition, studies were identified in the published literature in which lauric acid was fed to broilers (Londok *et al.*, 2018; Londok and Rompis, 2019) and lauric or myristic acid was fed to ruminants (Machmuller, 2006; Odongo *et al.*, 2007; Hristov *et al.*, 2009; Klop *et al.*, 2017). The dietary levels varied but were in the region of 0.1 to 0.3 g/kg complete feed in poultry and 65 g/kg DM in cattle. These studies were not designed as safety studies but were performed in healthy animals and included some limited parameters related to tolerability, such as performance. No adverse effects were reported in the studies under conditions comparable to the intended use of lauric and myristic acids in feed from C12/C14 lactylates of up to 0.8 and 0.3 g/kg complete feed, respectively. Therefore, the findings of the study provide corroborative evidence of the safety of the fatty acid components of C12/C14 lactylates to animals under the conditions of intended use.

6.1.3.7 Toxicological Information on Lauric and Myristic Acids

Summaries of the toxicological data on lauric and myristic acids evaluated by EFSA (2017a) and JECFA (1998) are provided in Tables 6.9 and 6.10, respectively. In addition, an acute toxicity study in rats using lauric acid was identified in the published literature and is included in Table 6.9 (Khan *et al.* 2020). An overview of the studies, primarily taken from the evaluations by EFSA and JECFA is provided below.

Acute Oral Toxicity Studies

In a modified acute toxicity in mice administered lauric acid for 3 days, the median lethal dose (LD_{50}) was reported to be >1,238 mg/kg body weight/day (Schafer and Bowles, 1985). Male Albino rats were administered increasing doses of lauric and myristic acid of up to 10,000 mg/kg body weight/day by gavage in another acute oral toxicity study ((b) (4), 1974). A dose of 10,000 mg/kg body weight/day of lauric acid resulted in one death. Congested lungs and kidneys and advanced autolytic changes were reported at necropsy. No deaths and no significant gross lesions were reported at necropsy on dosing with myristic acid. More recently, Khan *et al.* (2020) administered 2,000 mg/kg body weight of lauric acid orally to female SD rats. There were no mortalities or signs of toxicity after 15 days.

Subchronic Toxicity Studies

Male Osborne-Mendel Albino rats (5 rats/group; 40 to 50 kg body weight) were fed lauric acid in the diet at levels of 0 or 10% (equivalent to 0 and 9,000 mg/kg body weight/day) for 18 weeks (Fitzhugh *et al.*, 1960). Body weight, physical condition, behavior, appearance and mortality were monitored during the study. At the end of the study, blood samples were taken for hematological analysis, and necropsy and gross pathology was performed. No adverse findings were reported.

Stomach Irritation Studies

In a 150-day feeding study, Albino rats (10 rats/group; mixed sex and strain) were supplied with a rice diet containing 10% lauric acid (equivalent to 9,000 mg/kg body weight/day) (Mori, 1953). Interim

sacrifices were conducted during the study and stomachs were examined for gross lesions. No remarkable changes were reported in the forestomach or the glandular stomach.

Genotoxicity Studies

The mutagenicity of lauric acid was evaluated in a reverse mutation assay using *Salmonella typhimurium* strains TA1535, TA1537, TA97, TA98 and TA100 up to a maximum concentration of 666 µg/plate in the presence and absence of metabolic activation (Zeiger *et al*., 1988). The study complied with OECD TG 471 with the exception that tester strains *S. typhimurium* TA102 and *E. coli* WP2uvrA bearing AT mutation were not included. The same assay was also used to evaluate the mutagenicity of myristic acid up to a maximum concentration of 3,333 µg/plate in the presence and absence of metabolic activation. No mutagenicity was observed in either study.

A bacterial reverse mutation assay was conducted on myristic acid using *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 at concentrations of up to 10,000 µg/plate (Heck *et al*., 1989). The findings of the study were negative. Myristic acid was also not observed to induce gene mutations in the mouse lymphoma assay (L5178Y TK+/- cells) at concentrations of up to 62 µg/mL without metabolic activation, or 125 µg/mL with metabolic activation, or at 300 µg/mL in an unscheduled DNA synthesis (UDS) assay using hepatocytes obtained from male Fisher rats. The reporting of the study was noted to be limited (EFSA, 2017a).

Table 6.9: Summary of Toxicity Studies using Lauric Acid			
Study	**Details**	**Result**	**Reference**
Acute toxicity study	Mouse, oral	LD_{50} = 1,238 mg/kg bw	Schafer & Bowles, 1985 [Cited in JECFA, 1998]
Acute toxicity study	Rat, gavage	LD_{50} > 10,000 mg/kg bw	CIR, 1987
Acute toxicity study (OECD TG 423)	Female SD rat, oral	No mortality 15 days after a single dose of 2,000 mg/kg bw	Khan *et al.*, 2020
18-week toxicity study	Male rat, diet	NOEL >6,000 mg/kg bw per day (reported by JECFA, 1998) NOEL >9,000 mg/kg bw per day (reported by EFSA, 2017a)	Fitzhugh *et al.*, 1960 [Cited in EFSA, 2017a]
150-day toxicity study	Albino rat, both sexes, diet	No remarkable changes detected at 9,000 mg/kg bw per day	Mori, 1953 [Cited in JECFA, 1998]
Modified Ames test (pre-incubation method)	*S. typhimurium* TA98, TA100, TA1535, TA97 and TA1537	Negative[1] at up to 666 µg/plate	Zieger *et al.*, 1988 [Cited in JECFA, 1998]
Genotoxicity study	*Saccharomyces cerevisiae* D6	Aneuploidy at concentrations from 10 to 200 µg/ml,	Parry *et al.*, 1981 [Cited in EFSA, 2017a]

Abbreviations: bw = body weight; LD_{50} = median lethal dose; NOEL = no observed effect level; OECD = Organisation for Economic Co-operation and Development;
[1]Both with and without metabolic activation.

Table 6.10: Summary of Toxicity Studies using Myristic Acid			
Study	**Details**	**Result**	**Reference**
Acute toxicity study	Rat, oral	LD_{50} > 5,000 mg/kg bw	Moreno, 1977 [Cited in JECFA, 1998]
Acute toxicity study	Rat, gavage	LD_{50} > 10,000 mg/kg bw	CIR, 1987
Cell mutagenesis assay	Mouse lymphoma L5178Y TK +/-	Negative at 62.5 µg/ml[1] and 125 µg/ml[2]	Heck *et al.*, 1989 [Cited in JECFA, 1998]
Ames test (plate incorporation assay)	*S. typhimurium* TA98, TA100, TA1535, TA1537 and TA1538	Negative at 10 mg/plate[3]	Heck *et al.*, 1989 [Cited in JECFA, 1998]
Modified Ames test (pre-incubation method)	*S. typhimurium* TA97, TA98, TA100, TA1535 and TA1537	Negative at up to 3,333 µg/plate[3]	Zieger *et al.*, 1988 [Cited in JECFA, 1998]
UDS assay	Rat hepatocytes *in vitro*	Negative at 300 µg/ml	Heck *et al.*, 1989 [Cited in JECFA, 1998]

Abbreviations: bw = body weight; LD_{50} = median lethal dose; UDS = unscheduled DNA synthesis;
[1]Without metabolic activation;
[2]With metabolic activation;
[3]Both with and without metabolic activation.

Summary and Conclusions

The results of the available toxicity studies support the safety of lauric and myristic acids as components of C12/C14 lactylates under the intended conditions of use. Overall, lauric and myristic acid display low acute oral toxicity. Although irritation of the GI tract is unlikely on the basis that lauric and myristic acid in the free fatty acid and ester form in C12/C14 lactylates will only be present at relatively low levels in the feed (up to 1.8 g/kg complete feed of C12/C14 lactylates providing 0.8 and 0.3 mg lauric and myristic acids/kg complete feed), no irritation of the forestomach of rats was reported in animals fed diets containing 10% lauric acid. Only limited genotoxicity data are available and the studies conducted do not meet regulatory or OECD guidelines. However, no evidence of mutagenicity was observed in bacterial reverse mutation assays using lauric acid or myristic aid. The negative findings of UDS assay on myristic acid cannot be fully relied on due to limitations in the experimental design. No adverse findings were reported in rats fed 10% lauric acid in the diet for 18 weeks.

6.1.4 Information to Establish the Safety of Lactic Acid for the Target Animals

6.1.4.1 An Overview of Previous Safety Evaluations for Use in Feed and Food

Lactic acid and calcium lactate were recently re-evaluated by EFSA for use as preservatives in animal feed in the EU (EFSA, 2015, 2017b and 2019). A summary of these evaluations is provided in Table 6.11. In the original evaluation conducted in 2015, EFSA evaluated the safety of lactic acid and its calcium salt for ruminants and swine on the basis of the natural presence of the acid in the diet. On the basis that no adverse effects were observed in ruminants exposed to >100,000 mg lactic acid/kg DM, and swine exposed to 125,000 mg lactic acid/kg DM, no safety concerns were anticipated from preservative use at levels of up to 50,000 mg lactic acid/kg complete feed. However, in the absence of significant background consumption of lactic acid by poultry similar conclusions could not be drawn for this species. Furthermore, although published data in broilers and layers were reviewed, the studies were not designed for regulatory purposes and were considered insufficient by EFSA for the determination of a safe level of use of lactic acid in poultry. A lack of data in pre-ruminants also resulted in no conclusions being drawn for this category of animal.

Following publication of the initial opinion by EFSA, the applicant submitted additional published information in 2017 in an effort to address the gaps in the available data on poultry and pre-ruminants (EFSA, 2017b). EFSA again considered the information provided insufficient, and the applicant conducted a tolerance trial in broilers which was evaluated in an opinion published in 2019. On the basis of the 42-day feeding trial in broilers fed lactic acid (comprised of 90% L-lactic acid and 10% D-lactic acid) and D-lactic acid, EFSA concluded that no safety concerns were anticipated when lactic acid or calcium lactate were included in the diet of poultry at levels of up to 20,000 mg/kg complete feed (the applicant proposed use level). EFSA extrapolated the results of the study in broilers to support the safety of lactic acid and calcium lactate at levels of 20,000 mg/kg complete feed for all animals except swine and ruminants for which higher levels were acceptable, and pre-ruminants for which there continued to be a lack of data.

Table 6.11: Summary of Previous Scientific Evaluations of Lactic Acid for Feed Use		
Reference	**Scope of the Evaluation**	**Summary**
EFSA, 2015	Safety and efficacy of lactic acid and calcium lactate for use as technological additives for all animal species	The maximum levels of 50,000 mg lactic acid/kg complete feed and 30,000 mg calcium lactate/kg complete feed are considered safe for functional ruminants and pigs No conclusions on the safety for pre-ruminants or poultry could be drawn
EFSA, 2017b	Safety and efficacy of lactic acid and calcium lactate for use as technological additives for poultry and pre-ruminants	No safe concentration of lactic acid and calcium lactate in complete feed for chickens could be identified. No conclusions on the safety for lactic acid in milk replacer for pre-ruminants is possible. In the absence of data in poultry and pre-ruminants safety for all species cannot be established (i.e., only pigs and functional ruminants as detailed in EFSA, 2015)
EFSA, 2019	Safety and efficacy of lactic acid and calcium lactate for use as technological additives for poultry and all animal species except pre-ruminants	The maximum levels of 20,000 mg lactic acid/kg complete feed and 24,000 mg calcium lactate/kg complete feed are considered safe for all animals except swine, ruminants and pre-ruminants. Swine and ruminants can safely consume lactic acid and calcium lactate at higher levels (EFSA, 2015). No safe level is established for pre-ruminants.

Abbreviations: ADI = acceptable daily intake; EFSA = European Food Safety Authority.

A similar approach can be taken in evaluating the safety of the lactic acid component of C12/C14 lactylates under the intended conditions of use of not more than 1.8 g/kg complete feed, equivalent to 0.5 g lactic acid/kg complete feed. The metabolic fate of lactic acid in animals is considered in Section 6.1.4.2. Exposure by animals to lactic acid from C12/C14 lactylates is compared with that from the existing use as a preservative in feed in Section 6.1.4.3. In Section 6.1.4.4, the anticipated levels of intake of lactic acid from its natural presence in the diet are estimated. The results of the unpublished study in broilers which formed the basis for positive opinion issued by EFSA in 2019 on the safety of lactic acid for poultry are summarized in Section 6.1.4.5. In the same section, a brief outline of other published feeding trials in animals using lactic acid is provided.

Additionally, lactic acid and its ammonium, calcium, sodium and potassium salts have a long and established history of use as technological additives and flavorings in human food. The safety of lactic acid and its salts has been evaluated by the Scientific Committee on Food (SCF), EFSA and JECFA and a summary of these evaluations is provided in Table 6.12. The dietary exposure by humans to lactic acid from the regular diet of humans was determined to be significantly higher than that from use as a flavoring or food additive in the evaluations conducted by the SCF (1991) and JECFA (1974b). Although toxicological data were not considered critical to the safety determination on this basis, some limited toxicity studies were reviewed by the experts. The body of available toxicological information is summarized in Section 6.1.4.7.

Table 6.12: Summary of Previous Scientific Evaluations of Lactic Acid for Food Use			
Reference	**Scope of Evaluation**	**Purpose**	**Summary**
SCF, 1991	Dicarboxylic acids and their salts	Food additive	ADI of "not specified" was established Only L-lactic acid should be used in infant foods
EFSA, 2009	Aliphatic acyclic diols, triols and related substances	Flavoring substance	Insufficient information were available for EFSA to draw a conclusion
JECFA, 1974b	Lactic acid and its ammonium, calcium, potassium and sodium salts	Food additive	ADI of "not limited" was established Neither D-lactic acid nor DL-lactic acid should be used in infant foods

Abbreviations: ADI = acceptable daily intake; EFSA = European Food Safety Authority; JECFA = Joint FAO/WHO Expert Committee on Food Additives; SCF = Scientific Committee on Food.

6.1.4.2 ADME of Lactic Acid

Studies to evaluate the metabolic fate of lactic acid are available in the published literature and the majority have been evaluated by JECFA (1974b). A summary of these studies is provided below.

Lactic acid is an endogenous carboxylic acid and normal intermediary of carbohydrate and amino acid metabolism. Lactate produced by mammalian cells is L-lactate and this is the main isomer in blood whereas D-Lactate is normally present in very low concentrations (Ling *et al.*, 2012). L-Lactate is rapidly metabolized to pyruvate by L-lactate dehydrogenase, whereas D-lactate is converted to pyruvate by D-α-hydroxy acid dehydrogenase which metabolizes D-lactate at about one-fifth the rate that L-lactate dehydrogenase metabolizes L-lactate (Ewaschuk *et al.*, 2005). Absorbed lactic acid will ultimately be oxidized in animals to carbon dioxide and water.

Groups of rats (no further detail stated) were administered 1,700 mg/kg of L-, D-, or DL-lactate orally or by subcutaneous injection (Cori and Cori, 1929). The largest rise in liver glycogen was displayed following administration of L-lactate, with 40 to 95% of the absorbed dose being converted within 3 hours. Almost no glycogen was formed from the D-isomer. The highest blood lactate levels were observed following administration of D-lactate with 30% of the amount absorbed excreted into the urine. No L-lactate was identified in the urine. The authors concluded that both D- and L-isomers were absorbed at the same rate from the intestine, but D-lactate was utilized four times more slowly.

In another study, groups of male and female rats (6/sex/group) received sodium DL-lactate in the diet at a concentration of 215 mg/kg body weight (Cori, 1930). The absorption of sodium DL-lactate from the intestine was determined 1, 2, 3 and 4 hours after ingestion. The rate of absorption decreased with time and was roughly proportional to the amount of lactate present in the gut. The rate of absorption in some animals was limited by slow evacuation of the stomach.

Rabbits with blood levels of lactate of 200 or 250 mg/L, displayed excitation, dypnoea and tachycardia (Collazo *et al.*, 1933; cited in JECFA 1974b – limited details available). In another oral study in rabbits, animals were administered 600 to 1,600 mg/kg bodyweight of DL-lactate (Fürth and Engel, 1930). Most

animals were reported to die within three days. The urinary excretion of lactate varied from 0.26 to 0.31% and was not affected by alkalosis.

Although mammalian tissues only produce L-lactate, studies in tissues of the brain, heart, kidney and liver from ducks and rats indicate that both L- and D-isomers can be oxidized (Brin, 1965). Measurements of oxygen consumption and carbohydrate synthesis in rat liver showed that L-lactate was utilized almost entirely but virtually no D-isomer was used. Small but measurable amounts of D-lactate were utilized by rat kidney tissue, but none was used by the grey brain matter. L-Lactate was observed to stimulate oxygen consumption and carbon dioxide consumption in all rat tissues, whereas D-lactate only slightly stimulated respiration of the liver and heart tissue. The results of studies in duck tissue were similar, with L-lactate found to be oxidized three to five times more rapidly than D-lactate in heart and liver slices, and ten to twenty times more rapidly by brain tissue. D-Lactate was utilized to the same extent as the L-isomer in the duck and rat heart slices but to a lesser extent by the brain and liver tissues.

The lactic acid raw material used in the manufacture of C12/C14 lactylates is 98% L-isomer, with only minor levels of the D-isomer. Consequently, lactic acid from C12/C14 lactylates should be readily absorbed and utilized by animals.

6.1.4.3 History of Use of Lactic Acid in Feed

Lactic acid and calcium lactate are listed in 21 CFR §582.1061 and 21 CFR §582.1207 as GRAS for use as general purpose food additives in the feed and drinking water of animals when used in accordance with good manufacturing or feeding practice (U.S. FDA, 2021). No use levels of lactic acid or its calcium salt are specified in the U.S. beyond the minimum required to achieve the intended effect in accordance with good feeding or manufacturing practices. However lactic acid and its calcium salt are also authorized for use as preservatives in water and feed for animals in the EU (EC, 2022), and in the recent evaluations of these ingredients in feed in the EU outlined above (see Section 6.1.4.1), the applicant indicated that the maximum use level of lactic acid in feed for pigs and ruminants but excluding pre-ruminants, was 50 g/kg complete feed. For all other species except pre-ruminants, a maximum use level of 20 g/kg complete feed was proposed (EFSA, 2019). EFSA concluded that based on the available data, the levels of use proposed by the applicant do not pose a safety concern for animals, and therefore, it is reasonable to conclude that the amount of lactic acid in feed can be in the region of 50 g (pigs and ruminants) and 20 g (poultry and other animals)/kg complete feed in the EU. On the basis that lactic acid is a preservative and the amount added to feed will be that required to achieve the desired effect, it is also likely that the use levels in feed in the U.S. can reach similar amounts (FEFANA, 2014; (b) (4) , 2022).

By comparison, under the intended conditions of use of C12/C14 lactylates in feed proposed by Corbion of not more than 1.8 g/kg complete feed, animals will be exposed to 0.5 g lactic acid/kg complete feed. Animals consuming C12/C14 lactylate will therefore, be exposed to lactic acid levels which are 100-fold less than the level of 50 g lactic acid/kg complete feed reported in the EU as representing the practical use of the organic acid as a preservative for pigs and ruminants. Similarly, animals consuming 1.8 g C12/C14 lactylates/kg complete feed will be exposed to levels of lactic acid 40 times lower than from the use of the organic acid as a preservative at 20 g/kg complete feed for poultry and other non-ruminant and non-swine species. Thus, evidence of the safety of the lactic acid component of C12/C14 lactylates is

provided by the significantly higher levels of existing use of the acid and its calcium salt as a preservative in animal feed.

6.1.4.4 Background Exposure to Lactic Acid from the Diet

Swine

Fermented liquid feeds (FLFs), prepared by mixing dry feed with water and storing under conditions which allow lactic acid bacteria (LAB) and yeasts in the feed ingredients to act and reach steady state, have a history of use in animal feed, particularly swine feed, in the U.S. (EFSA, 2006; Plumed-Ferrer and von Wright, 2009; Missotten *et al*., 2015). The fermentation may be natural or controlled and the objective is to generate a feed with low pH which helps retard the growth of pathogenic microorganisms in feed and also can have a positive impact on the digestive health of the animal. The lactic acid content is generally recognized to be critical to the effectiveness of the FLF, with reports of desirable levels being in the range of 100 or 150 mmol/L primarily as L-lactic acid (EFSA, 2006; Missotten *et al*., 2015). Assuming that a weaned piglet of 20 kg consumes 4 L of FLF containing 150 mmol/L of lactic acid, the estimated exposure to the organic acid is 27 to 54 g/day (EFSA, 2006). By comparison, a piglet of 20 kg consuming 1 kg complete feed/day (EFSA, 2017c) containing C12/C14 lactylates at the maximum use level of 1.8 g/kg complete feed will be exposed to 0.5 g of lactic acid, which is >50-fold lower than from FLF. Thus, pigs can consume levels of lactic acid from the normal diet which are significantly higher than from the intended use of C12/C14 lactylates in feed.

Ruminants

Lactic acid bacteria present naturally in crops are responsible for the fermentation of water-soluble carbohydrates and the production of lactic acid during the ensiling process. Likewise, LAB can be utilized as additives to enhance the preservation and quality of the silage (Charmley, 2001; Muck *et al*., 2018). Lactic acid levels in corn silage for example, were reported to vary from 6.35 to 7.72% DM either untreated or treated with one of a number of *Lactobacillus* species (Ranjit and Kung, 2000). The ratio of L- and D-lactic acid was approximately 1:1 across all of the silages tested. Other studies indicate that silages which have undergone extensive homolactic fermentation can contain lactic acid at levels of 10 or 15% DM (McDonald *et al*., 1991; Charmley, 2001). The relative amounts of L- and D-lactic acid vary depending on the inoculant used, with up to 90% D-lactic acid produced by LAB isolates obtained from forages and crops by Cai *et al*. (1998). Assuming that silage represents around 35% DM of the TMR for cattle (NRC, 2001), animals can be exposed to levels of lactic acid of 2.5 to 5.3% (25 to 30 g/kg DM) from its natural presence in the feed. By comparison, ruminants are estimated to be exposed to 0.05% (or 0.5 g/kg DM) of lactic acid from the maximum intended use of C12/C14 lactylates in the diet of 1.8 g/kg complete feed, which is >50-fold lower. Thus, ruminants can consume levels of lactic acid from the normal diet which are significantly higher than from the intended use of C12/C14 lactylates in feed.

Summary and Conclusions

Swine and ruminants can be exposed to lactic acid from its natural presence in FLF or ensiled forages at significantly higher levels than anticipated from the use of C12/C14 lactylates as a source of lauric and myristic acids in animal feed. The safety of the lactic acid component of C12/C14 lactylates for swine

and ruminants can be established on the basis of the significantly higher background exposure from the normal diet.

6.1.4.5 Studies in the Target Animals

Other Target Animal Studies using Lactic acid

There are a number of studies in the published literature in which lactic acid was fed to poultry, swine and ruminants. A detailed evaluation of these studies was not considered necessary to establish the safety of the lactic acid component of C12/C14 lactylates on the basis of the information on background intakes outlined above. However, for completeness, a summary of the feeding studies identified in the literature are provided in Appendix 019 an the overall findings were briefly as follows:

- Broilers were fed diets of up to 30,000 mg/kg complete feed in the diet for 35 to 42 days with no adverse effects reported on performance or routine blood parameters. Under the conditions of intended use of C12/C14 lactylates in feed, broilers will be exposed to up to 1.8 g C12/C14 lactylates/kg complete feed equivalent to 0.5 g (or 500 mg) lactic acid/kg complete feed. Therefore, a margin of safety of 60 can be established from the results of these tolerance studies.
- Cats tolerated lactic acid when fed for one year at a level of 1.2% (12,000 mg/kg complete feed) in the diet. Under the conditions of intended use of C12/C14 lactylates in feed, broilers will be exposed to up to 1.8 g C12/C14 lactylates/kg complete feed equivalent to 0.5 g (or 500 mg) lactic acid/kg complete feed. Therefore, a margin of safety of 24 can be established from the results of these tolerance studies.
- One study in goldfish was identified in which lactic acid appeared to be well-tolerated at 0.2% in the feed.
- Swine were fed diets containing lactic acid at varying levels of lactic acid, including 1 or 2% in the diet with no reports of adverse effects. The studies were not designed to support safety but provide evidence of the ability of pigs to tolerate lactic acid.
- Only short-term studies were identified in ruminants which were not considered relevant to safety.

Taken together, these data along with the estimates of background exposure to lactic acid from the diet, indicate that the consumption of lactic acid as a component of C12/C14 lactylates under the conditions of intended use will not pose a safety concern.

Study in Broilers using Lactic Acid

An unpublished study was summarized by EFSA in its most recent opinion on lactic acid and calcium lactate (EFSA, 2019). The study was conducted using lactic acid comprising 90% L-lactic acid and 10% D-lactic acid. The lactic acid component of C12/C14 lactylates comprises 98% of the L-isomer with only low levels of the D-isomer. Thus, absorption and metabolism of lactic acid should be sufficiently similar for the results of the broiler study to be extrapolated to the acid component of C12/C14 lactylates.

In the study, 1,320 one-day old Ross 308 broilers were allocated to 60 floor pens each containing 22 chickens such that each treatment was replicated 10 times. The animals were fed corn/wheat/SBM-based diets containing 0 (control, Treatment 1), 10,000 (Treatment 2), 20,000 (Treatment 3) or 30,000

(Treatment 4) mg lactic acid/kg complete feed for 42 days. Two phase mash feeds were provided, a starter feed (days 1 to 21) and grower feed (days 22 to 42). General health and mortality were monitored daily. Growth, body weight, feed intake and FCR were evaluated at days 1, 21 and 42 of the study. At day 42, 20 chickens per treatment (2 birds/pen) were slaughtered and subject to necropsy. Organ weights and gross histopathology of tissues was performed. Blood samples were collected from the same birds and were analyzed for routine clinical chemistry and hematology parameters. Tibia bones also were collected from 20 birds per treatment (2 birds/pen) and analyzed for calcium and phosphorus. Feces excreted were collected over a 3-day period (days 40 to 42) and analyzed for DM content, calcium and phosphorus.

The health of the animals was considered normal throughout the study, and no adverse events were noted. Total mortality/cull ratio after 42 days was 66/1,320 birds (5%). There was no effect of lactic acid treatment on average daily feed intake (ADFI), average daily gain (ADG) or FCR. All blood parameters were within normal ranges/historic controls for the weight and age of the birds studied. Relative to the control group (T1), there were no significant differences in hematology parameters, metabolites or blood enzymes of electrolytes analyzed among treatments with the exception of hematocrit. A significantly elevated percent hematocrit (HCT) level was reported in birds fed diets containing 20,000 mg lactic acid/kg complete feed (Treatment 2) compared to the control birds (Treatment 1) but the same effect was not observed in birds fed 30,000 mg lactic acid/kg complete feed (Treatment 3). Thus, the effect was not considered to be related to treatment. No gross findings were observed in any organs and tissues examined. Tibia ash, Ca and P levels, as well as Ca and P digestibility was not affected by lactic acid treatment. Under the experimental conditions of the study, the authors concluded that the findings of the study indicate lactic acid is well-tolerated by broilers at levels of up to 30,000 mg lactic acid/kg complete feed.

Under the conditions of intended use of C12/C14 lactylates in feed, broilers will be exposed to up to 1.8 g C12/C14 lactylates/kg complete feed equivalent to 0.5 g (or 500 mg) lactic acid/kg complete feed. Therefore, a margin of safety of 60 can be established from the results of the tolerance study. The findings of the study provide corroborating evidence of the safety of the lactic acid component of C12/C14 lactylates for poultry under the conditions of intended use.

6.1.4.6 Safety for Pre-Ruminants

In the original EFSA opinion on lactic acid and calcium lactate for use in animals, it was concluded that: *"In the absence of data, no conclusions on the safety of lactic acid in pre-ruminants can be drawn."* Furthermore, it was recognized that: "*When the rate of D-lactate production exceeds the body's capacity for its metabolism and excretion, D-lactate accumulates in the blood causing metabolic acidosis. D-lactate acidosis is well known and described in veterinary medicine as a consequence of grain overfeeding in adult ruminants and in neonatal animals with diarrhea (Ewaschuk et al., 2005).*"

D-lactic acidosis is recognized as a contributory factor to acidemia in calves diagnosed as ruminal drinkers (Ewaschuk *et al*. 2005). Excessive consumption or a malfunctioning of the esophageal groove leads to the accumulation of milk in the rumen resulting in fermentation of lactose and D-lactic acidosis. Likewise, D-lactic acid production is a recognized secondary complication in diarrheic calves, representing around 64% of the total increase in organic acids.

C12/C14 lactylates is not intended for use in milk replacer and therefore, the concerns raised by EFSA largely do not apply to this assessment. However, for young ruminants transitioning to feed, exposure to C12/C14 lactylates may be up to 1.8 g/kg complete feed, equating to 0.5 g lactic acid/kg complete feed. As mentioned above, only 2% of the lactic acid component will be in the form of D-lactate and therefore, under practical conditions of use of C12/C14 lactylates, D-lactic acidosis is not expected to pose a safety concern for non-functional ruminants.

6.1.4.7 Toxicological Information on Lactic Acid and Its Salts

The acute toxicity of lactic acid has been assessed in several animal studies. The LD_{50} values determined in these studies ranged from 1,810 to 4,875 mg/kg body weight and are summarized in Table 6.13. Rats have been reported to survive subcutaneous doses of 2,000 to 4,000 mg lactic acid/kw body weight, but mice were killed by subcutaneous doses in the same range (Fürth and Engel, 1930; Cited in JECFA, 1974b).

Table 6.13: Acute LD_{50} Values Determined for Lactic Acid

Test Compound	Animal Model	Route	LD_{50} (mg/kg BW)	Reference
Sodium lactate	Rat	Intraperitoneal	2,000	Rhône-Poulenc, 1965 [Cited in JECFA, 1974b]
Lactic acid	Rat	Oral	3,730	Smyth *et al.*, 1941 [Cited in JECFA, 1974b]
Lactic acid	Guinea pig	Oral	1,810	Smyth *et al.*, 1941 [Cited in JECFA, 1974b]
Lactic acid	Mouse	Oral	4,875	Fitzhugh, 1945 [Cited in JECFA, 1974b]

Abbreviations: LD_{50} = median lethal dose.

Repeat dose studies indicate that short-term oral administration of lactic acid and its salts were not associated with adverse effects in rats and dogs at doses up to 2,000 or 1,600 mg/kg body weight/day, respectively. A short-term (14 to 16 day) feeding study in rats, in which animals received daily doses of 1,000 and 2,000 mg/kg body weight sodium lactate (as lactic acid) reported no accumulation effects (Fürth and Engel, 1930; cited in JECFA, 1974b). In another short-term study in dogs, 2 dogs received 600 to 1,600 mg 42 times during a two and half month period and no ill effects were reported (Faust, 1910; cited in JECFA, 1974b). Feeding 10% lactic acid to birds (species and duration not stated) was attributed to the development of polyneuritic crises resembling B1 deficiency on diets rich in carbohydrates, proteins or fats. No long term studies were available.

In summary, only limited toxicological data are available on lactic acid but relatively high levels of administration appear to be tolerated by animals. Overall, the findings from the toxicity studies support the safety of lactic acid as a component of C12/C14 lactylates under the intended conditions of use.

6.1.5 Information to Establish the Safety of the Sodium Component of C12/C14 Lactylates for the Target Animals

It is recognized that C12/C14 lactylates will also provide a source of sodium. The sodium content of C12/C14 lactylates is limited to not more than 8% by the product specifications for the ingredient (see Table 2.8). Under the conditions of intended use, the maximum C12/C14 lactylates content of complete feed is 1.8 g/kg, equating to a maximum of 0.14 g sodium/kg. The requirements of animals for sodium (or sodium chloride) varies with species but typically falls within the range of 1 to 2 g sodium/kg complete feed (Berger, 2006). Thus, the sodium component of C12/C14 lactylates will contribute around 7 to 14% to the sodium requirements of animals and will be taken into account by nutritionists in formulating the feed of animals. Overall, it may be concluded that the sodium content of C12/C14 lactylate will not pose a safety concern to target animals under the conditions of intended use.

6.1.6 Information to Establish the Safety of the Sulfate Component of C12/C14 Lactylates for the Target Animals

As mentioned in Part 2, sodium sulfate is added to the market formulation, ALOAPUR® PM as a processing aid at a level of less than (b)(4)%. The total sulfate content will be a combination of the contribution from the added sodium sulfate and the diatomaceous earth and mean values of sulfate and sulfur from 3 representative lots of ALOAPUR® PM were determined analytically to be 4,967 and 1,667 mg/kg, respectively.

Dietary sulfates are well absorbed by animals and ultimately excreted in the urine (Magee *et al.*, 2004; NRC, 2005). The NRC has established MTLs for sulfur in the complete feed of 0.4% DM for poultry and swine, 0.3% DM for cattle and sheep fed high concentrate diets and 0.5% DM for cattle and sheep fed high-forage diets (NRC, 2005). The MTL of sulfur in feed for horses is derived from interspecies extrapolation to be 0.5% DM and too little is known of sulfur toxicity in fish to calculate a limit (NRC, 2005). Notably, non-ruminants are far less likely to develop sulfur toxicosis than ruminants (NRC, 2005).

In non-ruminant and non-pseudo-ruminant feeds containing ALOAPUR® PM at the maximum level (5 g/kg as-fed), the sulfur content is estimated to be around 9.5 mg/kg (*ca.* 0.001%) DM[11] which is negligible compared to the MTLs of sulfur in poultry and swine feeds of 0.4% DM.

Likewise, 5 g ALOAPUR® PM/kg in the TMR (45% DM) of ruminants contains around 17.4 mg/kg DM (0.002%) DM[12] of sulfur which makes only a minor contribution to the MTLs of 0.3 to 0.5% DM for sulfur in cattle and sheep feeds.

It is also reported that 0.3% DM of sulfate in the diet of cattle can result in reduced feed intake (Knight, 1985). By comparison, the sulfate content of the TMR from the addition of 5 g/kg of ALOAPUR® PM containing 4,967 mg of sulfate will be in the region of 14 mg/kg as-fed or 31 mg/kg DM basis[13] (i.e.,

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[11] Calculation: mean sulfur content of 1,667 mg/kg in ALOAPUR® PM is equivalent to 8.3 mg/kg complete feed (12% moisture) or 9.5 mg/kg complete feed on a DM basis.
[12] Calculation: mean sulfur content of 1,667 mg/kg in ALOAPUR® PM is equivalent to 8.3 mg/kg complete feed or 17.4 mg/kg DM assuming a 45% DM content of the TMR.
[13] Calculation: mean sulfate content of 4,967 mg/kg in ALOAPUR® PM is equivalent to 14 mg/kg complete feed or 31 mg assuming a 45% DM content of the TMR.

around 100 times lower than 0.3% DM). Thus, the sulfate content of ALOAPUR® PM is not expected to pose a safety concern to target animals under the conditions of intended use.

6.2 INFORMATION TO ESTABLISH HUMAN FOOD SAFETY

As discussed in Part 3 of the GRAS Notice (Section 3.2), the use of C12/C14 lactylates as a source of lauric acid and myristic acid in the diet of animals should not lead to the deposition of substances not normally present, or at levels not usually observed, in the edible tissues of animals. Thus, no human food safety concerns are anticipated under the conditions of intended use of C12/C14 lactylates in the diets of food-producing animals at levels of up to 1.8 g/kg complete feed as-fed, corresponding to 5 g/kg complete feed of ALOAPUR® PM.

6.3 SUMMARY AND BASIS FOR GRAS CONCLUSION

Corbion intends to market C12/C14 lactylates for use as a source of lauric and myristic acids for animals at levels not exceeding 1.8 g/kg complete feed.

C12/C14 lactylates (b) (4) which is marketed under the trade name ALOAPUR® PM. The use of the diatomaceous carrier allows C12/C14 lactylates to be supplied in a homogenous powdered form which is readily mixed with finished feeds. The manufacturing process is conducted in accordance with cGMP, and a Hazard Analysis Critical Control Point plan is in place. The manufacturer will comply with the requirements for importing feed into the U.S. laid down by the Food Safety Modernization Act (FSMA) including the foreign supplier verification program (FSVP).

Appropriate feed grade specifications have been established for C12/C14 lactylates which include measurement of the AV and EV which confirm the mixture is an equilibrium mixture of free fatty acids and lactic acid esters, as well as criteria to control levels of heavy metal contaminants. Conformance with the product specifications was demonstrated by analytical data on 5 commercial lots of C12/C14 lactylates. Additional analytical data on 5 commercial lots of C12/C14 lactylates verified that no levels of dioxins or PCBs were present above detection limits.

Additionally, analytical data were provided on representative commercial lots of the market formulation, ALOAPUR® PM, demonstrating that a consistent product is manufactured which conforms with proposed compositional and contaminant specifications. Sodium sulfate is used as a processing aid in ALOAPUR® PM and the level is limited to less than (b) (4) %.

A shelf-life of 24 months is proposed for C12/C14 lactylates in the form of ALOAPUR® PM when stored unopened in the original packaging under cool and dry conditions. Stability data were provided for one representative commercial lot of ALOAPUR® PM to verify the shelf-life of C12/C14 lactylates under real-time conditions (25°C, 60% RH). A further 2 commercial lots of ALOAPUR® PM stored under warehouse conditions were shown to conform with product specifications after 5 to 6 years of storage. An accelerated shelf-life study was also conducted in which 3 representative lots of ALOAPUR® PM were stored for 6 months at 40°C and 75% RH. These studies confirm that the during prolonged storage, a

small amount of hydrolysis of the ester bond to form the free fatty acids can occur. These findings are not considered to impact the utility or safety of the product on the basis that hydrolysis of the esters occurs rapidly on ingestion of C12/C14 lactylates to yield free fatty acids and lactic acid which are absorbed and utilized by the animal.

Studies in feed demonstrate that C12/C14 lactylates can be mixed homogeneously into feed and exhibits acceptable stability to the pelleting process. Feed containing C12/C14 lactylates also displays acceptable stability when stored under ambient conditions for 3 months.

C12/C14 lactylates comprises an equilibrium mixture of sodium lauroyl-1-lactylate (*ca*. 33%), sodium myristoyl-1-lactylate (*ca*. 14%), sodium laurate (C12 fatty acid; *ca*. 18%), sodium myristate (*ca*. 8%), sodium lactate (*ca*. 9%), sodium lauroyl-2-lactylate (*ca*. 6%), sodium myristoyl-2-lactylate (*ca*. 2%), sodium lactoyl lactate (*ca*. 3%) and minor amounts of oligomers of lactate (not quantified). In this respect, animals will be provided with lauric (C12) and myristic (C14) acids as well as lactic acid in the free acid (or sodium salt) and esterified form. Based on this typical composition, C12/C14 lactylates is calculated to comprise around 42% lauric acid, 19% myristic acid and 28% lactic acid. The total fatty acids content is therefore, in the region of 61% (i.e., lauric acid + myristic acid). (b) (4)

C12/C14 lactylates is intended for use as a source of lauric and myristic acids in feed at levels in the region of 0.7 g/kg complete feed and not exceeding 1.8 g/kg complete feed. The equivalent levels of ALOAPUR® PM (the market formulation) are 2 g and 5 g/kg complete feed, respectively. At the maximum intended level of 1.8 g C12/C14 lactylates/kg complete feed, animals are estimated to be provided with 0.8 g lauric acid, 0.3 g myristic acid and 0.5 g lactic acid/kg complete feed. The combined lauric acid and myristic acid intake from C12/C14 lactylates is estimated to be around 1.1 g/kg complete feed. The target animals are all major and minor food-producing animals as well as companion animals.

Medium- and long-chain saturated fatty acids have recognized nutritional value for animals although no specific dietary requirements are set. C12/C14 lactylates are intended to provide a supplemental source of these fatty acids, contributing to the overall fatty acid profile of the complete feed. The fatty acid-containing ingredient will be included in the diet alongside other common fats and fat-containing nutrient sources. The fatty acid and energy requirements of the animal will largely be met by these other nutrient sources, and no detrimental impact on the nutritional quality of the feed is anticipated under the conditions of intended use of C12/C14 lactylates in feed.

The lactic acid esters of lauric and myristic acid will be enzymatically hydrolyzed to lauric acid, myristic acid and lactic acid in the GI tract of animals. Evidence for the hydrolysis of C12/C14 lactylates by gastric lipases is primarily provided by findings of a series of published *in vitro* studies (Phillips *et al*., 1981) using a structurally related substance, calcium stearoyl-lactylate. Unpublished *in vitro* studies provide corroborative evidence of hydrolysis by gastric lipases (Hodge, 1961). Lactic acid esters of lauric acid, myristic acid and stearic acid are expected to be hydrolyzed in an analogous manner in the GI tract of animals and it is reasonable to extrapolate available ADME data on calcium stearoyl-lactylate to C12/C14 lactylates. Direct evidence for the validity of this extrapolation is provided by an unpublished study in broilers conducted by Corbion demonstrating the absorption or digestion of C12/C14 lactylates from the GI tract.

The metabolism of the structurally related lactylate, calcium stearoyl (C18) lactylate also has been studied in the series of published ^{14}C-labeling experiments in rats, mice and guinea pigs conducted by Phillips *et al*., 1981. The findings of these studies indicate that lactylates are rapidly absorbed from the GI tract as their component parts and extensively metabolized by animals. Consistent with hydrolysis of lactylates to their constituents, the metabolism of a mixture of stearic and lactic acids was shown to be comparable to that of calcium stearoyl-lactylate in an unpublished radiolabeled study in rats (Hodge, 1955).

Together, the published data, supported by further body of unpublished information, demonstrate that C12/C14 lactylates will be hydrolyzed by gastric lipases in animals to lauric acid, myristic acid and lactic acid. Thus, the toxicological assessment of C12/C14 lactylates can be based on publicly available information on the individual components.

Medium- and long-chain saturated fatty acids such as lauric acid and myristic acid are well-documented in the published literature as normal components of vegetable and animal fats. No differences are anticipated in the metabolism of lauric and myristic acids as components of C12/C14 lactylates and as constituents of biological fats forming part of the normal diet. In general, fat products marketed in the U.S. under Section 33 of the AAFCO OP will potentially provide natural sources of these fatty acids in the animal feed. An assessment was conducted to estimate the potential exposure by animals to lauric and myristic acids from CO, CM and PKM at typical levels of inclusion in the diet of animals. These ingredients were identified to be particularly rich in lauric and myristic acids and although they are not the most common nutrient sources used in feed in the U.S., they have some recognized history of use and are widely utilized in other parts of the world. Overall, it was estimated that lauric and myristic acid from products of the coconut and palm kernel industries are present at significantly higher dietary levels in feed than will be provided by C12/C14 lactylates. Thus, the safety of the lauric and myristic acid components of C12/14 lactylates can be established from their natural presence in the background diet of animals.

Additionally, lauric and myristic acid have a long and established history of use as additives in food and as such have been the subject of evaluations by EFSA (2017a) and JECFA (1998). As part of these evaluations, EFSA and JECFA have reviewed a body of limited toxicological information on lauric and myristic acid, including the results of acute oral toxicity studies, *in vitro* mutagenicity assays and an 18-week feeding study in rats. Overall, the studies provide supporting evidence for the safety of the lauric and myristic acid components of C12/C14 lactylates under the conditions of intended use.

Lactic acid and calcium lactate have a long and established history of safe use as a technological additive (preservative) in feed and food in the U.S., the EU and elsewhere. The acid is rapidly absorbed by animals and ultimately metabolized to carbon dioxide and water. Animals will be exposed to lactic acid from the normal diet, particularly through the use of FLF for pigs and ensiled forages for ruminants. The estimated levels of exposure by swine and ruminants to lactic acid from these feeds is significantly higher than from the conditions of intended use of C12/C14 lactylates. Likewise, the use of lactic acid as a preservative in feed is estimated to be higher than the exposure by animals from the intended use of C12/C14 lactylates. Recognizing that exposure by poultry to lactic acid from the background diet to poultry is lower than for swine and ruminants, additional evidence of safety for these species are provided by a body of published data, as well as one unpublished study reported by EFSA (2019), in

which broilers tolerated levels of 30,000 mg/kg of lactic acid for 35 to 42 days. A margin of safety of 60 can be calculated from the study compared to the intended use of lactic acid as a component of C12/C14 lactylates in feed at levels of 1.8 g/kg complete feed, equivalent to 0.5 g lactic acid/kg complete feed. Thus, the findings of these studies support the extrapolation of data on the safety of lactic acid to all target animals, including avian species. Only limited toxicological information is available on lactic acid and its salts, including acute oral toxicity studies and a number of feeding studies in rodents and dogs. The results of these studies also provide supporting evidence for the safety the intended use of C12/C14 lactylates in feed.

It is recognized that C12/C14 lactylates also contains up to 8% sodium. Under the conditions of intended use, animals will be exposed to not more than 1.8 g C12/C14 lactylates/kg complete feed, equating to 0.14 g sodium/kg complete feed. The requirements of animals for sodium varies with species but is reported by the NRC to typically fall within the range of 1 to 2 g sodium/kg complete feed. Thus, the sodium component of C12/C14 lactylates will contribute around 7 to 14% of the dietary requirements for this mineral and is not therefore, expected to pose a safety concern to the target animals. Additionally, ALOAPUR® PM contains <(b) (4)% sodium sulfate and the contribution of this component to the MTL of sulfur in the feed of animals is negligible. Moreover, the amount of sulfate present in the feed from the intended use of C12/C14 lactylates falls 100 times below the level of 0.3% known to pose tolerability concerns to ruminants.

The data outlined above are considered sufficient to demonstrate the safety of C12/C14 lactylates for the intended use in animal feed. Moreover, sufficient data are available in the public domain to draw key conclusions on the safety of C12/C14 lactylates and these data, together with corroborative unpublished data, allow extrapolation to all categories and species of animal on the basis that (a) the metabolic fate of C12/C14 lactylates is common in all animals; (b) lauric acid, myristic acid and lactic acid are metabolized by well-established pathways; and (c) there is a long and established history of safe consumption of these components from the background diet as well as from additive use (lactic acid).

Considering that C12/C14 lactylates will be hydrolyzed into lauric acid, myristic acid and lactic acid which will be metabolized by established fatty acid and tricarboxylic acid pathways in animals, no deposition of new substances, or existing substances at higher levels normally observed, is anticipated in the edible tissues under the conditions of intended use. Thus, no human food safety concerns are anticipated under the conditions of intended use of C12/C14 lactylates in the diets of food-producing animals at levels of up to 1.8 g/kg complete feed as-fed.

Following a critical evaluation of the data and information summarized above, it can be concluded that C12/C14 lactylates produced by Corbion using suitable food-grade materials in accordance with cGMP and meeting appropriate feed grade specifications, is safe and suitable for the intended use as a source of lauric and myristic acids in animal feed. It is further concluded that Corbion's C12/C14 lactylates is generally recognized as safe (GRAS) for use in feed based on scientific procedures.

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

7.1 LIST OF APPENDICES

ALL APPENDICES EXCEPT APPENDICES 001, 008, 012A, B and C, 017 and 019 ARE CONSIDERED CONFIDENTIAL. NAMES OF CONFIDENTIAL APPENDICES ARE HIGHLIGHTED IN GREY.

In a number of Appendices, "ALOAPUR®" is used to describe the market formulation rather than the current name of "ALOAPUR® PM". These trade names describe the identical product (see Appendix 001).

PURAC 30S and PURAC 30L are preparations of C12/C14 lactylates (38-42%) on diatomaceous earth and monopropylene glycol (58-62%), respectively which were used as developmental products prior to the final market formulation (ALOAPUR® PM) being manufactured.

Appendix 001	Nomenclature (ALOAPUR® and ALOAPUR® PM)
Appendix 002A	(b) (4)
Appendix 002B	
Appendix 002C	
Appendix 002D	
Appendix 002E	
Appendix 002F	
Appendix 002G	
Appendix 002H	
Appendix 002I	
Appendix 003A	
Appendix 003B	
Appendix 004	
Appendix 005	
Appendix 006	
Appendix 007A	
Appendix 007B	
Appendix 007C	
Appendix 007D	
Appendix 007E	
Appendix 007F	
Appendix 007G	
Appendix 007H	
Appendix 007I	
Appendix 007J	
Appendix 007K	
Appendix 007L	
Appendix 007M	
Appendix 007N	
Appendix 007O	
Appendix 007P	
Appendix 007Q	

Appendix 007R	(b) (4)
Appendix 007S	
Appendix 007T	
Appendix 007U	
Appendix 007V	
Appendix 007W	
Appendix 007X	
Appendix 008	Ash Method
Appendix 009A	(b) (4)
Appendix 009B	
Appendix 009C	
Appendix 009D	
Appendix 009E	
Appendix 010	
Appendix 011	
Appendix 012A	
Appendix 012B	
Appendix 012C	
Appendix 013A	
Appendix 013B	
Appendix 014A	
Appendix 014B	
Appendix 015A	
Appendix 015B	
Appendix 016	
Appendix 017	Compositional Calculations (ALOAPUR® PM)
Appendix 018	Broiler Metabolism Study Raw Data (Study No. (b) (4)
Appendix 019	Summary of Published Studies in Target Animals using Lactic Acid

7.2 LIST OF ABBREVIATIONS

AAFCO	Association of American Feed Control Officials
AAS	Atomic Absorption Spectrometry
AC	Absorption Coefficient
ADFI	Average Daily Feed Intake
ADG	Average Daily Gain
ADI	Acceptable Daily Intake
ADME	Absorption, Distribution, Metabolism and Excretion
AV	Acid Value
BSFL	Black Soldier Fly Larvae
bw	Body Weight
BWG	Body Weight Gain
CAS No.	Chemical Abstracts Service Registry Number
CFR	Code of Federal Regulations
cGMP	current Good Manufacturing Practice
CM	Coconut Meal
CO	Coconut Oil
CSL	Calcium Stearoyl Lactate
CVs	Coefficients of Variation
DHA	Docosahexaenoic Acid
DM	Dry Matter
EFSA	European Food Safety Authority
EPA	Eicosapentaenoic Acid
EU	European Union
EV	Ester Value
FAO	Food and Agricultural Organization
FCR	Feed Conversion Ratio
FDA	Food and Drug Administration
FEMAS	Feed Materials Assurance Scheme
FFDCA	Federal Food, Drug and Cosmetic Act
FI	Feed Intake
FLF(s)	Fermented Liquid Feed(s)
FO	Fish Oil
FSMA	Food Safety Modernization Act
FSVP	Foreign Supplier Verification Program
GC	Gas Chromatography
GI	Gastrointestinal
GMP+	Good Manufacturing Practice
GPCR	G-Protein Coupled Receptors
GRAS	Generally Recognized as Safe
HACCP	Hazard Analysis and Critical Controls Point
HCT	Hematocrit
ICP-OES	Inductively Coupled Plasma-Optical Emission Spectrometry
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LAB	Lactic Acid Bacteria
LD_{50}	Median Lethal Dose

LDPE	Low Density Polyethylene
LLOQ	Lowest Level of Quantification
LSD	Least Significant Difference
MCT	Medium-Chain Triglycerides
MT	Metric Ton
MTL	Maximum Tolerable Limits
MW	Molecular Weight
NOEL	No Observed Effect Level
NRC	National Research Council
OECD	Organization for Economic Co-operation and Development
PCB	Polychlorinated Biphenyls
PCDD	Polychlorinated Dibenzo-p-Dioxins
PCDF	Polychlorinated Dibenzofurans
PKM	Palm Kernel Meal
PKO	Palm Kernel Oil
PO	Palm Oil
PP	Polypropylene
PUFAs	Polyunsaturated Fatty Acids
RH	Relative Humidity
SCF	Scientific Committee on Food
SI	Small Intestine
SO	Soy Oil
Spec.	Specification
SV	Saponification Value
T	Time
TEQ	Toxic Equivalency
TLC	Thin Layer Chromatography
TMR	Total Mixed Ration
UDS	Unscheduled DNA Synthesis
U.S.	United States
WHO	World Health Organization

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

7.3 REFERENCES

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Section/Definitions	Description
Section 33 and definitions therein	Definitions for fats and oils
33.2	Vegetable fat, or oil
33.3	Hydrolyzed _ fat, or oil, feed grade
57.109	Sodium sulfate
71.60	Coconut meal, mechanical extracted
71.61	Coconut meal, solvent extracted

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Section	Title
172.844	Calcium stearoyl-2-lactylate
172.846	Sodium stearoyl lactylate
172.848	Lactylic esters of fatty acids
573.340	Diatomaceous earth
582.1061	Lactic acid
582.1207	Calcium lactate
582.1763	Sodium hydroxide

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Declaration ALOAPUR equivalent to ALOAPUR PM

Declaration

Herewith we, Corbion bv – The Netherlands, declare that the product ALOAPUR® is an equivalent to ALOAPUR® PM. In 2020 ALOAPUR® was re-classificed from "Complementary Mineral Feed" to "PREMIXTURE" and renamed to ALOAPUR® PM due to European legislation.

The following batch numbers of ALOAPUR® item 7005400701 are equal to ALOAPUR® PM item 7005700701 but are not yet labeled as ALOAPUR®PM but should be considered as so:

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August 2th, 2021

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QA Manager Contract Manufacturing

REGISTERED UNDER NR. 23036930 CHAMBER OF COMMERCE
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Purac Biochem
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4200 AA Gorinchem
The Netherlands
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Purac Biochem bv
Arkelsedijk 46 • 4206 AC Gorinchem • P.O. Box 21 • 4200 AA Gorinchem • Netherlands
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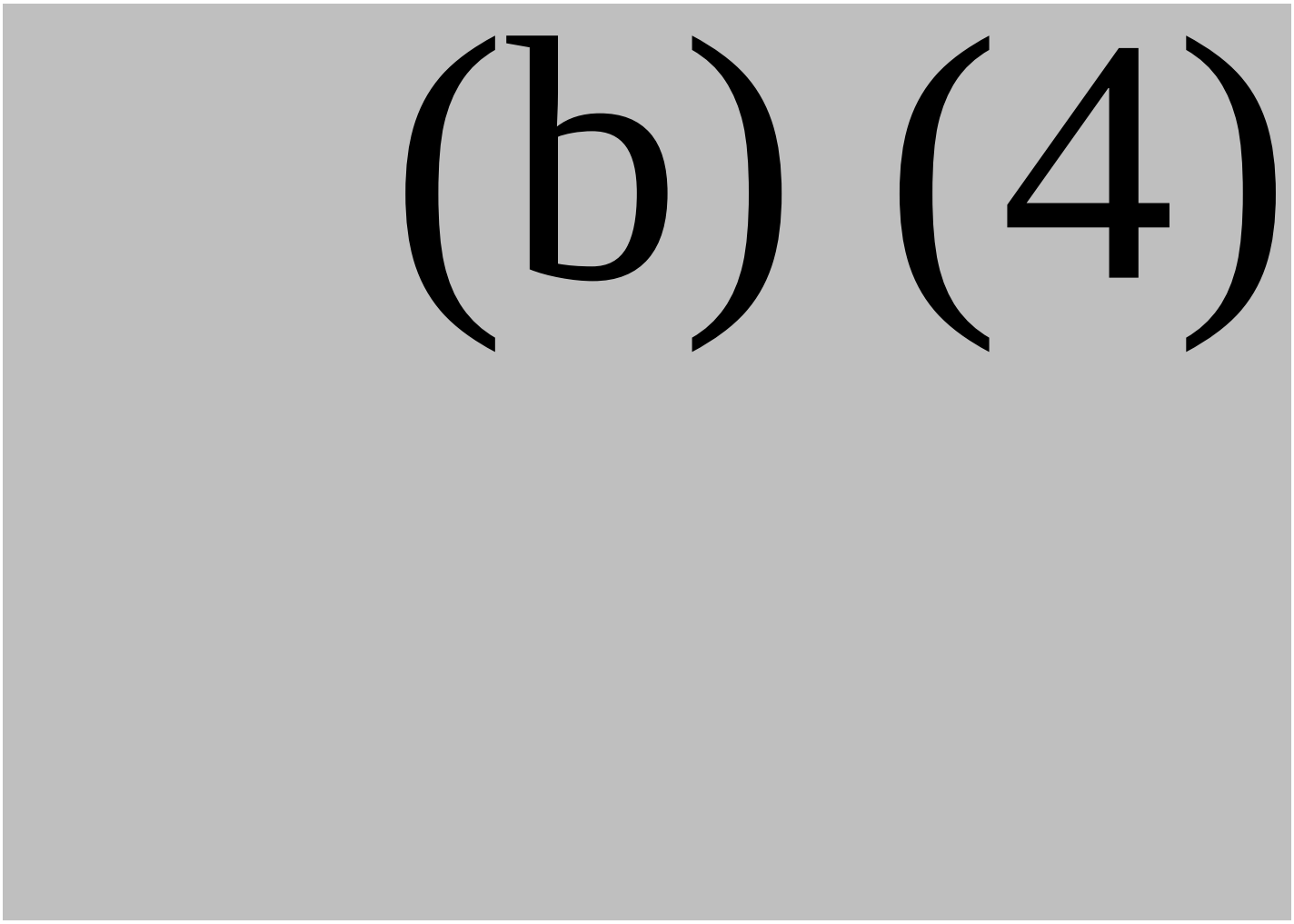
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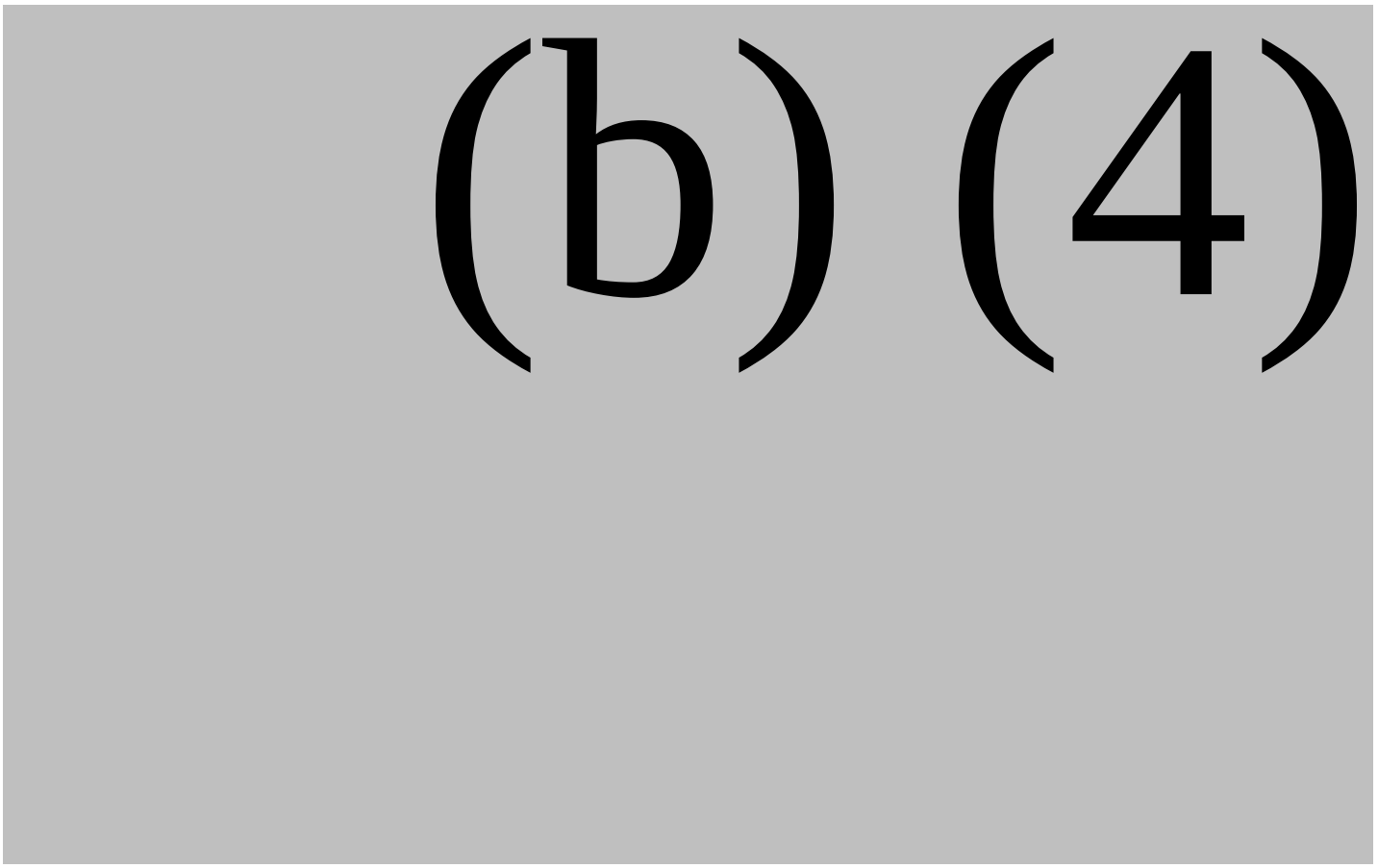
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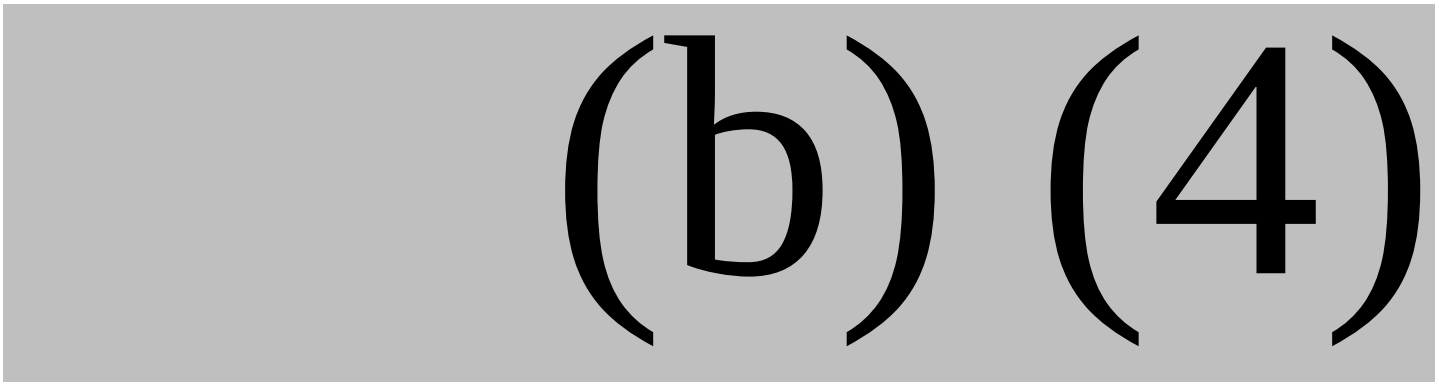
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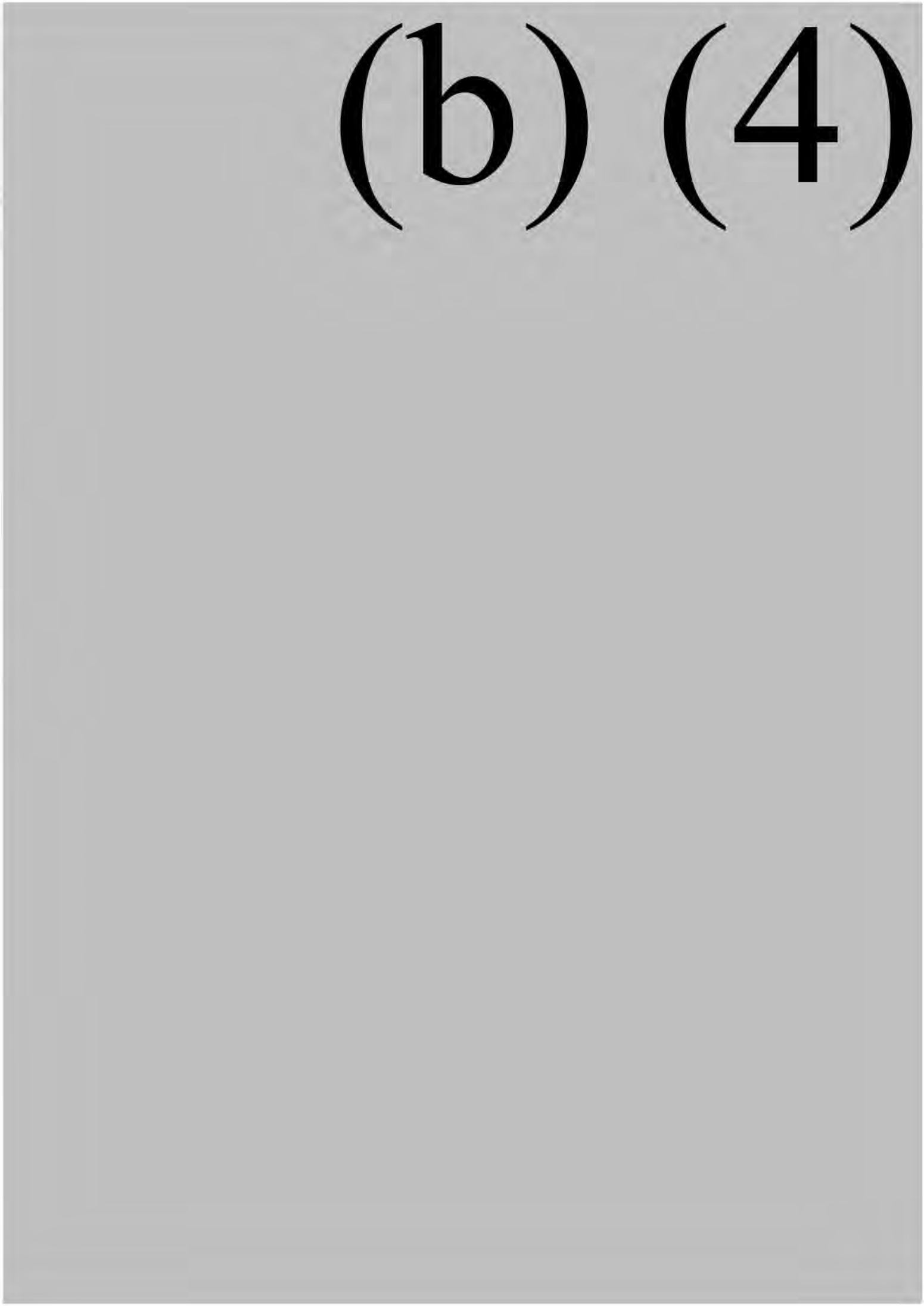
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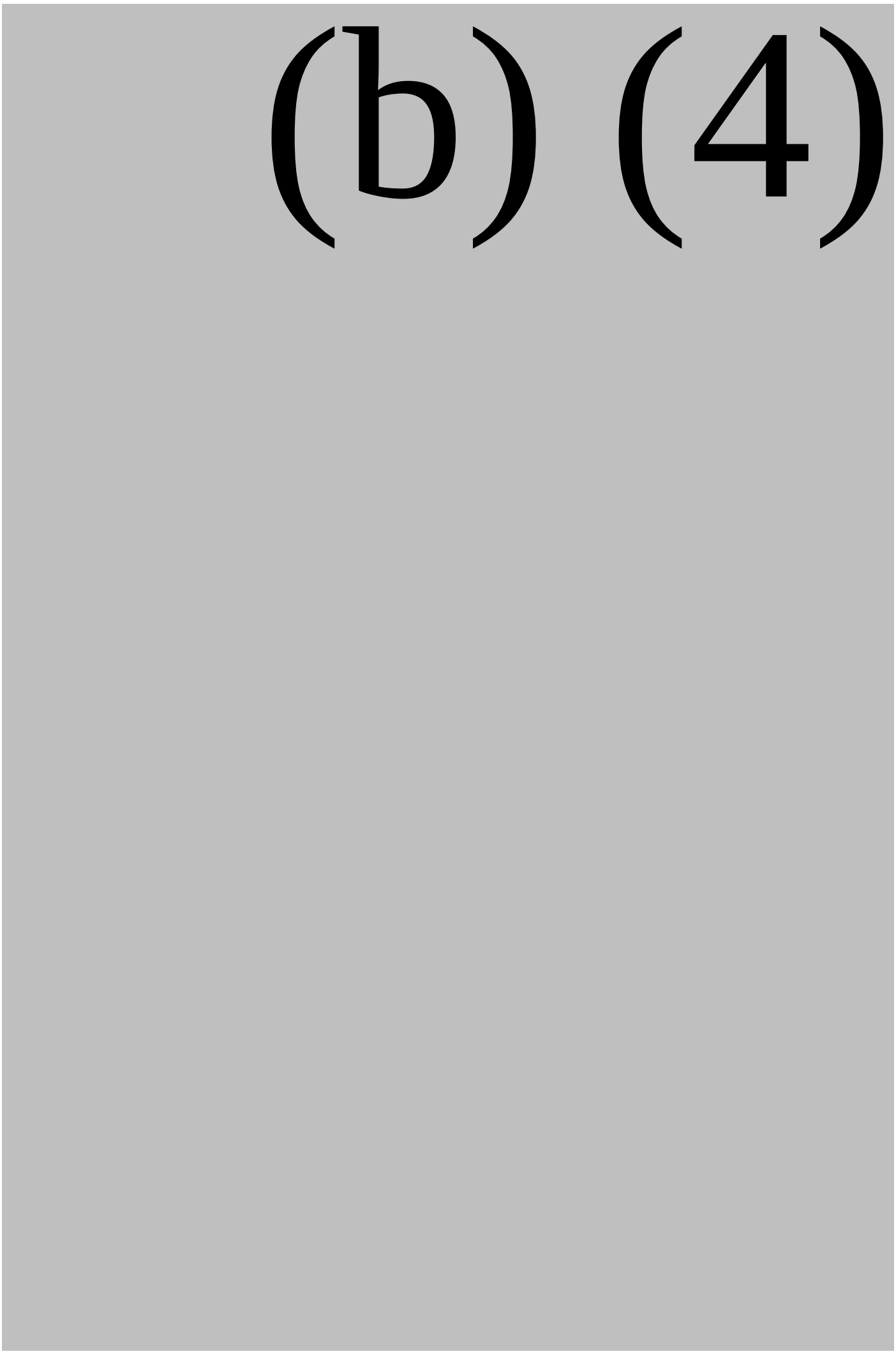
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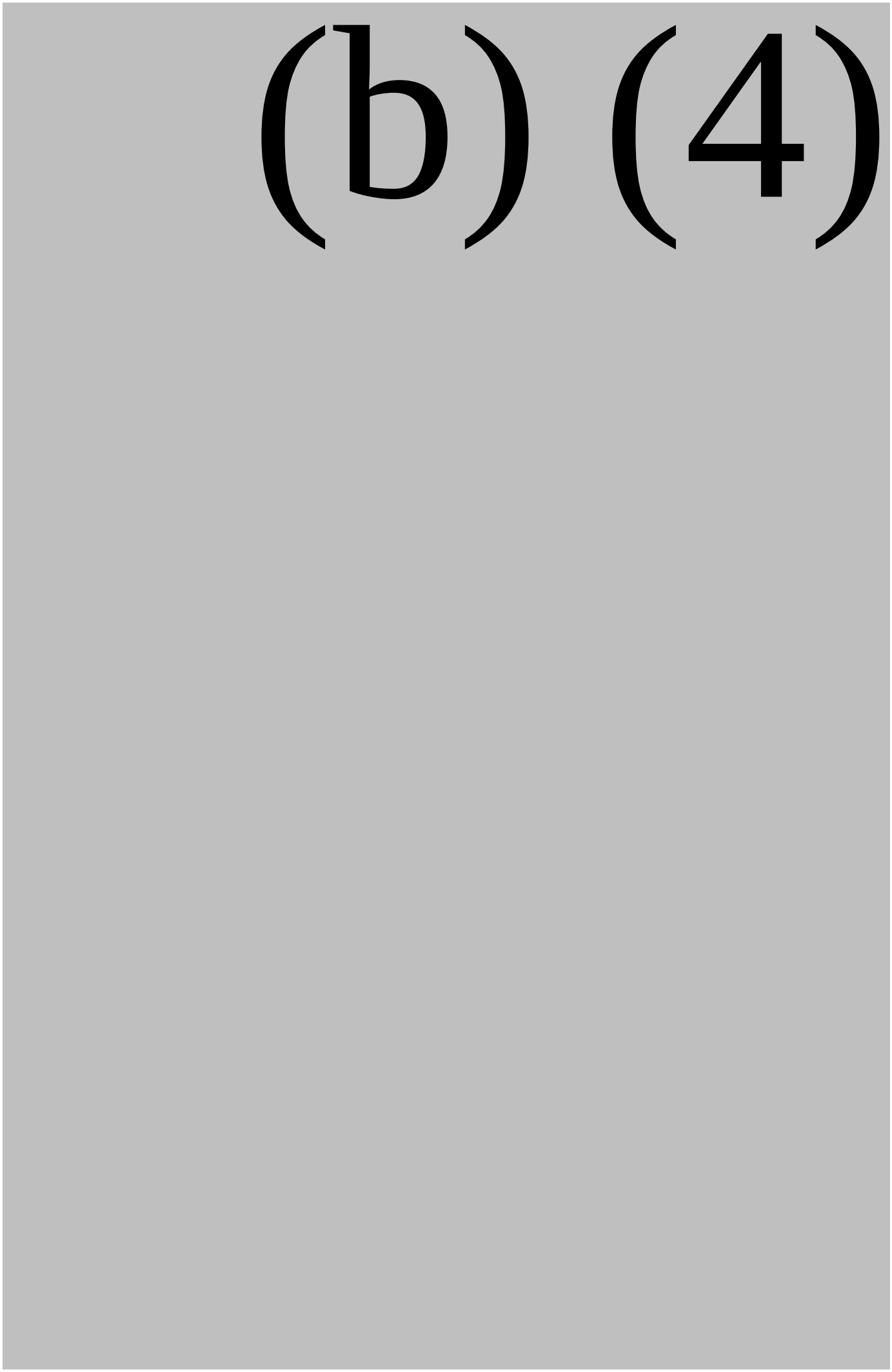
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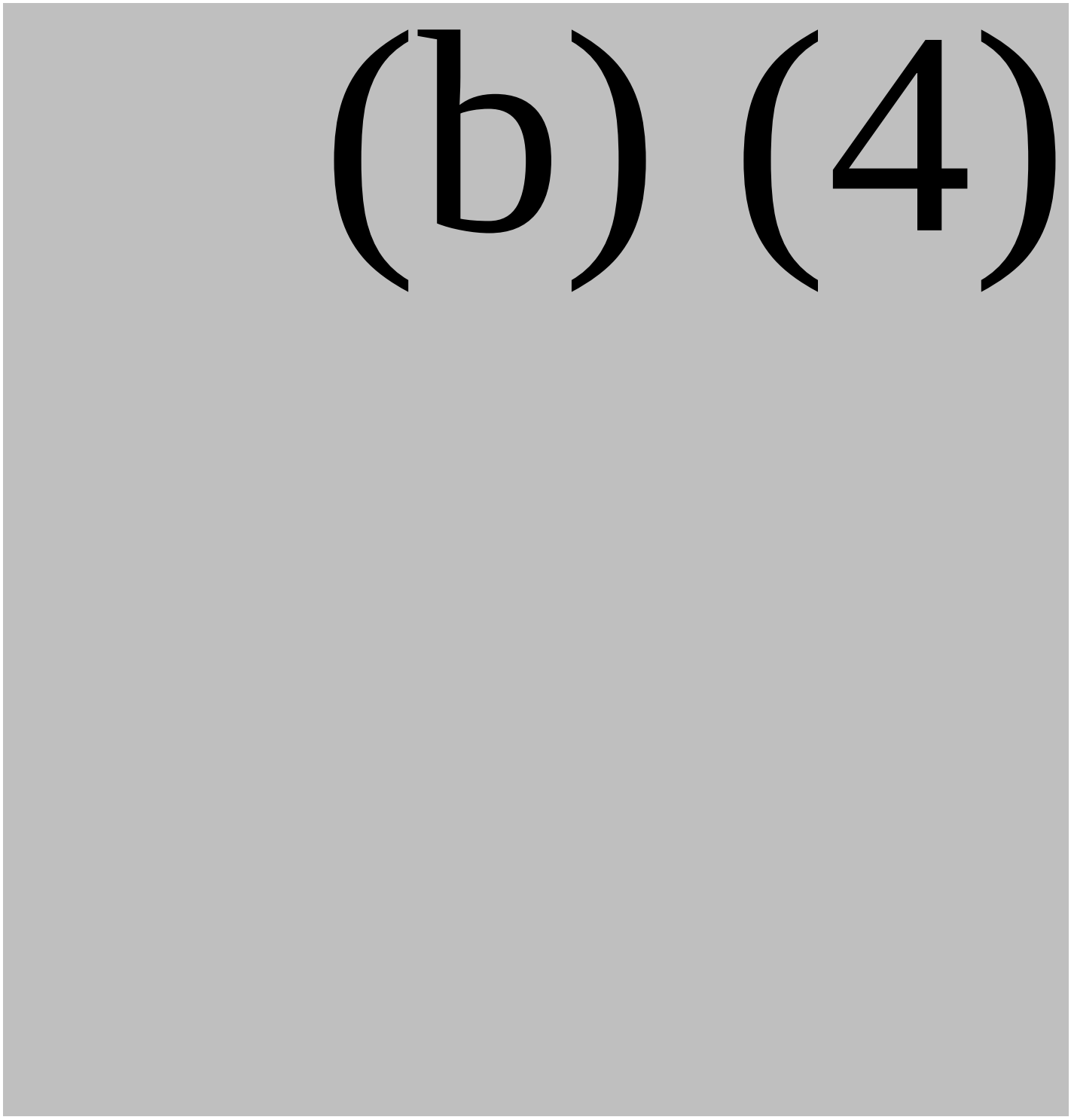
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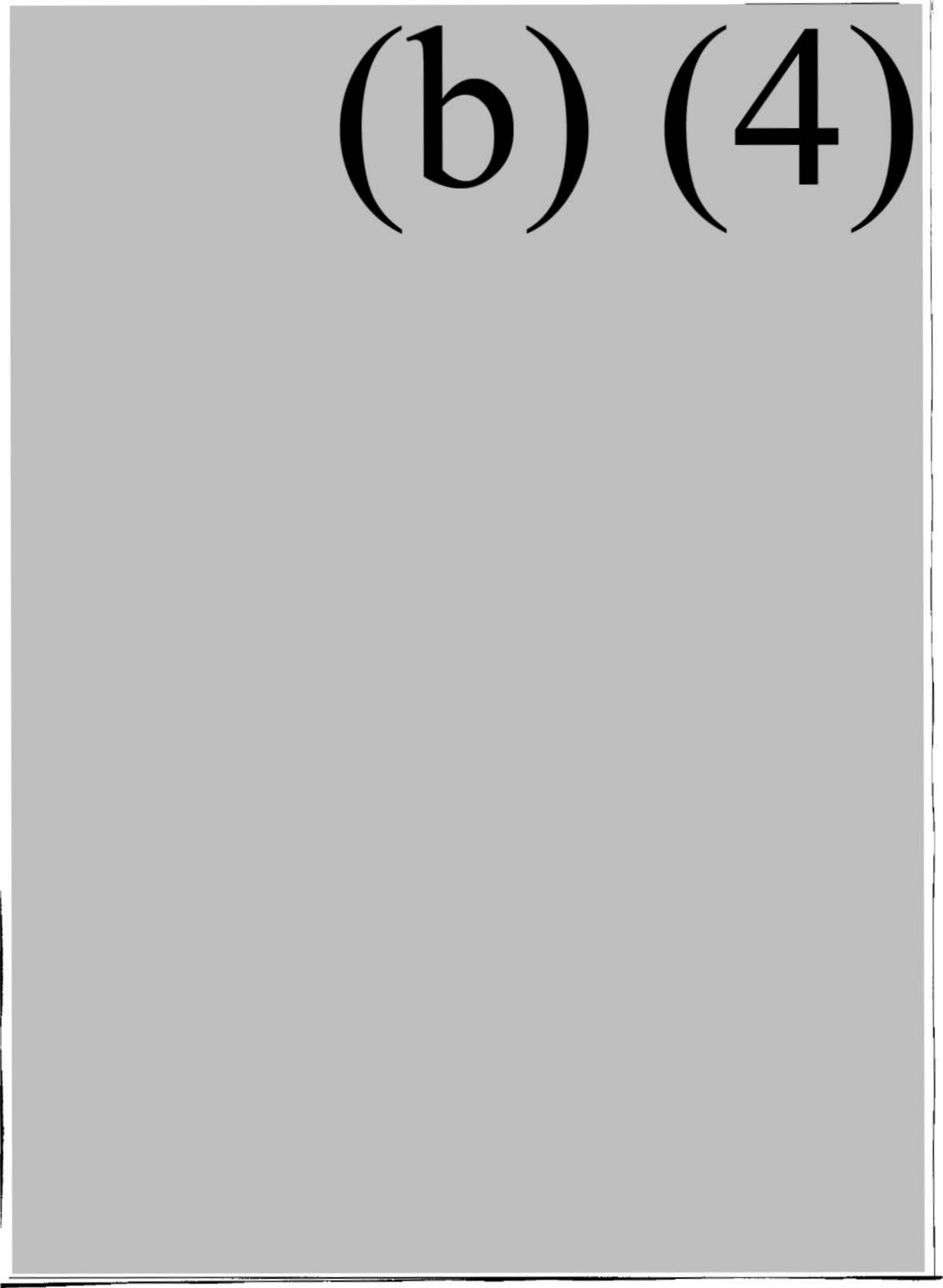
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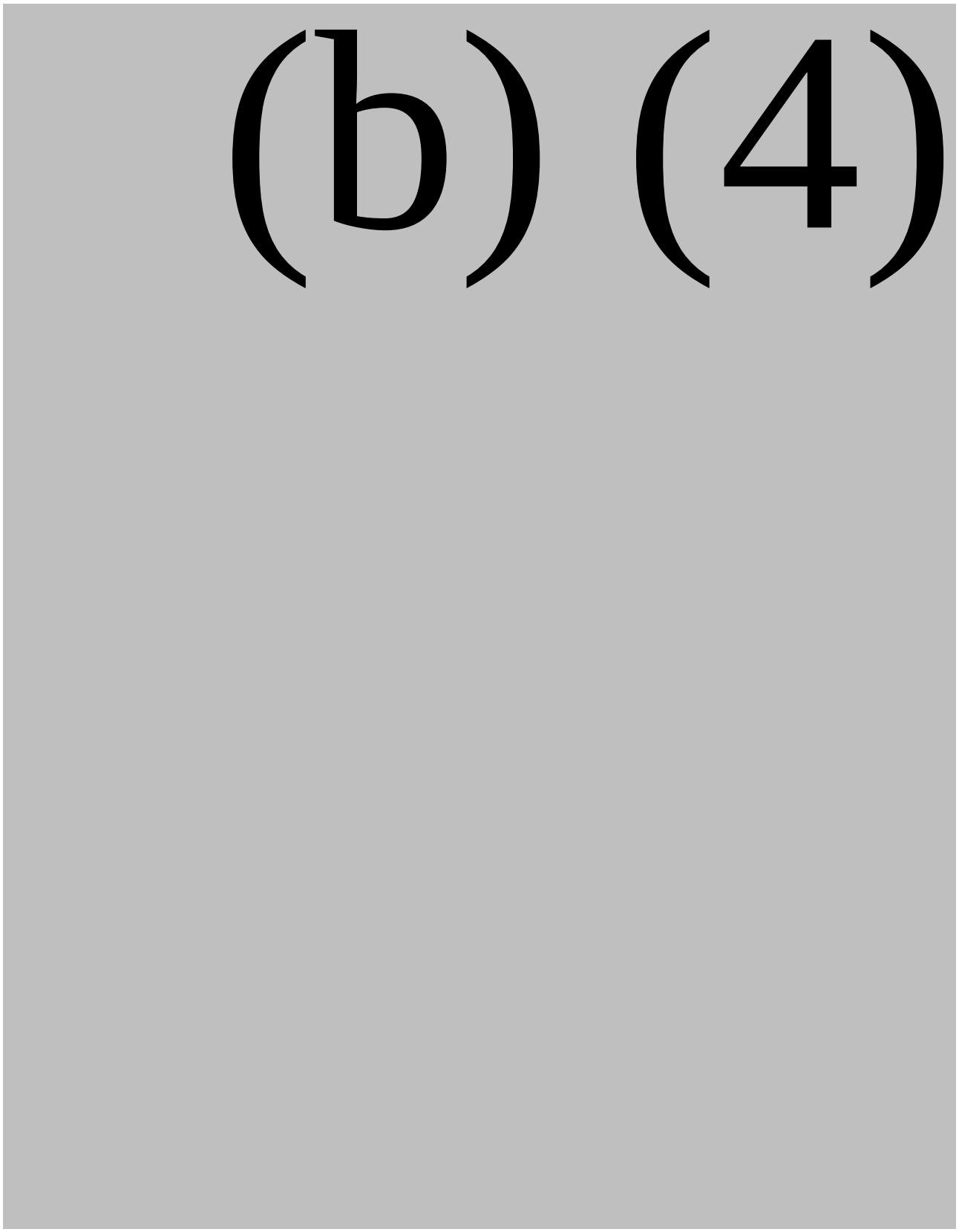

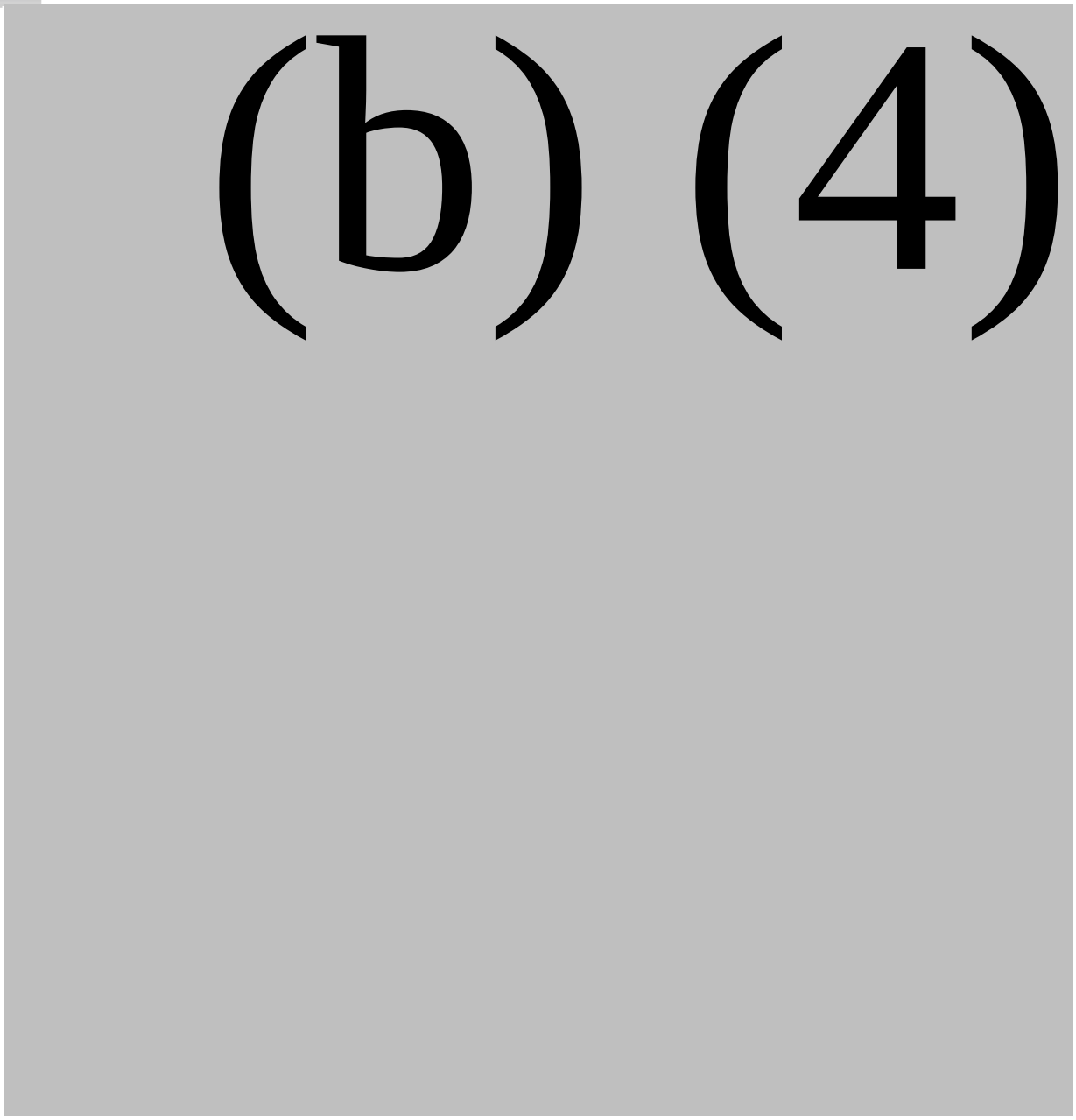

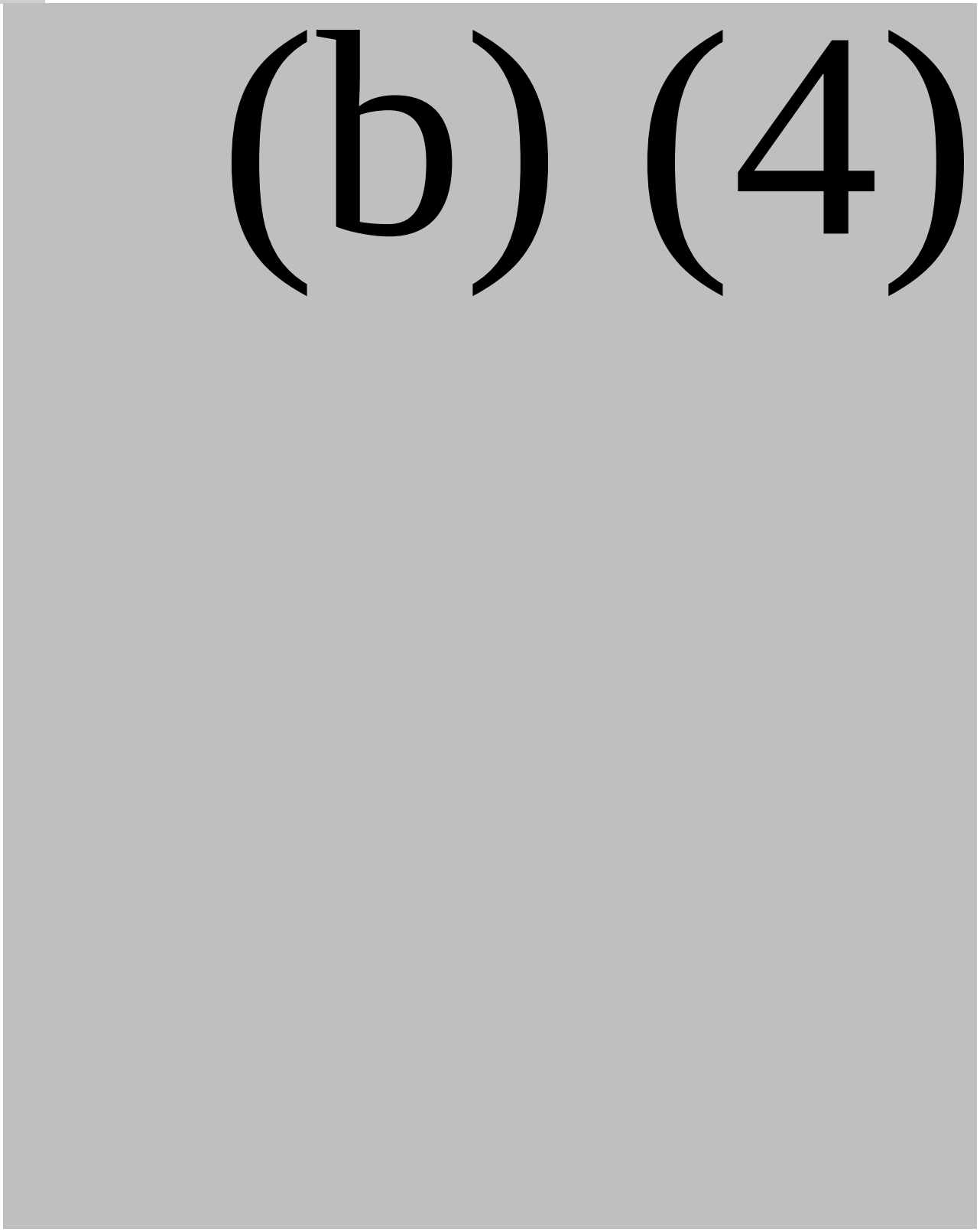

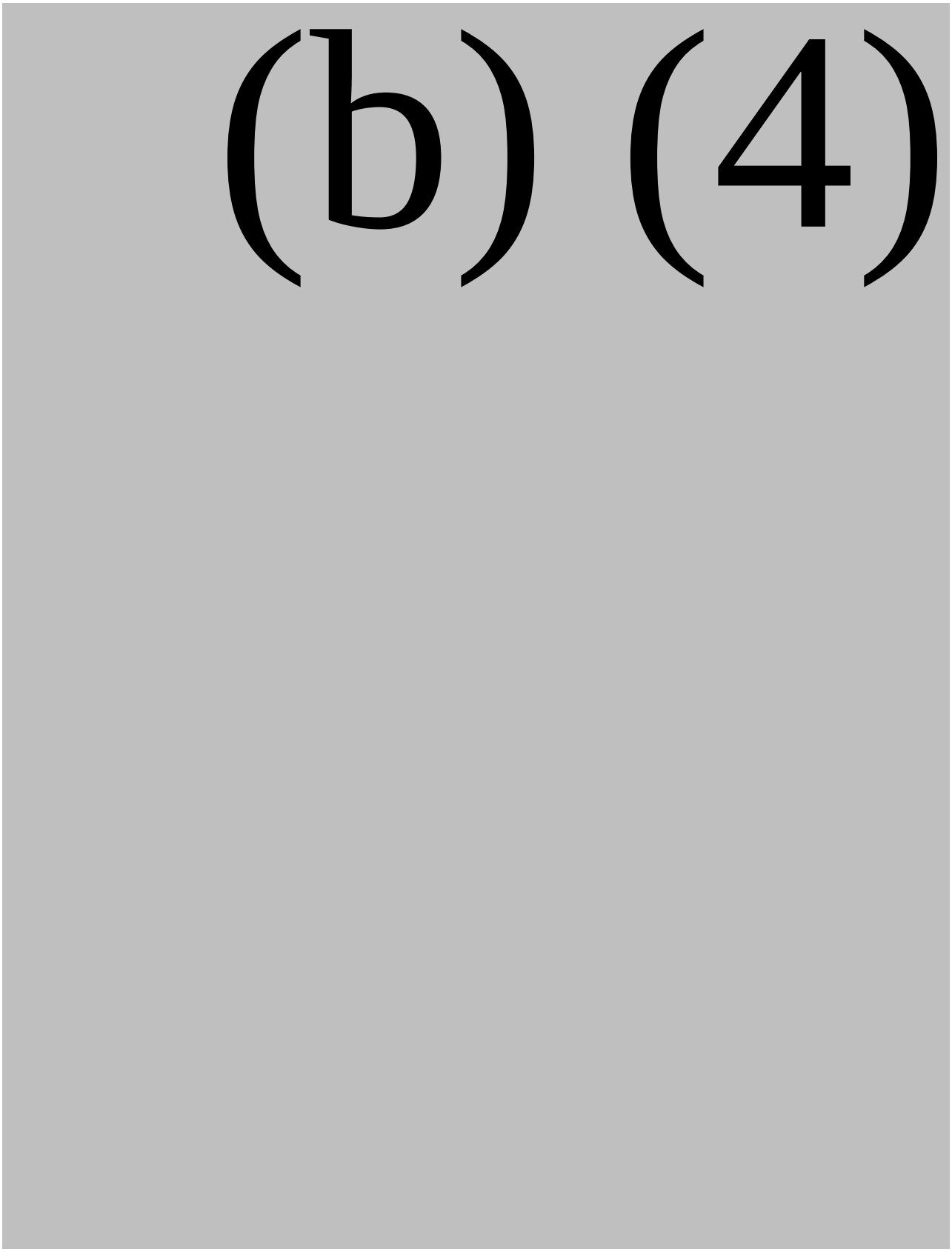

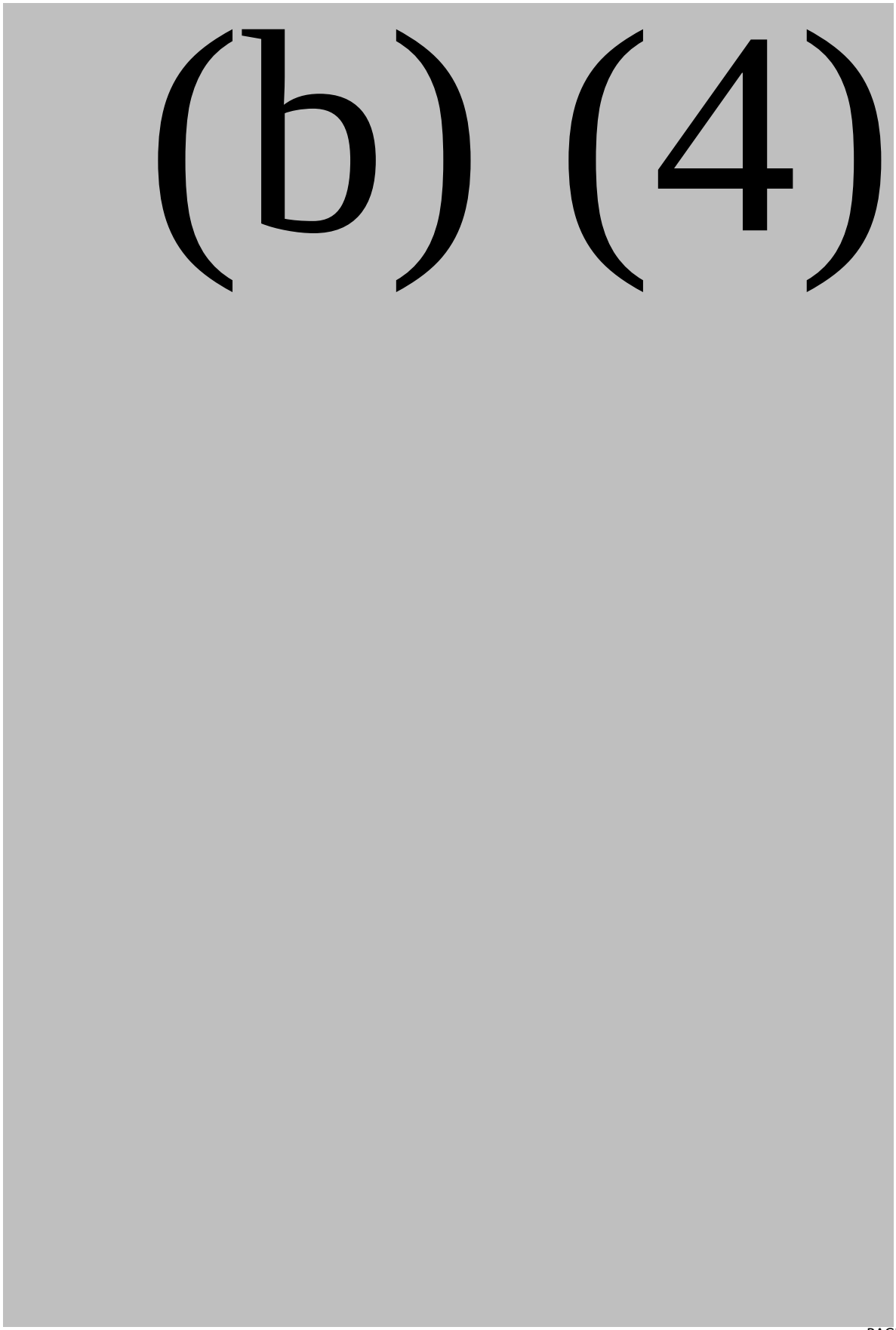

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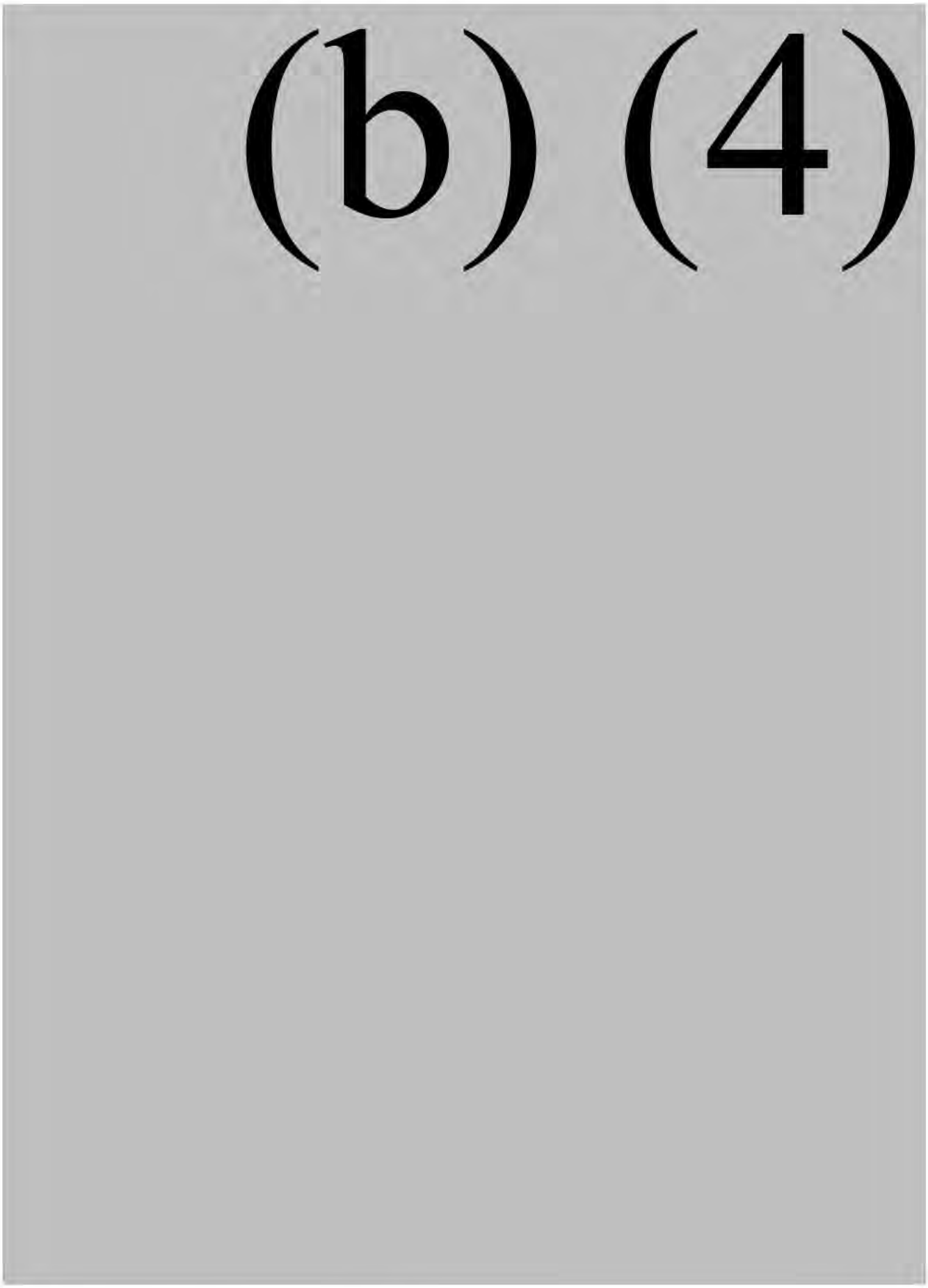

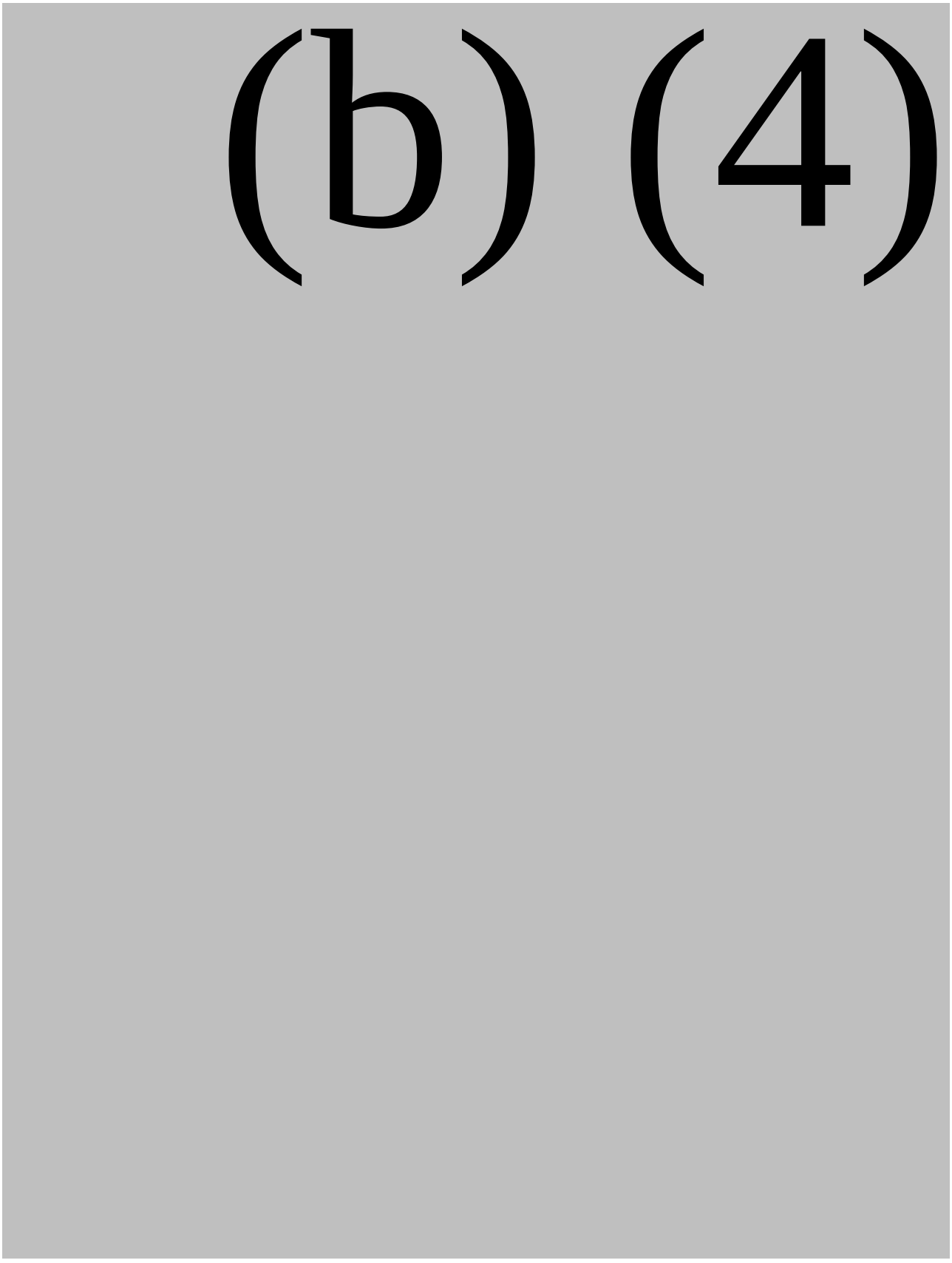

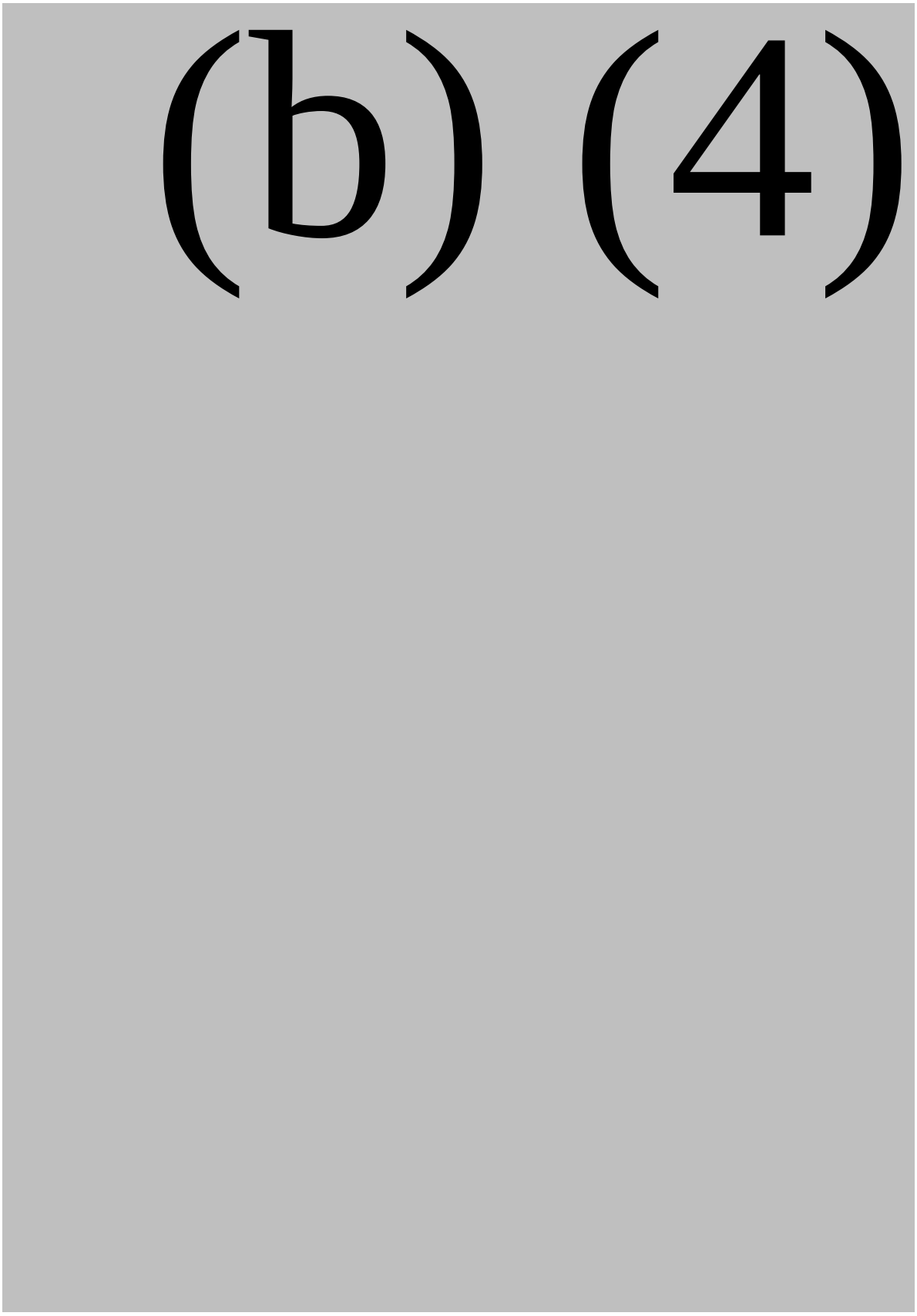

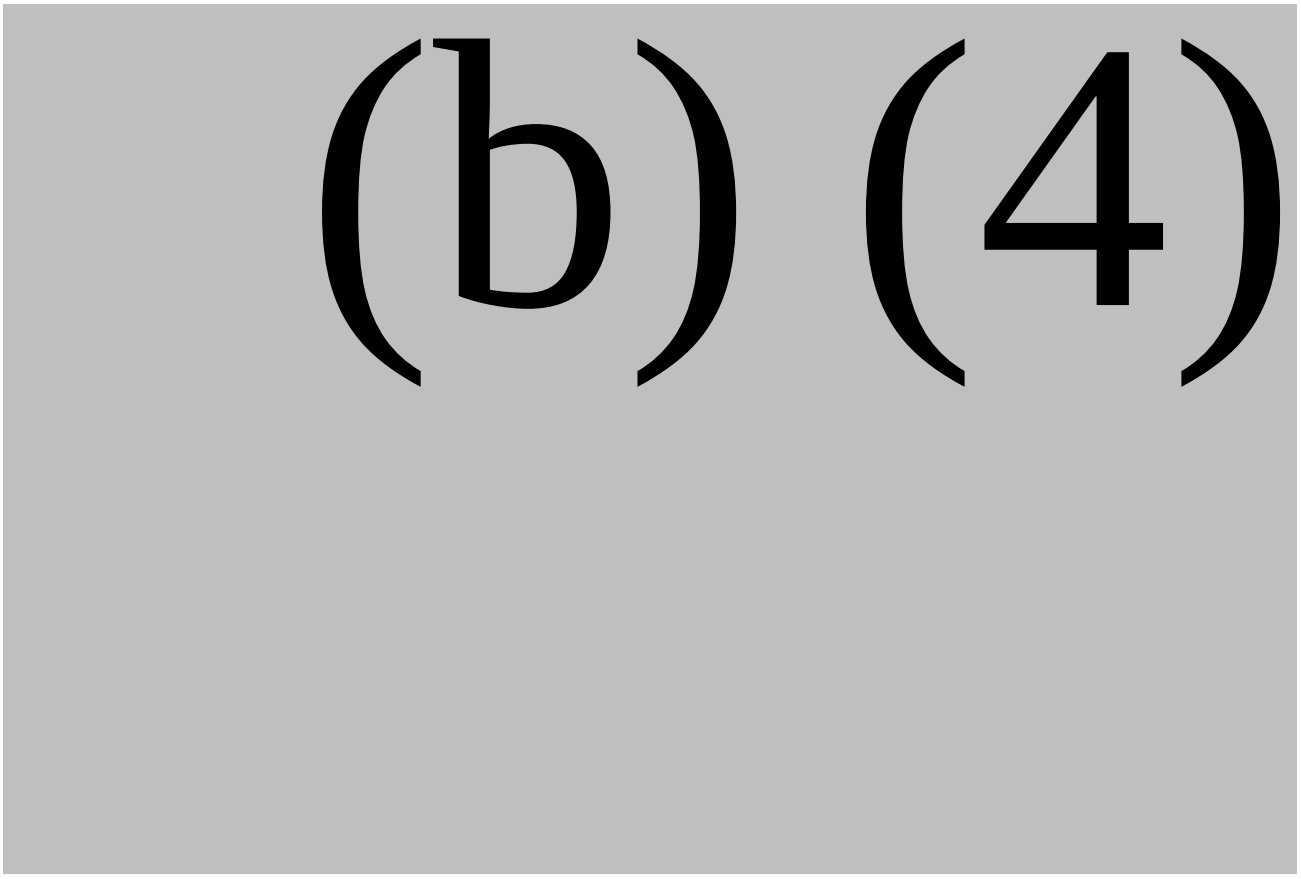

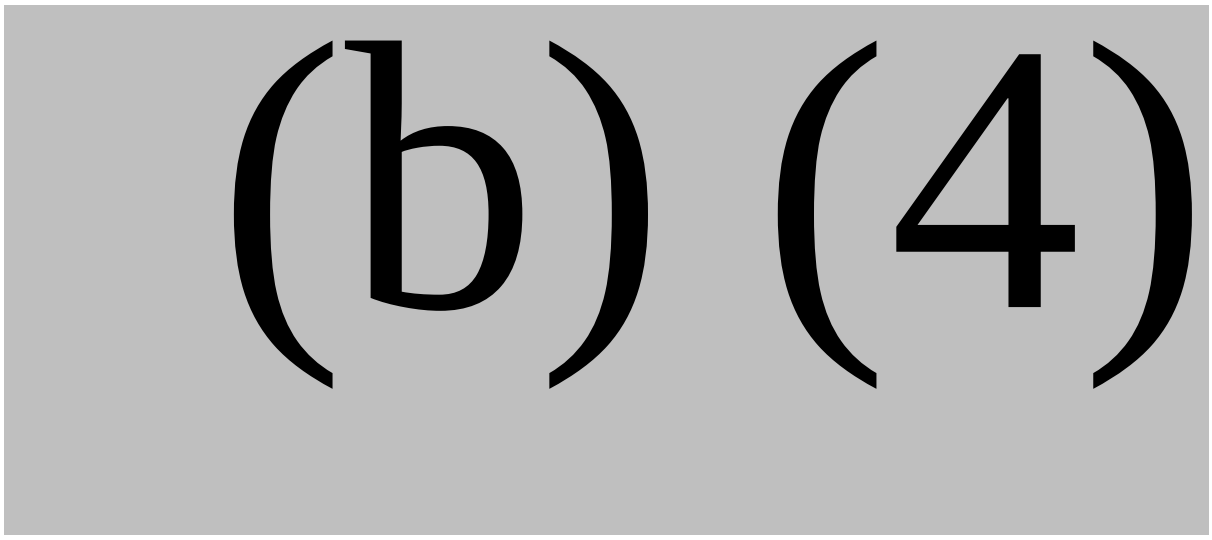

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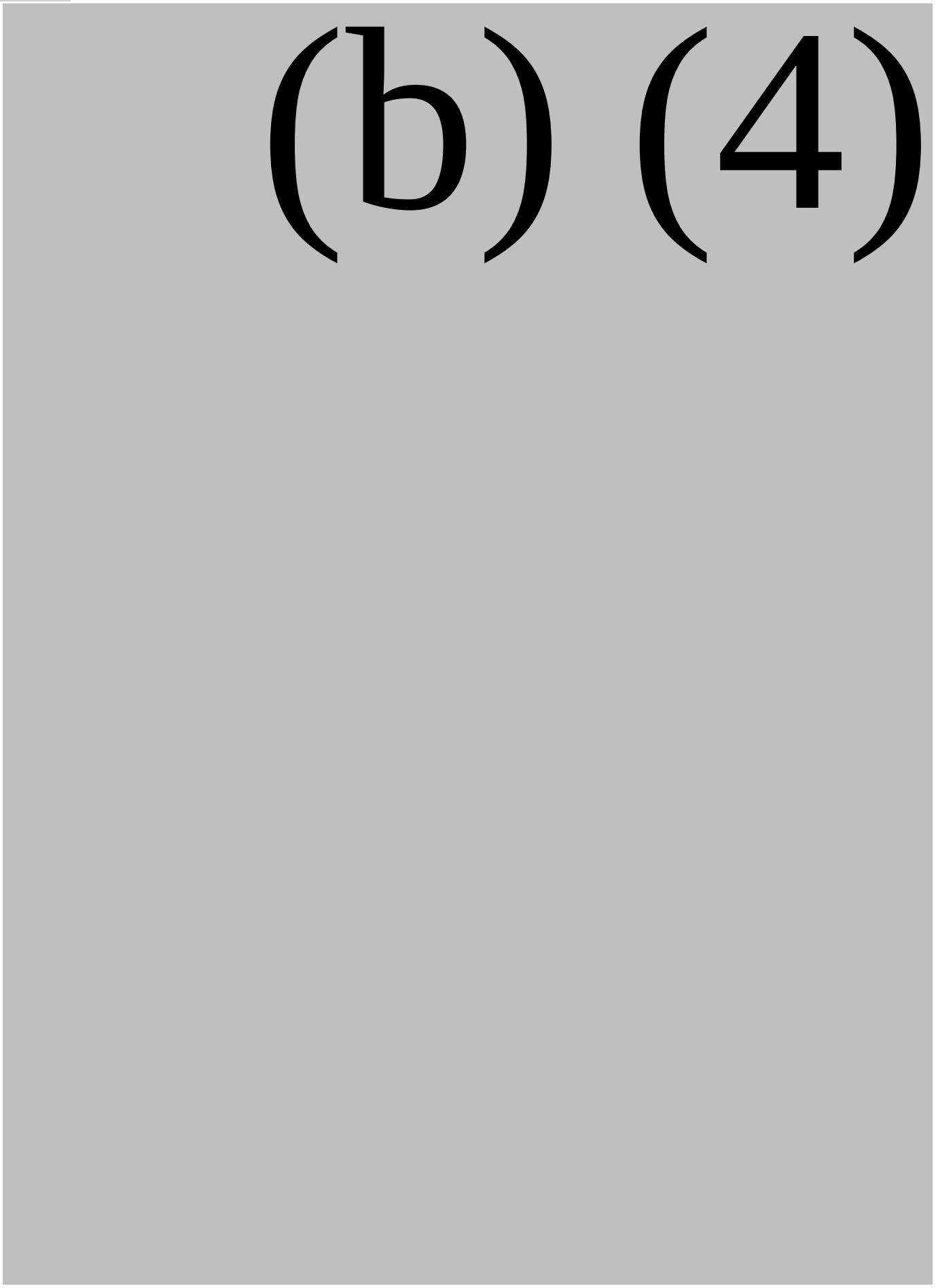

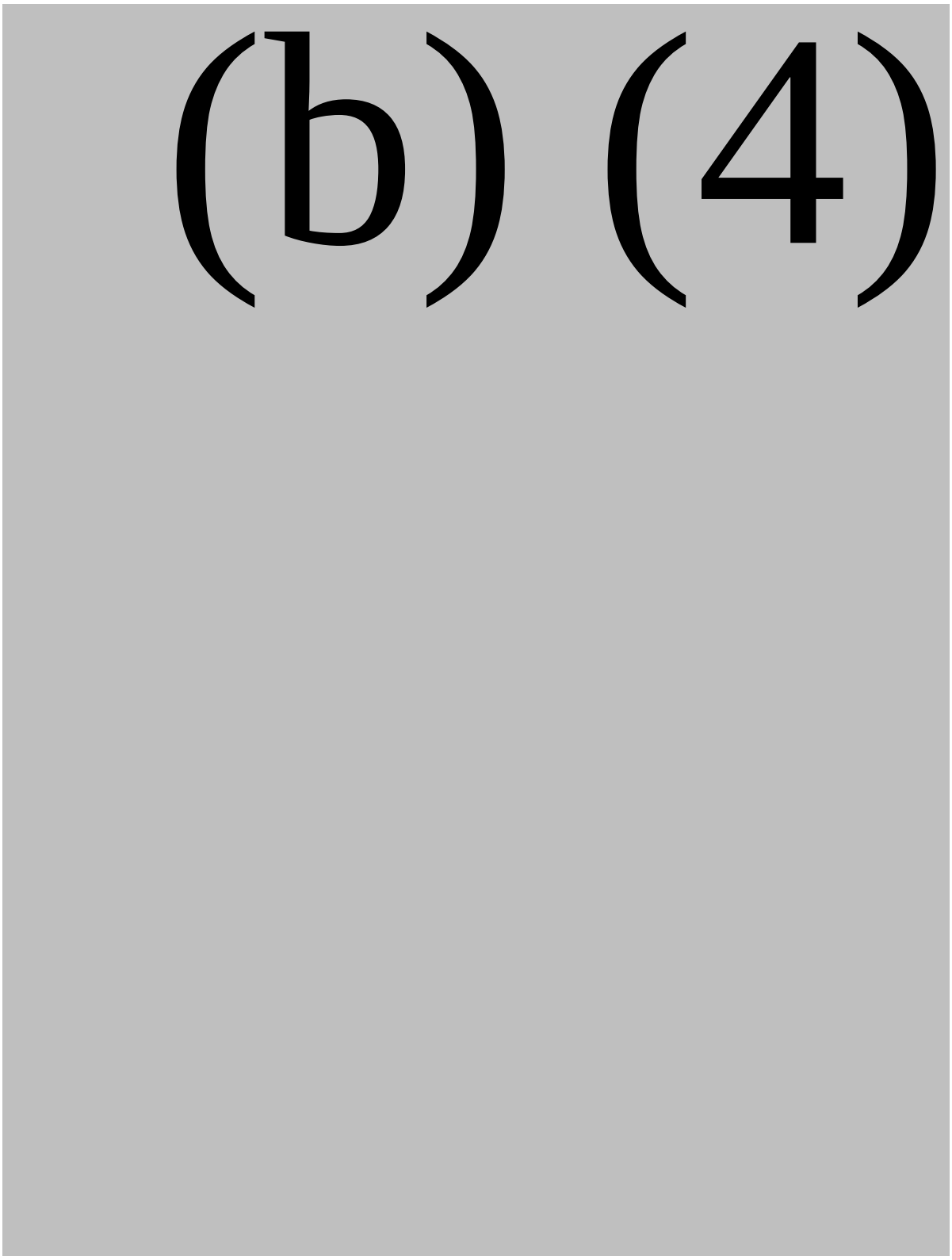

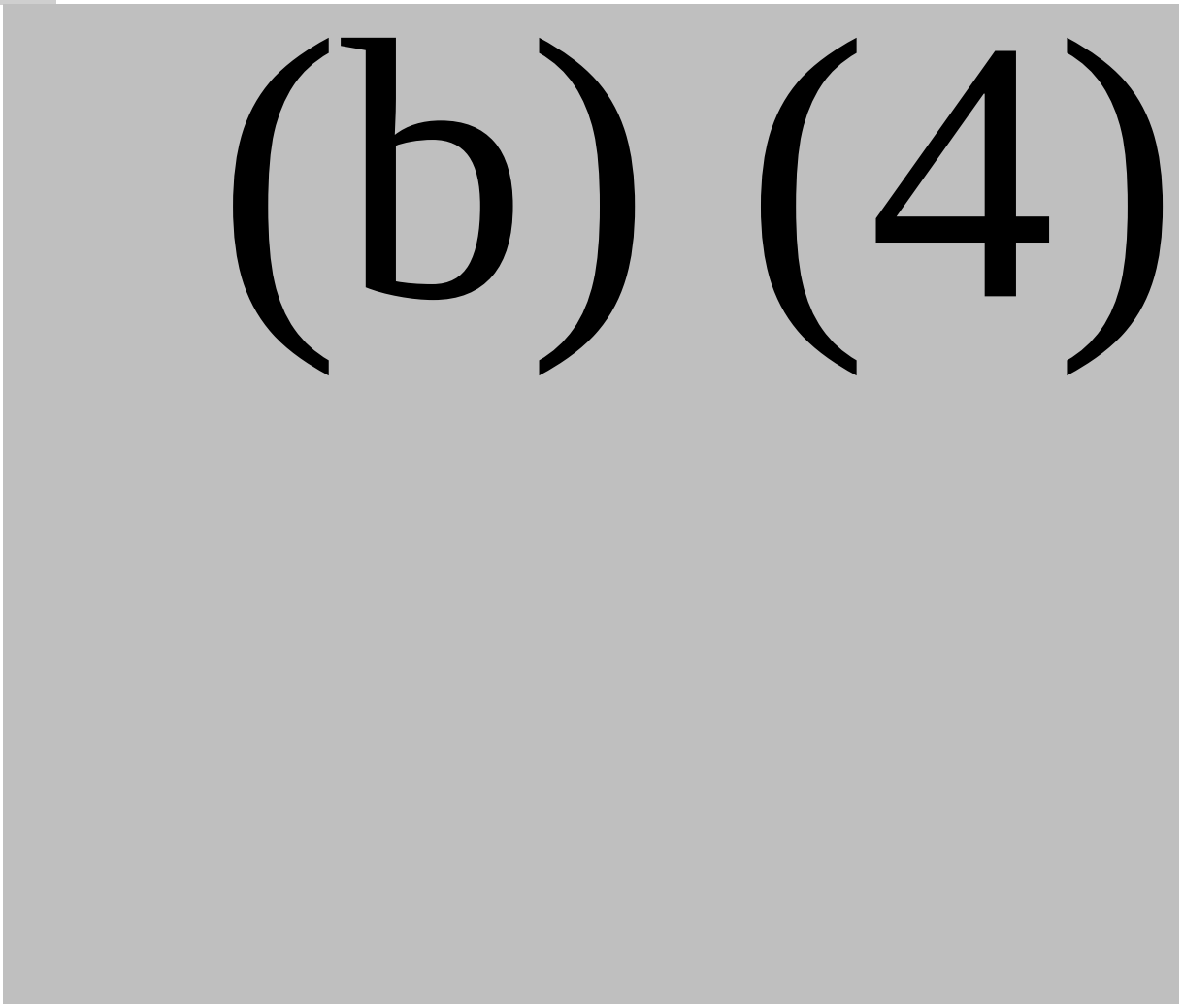

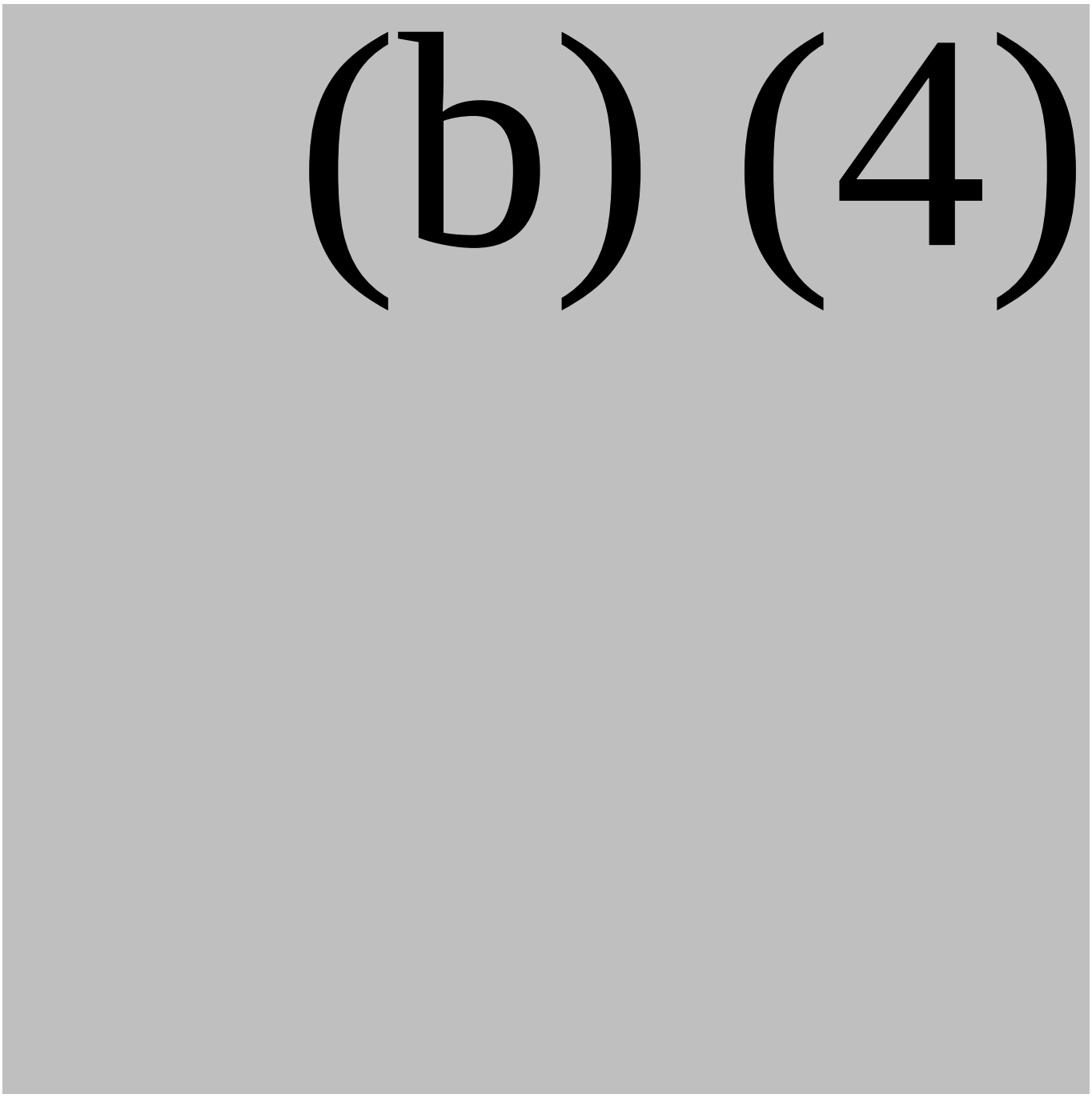
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Date 16/03/2020 9:58 AM

Certificate of Analysis

PURAC BIOCHEM BV

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Date 16/03/2020 4:18 PM

Certificate of Analysis

PURAC BIOCHEM BV

Shipment No. (b) (4)

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Certificate (b) (4)

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Date 17/03/2020 3:43 PM

Certificate of Analysis

PURAC BIOCHEM BV

Shipment No. (b) (4)

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Certificate (b) (4) (b) (4)

Date 16/03/2020 10:24 AM

Certificate of Analysis

PURAC BIOCHEM BV Shipment No. (b) (4)

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Certificate (b) (4)

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Date 19/03/2020 3:01 PM

Certificate of Analysis

PURAC BIOCHEM BV

Shipment No. (b) (4)

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Material PURAMIX 100

Material Code

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Date 20/05/2019 9:48 AM

Certificate of Analysis

PURAC BIOCHEM BV

Shipment No. (b) (4)

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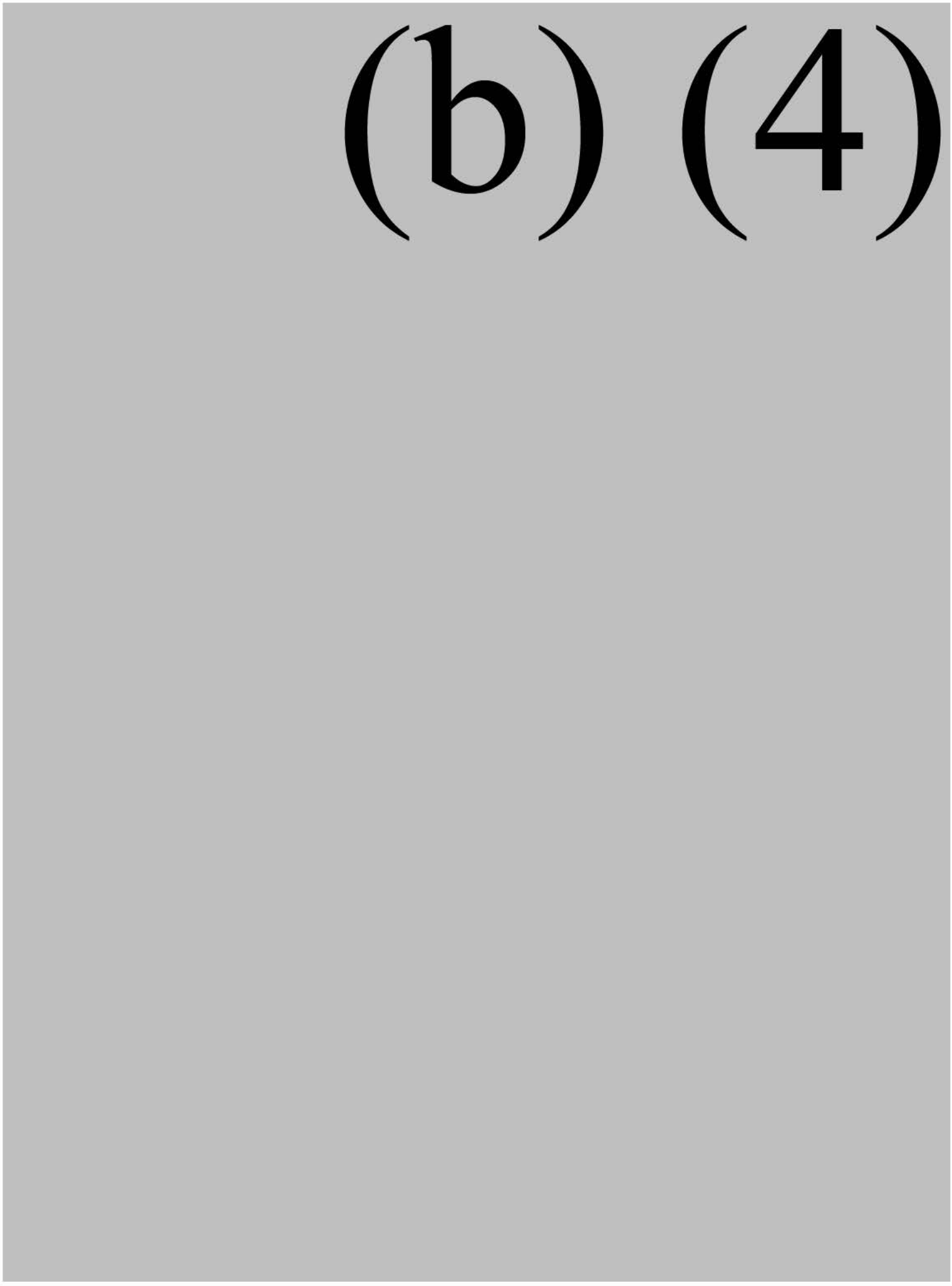
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Laboratory report

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(b) (4) **(R&D)**
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Postbus 21
4200 AA GORINCHEM

Sample number	:	(b) (4)
Customer number	:	D03531
Date Sample received	:	14-08-2020
Digital order ID	:	NC000066914003
Matrix (identified as)	:	Additive

Your sample characteristics

Subject	:	5 lactylaat samples Corbion
Productname	:	Puramix 100
External code	:	2020-000252-003
Product code customer	:	Puramix 100
Lot/batch/charge number	:	200300006
Additional info	:	Lactylaat
Sampling date	:	13-8-2020
Cost code	:	8420042

Parameter	Result	Unit	Method	Accr./cert.	
Mercury (Hg)	<0,100	mg/kg	10222	Q G	QS

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Laboratory report

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Purac Biochem BV (R&D)
attn. (b) (4)
Postbus 21
4200 AA GORINCHEM

Sample number	:	(b) (4)
Customer number	:	D03531
Date Sample received	:	14-08-2020
Digital order ID	:	NC000066914002
Matrix (identified as)	:	Additive

Your sample characteristics

Subject	:	5 lactylaat samples Corbion
Productname	:	Puramix 100
External code	:	2020-000252-002
Product code customer	:	Puramix 100
Lot/batch/charge number	:	2003000005
Additional info	:	Lactylaat
Sampling date	:	13-8-2020
Cost code	:	8420042

Parameter	Result	Unit	Method	Accr./cert.	
Mercury (Hg)	<0,100	mg/kg	10222	Q G	QS

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Laboratory report

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Purac Biochem BV (R&D)
attn. (b) (4)
Postbus 21
4200 AA GORINCHEM

Sample number	:	M20022985001
Customer number	:	D03531
Date Sample received	:	14-08-2020
Digital order ID	:	NC000066914001
Matrix (identified as)	:	Additive

Your sample characteristics

Subject	:	5 lactylaat samples Corbion
Productname	:	Puramix 100
External code	:	2020-000252-001
Product code customer	:	Puramix 100
Lot/batch/charge number	:	2003000004
Additional info	:	Lactylaat
Sampling date	:	13-8-2020
Cost code	:	8420042

Parameter	Result	Unit	Method	Accr./cert.	
Mercury (Hg)	<0,100	mg/kg	10222	Q G	QS

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Laboratory report

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Purac Biochem BV (R&D)
attn. (b) (4)
Postbus 21
4200 AA GORINCHEM

Sample number	:	M20030704002
Customer number	:	D03531
Date Sample received	:	27-10-2020
Digital order ID	:	NC000072385003

Your sample characteristics

Subject	:	Dioxine & Dioxine Like PCB's Puramix 100
Productname	:	PURAMIX 100
External code	:	2020-000252-004
Lot/batch/charge number	:	2003000007
Additional info	:	Mixture of lauroyl lactylate (C12) and myristoyl lactylate (C14)
Sampling date	:	10-6-2020
Cost code	:	10EXP056247

Parameter	Result	Unit	Method	Accr./cert.
Dioxine & Dioxine Like PCB's			10463	
WHO-TEQ (PCDD/F + DL-PCBs) incl. LOQ	0,222	ng TEQ/kg		Q G-B11 QS
Dioxine TEQ (WHO 2005) incl. LOQ	0,156	ng TEQ/kg		Q G-B11 QS
2,3,7,8-TCDD	<0,04	ng/kg		Q G-B11 QS
1,2,3,7,8-PeCDD	<0,04	ng/kg		Q G-B11 QS
1,2,3,4,7,8-HxCDD	<0,08	ng/kg		Q G-B11 QS
1,2,3,6,7,8-HxCDD	<0,08	ng/kg		Q G-B11 QS
1,2,3,7,8,9-HxCDD	<0,08	ng/kg		Q G-B11 QS
1,2,3,4,6,7,8-HpCDD	<0,10	ng/kg		Q G-B11 QS
OCDD	<0,20	ng/kg		Q G-B11 QS
2,3,7,8-TCDF	<0,04	ng/kg		Q G-B11 QS
1,2,3,7,8-PeCDF	<0,04	ng/kg		Q G-B11 QS
2,3,4,7,8-PeCDF	<0,04	ng/kg		Q G-B11 QS
1,2,3,4,7,8-HxCDF	<0,08	ng/kg		Q G-B11 QS
1,2,3,6,7,8-HxCDF	<0,08	ng/kg		Q G-B11 QS
2,3,4,6,7,8-HxCDF	<0,08	ng/kg		Q G-B11 QS
1,2,3,7,8,9-HxCDF	<0,08	ng/kg		Q G-B11 QS
1,2,3,4,6,7,8-HpCDF	<0,10	ng/kg		Q G-B11 QS
1,2,3,4,7,8,9-HpCDF	<0,10	ng/kg		Q G-B11 QS
OCDF	<0,20	ng/kg		Q G-B11 QS
DL-PCB TEQ (WHO 2005) incl. LOQ	0,065	ng TEQ/kg		Q G-B11 QS
PCB-77	<0,80	ng/kg		Q G-B11 QS
PCB-81	<0,80	ng/kg		Q G-B11 QS
PCB-126	<0,40	ng/kg		Q G-B11 QS
PCB-169	<0,80	ng/kg		Q G-B11 QS
PCB-105	<4,0	ng/kg		Q G-B11 QS
PCB-114	<4,0	ng/kg		Q G-B11 QS
PCB-118	6,1	ng/kg		Q G-B11 QS

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Parameter	Result	Unit	Method	Accr./cert.
PCB-123	<4,0	ng/kg		Q G-B11 QS
PCB-156	<4,0	ng/kg		Q G-B11 QS
PCB-157	<4,0	ng/kg		Q G-B11 QS
PCB-167	<4,0	ng/kg		Q G-B11 QS
PCB-189	<4,0	ng/kg		Q G-B11 QS
NDL-PCB's (Polychlorinated biphenyls)			10463	
Polychlorinated biphenyl-28	<0,5	µg/kg		Q G-B11
Polychlorinated biphenyl-52	<0,5	µg/kg		Q G-B11
Polychlorinated biphenyl-101	<0,5	µg/kg		Q G-B11
Polychlorinated biphenyl-138	<0,5	µg/kg		Q G-B11
Polychlorinated biphenyl-153	<0,5	µg/kg		Q G-B11
Polychlorinated biphenyl-180	<0,5	µg/kg		Q G-B11
Sum NDL-PCBs (upperbound)	3,0	µg/kg		Q G-B11

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Laboratory report

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Purac Biochem BV (R&D
attn. (b) (4)
Postbus 21
4200 AA GORINCHEM

Sample number	:	M20030704001
Customer number	:	D03531
Date Sample received	:	27-10-2020
Digital order ID	:	NC000072385002

Your sample characteristics

Subject	:	Dioxine & Dioxine Like PCB's Puramix 100
Productname	:	PURAMIX 100
External code	:	2020-000252-005
Lot/batch/charge number	:	2003000008
Additional info	:	Mixture of lauroyl lactylate (C12) and myristoyl lactylate (C14)
Sampling date	:	14-5-2020
Cost code	:	10EXP056247

Parameter	Result	Unit	Method	Accr./cert.
Dioxine & Dioxine Like PCB's			10463	
WHO-TEQ (PCDD/F + DL-PCBs) incl. LOQ	0,222	ng TEQ/kg		Q G-B11 QS
Dioxine TEQ (WHO 2005) incl. LOQ	0,156	ng TEQ/kg		Q G-B11 QS
2,3,7,8-TCDD	<0,04	ng/kg		Q G-B11 QS
1,2,3,7,8-PeCDD	<0,04	ng/kg		Q G-B11 QS
1,2,3,4,7,8-HxCDD	<0,08	ng/kg		Q G-B11 QS
1,2,3,6,7,8-HxCDD	<0,08	ng/kg		Q G-B11 QS
1,2,3,7,8,9-HxCDD	<0,08	ng/kg		Q G-B11 QS
1,2,3,4,6,7,8-HpCDD	<0,10	ng/kg		Q G-B11 QS
OCDD	<0,20	ng/kg		Q G-B11 QS
2,3,7,8-TCDF	<0,04	ng/kg		Q G-B11 QS
1,2,3,7,8-PeCDF	<0,04	ng/kg		Q G-B11 QS
2,3,4,7,8-PeCDF	<0,04	ng/kg		Q G-B11 QS
1,2,3,4,7,8-HxCDF	<0,08	ng/kg		Q G-B11 QS
1,2,3,6,7,8-HxCDF	<0,08	ng/kg		Q G-B11 QS
2,3,4,6,7,8-HxCDF	<0,08	ng/kg		Q G-B11 QS
1,2,3,7,8,9-HxCDF	<0,08	ng/kg		Q G-B11 QS
1,2,3,4,6,7,8-HpCDF	<0,10	ng/kg		Q G-B11 QS
1,2,3,4,7,8,9-HpCDF	<0,10	ng/kg		Q G-B11 QS
OCDF	<0,20	ng/kg		Q G-B11 QS
DL-PCB TEQ (WHO 2005) incl. LOQ	0,065	ng TEQ/kg		Q G-B11 QS
PCB-77	<0,80	ng/kg		Q G-B11 QS
PCB-81	<0,80	ng/kg		Q G-B11 QS
PCB-126	<0,40	ng/kg		Q G-B11 QS
PCB-169	<0,80	ng/kg		Q G-B11 QS
PCB-105	<4,0	ng/kg		Q G-B11 QS
PCB-114	<4,0	ng/kg		Q G-B11 QS
PCB-118	<4,0	ng/kg		Q G-B11 QS

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Parameter	Result	Unit	Method	Accr./cert.
PCB-123	<4,0	ng/kg		Q G-B11 QS
PCB-156	<4,0	ng/kg		Q G-B11 QS
PCB-157	<4,0	ng/kg		Q G-B11 QS
PCB-167	<4,0	ng/kg		Q G-B11 QS
PCB-189	<4,0	ng/kg		Q G-B11 QS
NDL-PCB's (Polychlorinated biphenyls)			10463	
Polychlorinated biphenyl-28	<0,5	µg/kg		Q G-B11
Polychlorinated biphenyl-52	<0,5	µg/kg		Q G-B11
Polychlorinated biphenyl-101	<0,5	µg/kg		Q G-B11
Polychlorinated biphenyl-138	<0,5	µg/kg		Q G-B11
Polychlorinated biphenyl-153	<0,5	µg/kg		Q G-B11
Polychlorinated biphenyl-180	<0,5	µg/kg		Q G-B11
Sum NDL-PCBs (upperbound)	3,0	µg/kg		Q G-B11

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Laboratory report

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Purac Biochem BV (R&D)
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Postbus 21
4200 AA GORINCHEM

Sample number	:	M20030704003
Customer number	:	D03531
Date Sample received	:	27-10-2020
Digital order ID	:	NC000072385004

Your sample characteristics

Subject	:	Dioxine & Dioxine Like PCB's Puramix 100
Productname	:	PURAMIX 100
External code	:	2020-000252-006
Lot/batch/charge number	:	1905000027
Additional info	:	Mixture of lauroyl lactylate (C12) and myristoyl lactylate (C14)
Sampling date	:	29-8-2019
Cost code	:	10EXP056247

Parameter	Result	Unit	Method	Accr./cert.
Dioxine & Dioxine Like PCB's			10463	
WHO-TEQ (PCDD/F + DL-PCBs) incl. LOQ	0,222	ng TEQ/kg		Q G-B11 QS
Dioxine TEQ (WHO 2005) incl. LOQ	0,156	ng TEQ/kg		Q G-B11 QS
2,3,7,8-TCDD	<0,04	ng/kg		Q G-B11 QS
1,2,3,7,8-PeCDD	<0,04	ng/kg		Q G-B11 QS
1,2,3,4,7,8-HxCDD	<0,08	ng/kg		Q G-B11 QS
1,2,3,6,7,8-HxCDD	<0,08	ng/kg		Q G-B11 QS
1,2,3,7,8,9-HxCDD	<0,08	ng/kg		Q G-B11 QS
1,2,3,4,6,7,8-HpCDD	<0,10	ng/kg		Q G-B11 QS
OCDD	<0,20	ng/kg		Q G-B11 QS
2,3,7,8-TCDF	<0,04	ng/kg		Q G-B11 QS
1,2,3,7,8-PeCDF	<0,04	ng/kg		Q G-B11 QS
2,3,4,7,8-PeCDF	<0,04	ng/kg		Q G-B11 QS
1,2,3,4,7,8-HxCDF	<0,08	ng/kg		Q G-B11 QS
1,2,3,6,7,8-HxCDF	<0,08	ng/kg		Q G-B11 QS
2,3,4,6,7,8-HxCDF	<0,08	ng/kg		Q G-B11 QS
1,2,3,7,8,9-HxCDF	<0,08	ng/kg		Q G-B11 QS
1,2,3,4,6,7,8-HpCDF	<0,10	ng/kg		Q G-B11 QS
1,2,3,4,7,8,9-HpCDF	<0,10	ng/kg		Q G-B11 QS
OCDF	<0,20	ng/kg		Q G-B11 QS
DL-PCB TEQ (WHO 2005) incl. LOQ	0,065	ng TEQ/kg		Q G-B11 QS
PCB-77	<0,80	ng/kg		Q G-B11 QS
PCB-81	<0,80	ng/kg		Q G-B11 QS
PCB-126	<0,40	ng/kg		Q G-B11 QS
PCB-169	<0,80	ng/kg		Q G-B11 QS
PCB-105	<4,0	ng/kg		Q G-B11 QS
PCB-114	<4,0	ng/kg		Q G-B11 QS
PCB-118	<4,0	ng/kg		Q G-B11 QS

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Parameter	Result	Unit	Method	Accr./cert.
PCB-123	<4,0	ng/kg		Q G-B11 QS
PCB-156	<4,0	ng/kg		Q G-B11 QS
PCB-157	<4,0	ng/kg		Q G-B11 QS
PCB-167	<4,0	ng/kg		Q G-B11 QS
PCB-189	<4,0	ng/kg		Q G-B11 QS
NDL-PCB's (Polychlorinated biphenyls)			10463	
Polychlorinated biphenyl-28	<0,5	µg/kg		Q G-B11
Polychlorinated biphenyl-52	<0,5	µg/kg		Q G-B11
Polychlorinated biphenyl-101	<0,5	µg/kg		Q G-B11
Polychlorinated biphenyl-138	<0,5	µg/kg		Q G-B11
Polychlorinated biphenyl-153	<0,5	µg/kg		Q G-B11
Polychlorinated biphenyl-180	<0,5	µg/kg		Q G-B11
Sum NDL-PCBs (upperbound)	3,0	µg/kg		Q G-B11

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COMMISSION REGULATION (EC) No 152/2009

of 27 January 2009

laying down the methods of sampling and analysis for the official control of feed

(Text with EEA relevance)

(OJ L 54, 26.2.2009, p. 1)

Amended by:

		Official Journal		
		No	page	date
►**M1**	Commission Regulation (EU) No 278/2012 of 28 March 2012	L 91	8	29.3.2012
►**M2**	Commission Regulation (EU) No 51/2013 of 16 January 2013	L 20	33	23.1.2013
►**M3**	Commission Regulation (EU) No 691/2013 of 19 July 2013	L 197	1	20.7.2013
►**M4**	Commission Regulation (EU) No 709/2014 of 20 June 2014	L 188	1	27.6.2014
►**M5**	Commission Regulation (EU) 2017/645 of 5 April 2017	L 92	35	6.4.2017
►**M6**	Commission Regulation (EU) 2017/771 of 3 May 2017	L 115	22	4.5.2017
►**M7**	Commission Implementing Regulation (EU) 2020/1560 of 26 October 2020	L 357	17	27.10.2020
►**M8**	Commission Implementing Regulation (EU) 2022/893 of 7 June 2022	L 155	24	8.6.2022

Corrected by:

►**C1**	Corrigendum, OJ L 62, 6.3.2013, p. 36 (51/2013)

▼B

COMMISSION REGULATION (EC) No 152/2009

of 27 January 2009

laying down the methods of sampling and analysis for the official control of feed

(Text with EEA relevance)

▼M3

Article 1

Sampling for the official control of feed, in particular as regards the determination of constituents, including material which contains or consists of or is produced from genetically modified organisms (GMOs), feed additives as defined by Regulation (EC) No 1831/2003 of the European Parliament and of the Council [1], undesirable substances as defined by Directive 2002/32/EC of the European Parliament and of the Council [2] shall be carried out in accordance with the methods set out in Annex I.

The method of sampling set out in Annex I is applicable for the control of feed as regards the determination of pesticide residues as defined in Regulation (EC) No 396/2005 of the European Parliament and of the Council [3] and control of compliance with Regulation (EU) No 619/2011.

▼B

Article 2

Preparation of samples for analysis and expression of results shall be carried out in accordance with the methods set out in Annex II.

Article 3

Analysis for the official control of feed shall be carried out using the methods set out in Annex III (Methods of analysis to control the composition of feed materials and compound feed, Annex IV (Methods of analysis to control the level of authorised additives in feed), Annex V (Methods of analysis to control undesirable substances in feed) and Annex VI (Methods of analysis for the determination of constituents of animal origin for the official control of feed).

Article 4

The energy value of compound poultry feed shall be calculated in accordance with Annex VII.

Article 5

The methods of analysis to control illegal presence of no longer authorised additives in feed set out in Annex VIII shall be used for confirmatory purposes.

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[1] OJ L 268, 18.10.2003, p. 29.
[2] OJ L 140, 30.5.2002, p. 10.
[3] OJ L 70, 16.3.2005, p. 1.

▼B

Article 6

Directives 71/250/EEC, 71/393/EEC, 72/199/EEC, 73/46/EEC, 76/371/EEC, 76/372/EEC, 78/633/EEC, 81/715/EEC, 84/425/EEC, 86/174/EEC, 93/70/EEC, 93/117/EC, 98/64/EC, 1999/27/EC, 1999/76/EC, 2000/45/EC, 2002/70/EC and 2003/126/EC are repealed.

References to the repealed Directives shall be construed as references to this Regulation and shall be read in accordance with the correlation tables in Annex IX.

Article 7

This Regulation shall enter into force on the twentieth day following that of 20th day following its publication in the ***Official Journal of the European Union.***

It shall apply from 26 August 2009.

This Regulation shall be binding in its entirety and directly applicable in all Member States.

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ANNEX I

METHODS OF SAMPLING

1. PURPOSE AND SCOPE

Samples intended for the official control of feed shall be taken according to the methods described below. Samples thus obtained shall be considered as representative of the sampled portions.

The purpose of representative sampling is to obtain a small fraction from a lot in such a way that a determination of any particular characteristic of this fraction will represent the mean value of the characteristic of the lot. The lot shall be sampled by repeatedly taking incremental samples at various single positions in the lot. These incremental samples shall be combined by mixing to form an aggregate sample from which representative final samples shall be prepared by representative dividing.

If by a visual inspection, portions of the feed to be sampled show a difference in quality from the rest of the feed from the same lot, such portions shall be separated from the rest of the feed and treated as a separate sublot. If it is not possible to divide the feed into separate sublots, the feed shall be sampled as one lot. In such cases, mention shall be made of this fact in the sampling report.

Where a feed sampled in accordance with the provisions of this Regulation is identified as not satisfying EU requirements, is part of a lot of feed of the same class or description, it shall be presumed that all of the feed in that lot is so affected, unless following a detailed assessment there is no evidence that the rest of the lot fails to satisfy the EU requirements.

2. DEFINITIONS

— Lot (or batch): an identified quantity of feed determined to have common characteristics, such as origin, variety, type of packaging, packer, consignor or labelling, and in case of a production process, a unit of production from a single plant using uniform production parameters or a number of such units, when produced in continuous order and stored together.

— Sampled portion: A lot or an identified part of the lot or sublot.

— Sealed sample: a sample sealed in such a manner as to prevent any access to the sample without breaking or removing the seal.

— Incremental sample: A quantity taken from one point in the sampled portion.

— Aggregate sample: An aggregate of incremental samples taken from the same sampled portion.

— Reduced sample: A part of the aggregate sample, obtained from the latter by a process of representative reduction.

— Final sample: A part of the reduced sample or of the homogenised aggregate sample.

— Laboratory sample: a sample intended for the laboratory (as received by the laboratory) and can be the final, reduced or aggregate sample.

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3. GENERAL PROVISIONS

— Sampling personnel: the samples shall be taken by persons authorised for that purpose by the competent authority.

— The sample has to be sealed in such a manner as to prevent any access to the sample without breaking or removing the seal. The seal's mark should be clearly identifiable and clearly visible. Alternatively, the sample can be put in a recipient which can be closed in such a manner that it cannot be opened without irreversibly damaging the receptacle or container, avoiding the re-use of the receptacle or container.

— Identification of the sample: the sample has to be indelibly marked and must be identified in such a way that there is an unambiguous link to the sampling report.

— From each aggregate sample at least two final samples are taken: at least one for control (enforcement) and one for the feed business operator (defence). Eventually, one final sample may be taken for reference. In case the complete aggregate sample is homogenized, the final samples are taken from the homogenized aggregate sample, unless such procedure conflicts with Member States' rules as regards the right of the feed business operator.

4. APPARATUS

4.1. The sampling apparatus must be made of materials which cannot contaminate the products to be sampled. Apparatus which is intended to be used multiple times must be easy to clean to avoid any cross-contamination.

4.2. **Apparatus recommended for the sampling of solid feed**

4.2.1. ***Manual sampling***

4.2.1.1. Flat-bottomed shovel with vertical sides

4.2.1.2. Sampling spear with a long split or compartments. The dimensions of the sampling spear must be appropriate to the characteristics of the sampled portion (depth of container, dimensions of sack, etc.) and to the particle size of the feed.

In case the sampling spear has several apertures, in order to ensure that the sample is taken at the different locations alongside the spear, the apertures should be separated by compartments or sequentially staggered apertures.

4.2.2. ***Mechanical sampling***

Appropriate mechanical apparatus may be used for the sampling of moving feed. Appropriate means that at least the whole section of the flow is sampled.

Sampling of feed in motion (at high flow rates) can be performed by automatic samplers.

4.2.3. ***Divider***

If possible and appropriate, apparatus designed to divide the sample into approximately equal parts should be used for the preparation of reduced samples in a representative way.

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5. QUANTITATIVE REQUIREMENTS AS REGARDS NUMBER OF INCREMENTAL SAMPLES

— The quantitative requirements in points 5.1 and 5.2 as regards the number of incremental samples are applicable for sampled portion sizes up to a maximum of 500 tonnes and which can be sampled in a representative way. The sampling procedure described is equally valid for quantities larger than prescribed maximum sampled portion size provided that the maximum number of incremental samples given in the tables below is ignored, the number of incremental samples being determined by the square-root formula given in the appropriate part of the procedure (see point 5.3) and the minimum aggregate sample size increased proportionally. This does not prevent a large lot being divided into smaller sublots and each sublot sampled in accordance with the procedure described in points 5.1 and 5.2.

— The size of the sampled portion must be such that each of its constituent parts can be sampled.

— For very large lots or sublots (> 500 tonnes) and for lots which are transported or stored in such a way that sampling cannot be done in accordance with the sampling procedure provided for in points 5.1 and 5.2 of this chapter, the sampling procedure as provided for in point 5.3 is to be applied.

— In case the feed business operator is required by legislation to comply with this Regulation within the frame of a mandatory monitoring system, the feed business operator may deviate from the quantitative requirements as provided for in this chapter to take into account operational characteristics on the condition that the feed business operator has demonstrated to the satisfaction of the competent authority the equivalence of the sampling procedure as regards representativeness and after authorisation from the competent authority.

— In exceptional cases, if it is not possible to carry out the method of sampling set out as regards the quantitative requirements because of the unacceptable commercial damage to the lot (because of packaging forms, means of transport, way of storage etc.) an alternative method of sampling may be applied provided that it is as representative as possible and is fully described and documented.

5.1. **Quantitative requirements as regards incremental samples in relation to the control of substances or products uniformly distributed throughout the feed**

5.1.1. ***Loose solid feed***

Size of sampled portion	Minimum number of incremental samples
≤ 2,5 tonnes	7
> 2,5 tonnes	√ 20 times the number of tonnes making up the sampled portion (*), up to 40 incremental samples

(*) Where the number obtained is a fraction, it shall be rounded up to the next whole number.

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5.1.2. ***Loose liquid feed***

Size of sampled portion	Minimum number of incremental samples
≤ 2,5 tonnes or ≤ 2 500 litres	4 (*)
> 2,5 tonnes or > 2 500 litres	7 (*)

(*) In case it is not possible to make the liquid homogeneous, the number of incremental samples has to be increased.

5.1.3. ***Packaged feed***

Feed (solid and liquid) can be packaged in bags, sacks, cans, barrels etc. which are referred to in the table as units. Large units (≥ 500 kg or litres) have to be sampled in accordance with the provisions foreseen for loose feed (see points 5.1.1 and 5.1.2).

Size of sampled portion	Minimum number of units from which (at least) one incremental sample has to be taken (*)
1 to 20 units	1 unit (**)
21 to 150 units	3 units (**)
151 to 400 units	5 units (**)
> 400 units	¼ of the √ number of units making up the sampled portion (***), up to 40 units

(*) In the case where opening of an unit might affect the analysis (e.g. perishable wet feeds) an incremental sample shall be the unopened unit.
(**) For units whose contents do not exceed 1 kg or one litre, an incremental sample shall be the contents of one original unit.
(***) Where the number obtained is a fraction, it shall be rounded up to the next whole number.

5.1.4. ***Feed blocks and mineral licks***

Minimum one block or lick to be sampled per sampled portion of 25 units, up to a maximum of four blocks or licks.

For blocks or licks weighing not more than 1 kg each, an incremental sample shall be the contents of one block or one lick.

5.1.5. ***Roughages/forage***

Size of sampled portion	Minimum number of incremental samples (*)
≤ 5 tonnes	5
> 5 tonnes	√ 5 times the number of tonnes making up the sampled portion (**), up to 40 incremental samples

(*) It is acknowledged that in certain situations (e.g. silages) it is not possible to take the required incremental samples, without causing unacceptable damage to the lot. An alternative method of sampling may be applied in such situations and a guidance for sampling such lots will be elaborated before the entry into application of this Regulation.
(**) Where the number obtained is a fraction, it shall be rounded up to the next whole number.

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5.2. **Quantitative requirements as regards incremental samples in relation to the control of constituents or substances likely to be distributed non-uniformly in feed**

These quantitative requirements as regards incremental samples are to be used in the following situations:

— control of aflatoxins, rye ergot, other mycotoxins and harmful botanical impurities in feed materials;

— control of cross contamination by a constituent, including GM material, or substance for which non-uniform distribution is expected in feed materials.

In case the control authority has strong suspicion that such a non-uniform distribution occurs also in case of cross contamination by a constituent or substance in a compound feed, the quantitative requirements as provided for in the table below can be applied.

Size of sampled portion	Minimum number of incremental samples
< 80 tonnes	See quantitative requirements under point 5.1. The number of incremental samples to be taken has to be multiplied by 2,5.
≥ 80 tonnes	100

5.3. **Quantitative requirements as regards the incremental samples in the case of very large lots**

In the case of large sampled portions (sampled portions > 500 tonnes), the number of incremental samples to be taken = 40 incremental samples + √ tonnes in relation to the control of substances or products uniformly distributed throughout the feed or 100 incremental samples + √ tonnes in relation to the control of constituents or substances likely to be distributed non-uniformly in feed materials.

6. QUANTITATIVE REQUIREMENTS AS REGARDS AGGREGATE SAMPLE

A single aggregate sample per sampled portion is required.		
	Nature of feed	Minimum size of aggregate sample (*) (**)
6.1.	Loose feed	4 kg
6.2.	Packaged feed:	4 kg (***)

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A single aggregate sample per sampled portion is required.		
	Nature of feed	Minimum size of aggregate sample (*) (**)
6.3.	Liquid or semi-liquid feed:	4 litres
6.4.	Feed blocks or mineral licks:	
6.4.1.	each weighing more than 1 kg	4 kg
6.4.2.	each weighing not more than 1 kg	weight of four original blocks or licks
6.5.	Roughage/forage	4 kg (****)

(*) In case the sampled feed is of high value, a smaller quantity of aggregate sample can be taken on the condition this is described and documented in the sampling report.

(**) In accordance with the provisions of Commission Regulation (EU) No 619/2011 of 24 June 2011 laying down the methods of sampling and analysis for the official control of feed as regards presence of genetically modified material for which an authorisation procedure is pending or the authorisation of which has expired (OJ L 166, 25.6.2011, p. 9), the aggregate sample for the control of the presence of genetically modified material must contain at least 35 000 seeds/grains. This means that for maize the size of the aggregate sample must be at least 10,5 kg and for soybean 7 kg. For other seeds and grains such as barley, millet, oat, rice, rye, wheat and rapeseed, the aggregate sample size of 4 kg corresponds to more than 35 000 seeds.

(***) In case of packaged feed, it may also not be possible to achieve the size of 4 kg for the aggregate sample depending of the size of the individual units.

(****) In case it concerns roughage or forage with a low specific gravity (e.g. hay, straw), the aggregate sample should have a minimum size of 1 kg.

7. QUANTITATIVE REQUIREMENTS AS REGARDS FINAL SAMPLES

Final samples

Analysis of at least one final sample is required. The amount in the final sample for analysis shall be not less than the following:

Solid feed	500 g (*) (**) (***)
Liquid or semi-liquid feed	500 ml (*)

(*) In accordance with the provisions of Regulation (EU) No 619/2011, the final sample for the control of the presence of genetically modified material must contain at least 10 000 seeds/grains. This means that for maize the size of the final sample must be at least 3 000 g and for soybean 2 000 g. For other seeds and grains such as barley, millet, oat, rice, rye, wheat and rapeseed, the final sample size of 500 g corresponds to more for 10 000.

(**) In case the size of the aggregate sample is significantly less than 4 kg or litre (see footnotes point (6), also a smaller quantity of final sample can be taken on the condition this is described and documented in the sampling report.

(***) In case of sampling pulses, cereal grains and tree nuts for the determination of pesticide residues, the minimum size of the final sample shall be 1 kg in accordance with the provisions of Commission Directive 2002/63/EC (OJ L 187, 16.7.2002, p. 30).

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ANNEX II

GENERAL PROVISIONS ON METHODS OF ANALYSIS FOR FEED

A. PREPARATION OF SAMPLES FOR ANALYSIS

1. **Purpose**

The procedures described below concern the preparation for analysis of samples, sent to the control laboratories after sampling in accordance with the provisions laid down in Annex I.

The laboratory samples must be prepared in such a way that the amounts weighed out, as provided for in the methods of analysis, are homogeneous and representative of the final samples.

2. **Precautions to be taken**

The sample preparation procedure to be followed is dependent on the methods of analysis to be used and the constituents or substances to be controlled. It is therefore of major importance that it is ensured that the followed sample preparation procedure is appropriate for the used method of analysis and for constituents or substances to be controlled.

All the necessary operations must be performed in such a way as to avoid as far as possible contamination of the sample and changes of its composition.

Grinding, mixing and sieving shall be carried out without delay with minimal exposure of the sample to the air and light. Mills and grinders likely to appreciably heat the sample shall not be used.

Manual grinding is recommended for feed which are particularly sensitive to heat. Care shall also be taken to ensure that the apparatus itself is not a source of contamination.

If the preparation cannot be carried out without significant changes in the moisture content of the sample, determine the moisture content before and after preparation according to the method laid down in Part A of Annex III.

3. **Procedure**

3.1. ***General procedure***

The test aliquot is taken from the final sample. Coning and quartering is not recommended because this might provide test aliquots with high splitting error.

3.1.1. Feed which can be ground as such

— Mix the sieved final sample and collect it in a suitable clean, dry container fitted with an air-tight stopper. Mix again in order to ensure full homogenisation, immediately before weighing out the amount for analysis (test aliquot).

3.1.2. Feed which can be ground after drying

— Unless otherwise specified in the methods of analysis, dry the final sample to bring its moisture content down to a level of 8 to 12 %, according to the preliminary drying procedure described under point 4.3 of the method of determination of moisture mentioned in Part A of Annex III). Then proceed as indicated in section 3.1.1.

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3.1.3. Liquid or semi-liquid feed

— Collect the final sample in a suitable clean, dry container, fitted with an air-tight stopper. Mix thoroughly in order to ensure full homogenisation immediately before weighing out the amount for analysis (test aliquot).

3.1.4. Other feed

— Final samples which cannot be prepared according to one of the above procedures shall be treated by any other procedure which ensures that the amounts weighed out for the analysis (test aliquot) are homogeneous and representative of the final samples.

3.2. ***Specific procedure in case of examination by visual inspection or by microscopy or in cases where the whole aggregate sample is homogenised***

— In case of an examination by visual inspection (without making use of microscope), the whole laboratory sample is used for examination.

— In case of a microscopic examination, the laboratory may reduce the aggregate sample, or further reduce the reduced sample. The final samples for defence and eventually reference purposes are taken following a procedure equivalent to the procedure followed for the final sample for enforcement.

— In case the whole aggregate sample is homogenized, the final samples are taken from the homogenized aggregate sample.

4. **Storage of samples**

Samples must be stored at a temperature that will not alter their composition. Samples intended for the analysis of vitamins or substances which are particularly sensitive to light shall be stored in such conditions that the sample is not adversely affected by light.

B. PROVISIONS RELATING TO REAGENTS AND APPARATUS USED IN METHODS OF ANALYSIS

1. Unless otherwise specified in the methods of analysis, all analytical reagents must be analytically pure (a.p.). When trace analysis is carried out, the purity of the reagents must be checked by a blank test. Depending upon the results obtained, further purification of the reagents may be required.

2. Any operation involving preparation of solutions, dilution, rinsing or washing, mentioned in the methods of analysis without indication as to the nature of the solvent or diluent employed, implies that water must be used. As a general rule, water shall be demineralised or distilled. In particular cases, which are indicated in the methods of analysis, it must be submitted to special procedures of purification.

3. In view of the equipment normally found in control laboratories, only those instruments and apparatus which are special or require specific usage are referred to in the methods of analysis. They must be clean, especially when very small amounts of substances have to be determined.

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C. APPLICATION OF METHODS OF ANALYSIS AND EXPRESSION OF THE RESULTS

1. **Extraction procedure**

Several methods determine a specific extraction procedure. As a general rule, other extraction procedures than the procedure referred to in the method can be applied on the condition that the used extraction procedure has been proven to have the equivalent extraction efficiency for the matrix analysed as the procedure mentioned in the method.

2. **Clean-up procedure**

Several methods determine a specific clean-up procedure. As a general rule, other clean-up procedures than the procedure referred to in the method can be applied on the condition that the used clean-up procedure has been proven to result in equivalent analytical results for the matrix analysed as the procedure mentioned in the method.

3. **Number of determinations**

In case of the analysis of undesirable substances, if the result of the first determination is significantly (> 50 %) lower than the specification to be controlled, no additional determinations are necessary, on the condition that the appropriate quality procedures are applied. In other cases a duplicate analysis (second determination) is necessary to exclude the possibility of internal cross-contamination or an accidental mix-up of samples. The mean of the two determinations, taking into account the measurement uncertainty is used for verification of compliance.

In case of the control of the declared content of a substance or ingredient, if the result of the first determination confirms the declared content, i.e. the analytical result falls within the acceptable range of variation of the declared content, no additional determinations are necessary, on the condition that the appropriate quality procedures are applied. In other cases a duplicate analysis (second determination) is necessary to exclude the possibility of internal cross-contamination or an accidental mix-up of samples. The mean of the two determinations, taking into account the measurement uncertainty is used for verification of compliance.

In some cases this acceptable range of variation is defined in legislation such as in Regulation (EC) No 767/2009 of the European Parliament and of the Council of 13 July 2009 on the placing on the market and use of feed, amending European Parliament and Council Regulation (EC) No 1831/2003 and repealing Council Directive 79/373/EEC, Commission Directive 80/511/EEC, Council Directives 82/471/EEC, 83/228/EEC, 93/74/EEC, 93/113/EC and 96/25/EC and Commission Decision 2004/217/EC ([1]).

4. **Reporting of the method of analysis used**

The analysis report shall mention the method of analysis used.

5. **Reporting of the analytical result**

The analytical result shall be expressed in the manner laid down in the method of analysis to an appropriate number of significant figures and shall be corrected, if necessary, to the moisture content of the final sample prior to preparation.

([1]) OJ L 229, 1.9.2009, p. 1.

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6. **Measurement uncertainty and recovery rate in case of analysis of undesirable substances**

As regards undesirable substances within the meaning of Directive 2002/32/EC, a product intended for animal feed shall be considered as non-compliant with the established maximum content, if the analytical result, relative to a feed with a moisture content of 12 %, is deemed to exceed the maximum content taking into account expanded measurement uncertainty and correction for recovery. In order to assess compliance, the analysed concentration is used after being corrected for recovery and after deduction of the expanded measurement uncertainty. This procedure is only applicable in cases where the method of analysis enables the estimation of measurement uncertainty and correction for recovery (e.g. not possible in case of microscopic analysis).

The analytical result shall be reported as follows (in so far the used method of analysis enables to estimate the measurement uncertainty and recovery rate):

(a) corrected for recovery, the level of recovery being indicated. The correction for recovery is not necessary in case the recovery rate is between 90-110 %.

(b) as ‘x +/– U’, whereby x is the analytical result and U is the expanded measurement uncertainty, using a coverage factor of 2 which gives a level of confidence of approximately 95 %.

However, if the result of the analysis is significantly (> 50 %) lower than the specification to be controlled, and on the condition that the appropriate quality procedures are applied and the analysis serves only the purpose of checking compliance with legal provisions, the analytical result might be reported without correction for recovery and the reporting of the recovery rate and measurement uncertainty might be omitted in these cases.

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S = Total optical rotation in saccharimeter degrees

S' = Optical rotation in saccharimeter degrees of the substances soluble in 40 % (v/v) ethanol

N = weight (g) of saccharose in 100 ml of water yielding an optical rotation of 100 saccharimeter degrees when measured using a 200 mm tube

16,29 g for the French saccharimeters

26,00 g for the German saccharimeters

20,00 g for mixed saccharimeters.

$[\alpha]_D^{20°}$ = Specific optical rotation of pure starch (see 6.1)

6.3. ***Repeatability***

The difference between the results of two parallel determinations carried out on the same sample must not exceed 0,4 in absolute value for a starch content lower than 40 % and 1 % relative for starch contents equal to or greater than 40 %.

7. **Observations**

7.1. If the sample contains more than 6 % of carbonates, calculated in terms of calcium carbonate, they must be destroyed by treatment with an exactly appropriate quantity of dilute sulphuric acid before determination of the total optical rotation.

7.2. In the case of products with a high lactose content, such as powdered milk serum or skimmed milk powder, proceed as follows after adding 80 ml of ethanol (3.5). Fit a reflux condenser to the flask and immerse the latter in a water bath at 50 °C for 30 minutes. Leave to cool and continue the analysis as indicated in 5.3.

7.3. The following feed materials, where they are present in significant amounts in feed, are known to give rise to interferences when determining the starch content by the polarimetric method and thereby incorrect results could be yielded:

— (sugar) beet products such as (sugar)beet pulp, (sugar) beet molasses, (sugar) beet pulp — molassed, (sugar) beet vinasse, (beet) sugar,

— citrus pulp,

— linseed; linseed expeller; linseed extracted,

— rape seed; rape seed expeller; rape seed extracted; rape seed hulls,

— sunflower seed; sunflower seed extracted; sunflower seed, partially decorticated, extracted,

— copra expeller; copra extracted,

— potato pulp,

— dehydrated yeast,

— products rich in inulin (e.g. Chips and meal of Jerusalem artichokes),

— greaves.

M. DETERMINATION OF CRUDE ASH

1. **Purpose and Scope**

This method makes it possible to determine the crude ash content of feed.

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2. **Principle**

The sample is ashed at 550 °C; the residue is weighed.

3. **Reagents**

Ammonium nitrate, solution 20 % (w/v).

4. **Apparatus**

4.1. Hot-plate.

4.2. Electric muffle-furnace with thermostat.

4.3. Crucibles for ashing made of silica, porcelain or platinum either rectangular (approx. 60 × 40 × 25 mm) or circular (diameter: 60 to 75 mm, height: 20 to 40 mm).

5. **Procedure**

Weigh out to the nearest mg approximately 5 g of the sample (2,5 in the case of products which have a tendency to swell) and place in a crucible for ashing which has first been heated at 550 °C, cooled down and tared. Place the crucible on the hot-plate and heat gradually until the substance carbonises. Ash according to 5.1 or 5.2.

5.1. Put the crucible into the calibrated muffle furnace set at 550 °C. Keep at this temperature until white, light grey or reddish ash is obtained which appears to be free from carbonaceous particles. Place the crucible in a desiccator, leave to cool and weigh immediately.

5.2. Put the crucible into the calibrated muffle-furnace set at 550 °C. Ash for 3 hours. Place the crucible in a desiccator, leave to cool and weigh immediately. Ash again for 30 minutes to ensure that the weight of the ash remains constant (loss in weight between two successive weightings must be less than or equal to 1 mg).

6. **Calculation of results**

Calculate the weight of the residue by deducting the tare.

Express the result as a percentage of the sample.

7. **Observations**

7.1. The ash of ***substances which are difficult to ash*** must be subjected to an initial ashing of at least three hours, cooled and then a few drops of 20 % solution of ammonium nitrate or water added to it (carefully, to avoid dispersal of the ash or the formation of lumps). Continue calcining after drying in the oven. Repeat the operation as necessary until ashing is complete.

7.2. In the case of ***substances resistant to the treatment*** described under 7.1, proceed as follows: after ashing for three hours, place the ash in warm water and filter through a small, ash-free filter. Ash the filter and its contents in the original crucible. Place the filtrate in the cooled crucible, evaporate until dry, ash and weigh.

7.3. In the case of ***oils and fats***, weigh accurately a sample of 25 g in a suitably sized crucible. Carbonise by setting light to the substance with a strip of ash-free filter paper. After combustion, moisten with as little water as possible. Dry and ash as described under 5.

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N. DETERMINATION OF ASH WHICH IS INSOLUBLE IN HYDROCHLORIC ACID

1. **Purpose and Scope**

This method makes it possible to determine the level in feed of mineral substances which are insoluble in hydrochloric acid. Two methods can be used, depending on the nature of the sample.

1.1. ***Method A:*** applicable to organic feed materials and to most compound feed.

1.2. ***Method B:*** applicable to mineral compounds and mixtures and to compound feed, whose content in substances insoluble in hydrochloric acid, as determined by Method A, is greater than 1 %.

2. **Principle**

2.1. ***Method A:*** the sample is ashed, the ash boiled in hydrochloric acid and the insoluble residue filtered and weighed.

2.2. ***Method B:*** the sample is treated with hydrochloric acid. The solution is filtered, the residue ashed and the ash thus obtained treated in accordance with Method A.

3. **Reagents**

3.1. Hydrochloric acid 3 mol/litre.

3.2. Trichloroacetic acid, solution 20 % solution (w/v).

3.3. Trichloroacetic acid, solution 1 % (w/v).

4. **Apparatus**

4.1. Hot plate.

4.2. Electric muffle-furnace with thermostat.

4.3. Crucibles for ashing made of silica, porcelain or platinum, either rectangular (approx. 60 × 40 × 25 mm) or circular (diameter: 60 to 75 mm, height: 20 to 40 mm).

5. **Procedure**

5.1. ***Method A***

Ash the sample using the method described for the determination of crude ash. Ash obtained from that analysis may also be used.

Place the ash in a 250 to 400 ml beaker using 75 ml of hydrochloric acid (3.1). Bring slowly to the boil and boil gently for 15 minutes. Filter the warm solution through an ash-free filter paper and wash the residue with warm water until the acid reaction is no longer visible. Dry the filter containing the residue and ash in a tared crucible at a temperature of not less than 550 ^{o}C and not more than 700 ^{o}C. Cool in a desiccator and weigh.

5.2. ***Method B***

Weigh 5 g of the sample to the nearest mg and place in a 250 to 400 ml beaker. Add 25 ml of water and 25 ml of hydrochloric acid (3.1) successively, mix and wait for effervescence to cease. Add a further 50 ml of hydrochloric acid (3.1). Wait for any release of gas to cease then place the beaker in a boiling water bath and keep it there for 30 minutes or longer, if necessary, in order to hydrolyse thoroughly any starch which may be present. Filter while warm through an ash-free filter

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and wash the filter in 50 ml of warm water (see observation 7). Place the filter containing the residue in a crucible for ashing, dry and ash at a temperature of not less than 550 °C and not more than 700 °C. Place the ash in a 250 to 400 ml beaker using 75 ml of hydrochloric acid (3.1); continue as described in the second subparagraph of 5.1.

6. **Calculation of results**

Calculate the weight of the residue by deducting the tare. Express the result as a percentage of the sample.

7. **Observation**

If filtration proves difficult recommence the analysis, replacing the 50 ml of hydrochloric acid (3.1) by 50 ml of 20 % trichloroacetic acid (3.2) and washing the filter in a warm solution of 1 % trichloroacetic acid (3.3).

O. DETERMINATION OF CARBONATES

1. **Purpose and Scope**

This method makes it possible to determine the amount of carbonates, conventionally expressed as calcium carbonate, in most feed.

However in certain cases (for example, with iron carbonate) a special method must be used.

2. **Principle**

The carbonates are decomposed in hydrochloric acid; the carbon dioxide released is collected in a graduated tube, and its volume compared with that released under the same conditions by a known quantity of calcium carbonate.

3. **Reagents**

3.1. Hydrochloric acid, density 1,10 g/ml.

3.2. Calcium carbonate.

3.3. Sulphuric acid, approximately 0,05 mol/litre, coloured with methyl red.

4. **Apparatus**

Scheibler-Dietrich apparatus (see diagram) or equivalent apparatus.

5. **Procedure**

According to the sample's carbonate content, weigh a portion of the sample as shown below:

— 0,5 g for products containing from 50 % to 100 % of carbonates, expressed as calcium carbonate,

— 1 g for products containing from 40 % to 50 % of carbonates, expressed as calcium carbonate,

— 2 to 3 g for other products.

Place the portion of the sample in the special flask (4) of the apparatus, fitted with a small tube of unbreakable material containing 10 ml of hydrochloric acid (3.1), and connect the flask to the apparatus. Turn the three-way cock (5) so that the tube (1) connects with the outside. Using the mobile tube (2), which is filled with coloured sulphuric acid (3.3) and connected to the graduated tube (1), bring the level of the liquid up to the zero mark. Turn the cock (5) in order to connect up tubes (1) and (3) and check that the level is at zero.

Run the hydrochloric acid (3.1) slowly over the portion of the sample, tilting the flask (4). Make the pressure equal by lowering the tube (2). Shake the flask (4) until the release of carbon dioxide has stopped completely.

Restore pressure by bringing the liquid back to the same level in tubes (1) and (2). After a ***few minutes***, when the volume of gas has become constant, take the reading.

Carry out a control test in the same conditions on 0,5 g of calcium carbonate (3.2).

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(b) (4)

CERTIFICATE OF ANALYSIS

Order Nr **Cust Order Ref**

Product ALOAPUR
ALOAPUR

Lot No (b) (4)

Manufacturing Date 14-Dec-2018 **Retest Date** 13-Dec-2020

Test	Units	Specification	Results
Ester value	mg KOH per	>=40	(b) (4)
Ash, residue on ignition	%	<=67	

Parameters not tested in all lots but validated through in-process or final testing.

Test	Units	Specification
Arsenic	ppm	<=12
Fluorine	ppm	<=500
Lead	ppm	<=15
Mercury	ppm	<=0.2
Dioxins	ppt	<=0.75
Dioxins + Dioxins like PCBs	ppt	<=1.5
non Dioxin like PCBs	ppb	<=10
Aflatoxin B1	ppm	<=0.01

This lot complies with: 2002/32/EC
This document is generated by a validated system and therefore not signed.

(b) (4)
Manager Quality Control

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www.corbion.nl
VAT nr. (b) (4)

Corbion

CERTIFICATE OF ANALYSIS

Order Nr **Cust Order Ref**

Product ALOAPUR
ALOAPUR

Lot No (b) (4)

Manufacturing Date 10-May-2019 **Retest Date** 09-May-2021

Test	Units	Specification	Results
Ester value	mg KOH per	>=40	(b) (4)
Ash, residue on ignition	%	<=67	

Parameters not tested in all lots but validated through in-process or final testing.

Test	Units	Specification
Arsenic	ppm	<=12
Fluorine	ppm	<=500
Lead	ppm	<=15
Mercury	ppm	<=0.2
Dioxins	ppt	<=0.75
Dioxins + Dioxins like PCBs	ppt	<=1.5
non Dioxin like PCBs	ppb	<=10
Aflatoxin B1	ppm	<=0.01

This lot complies with: 2002/32/EC
This document is generated by a validated system and therefore not signed.

(b)(6)

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VAT nr. (b) (4)

CERTIFICATE OF ANALYSIS

Order Nr **Cust Order Ref**

Product ALOAPUR
ALOAPUR

Lot No (b) (4)

Manufacturing Date 22-Dec-2019 **Retest Date** 21-Dec-2021

Test	Units	Specification	Results
Ester value	mg KOH per	>=40	(b) (4)
Ash, residue on ignition	%	<=67	

Parameters not tested in all lots but validated through in-process or final testing.

Test	Units	Specification
Arsenic	ppm	<=12
Fluorine	ppm	<=500
Lead	ppm	<=15
Mercury	ppm	<=0.2
Dioxins	ppt	<=0.75
Dioxins + Dioxins like PCBs	ppt	<=1.5
non Dioxin like PCBs	ppb	<=10
Aflatoxin B1	ppm	<=0.01

This lot complies with: 2002/32/EC
This document is generated by a validated system and therefore not signed.

(b)(6)

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VAT nr. (b) (4)

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CERTIFICATE OF ANALYSIS

Order Nr **Cust Order Ref**

Product ALOAPUR
ALOAPUR

Lot No (b) (4)

Manufacturing Date 01-Jan-2020 **Retest Date** 31-Dec-2021

Test	Units	Specification	Results
Ester value	mg KOH per	>=40	(b) (4)
Ash, residue on ignition	%	<=67	

Parameters not tested in all lots but validated through in-process or final testing.

Test	Units	Specification
Arsenic	ppm	<=12
Fluorine	ppm	<=500
Lead	ppm	<=15
Mercury	ppm	<=0.2
Dioxins	ppt	<=0.75
Dioxins + Dioxins like PCBs	ppt	<=1.5
non Dioxin like PCBs	ppb	<=10
Aflatoxin B1	ppm	<=0.01

This lot complies with: 2002/32/EC
This document is generated by a validated system and therefore not signed.

(b)(6)

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VAT nr. (b) (4)

CERTIFICATE OF ANALYSIS

Order Nr **Cust Order Ref**

Product ALOAPUR
ALOAPUR

Lot No (b) (4)

Manufacturing Date 07-Jan-2020 **Retest Date** 06-Jan-2022

Test	Units	Specification	Results
Ester value	mg KOH per	>=40	(b) (4)
Ash, residue on ignition	%	<=67	

Parameters not tested in all lots but validated through in-process or final testing.

Test	Units	Specification
Arsenic	ppm	<=12
Fluorine	ppm	<=500
Lead	ppm	<=15
Mercury	ppm	<=0.2
Dioxins	ppt	<=0.75
Dioxins + Dioxins like PCBs	ppt	<=1.5
non Dioxin like PCBs	ppb	<=10
Aflatoxin B1	ppm	<=0.01

This lot complies with: 2002/32/EC
This document is generated by a validated system and therefore not signed.

(b)(6)

To	: QC lab
From	: (b)(6)
Date	: 05 July 2022
AS-code	: 2021-000472
Project	: 100208 Esters Support NON WBSO
Filename	: AS2021-000472
Version	: 1
Keywords	: Lactylates, Aloapur PM, QC, Stability

Analysis for stability testing Aloapur PM QC lab

Introduction:
Analysis for stability testing Aloapur PM QC lab

Samples:

(b) (4)

(b) (4)

Results:

Table 1: GLC lactylates:

Sample		1 x68230	2 x56376	3 x02765	4 x68230	5 x56376	6 x02765	7 x68230	8 x56376	9 x02765
Time [months]		0	0	0	3	3	3	6	6	6
lactic acid	[% (w/w)]	(b) (4)								
lactoyl lactic acid	[% (w/w)]									
C12 fatty acid	[% (w/w)]									
C12-1 lactylate	[% (w/w)]									
C12-2 lactylate	[% (w/w)]									
C14 fatty acid	[% (w/w)]									
C14-1 lactylate	[% (w/w)]									
C14-2 lactylate	[% (w/w)]									

Remark:

A)

B) (b) (4)

References:

ELN-reference	Analyst	Requested analysis
(b) (4)	(b)(6)	GC lactylates
		GC lactylates
		GC lactylates

Appendix A: Test methods.

- GC lactylates:

As described in analytical method "AS-A-0403 - GC - Quantification of lactic- and lactoyl acid, fatty acids and individual lactylates"

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REPORT

ALOAPUR PM - sodium sulfate content

FROM (b)(6)

DATE January 27, 2022
REFERENCE Reference

SUBJECT Registration of ALOAPUR PM in The USA

(b) (4)

TO (b) (4)
CC

VERSION 1.0

AUTHOR (b) (4)

Report CONFIDENTIAL
REPORT

Contents

1 Introduction

ALOAPUR PM is a free flowing powder product. It consists of 2 main components:

(1) The Active Ingredient: (b) (4)

(2) The Carrier: (b) (4)

ALOAPUR PM has received the GRAS Status as was established by an expert panel. However, in order to be listed in the AAFCO, a FDA notification is required. It has been requested by the FDA that Corbion clarifies the amount of sodium sulfate that is used in the market formulation and quantify the level of sulfur in the final marketed product. This information is needed for the composition of the market formulation and for the safety assessment for targeted animal species, especially for ruminants."

(b) (4)

2 Batch information and specification

(b) (4)

Table 2. Relation between sodium sulfate and packaging size

Packaging size	Sodium sulfate [kg]	Batch volume [kg]	Concentration
(b) (4)			

(b) (4)

3 Analytical results

The analytical results are obtained via ICP-MS and are summarized in Table 2.

Table 3. Results from Eurofins using ICP-MS

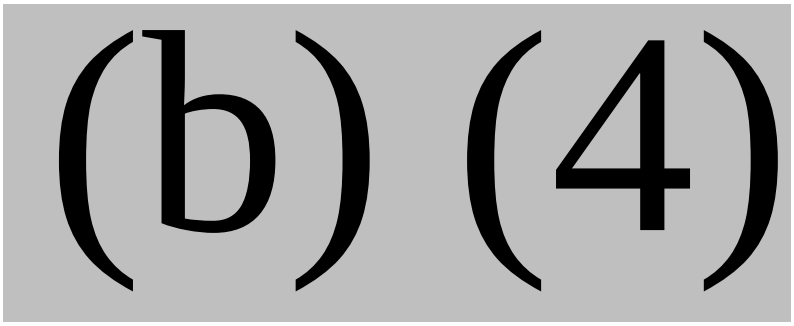
Sample	Lot number	Sulphur (S)	Sulfate (SO4)
		mg/kg = ppm	mg/kg = ppm
Aloapur PM	(b) (4)		
Aloapur PM			
Aloapur PM			

(b) (4)

4 Conclusions

(b) (4)

5 Appendix – Analytical reports



Analyserapport

(b) (4)

(b) (4)

Purac Biochem BV (QC)
t.a.v. (b) (4)
Postbus 21
4200 AA GORINCHEM

(b) (4)

Analyseparameter	Analyseuitslag	Eenheid	Methode	Accr./cert.
Fluoride	<20	mg/kg	10012	Q G-B10
Arseen	13,7	mg/kg	10222	Q G-B11 QS
Cadmium	1,04	mg/kg	10222	Q G-B11 QS
Kwik	<0,100	mg/kg	10222	Q G-B10 QS
Lood	6,02	mg/kg	10222	Q G-B11 QS
Aflatoxine B1 (LC-MS/MS)	<0,0010	mg/kg	10370	
Dioxine & Dioxine Like PCB's			10463	
WHO-TEQ (PCDD/F + DL-PCBs) incl. LOQ	0,222	ng TEQ/kg		Q G-B11 QS
Dioxine TEQ (WHO 2005) incl. LOQ	0,156	ng TEQ/kg		Q G-B11 QS
2,3,7,8-TCDD	<0,04	ng/kg		Q G-B11 QS
1,2,3,7,8-PeCDD	<0,04	ng/kg		Q G-B11 QS
1,2,3,4,7,8-HxCDD	<0,08	ng/kg		Q G-B11 QS
1,2,3,6,7,8-HxCDD	<0,08	ng/kg		Q G-B11 QS
1,2,3,7,8,9-HxCDD	<0,08	ng/kg		Q G-B11 QS
1,2,3,4,6,7,8-HpCDD	<0,10	ng/kg		Q G-B11 QS
OCDD	<0,20	ng/kg		Q G-B11 QS
2,3,7,8-TCDF	<0,04	ng/kg		Q G-B11 QS
1,2,3,7,8-PeCDF	<0,04	ng/kg		Q G-B11 QS
2,3,4,7,8-PeCDF	<0,04	ng/kg		Q G-B11 QS
1,2,3,4,7,8-HxCDF	<0,08	ng/kg		Q G-B11 QS
1,2,3,6,7,8-HxCDF	<0,08	ng/kg		Q G-B11 QS
2,3,4,6,7,8-HxCDF	<0,08	ng/kg		Q G-B11 QS
1,2,3,7,8,9-HxCDF	<0,08	ng/kg		Q G-B11 QS
1,2,3,4,6,7,8-HpCDF	<0,10	ng/kg		Q G-B11 QS
1,2,3,4,7,8,9-HpCDF	<0,10	ng/kg		Q G-B11 QS
OCDF	<0,20	ng/kg		Q G-B11 QS
DL-PCB TEQ (WHO 2005) incl. LOQ	0,065	ng TEQ/kg		Q G-B11 QS
PCB-77	<0,80	ng/kg		Q G-B11 QS
PCB-81	<0,80	ng/kg		Q G-B11 QS
PCB-126	<0,40	ng/kg		Q G-B11 QS

(b) (4)

Analyseparameter	Analyseuitslag	Eenheid	Methode	Accr./cert.
PCB-169	<0,80	ng/kg		Q G-B11 QS
PCB-105	<4,0	ng/kg		Q G-B11 QS
PCB-114	<4,0	ng/kg		Q G-B11 QS
PCB-118	<4,0	ng/kg		Q G-B11 QS
PCB-123	<4,0	ng/kg		Q G-B11 QS
PCB-156	<4,0	ng/kg		Q G-B11 QS
PCB-157	<4,0	ng/kg		Q G-B11 QS
PCB-167	<4,0	ng/kg		Q G-B11 QS
PCB-189	<4,0	ng/kg		Q G-B11 QS
NDL-PCB's (polychloorbifenylen)			10463	
Polychloorbifenyl-28	<0,5	µg/kg		Q G-B11 QS
Polychloorbifenyl-52	<0,5	µg/kg		Q G-B11 QS
Polychloorbifenyl-101	<0,5	µg/kg		Q G-B11 QS
Polychloorbifenyl-138	<0,5	µg/kg		Q G-B11 QS
Polychloorbifenyl-153	<0,5	µg/kg		Q G-B11 QS
Polychloorbifenyl-180	<0,5	µg/kg		Q G-B11 QS
NDL-PCB Totaal (upper bound)	3,0	µg/kg		Q G-B11 QS

Methode omschrijvingen

Methode/analyse	Norm
10012	Eigen methode
10222	Eigen methode
10463	Eigen methode

(b) (4)

Analyserapport

(b) (4)

Purac Biochem BV (QC)
t.a.v. (b) (4)
Postbus 21
4200 AA GORINCHEM

Monsternummer	:	M22000310003
Klantnummer	:	D05421
Monsterontvangstdatum	:	05-01-2022
Digitale order ID	:	NC000106011003

Uw monsterkenmerken

Reden onderzoek	:	2668
Productnaam	:	7500068230/T=0
Omschrijving	:	Aloapur PM
Monsternamedatum	:	3-1-2022
Kostencode	:	4700013971

Analyseparameter	Analyseuitslag	Eenheid	Methode	Accr./cert.
Fluoride	<20	mg/kg	10012	Q G-B10
Arseen	13,8	mg/kg	10222	Q G-B11 QS
Cadmium	0,850	mg/kg	10222	Q G-B11 QS
Kwik	<0,100	mg/kg	10222	Q G-B10 QS
Lood	6,01	mg/kg	10222	Q G-B11 QS
Aflatoxine B1 (LC-MS/MS)	<0,0010	mg/kg	10370	
Dioxine & Dioxine Like PCB's			10463	
WHO-TEQ (PCDD/F + DL-PCBs) incl. LOQ	0,222	ng TEQ/kg		Q G-B11 QS
Dioxine TEQ (WHO 2005) incl. LOQ	0,156	ng TEQ/kg		Q G-B11 QS
2,3,7,8-TCDD	<0,04	ng/kg		Q G-B11 QS
1,2,3,7,8-PeCDD	<0,04	ng/kg		Q G-B11 QS
1,2,3,4,7,8-HxCDD	<0,08	ng/kg		Q G-B11 QS
1,2,3,6,7,8-HxCDD	<0,08	ng/kg		Q G-B11 QS
1,2,3,7,8,9-HxCDD	<0,08	ng/kg		Q G-B11 QS
1,2,3,4,6,7,8-HpCDD	<0,10	ng/kg		Q G-B11 QS
OCDD	<0,20	ng/kg		Q G-B11 QS
2,3,7,8-TCDF	<0,04	ng/kg		Q G-B11 QS
1,2,3,7,8-PeCDF	<0,04	ng/kg		Q G-B11 QS
2,3,4,7,8-PeCDF	<0,04	ng/kg		Q G-B11 QS
1,2,3,4,7,8-HxCDF	<0,08	ng/kg		Q G-B11 QS
1,2,3,6,7,8-HxCDF	<0,08	ng/kg		Q G-B11 QS
2,3,4,6,7,8-HxCDF	<0,08	ng/kg		Q G-B11 QS
1,2,3,7,8,9-HxCDF	<0,08	ng/kg		Q G-B11 QS
1,2,3,4,6,7,8-HpCDF	<0,10	ng/kg		Q G-B11 QS
1,2,3,4,7,8,9-HpCDF	<0,10	ng/kg		Q G-B11 QS
OCDF	<0,20	ng/kg		Q G-B11 QS
DL-PCB TEQ (WHO 2005) incl. LOQ	0,065	ng TEQ/kg		Q G-B11 QS
PCB-77	<0,80	ng/kg		Q G-B11 QS
PCB-81	<0,80	ng/kg		Q G-B11 QS
PCB-126	<0,40	ng/kg		Q G-B11 QS

(b) (4)

Analyseparameter	Analyseuitslag	Eenheid	Methode	Accr./cert.
PCB-169	<0,80	ng/kg		Q G-B11 QS
PCB-105	<4,0	ng/kg		Q G-B11 QS
PCB-114	<4,0	ng/kg		Q G-B11 QS
PCB-118	<4,0	ng/kg		Q G-B11 QS
PCB-123	<4,0	ng/kg		Q G-B11 QS
PCB-156	<4,0	ng/kg		Q G-B11 QS
PCB-157	<4,0	ng/kg		Q G-B11 QS
PCB-167	<4,0	ng/kg		Q G-B11 QS
PCB-189	<4,0	ng/kg		Q G-B11 QS
NDL-PCB's (polychloorbifenylen)			10463	
Polychloorbifenyl-28	<0,5	µg/kg		Q G-B11 QS
Polychloorbifenyl-52	<0,5	µg/kg		Q G-B11 QS
Polychloorbifenyl-101	<0,5	µg/kg		Q G-B11 QS
Polychloorbifenyl-138	<0,5	µg/kg		Q G-B11 QS
Polychloorbifenyl-153	<0,5	µg/kg		Q G-B11 QS
Polychloorbifenyl-180	<0,5	µg/kg		Q G-B11 QS
NDL-PCB Totaal (upper bound)	3,0	µg/kg		Q G-B11 QS

Methode omschrijvingen

Methode/analyse	Norm
10012	Eigen methode
10222	Eigen methode
10463	Eigen methode

(b) (4)

Analyserapport

(b) (4)

(b) (4)

Purac Biochem BV (QC)
t.a.v. (b)(6)
Postbus 21
4200 AA GORINCHEM

Monsternummer	:	M22000310002
Klantnummer	:	D05421
Monsterontvangstdatum	:	05-01-2022
Digitale order ID	:	NC000106011002

Uw monsterkenmerken

Reden onderzoek	:	2668
Productnaam	:	2102002765/T=0
Omschrijving	:	Aloapur PM
Monsternamedatum	:	3-1-2022
Kostencode	:	4700013971

Analyseparameter	Analyseuitslag	Eenheid	Methode	Accr./cert.
Fluoride	<20	mg/kg	10012	Q G-B10
Arseen	11,8	mg/kg	10222	Q G-B11 QS
Cadmium	0,620	mg/kg	10222	Q G-B11 QS
Kwik	<0,100	mg/kg	10222	Q G-B10 QS
Lood	6,17	mg/kg	10222	Q G-B11 QS
Aflatoxine B1 (LC-MS/MS)	<0,0010	mg/kg	10370	
Dioxine & Dioxine Like PCB's			10463	
WHO-TEQ (PCDD/F + DL-PCBs) incl. LOQ	0,222	ng TEQ/kg		Q G-B11 QS
Dioxine TEQ (WHO 2005) incl. LOQ	0,156	ng TEQ/kg		Q G-B11 QS
2,3,7,8-TCDD	<0,04	ng/kg		Q G-B11 QS
1,2,3,7,8-PeCDD	<0,04	ng/kg		Q G-B11 QS
1,2,3,4,7,8-HxCDD	<0,08	ng/kg		Q G-B11 QS
1,2,3,6,7,8-HxCDD	<0,08	ng/kg		Q G-B11 QS
1,2,3,7,8,9-HxCDD	<0,08	ng/kg		Q G-B11 QS
1,2,3,4,6,7,8-HpCDD	<0,10	ng/kg		Q G-B11 QS
OCDD	<0,20	ng/kg		Q G-B11 QS
2,3,7,8-TCDF	<0,04	ng/kg		Q G-B11 QS
1,2,3,7,8-PeCDF	<0,04	ng/kg		Q G-B11 QS
2,3,4,7,8-PeCDF	<0,04	ng/kg		Q G-B11 QS
1,2,3,4,7,8-HxCDF	<0,08	ng/kg		Q G-B11 QS
1,2,3,6,7,8-HxCDF	<0,08	ng/kg		Q G-B11 QS
2,3,4,6,7,8-HxCDF	<0,08	ng/kg		Q G-B11 QS
1,2,3,7,8,9-HxCDF	<0,08	ng/kg		Q G-B11 QS
1,2,3,4,6,7,8-HpCDF	<0,10	ng/kg		Q G-B11 QS
1,2,3,4,7,8,9-HpCDF	<0,10	ng/kg		Q G-B11 QS
OCDF	<0,20	ng/kg		Q G-B11 QS
DL-PCB TEQ (WHO 2005) incl. LOQ	0,065	ng TEQ/kg		Q G-B11 QS
PCB-77	<0,80	ng/kg		Q G-B11 QS
PCB-81	<0,80	ng/kg		Q G-B11 QS
PCB-126	<0,40	ng/kg		Q G-B11 QS

(b) (4)

Analyseparameter	Analyseuitslag	Eenheid	Methode	Accr./cert.
PCB-169	<0,80	ng/kg		Q G-B11 QS
PCB-105	<4,0	ng/kg		Q G-B11 QS
PCB-114	<4,0	ng/kg		Q G-B11 QS
PCB-118	<4,0	ng/kg		Q G-B11 QS
PCB-123	<4,0	ng/kg		Q G-B11 QS
PCB-156	<4,0	ng/kg		Q G-B11 QS
PCB-157	<4,0	ng/kg		Q G-B11 QS
PCB-167	<4,0	ng/kg		Q G-B11 QS
PCB-189	<4,0	ng/kg		Q G-B11 QS
NDL-PCB's (polychloorbifenylen)			10463	
Polychloorbifenyl-28	<0,5	µg/kg		Q G-B11 QS
Polychloorbifenyl-52	<0,5	µg/kg		Q G-B11 QS
Polychloorbifenyl-101	<0,5	µg/kg		Q G-B11 QS
Polychloorbifenyl-138	<0,5	µg/kg		Q G-B11 QS
Polychloorbifenyl-153	<0,5	µg/kg		Q G-B11 QS
Polychloorbifenyl-180	<0,5	µg/kg		Q G-B11 QS
NDL-PCB Totaal (upper bound)	3,0	µg/kg		Q G-B11 QS

Methode omschrijvingen

Methode/analyse	Norm
10012	Eigen methode
10222	Eigen methode
10463	Eigen methode

(b) (4)

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The Netherlands

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www.corbion.com

REPORT

FROM (b)(6)

DATE August 21, 2020
REFERENCE QA-Stability-2020-07 PvT
SUBJECT Stability results Aloapur (b) (4)

VERSION 1.0
AUTHOR (b)(6)

Author	Function	Signature and date
(b)(6)	QA Manager Food	ValidSigned door (b)(6) op 21-08-2020

REPORT

Stability results (b) (4)

Contents

Corbion

Report INTERNAL USE ONLY

REPORT

Stability results (b) (4)

1 Introduction and goal

ALOAPUR is a powder formulation of sodium salts of lactylates of fatty acids. (b) (4) Its intended use is as a mineral feed in animal nutrition (EU). The current shelf-life 730 days. Goal is to have evidence to guarantee the shelf-life of 730 days. The climate chamber is monitored for temperature and relative humidity, the conditions are (b) (4) and (b) (4) relative humidity.

2 Batch information

Product	Protocol number	Item number	Lot number	Mfg date
Aloapur	(b) (4)	7005400701	1603003760	(b) (4)

3 Analytical results

Lotnummer	Ester value (%)	Sodium (%)	Bulk Density (kg/m3)	Ash (%)	Particle size >1000 µm (%)	Pasticle size <125 µm (%)
Specification	≥ 40	≤ 3.0	567 - 771	≤ 67	≤ 2	≤ 2
1603003760/T=0	(b) (4)					
1603003760/T=3						
1603003760/T=6						
1603003760/T=9						
1603003760/T=12						
1603003760/T=18						
1603003760/T=24						

4 Conclusion and recommendations

Based on the data the following can be concluded and recommended.

None of the parameters is out of external specification after a period of 24 months.
The shelf-life of 730 days can be guaranteed.

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REPORT

ALOAPUR PM stability tests 2021

FROM (b)(6)
Sr. Application Specialist
LAS

(b)(6)

DATE July 5, 2021
REFERENCE (b) (4)

SUBJECT Analytical results

TO (b)(6)
CC

VERSION 1.0

AUTHOR (b)(6)

Report CONFIDENTIAL

REPORT

ALOAPUR PM stability tests 2021

Contents

1 Introduction

ALOAPUR PM has received GRAS independent conclusions status as established by an Expert Panel. However, in order to be listed in the AAFCO, a FDA notification is required. Within this notification, a shelf life study of at least 3 batches is required. However, Corbion has performed a shelf life study for only 1 batch. Therefore, two old ALOAPUR PM batches were analyzed to check if those batches still meet the specifications.

The goal of this research is to verify if ALOAPUR PM meets the specifications after storage in a warehouse for 5-6 years.

2 Batch information and specifications

The commercial ALOAPUR PM batches as used for this study were produced in 2015 and 2016. These batches have been stored in a warehouse with non specified conditions (in contrast to the conditions as usually applied in controlled shelf life studies).
These batches are between 5-6 years old which is significantly longer than the official minimal 2 years that is required for a shelf life test. Therefore, the results as described in this report should represent a shelf life of more than two years.

Table 1. Selected ALOAPUR PM Samples

Sample number	Lot number	Item number
		(b) (4)

Table 2. Specifications for ALOAPUR PM

Parameter	Specification
Ester value	(b) (4)
Sodium content	
Bulk density	
Ash, residue on ignition	
Particle size > 1000 µm	
Particle size < 125 µm	

3 Results

Table 3: Analytical results of 2 ALOAPUR PM batches

Sample	Ester Value [mg KOH/g]	Na [%(w/w)]	Bulk density [kg/m³]	Particle Size Distribution <106 [µm]	Particle Size Distribution >1000 [µm]	Ash residue [% (w/w)]
						(b) (4)
Spec						(b) (4)

4 Conclusions and recommendations

Analyses on two ALOAPUR PM samples from 2015 and 2016 show that these samples are still in spec. The properties did not change over the past 5 – 6 years. It can therefore be assumed that ALOAPUR PM is a stable product with a shelf life of at least more than 2 years.

Corbion

Confidential

Long term protocol ALOAPUR PM

Authorization Long Term Stability				
	Name	Function	Signature	Date
Author	(b)(6)	Manager QA	ValidSigned b (b)(6) on 16-1	
Review and QC approval	(b)(6)	Manager QC	ValidSigned by (b)(6) on 22-1	

Product particulars	
Revision number	0 new
Lot number	(b) (4)
Brand name	ALOAPUR® PM
Start Date	
Material number	(b) (4)
Production location	(b) (4)
Packaging material	ORIGINAL bag ((b) (4)
Storage conditions	40°C / 75% humidity
Time intervals	0 3, 6, 9, 12, 18, 24, 36 months 40 °C

Analysis 40 °C / 75% humidity

Analysis	UOM	Specification	0	1	2	3	6	9	12	18	24	36
Ash / residue on ignition	% (w/w)	≤ 68	X	(b) (4)								
Ester value	mg KOH/g	≥ 45	X									
Sodium	%	2.4 – 3.2	X									
Bulkdensity	Kg/m³	600 - 900	X									
Aflatoxin B1	mg/Kg	≤ 0.01	X									
Arsenic	mg/Kg	≤ 30	X									
Cadmium	mg/Kg	≤ 15	X									
Dioxines (NgWHO-PCDD/F/kg)	ug/Kg	≤ 0.5	X									
Dioxins + Dioxin like PCBs	ng/Kg	≤ 1.5	X									
Fluoride	mg/Kg	≤ 500	X									
Lead	mg/Kg	≤ 15	X									
Mercury	mg/Kg	≤ 0.2	X									
Non Dioxin like PCB's	µg/Kg	≤ 10	X									
lactylaten (R&D analysis)	% (w/w)	information	X									
Cakiness		information	X									

(b) (4)

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REPORT

ALOAPUR PM - accelerated ageing tests

FROM (b)(6)

DATE September 30, 2022

REFERENCE

SUBJECT Analytical results

TO (b)(6)

CC

VERSION 2.0

AUTHOR (b)(6)

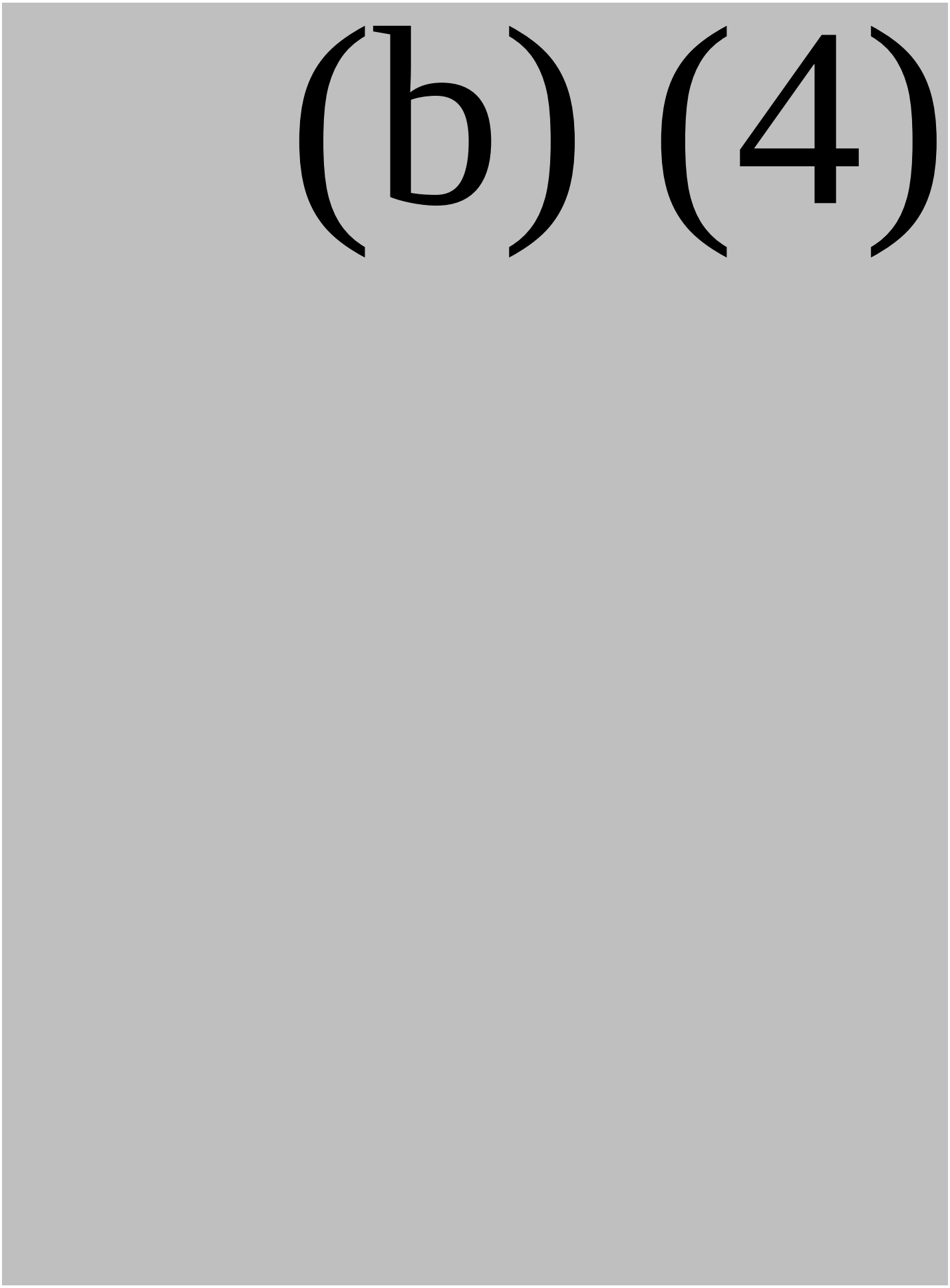
Report CONFIDENTIAL

REPORT

ALOAPUR PM - accelerated ageing tests

Contents

Corbion



2 Batch information and specifications

The commercial ALOAPUR PM batches as used for this study are produced in 2021, see **Error! Reference source not found.**.

Table 1. Selected ALOAPUR® PM Samples

Lot number	Item number	Number of bags	Packaging type
			(b) (4)

The test parameters for ALOAPUR® PM are shown in Table 2, while Table 3 shows the argumentation for the different parameters that will be tested.[2]

Table 2. Test parameters for the accelerated shelf life test of ALOAPUR® PM at 40 °C and 75% relative humidity

Parameter	UOM	Specification	0	1	2	3	6
Ash / residue on ignition	% (w/w)	(b) (4)					
Ester value	mg KOH/g						
Sodium	%						
Bulk density	kg/m³						
Aflatoxin B1*	mg/kg						
Arsenic *	mg/kg						
Cadmium *	mg/kg						
Dioxins *	ng WHO-PCDD/F-TEQ/kg						
Sum of Dioxins + Dioxin-like PCBs *	ng WHO-PCDD/F-PCB-TEQ/kg						
Fluorine *	mg/kg						
Lead *	mg/kg						
Mercury *	mg/kg						
Non-dioxin like PCB's *	µg/kg						
Lactylates (R&D analysis)	See remarks						(b) (4)
Cakiness	See remarks						

** Measured externally by* (b) (4)

Remarks:

- (b) (4)

[2] (b) (4)

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Report **CONFIDENTIAL**

REPORT

ALOAPUR PM - accelerated ageing tests

(b) (4)

3 Results

(b) (4)

*Table 4. Analytical results of the accelerated shelf life test of ALOAPUR® PM **LOT** (b) (4) (0 ≤ t ≤ 6 months).*

Analysis LOT 7500056376	Unit	Spec	t = 0	t = 1	t = 2	t = 3	t = 6
Ash / residue on ignition	% (w/w)	(b) (4)					
Ester Value	mg KOH/g						
Bulk density	kg/m³						
Sodium	%						
Aflatoxin B1	mg/kg						
Arsenic	mg/kg						
Cadmium	mg/kg						
Dioxins (ng WHO-PCDD/F-TEQ/kg)	ng/kg						
Sum of Dioxins + Dioxin-like PCBs (ng WHO-PCDD/F-PCB-TEQ/kg)	ng/kg						
Non-dioxin like PCB's	µg/kg						
Fluoride	mg/kg						
Lead	mg/kg						
Mercury	mg/kg						
Lactylates (R&D)	% (w/w)						
Moisture content (R&D)	% (w/w)						
Caking	-						

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REPORT

ALOAPUR PM - accelerated ageing tests

Table 5. Analytical results of the accelerated shelf life test of ALOAPUR® PM ***LOT*** (b) (4) *(0 ≤ t ≤ 6 months).*

Analysis LOT 2102002765	Unit	Spec	t = 0	t = 1	t = 2	t = 3	t = 6
Ash / residue on ignition	% (w/w)	(b) (4)					
Ester Value	mg KOH/g						
Bulk density	kg/m³						
Sodium	%						
Aflatoxin B1	mg/kg						
Arsenic	mg/kg						
Cadmium	mg/kg						
Dioxins (ng WHO-PCDD/F-TEQ/kg)	ng/kg						
Sum of Dioxins + Dioxin-like PCBs (ng WHO-PCDD/F-PCB-TEQ/kg)	ng/kg						
Non-dioxin like PCB's	µg/kg						
Fluoride	mg/kg						
Lead	mg/kg						
Mercury							
Lactylates (R&D)	% (w/w)						
Moisture content (R&D)	% (w/w)						
Caking	µg/kg						

Table 6. Analytical results of the accelerated shelf life test of ALOAPUR® PM LOT (b) (4) (0 ≤ t ≤ 6 months).

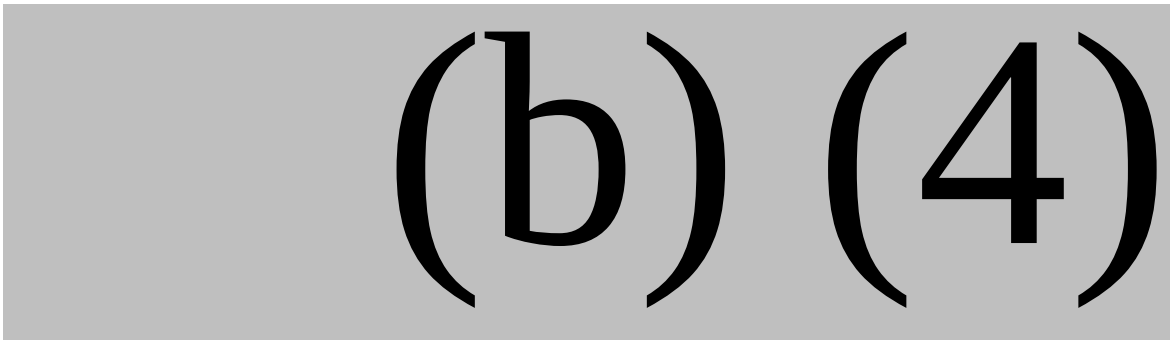
Analysis LOT 7500068230	Unit	Spec	t = 0	t = 1	t = 2	t = 3	t = 6
Ash / residue on ignition	% (w/w)	(b) (4)					
Ester Value	mg KOH/g						
Bulk density	kg/m³						
Sodium	%						
Aflatoxin B1	mg/kg						
Arsenic	mg/kg						
Cadmium	mg/kg						
Dioxins (ng WHO-PCDD/F-TEQ/kg)	ng/kg						
Sum of Dioxins + Dioxin-like PCBs (ng WHO-PCDD/F-PCB-TEQ/kg)	ng/kg						
Non-dioxin like PCB's	µg/kg						
Fluoride	mg/kg						
Lead	mg/kg						
Mercury	mg/kg						
Lactylates (R&D)	% (w/w)						
Moisture content (R&D)	% (w/w)						
Caking	-						

The composition of the tested ALOAPUR® PM LOTS is shown in Tables 7-9.[4]

Table 7: Composition of LOT LOT (b) (4) as a function of storage time at T = 40 °C and RH = 75%

(b) (4)

[4] Internal Report: (b) (4)



4 Conclusions and recommendations

(b) (4)

Corbion

To	(b)(6)
From	
Date	: 2 May 2012
Project	: (b) (4)
Version	: 1.1

In-feed stability of Puramix 30S

Introduction:

For market introduction of the Puramix 30S product, it is required to assess the in-feed stability. In cooperation with (b) (4) and (b) (4) a study was designed to assess homogeneity of the product in the feed, stability of the lactylates under conditions of pelleting and over time stability in both mash and pellets. For these assessments, C12-1 and C14-1 lactylates were measured, assuming them as indicator substances for the product. The overall protocol (b) (4) is attached as Annex 1

Sample(s):

Samples were produced by (b) (4). The composition of the feeds and a list of the samples are attached as Annex 2 and 3

Analytical methods:

Samples were analysed by (b) (4) The analytical protocol is attached as Annex 4.

Results at T=0:

(b) (4)

(b) (4)

Conclusions at T=0:

(b) (4)

			Average (ppm)		Variation (%)	
Sample	Code	No Samples	C12	C14	C12	C14
Wheat blank mash	EU BM					
Wheat dosed mash	EU DM					
Wheat dosed pellets 65C	EU P65					
Wheat dosed pellets 75C	EU P75					
Wheat dosed pellets 85C	EU P85					
Average						
			Average (ppm)		Variation (%)	
Sample	Code	No Samples	C12	C14	C12	C14
Corn blank mash	US BM					
Corn dosed mash	US DM					
Corn dosed pellets 65C	US P65					
Corn dosed pellets 75C	US P75					
Corn dosed pellets 85C	US P85					
Average						

(b) (4)

(b) (4)

Table 1: Measured lactylates concentrations and coefficients of variation

	Ratio C12 / Total (%)	Recovery C12/DM (%)	Recovery C14/DM (%)	Recovery C14/C12	Recovery C12+C14/DM	Recovery C12/Calc	Recovery C14/Calc	Recovery C12+C14/Calc
EU BM								
EU DM	73%							
EU P65	71%							
EU P75	71%							
EU P85	71%							

(b) (4)

	Ratio C12 / Total (%)	Recovery C12/DM (%)	Recovery C14/DM (%)	Recovery C14/C12	Recovery C12+C14/DM	Recovery C12/Calc	Recovery C14/Calc	Recovery C12+C14/Calc
US BM	49%							
US DM	72%							
US P65	71%							
US P75	71%							
US P85	71%							

(b) (4)

Table 2: Relative recoveries: C12-1 Lactylate over Total Lactylate ratios, Lactylate levels over Dosed Mash levels, C14 levels over C12 levels, Lactylate levels of Dosed Mash over calculated levels.

(b) (4)

Results over time:

tbd

Conclusions over time:

tbd

Annex 1:
Protocol (b) (4)

Annex 2:
Feed compositions designed by (b)(6)

Annex 3:
List of all samples taken by (b)(6)

Annex 4:
Protocol of Lactylate analysis of feed by (b) (4)

To : (b) (4)

From : Purac ((b)(6))

Copy : Purac ((b)(6))

February 1, 2012

Re : **In-Feed stability of PURAC PRMX 30S**

To assess the stability of our new product PURAC PRMX 30S, we have designed the following study:

Scope:

The study evaluates the stability of the product in broiler feed. Samples will be taken during pelleting, storage and the homogeneity within the feed will be established. (b) (4)

General information:

Code: (b) (4)
Status protocol: Draft
Protocol date: 1 February 2012
Study start: tbd
Completion date: tbd +3months

Type of study:
(b) (4).

Quality Assurance:
According to Standard Operating Procedures at feed production site and according to GLP at analytical laboratory.

Contacts:

Sponsor: Purac Biochem
Arkelsedijk 46
4206AC Gorinchem

(b)(6)

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

Feed design and production

(b) (4)

Feed sampling

(b) (4)

(b) (4)

(b) (4)

(b) (4)

Sample shipping and storage

(b) (4)

Immediate sample analysis

(b) (4)

In-time sample analysis

(b) (4)

Planning

Day	Who/where	
0		(b) (4)
0(+<2 days)		
0+4 weeks		
0+8 weeks		
0+12 weeks		
0+3 months		

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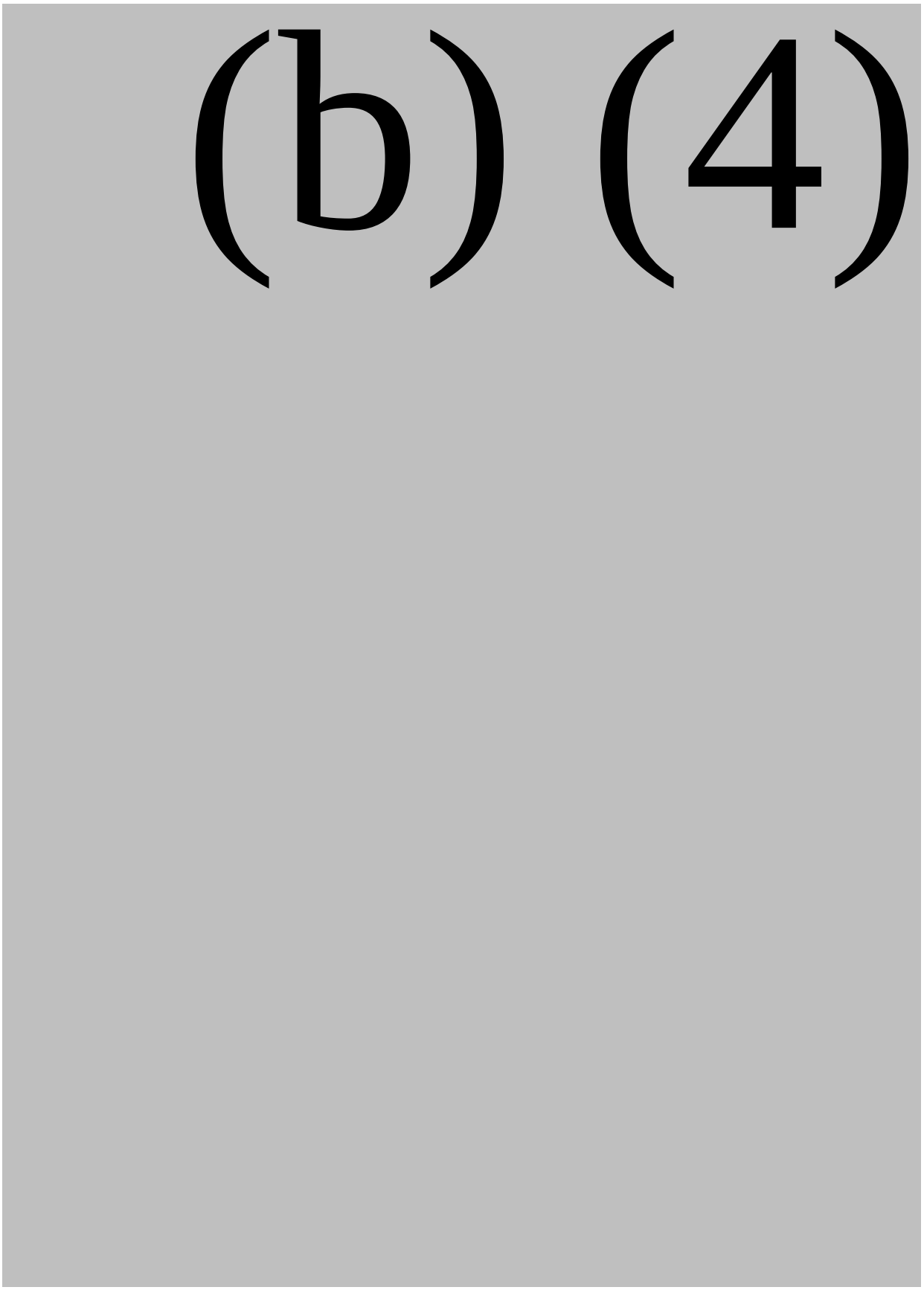

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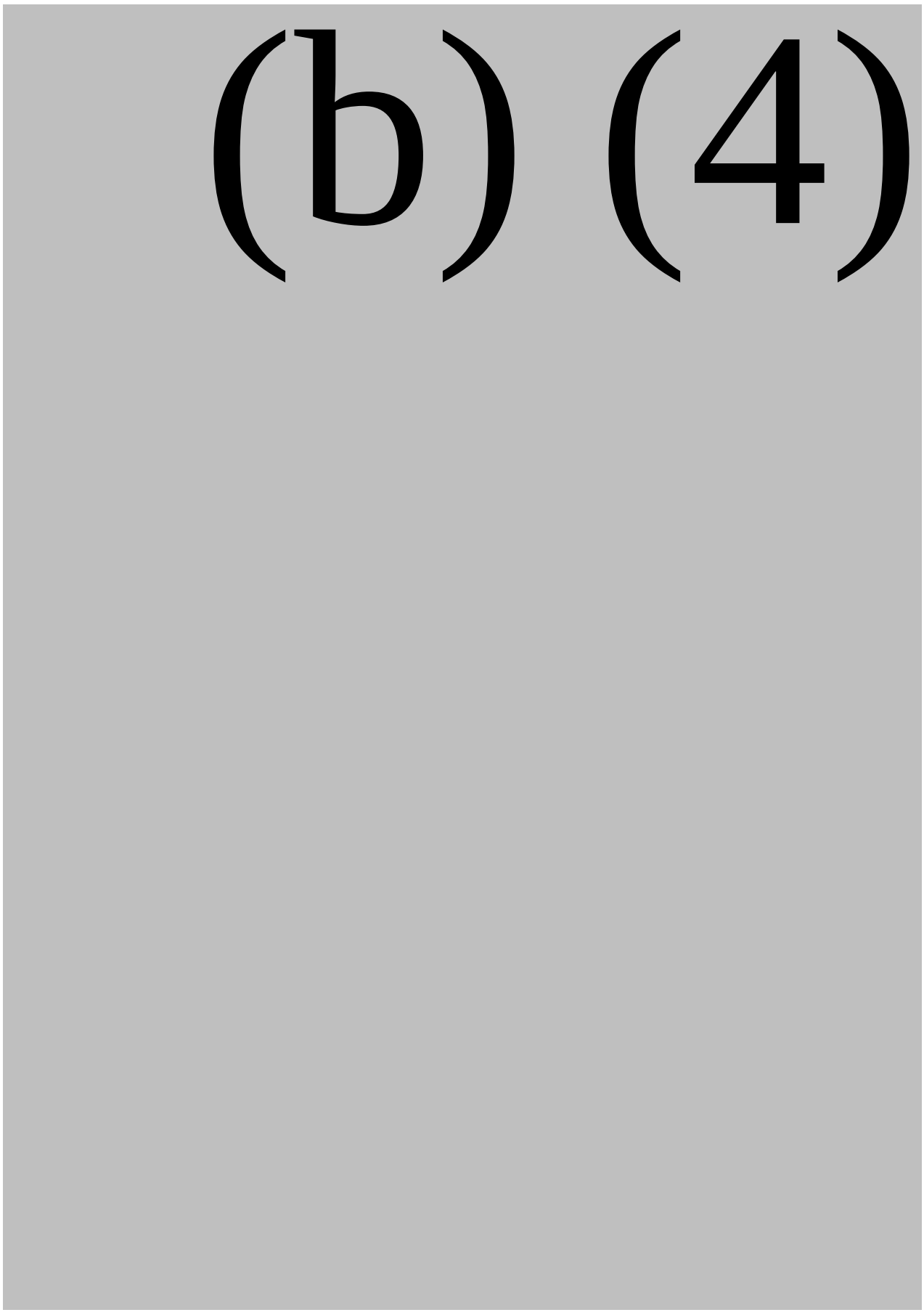

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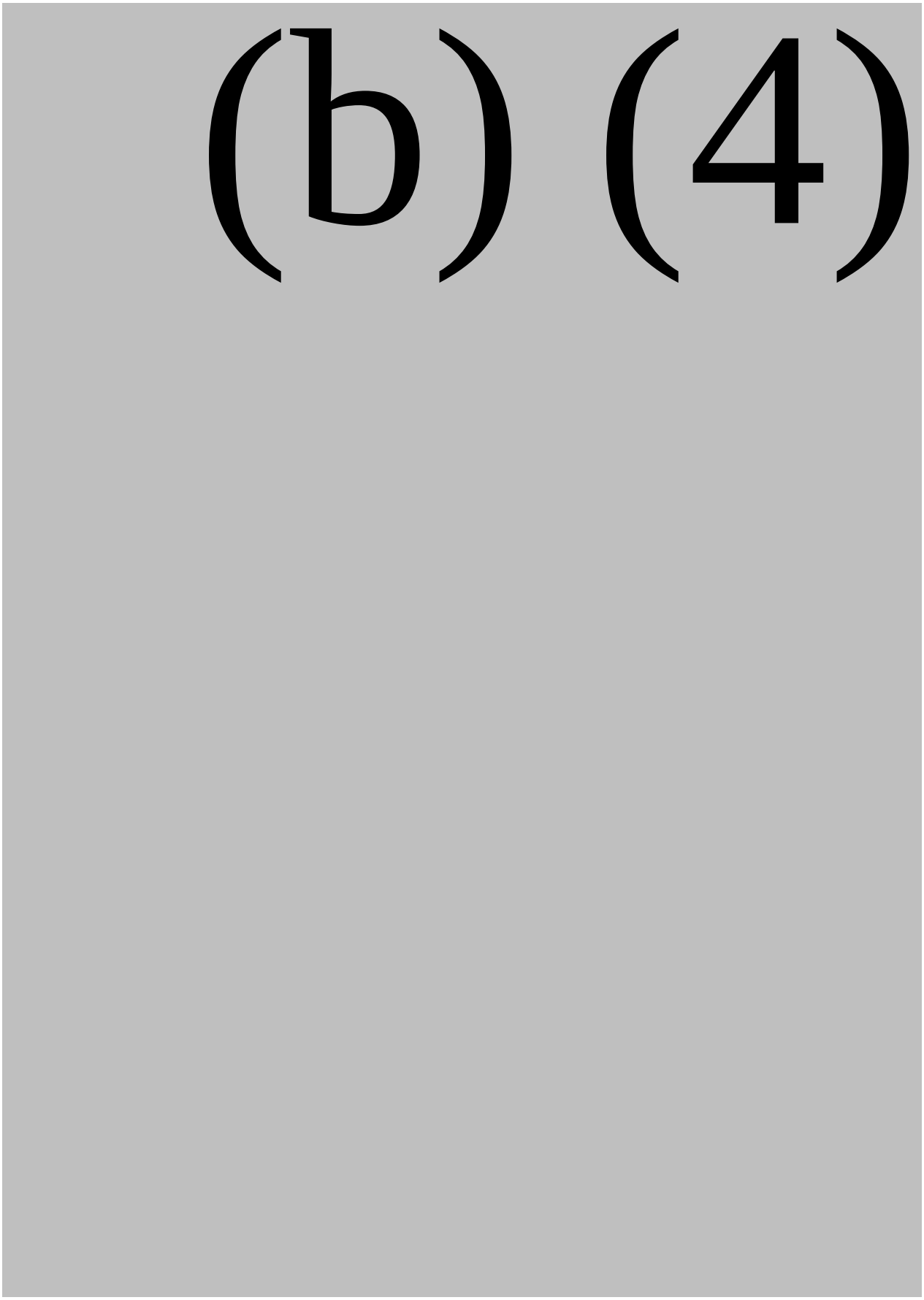

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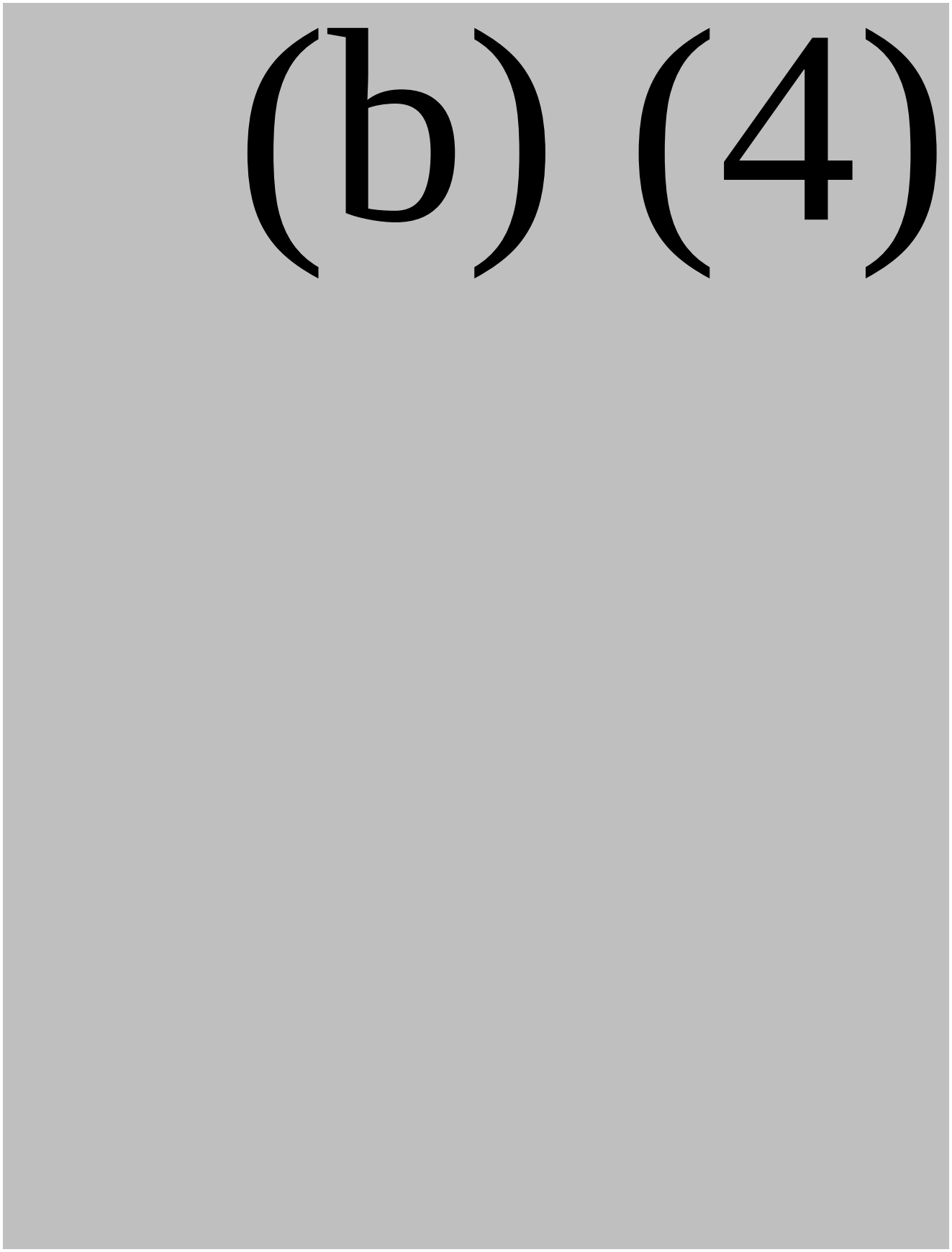


(b) (4)

(b) (4)

(b) (4)

FINAL REPORT

Study Title

ANALYSIS OF POULTRY FEED SAMPLES FOR C-12-LACTYLATE, C-14-LACTYLATE AND C-16-LACTYLATE CONTENT INCLUDING DETERMINATION OF STABILITY OVER 3 MONTHS

Author

(b)(6)

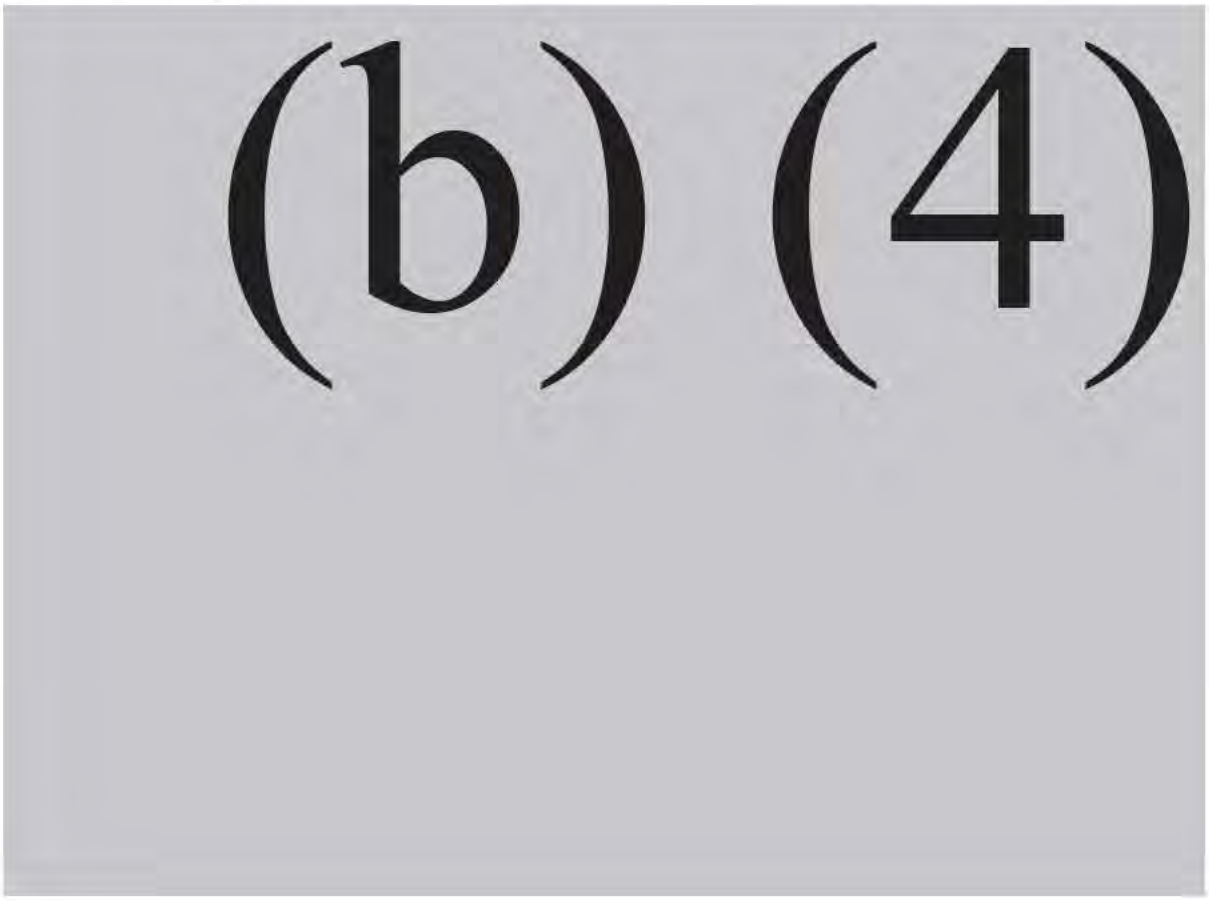
Test facility

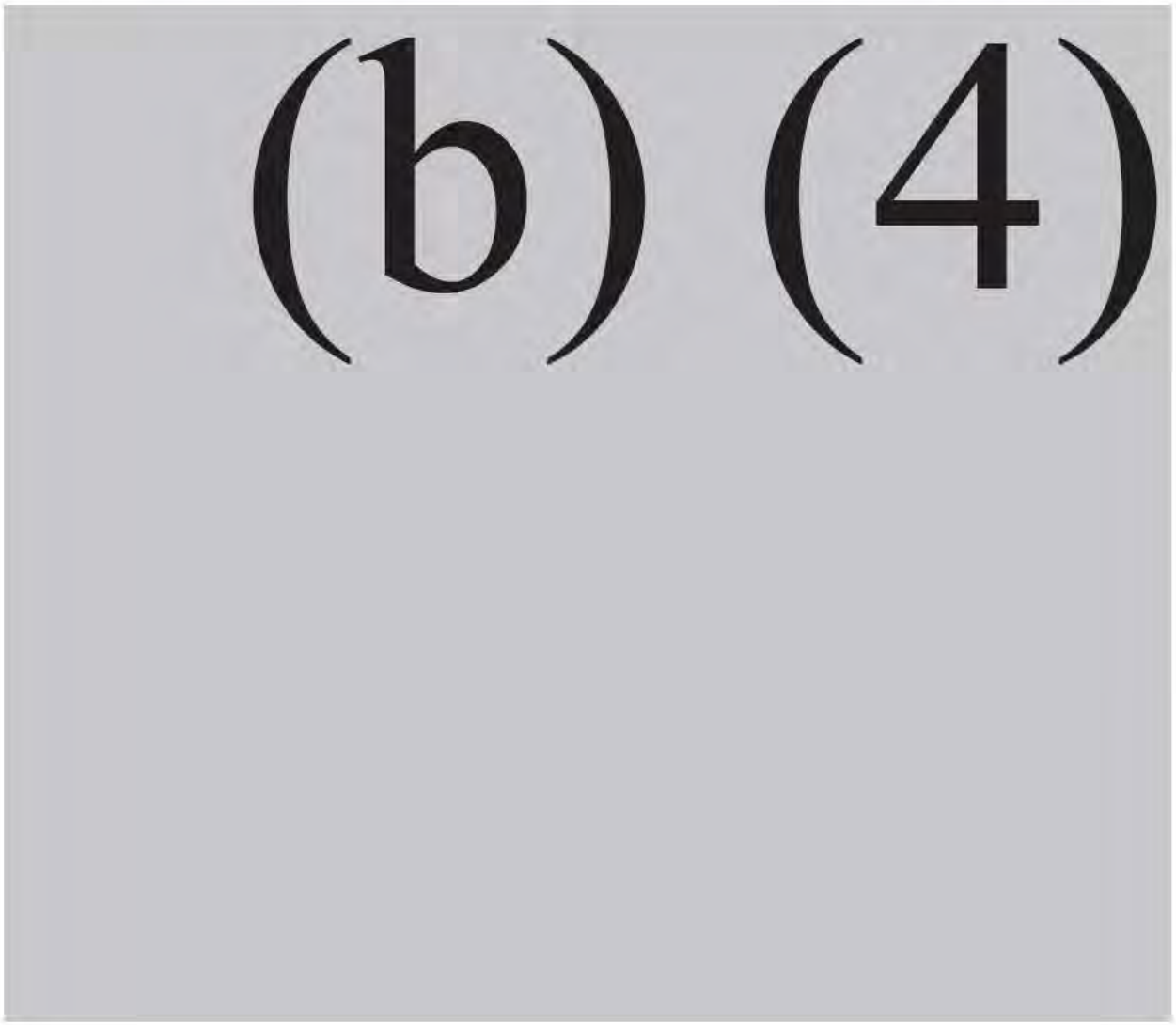
(b) (4)

Laboratory Project Identification

(b) (4)

1. CONTENTS




(b) (4)

2. STATEMENT OF GLP COMPLIANCE

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

Date: 28 January 2013 Date: 16 January 2013

3. QUALITY ASSURANCE STATEMENT

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

Date: 21 January, 2013

4. SUMMARY

(b) (4)

5. INTRODUCTION

5.1. Preface

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

Study Monitor

Test Facility

Study Director

Study schedule bioanalysis (Experimental work)
Start : 30 March 2012
Completed : 10 October 2012

5.2. Aims of study

(b) (4)

5.3. Storage and retention of records and materials

(b) (4)

6. MATERIALS AND METHODS

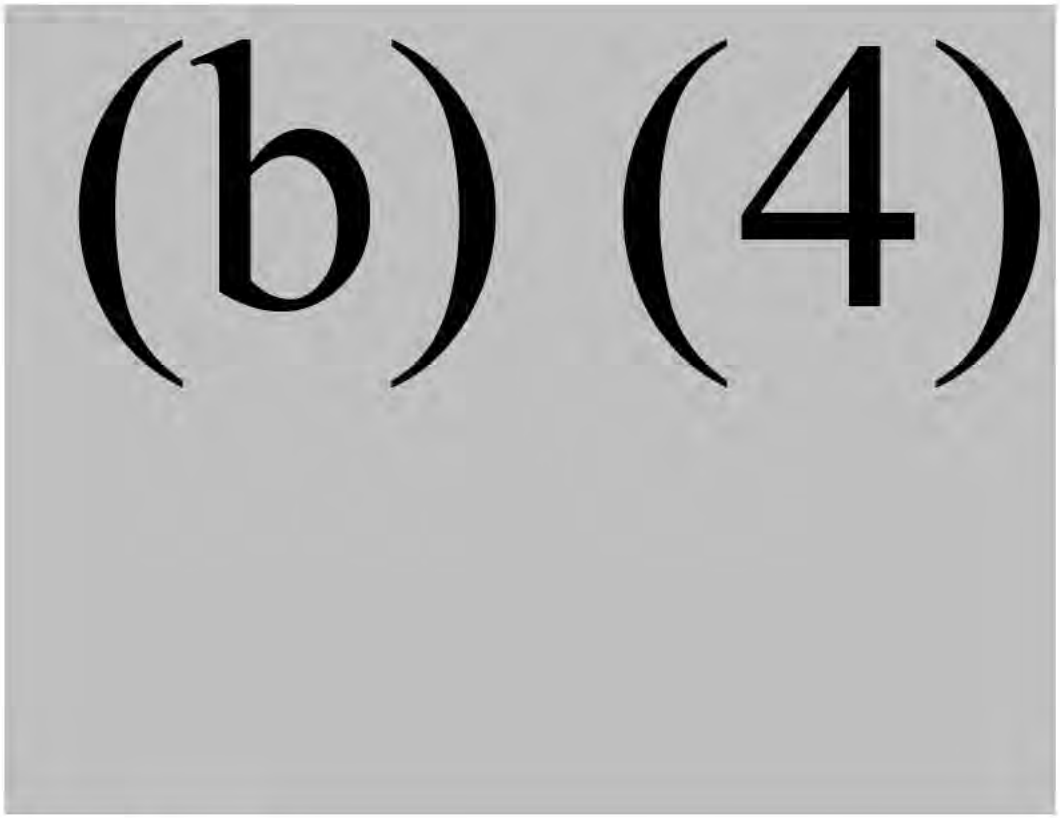
(b) (4)

6.1. Test substances

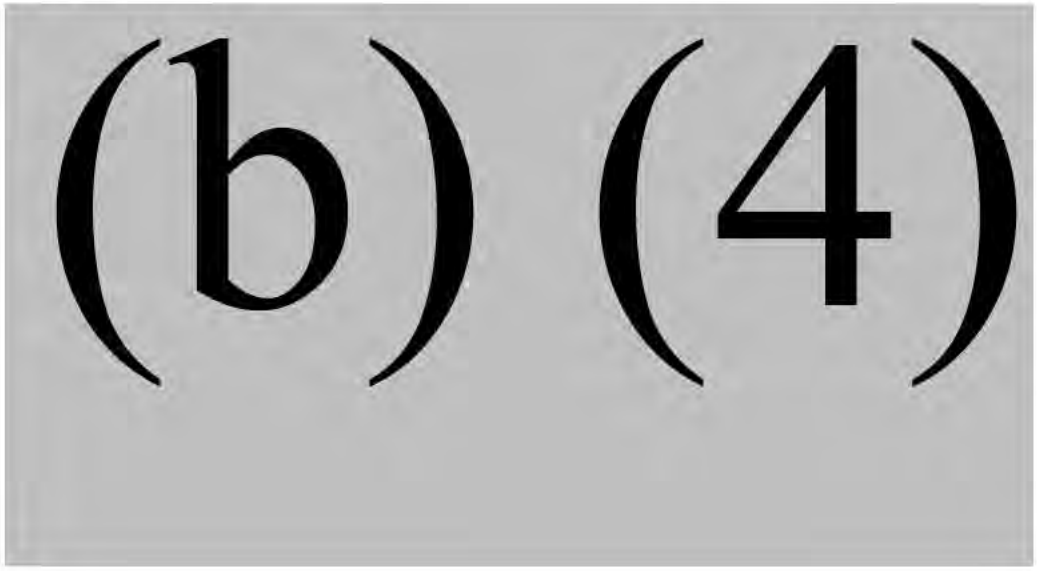
6.1.1. C-12-Lactylate

(b) (4)

6.1.2. C-14-Lactylate



6.1.3. C-16-Lactylate



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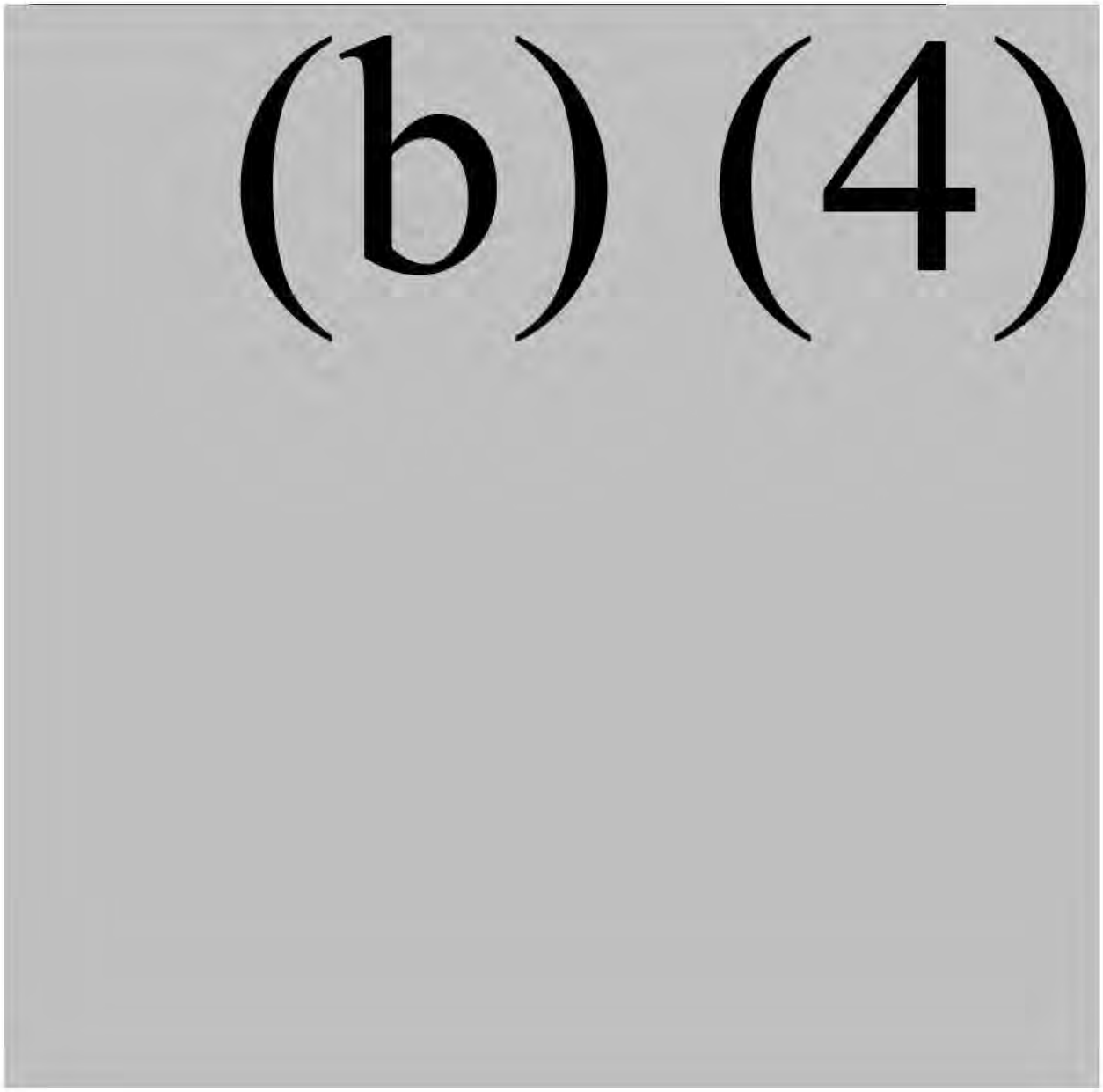
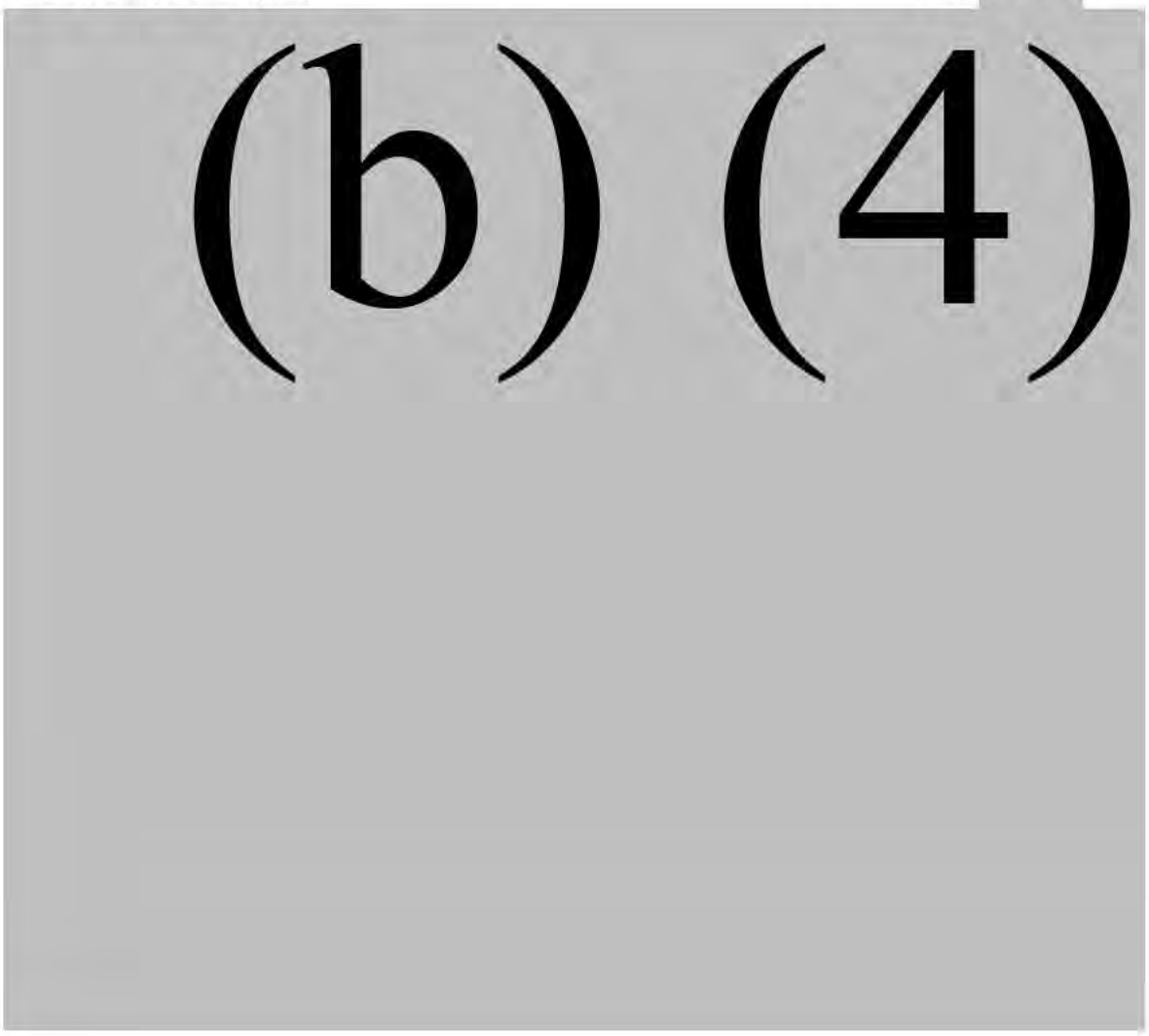


7. SAMPLE ANALYSIS/PREPARATION

7.1. Feed samples

(b) (4)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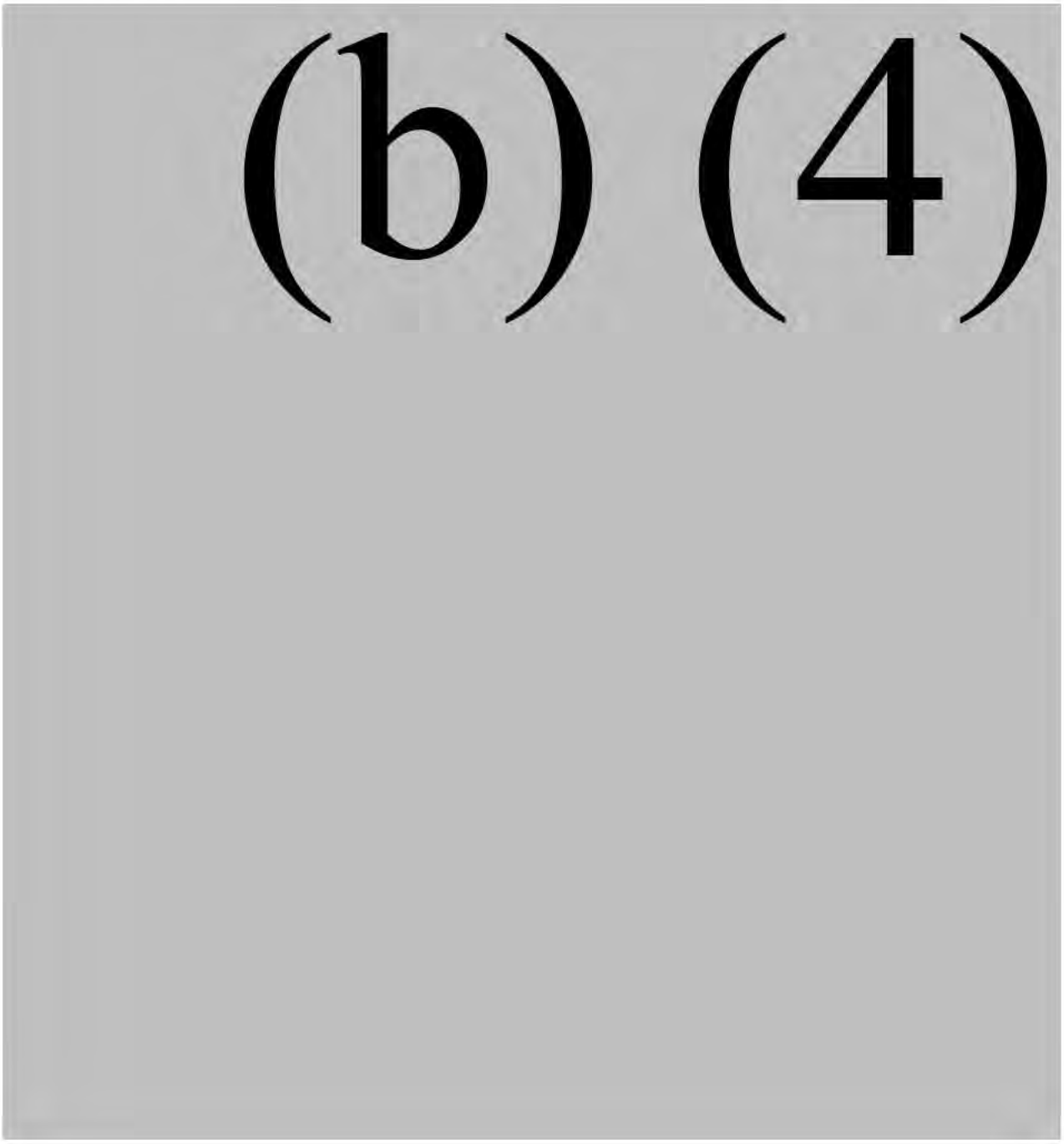
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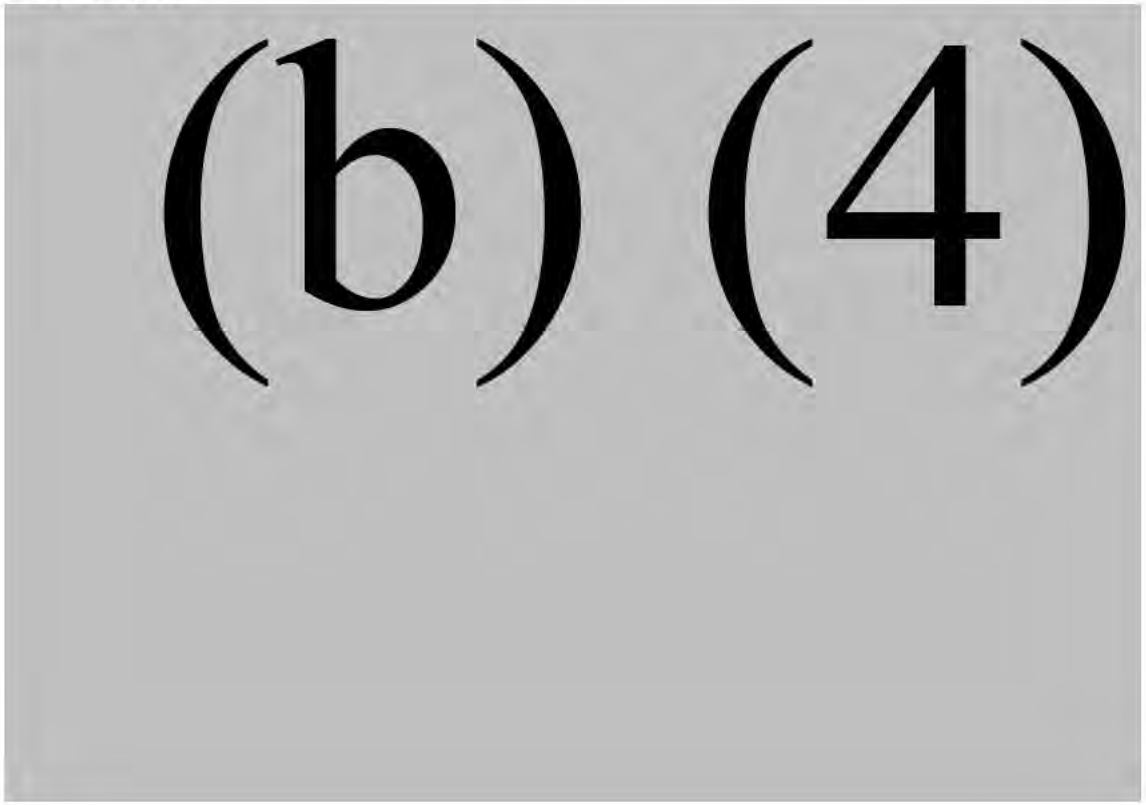
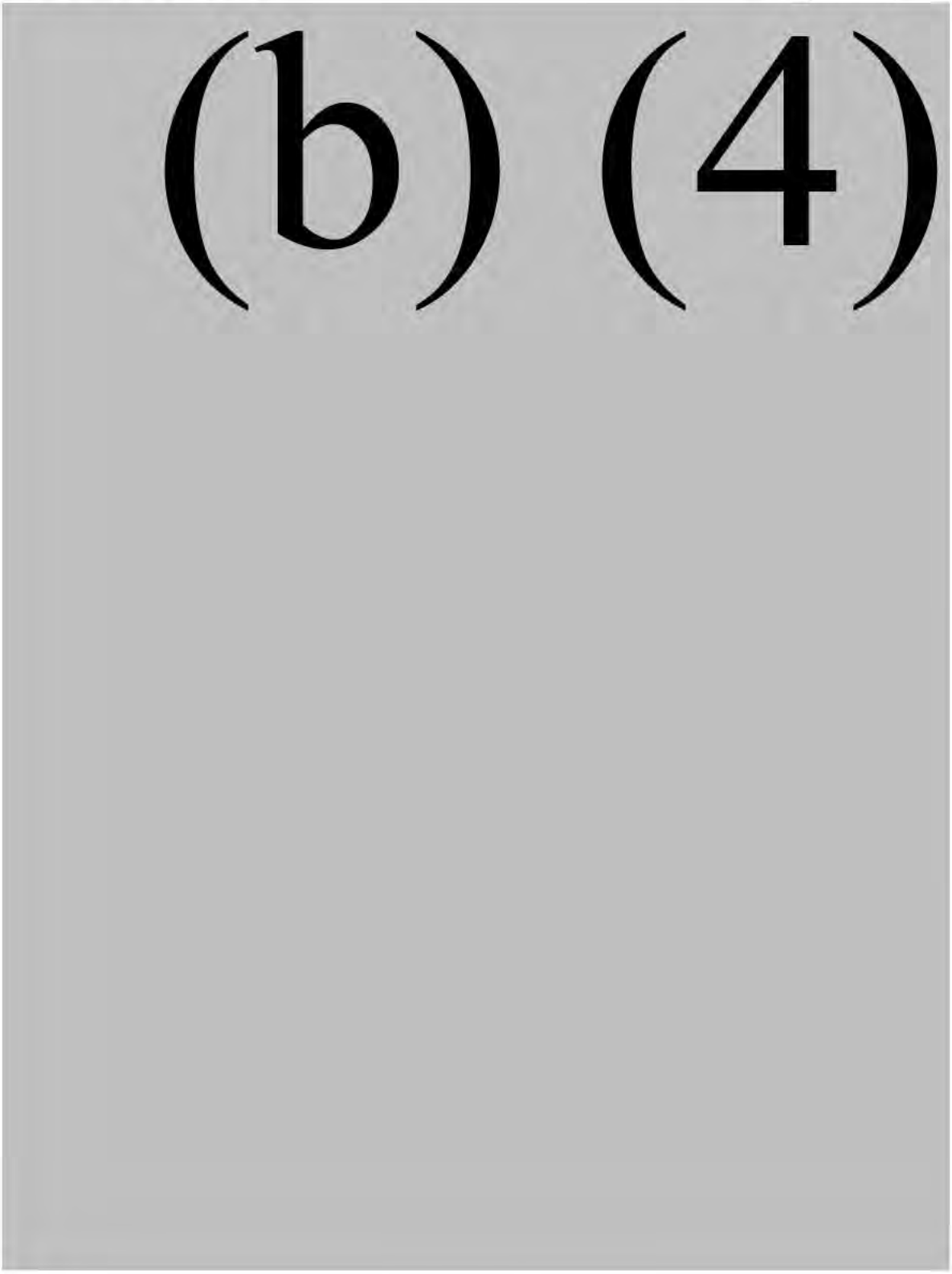
10. RESULTS

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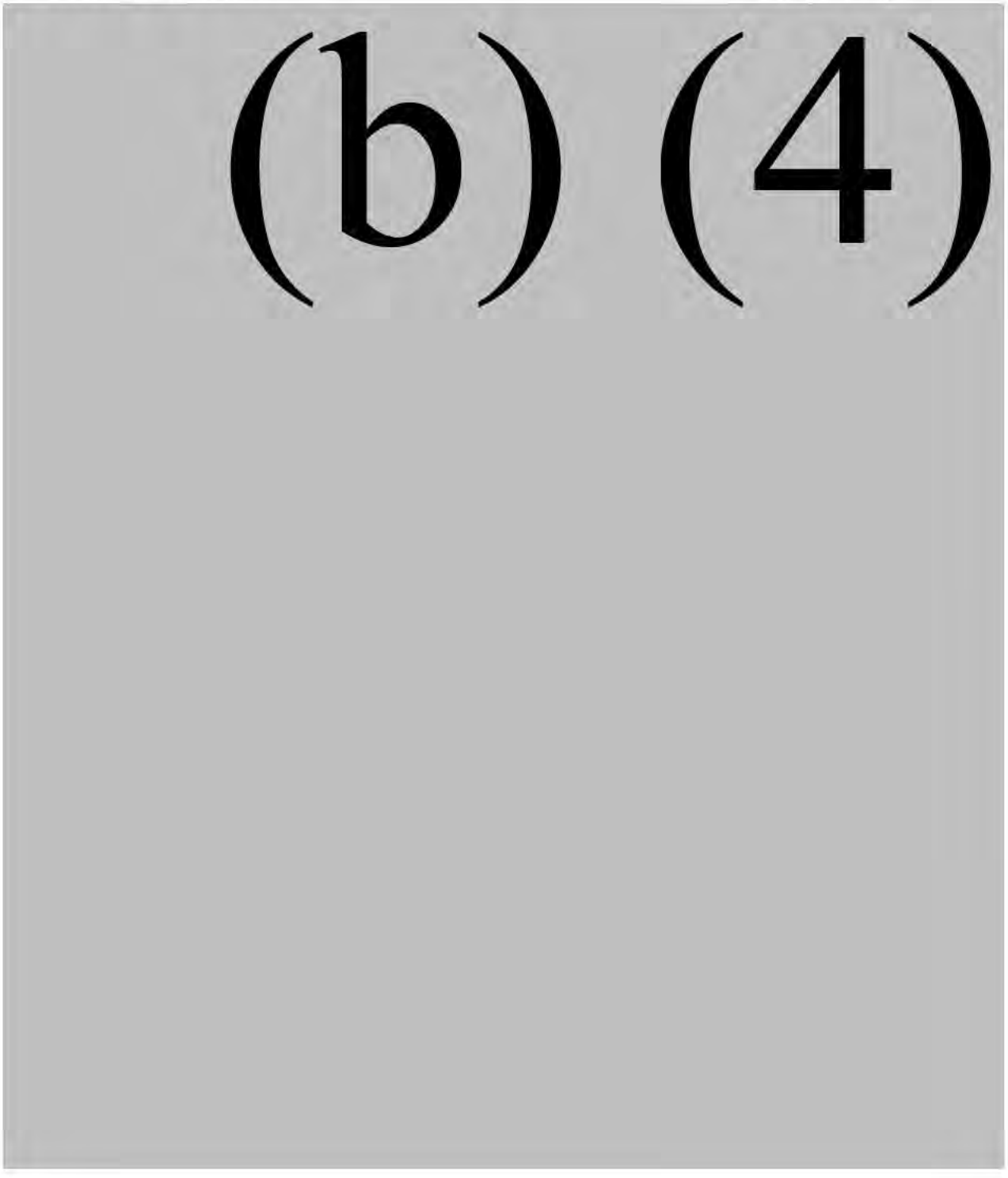
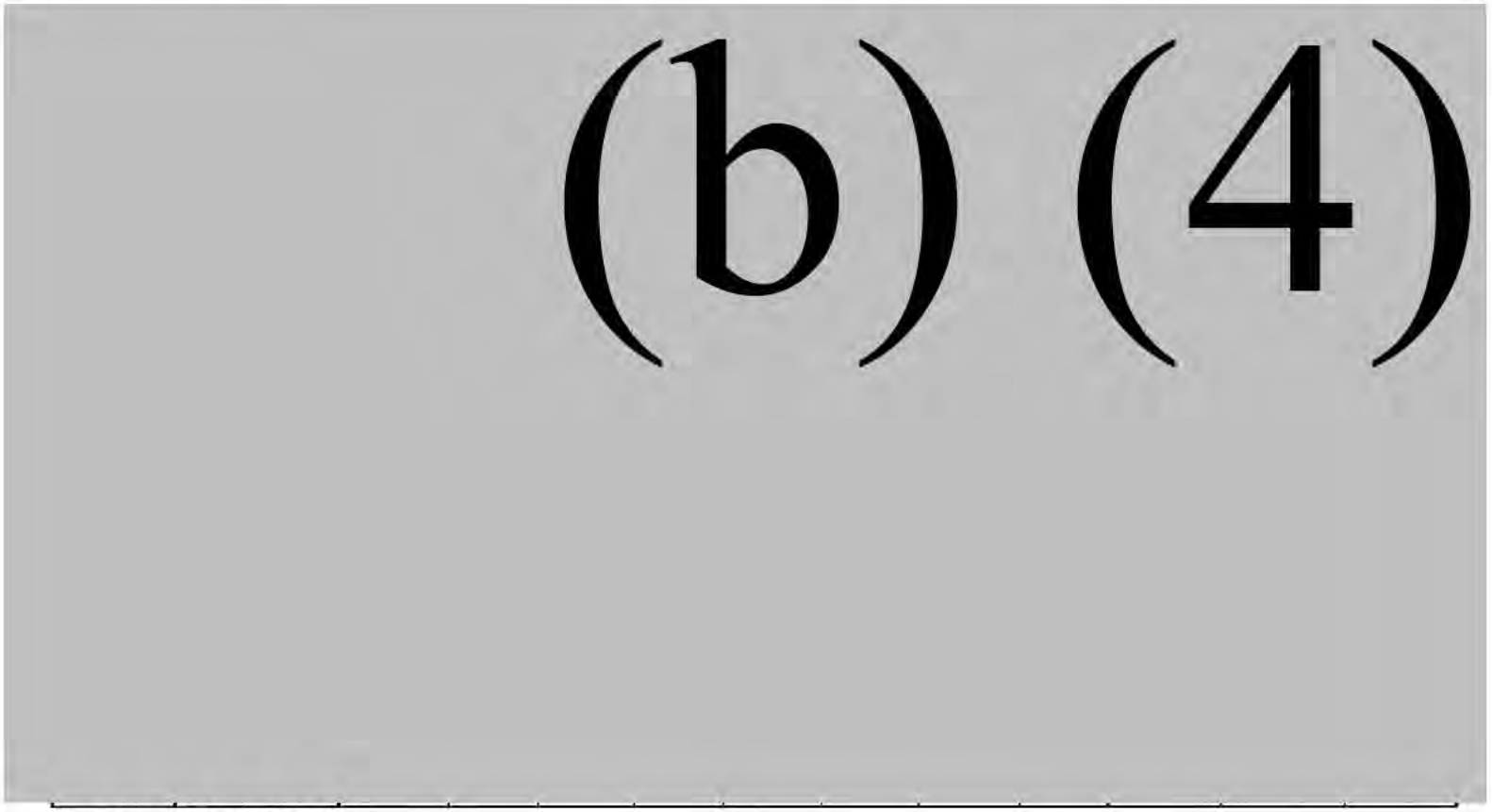

(b) (4)

(b) (4)

10.3. Linearity

(b) (4)



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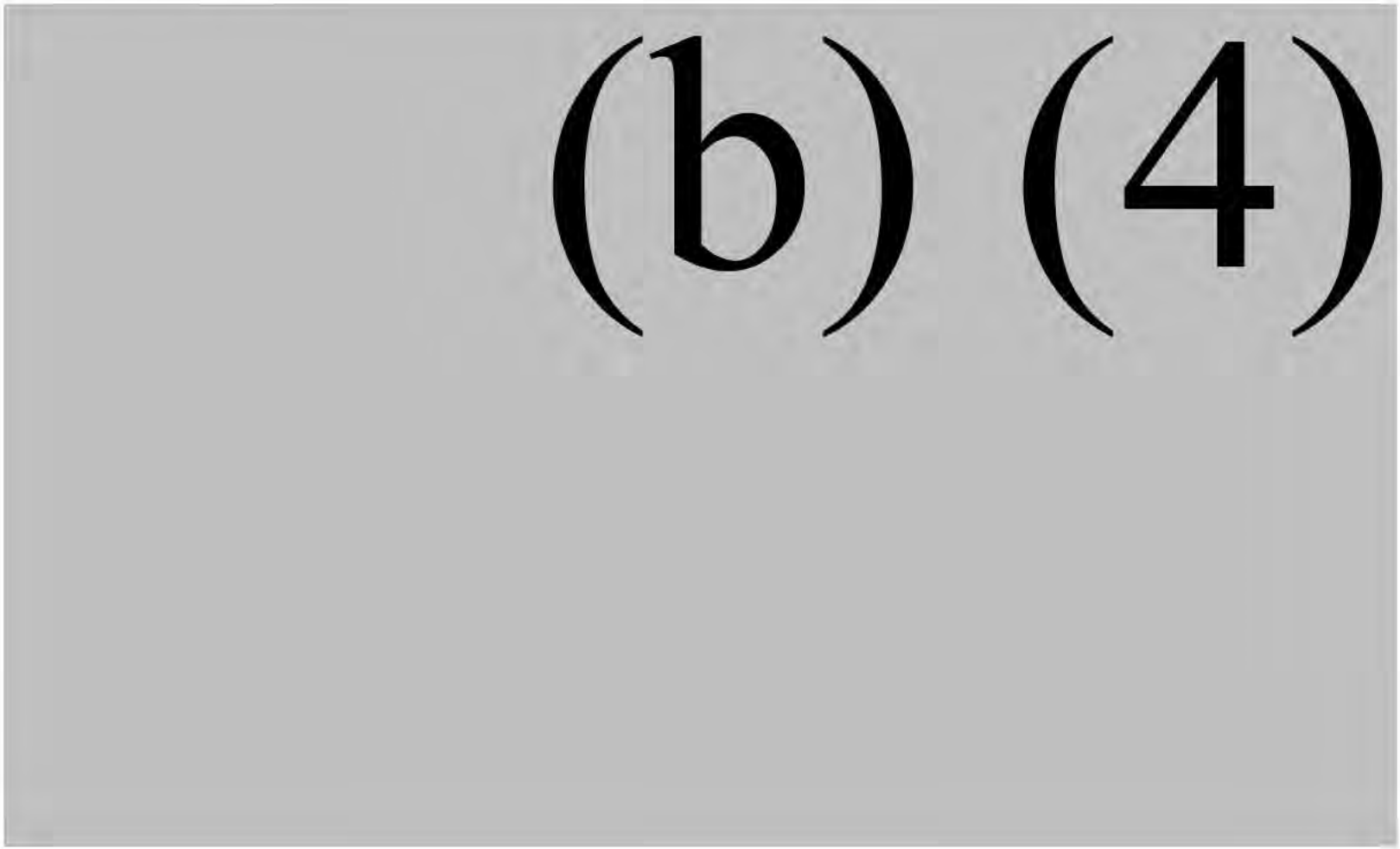
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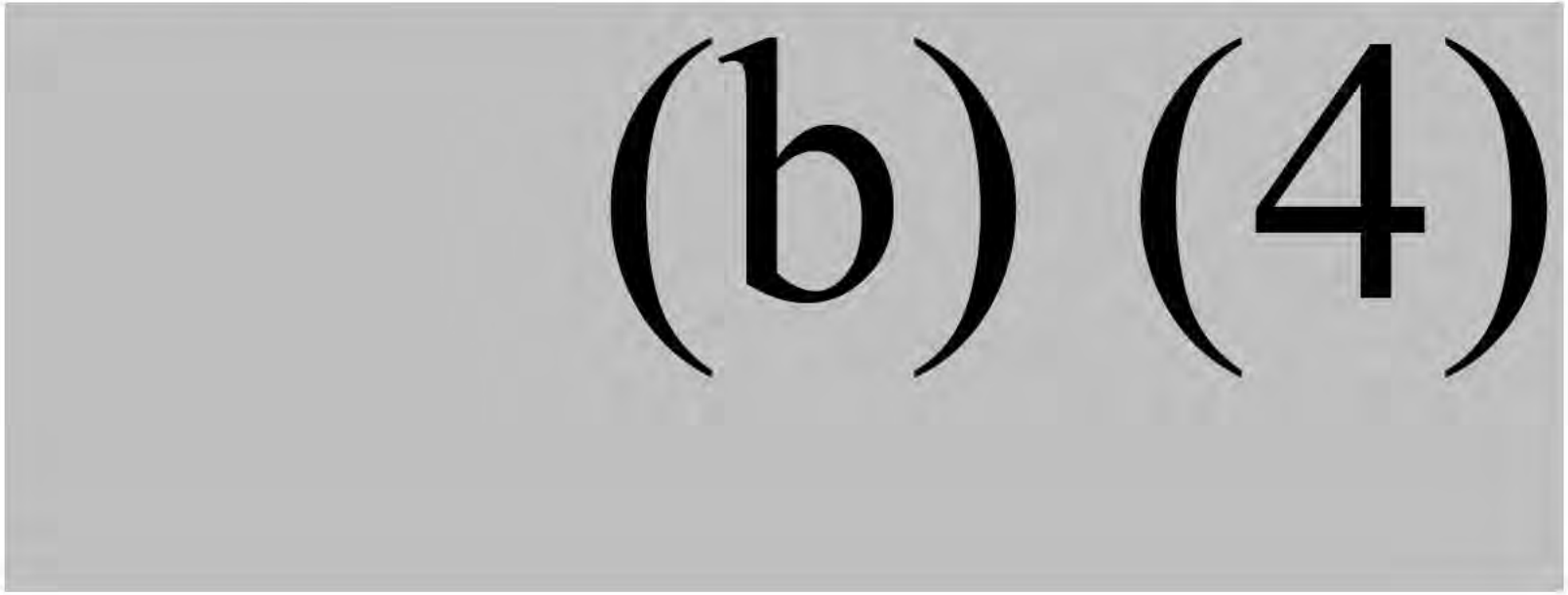
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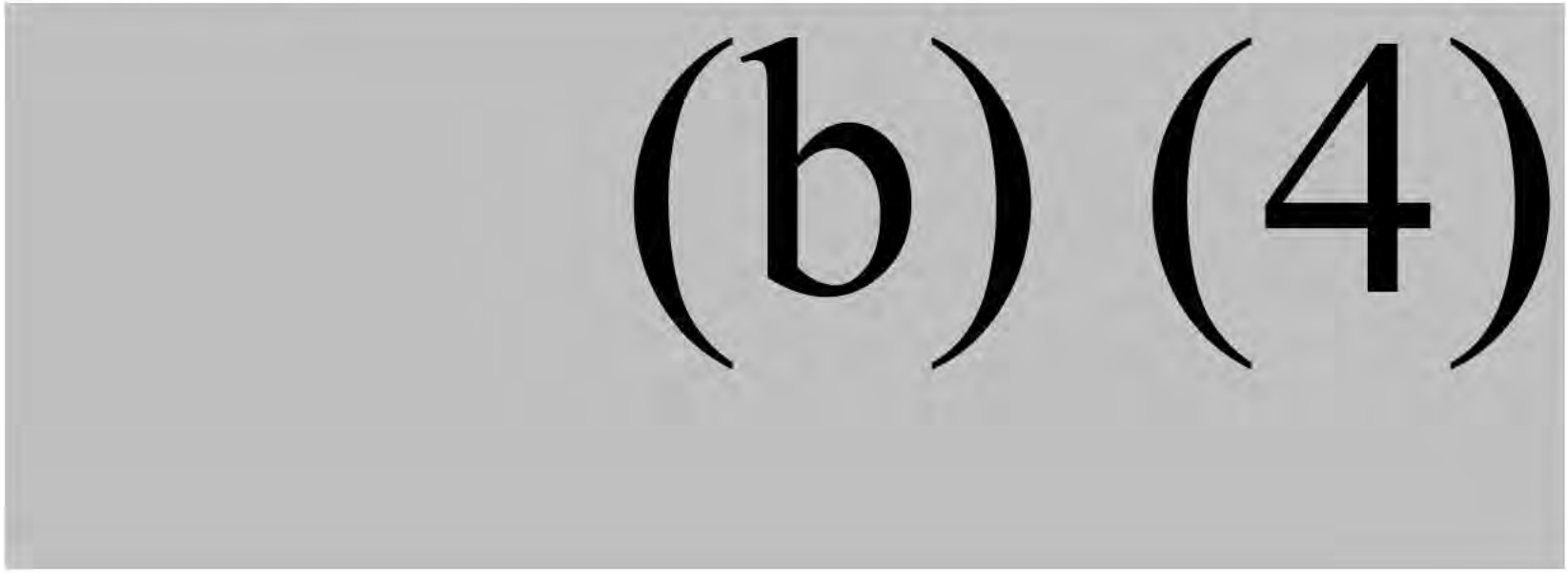
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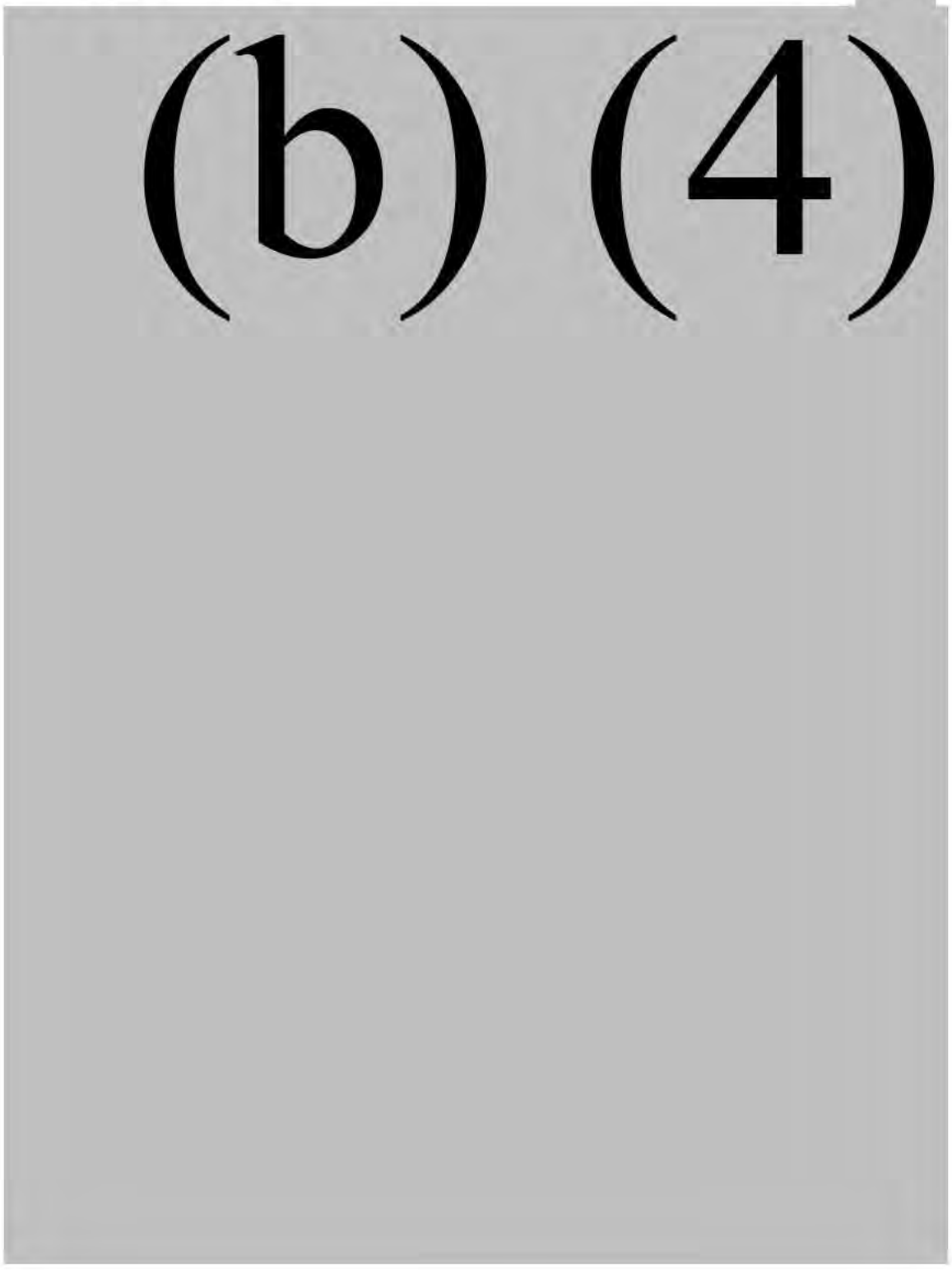
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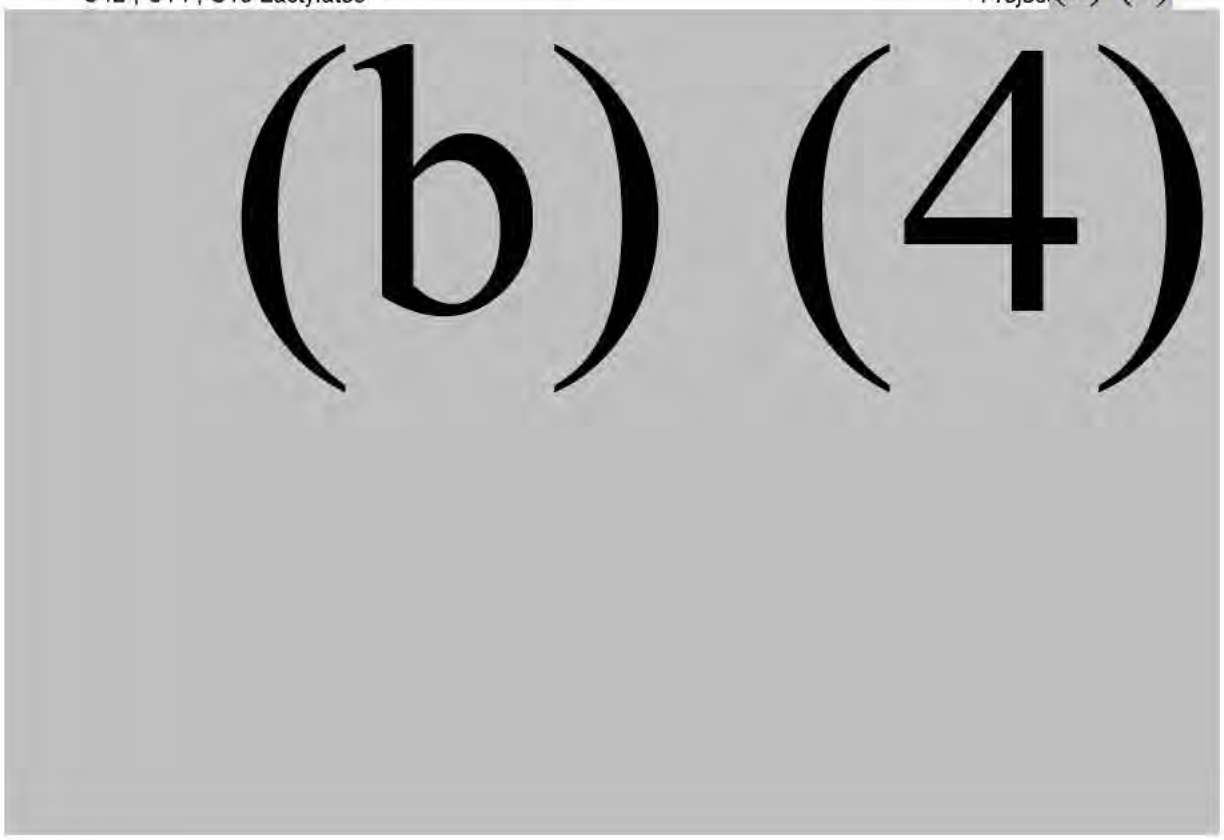
(b) (4)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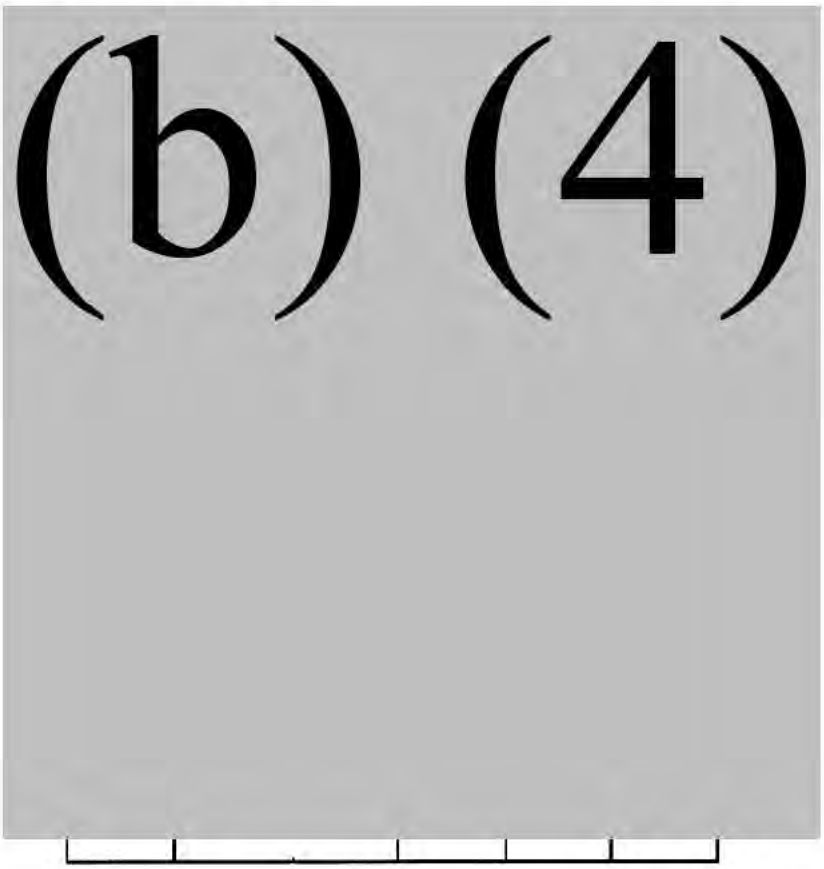
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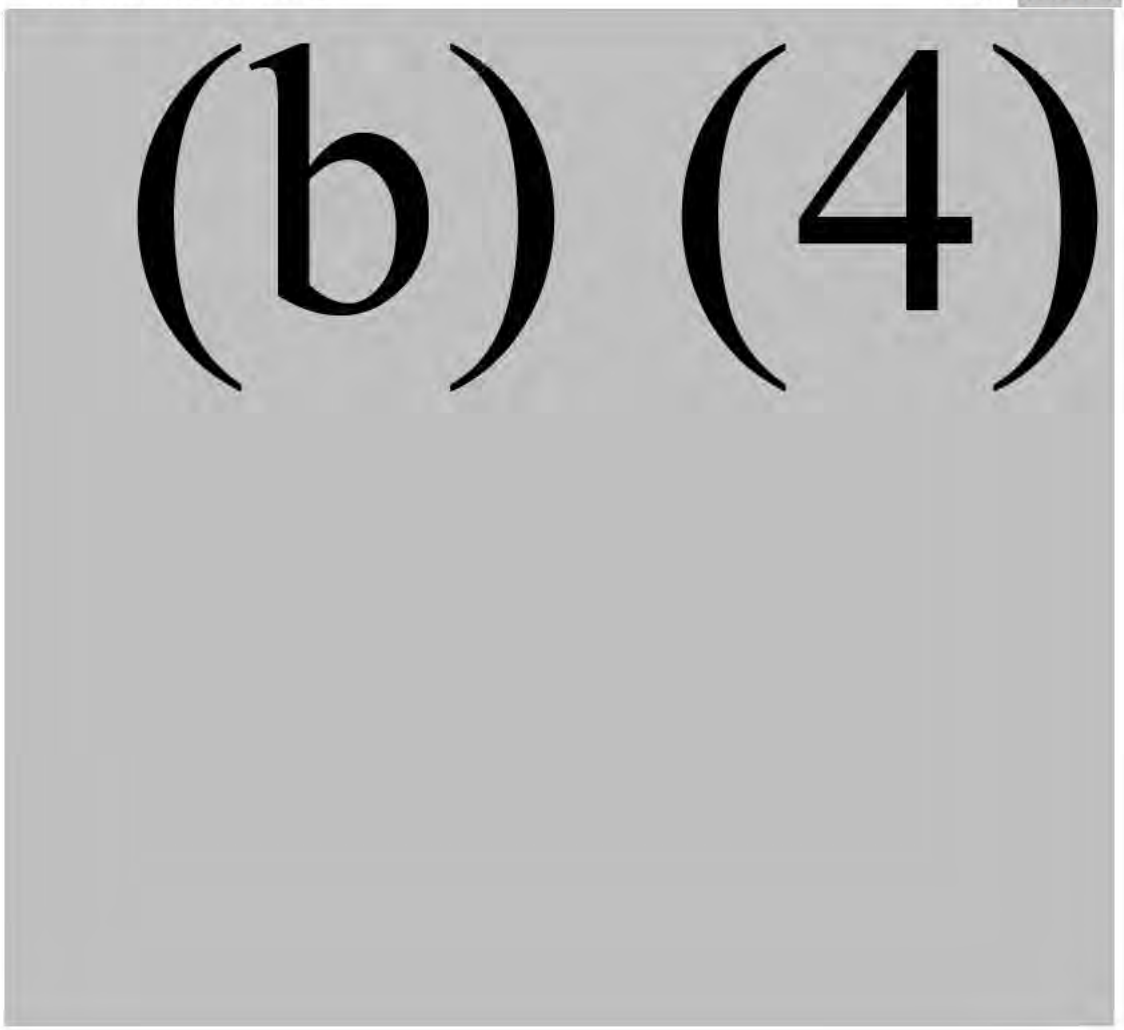
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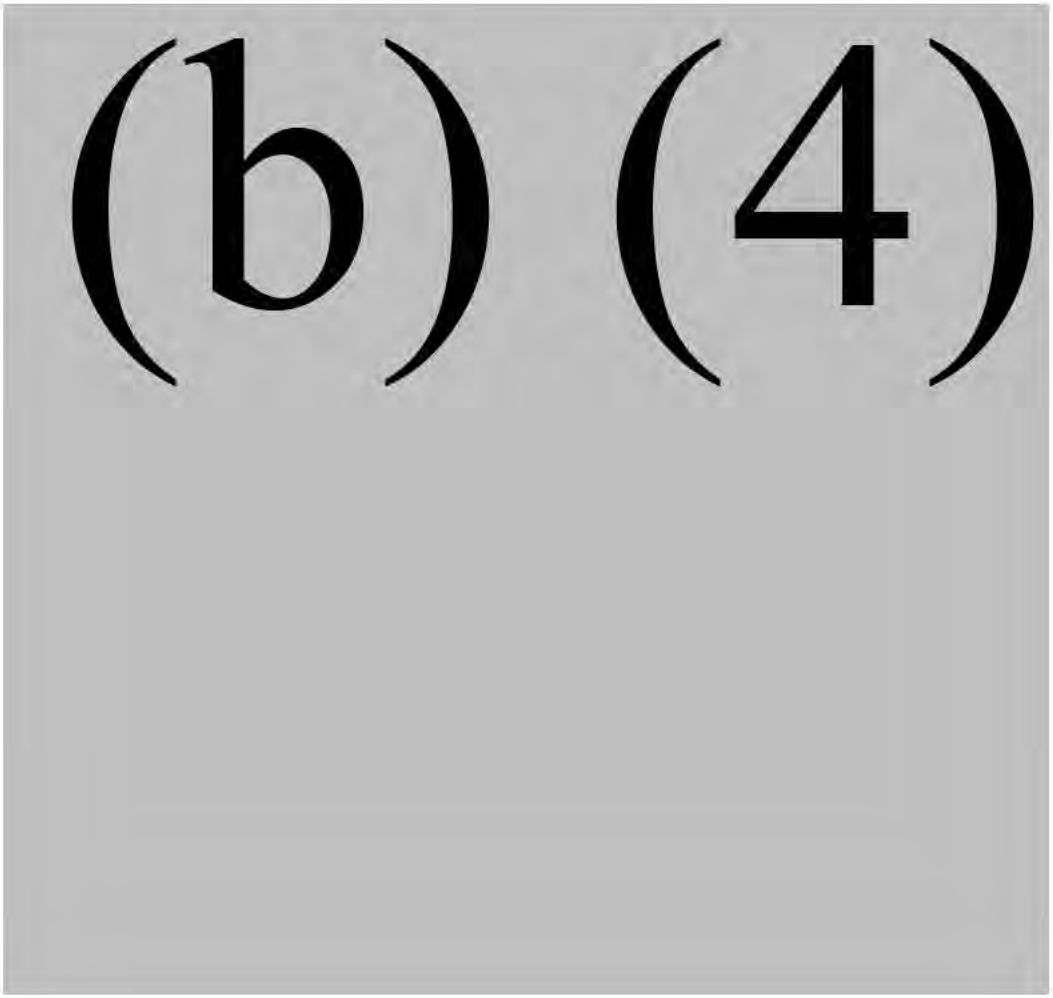
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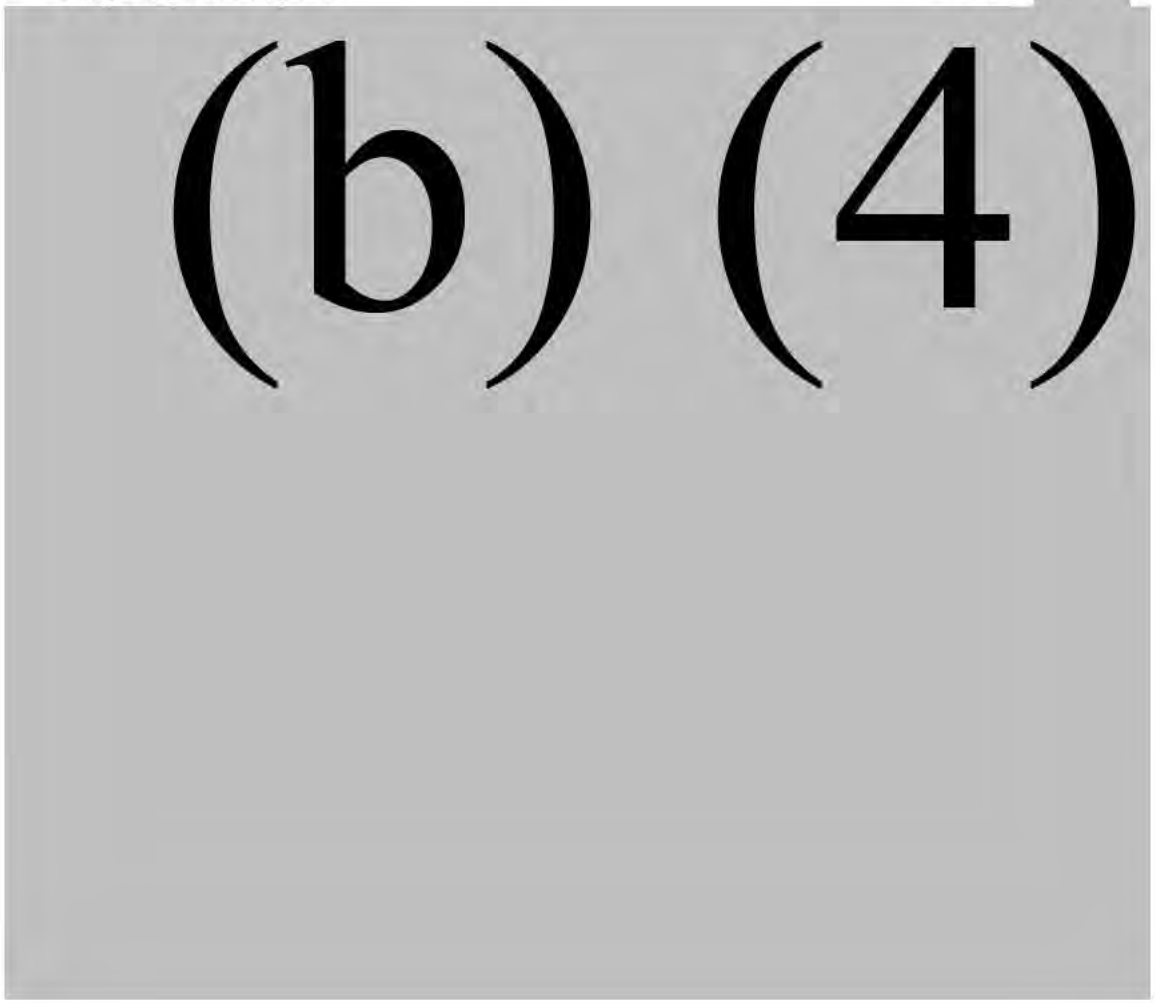
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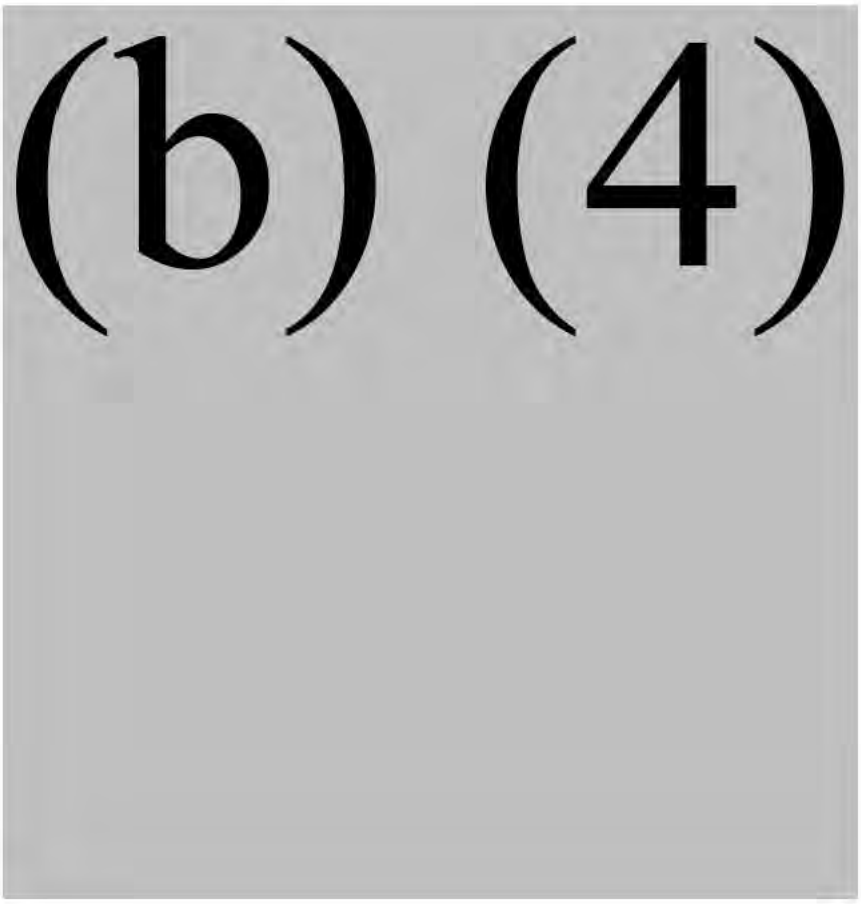
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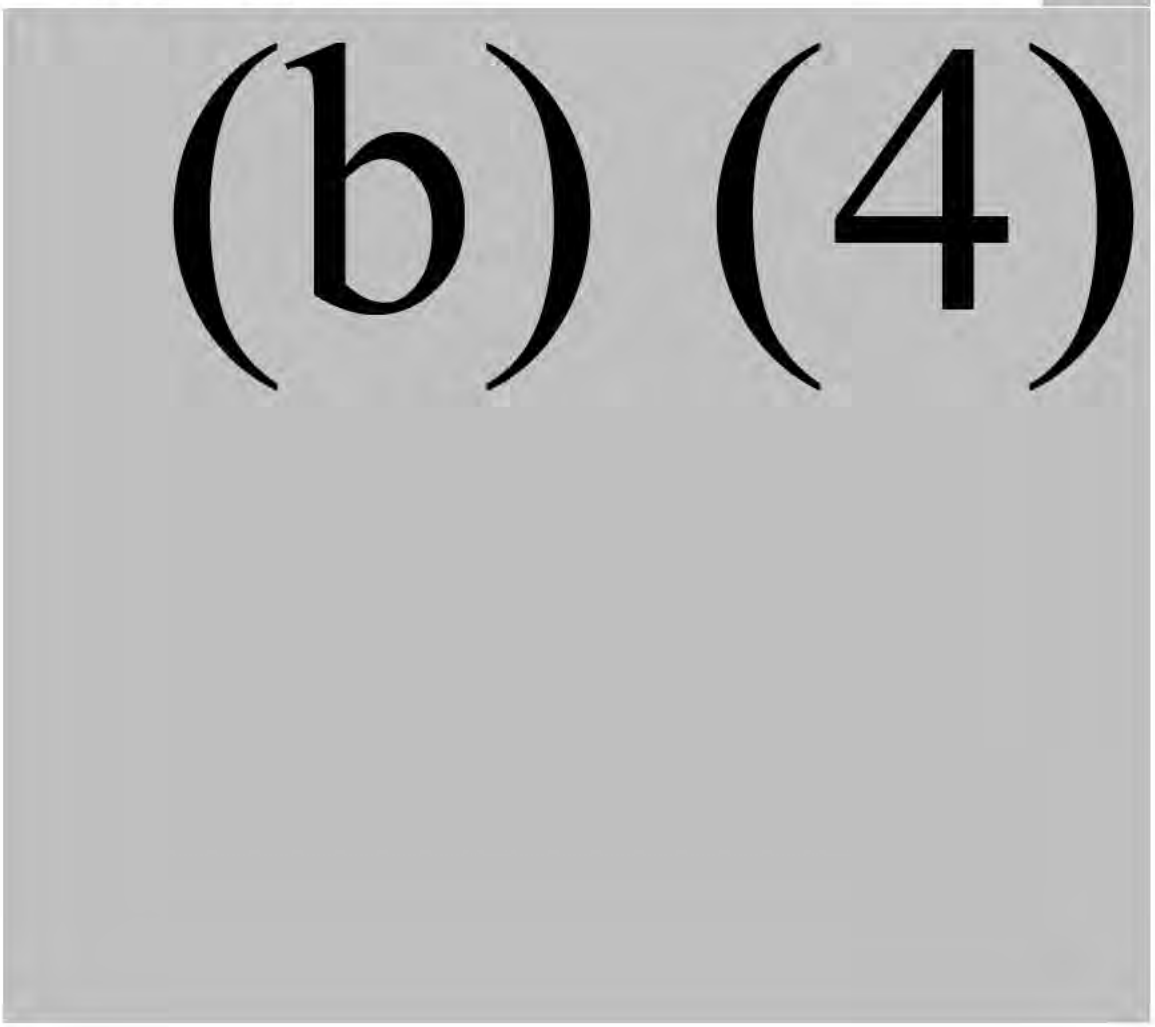
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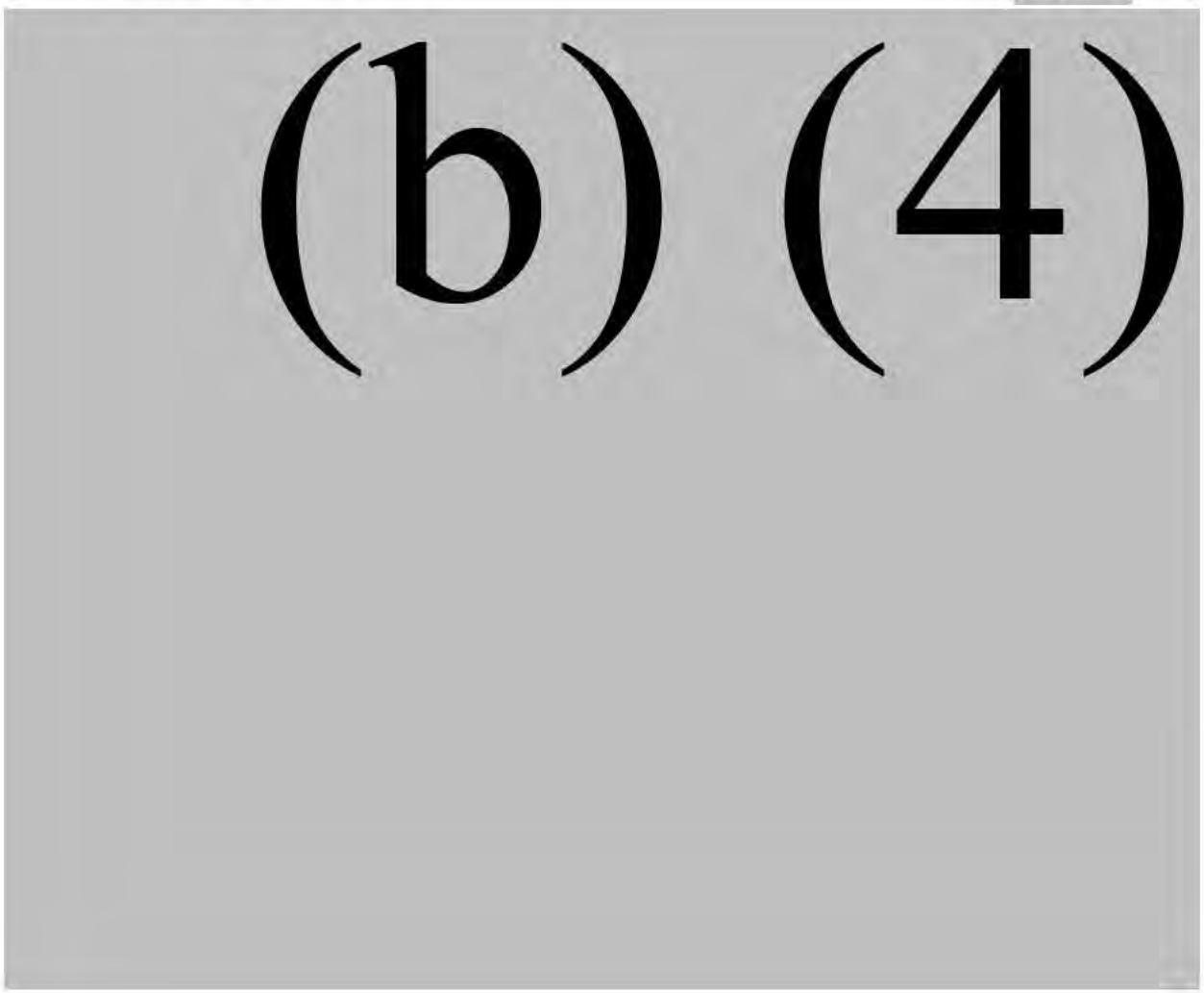
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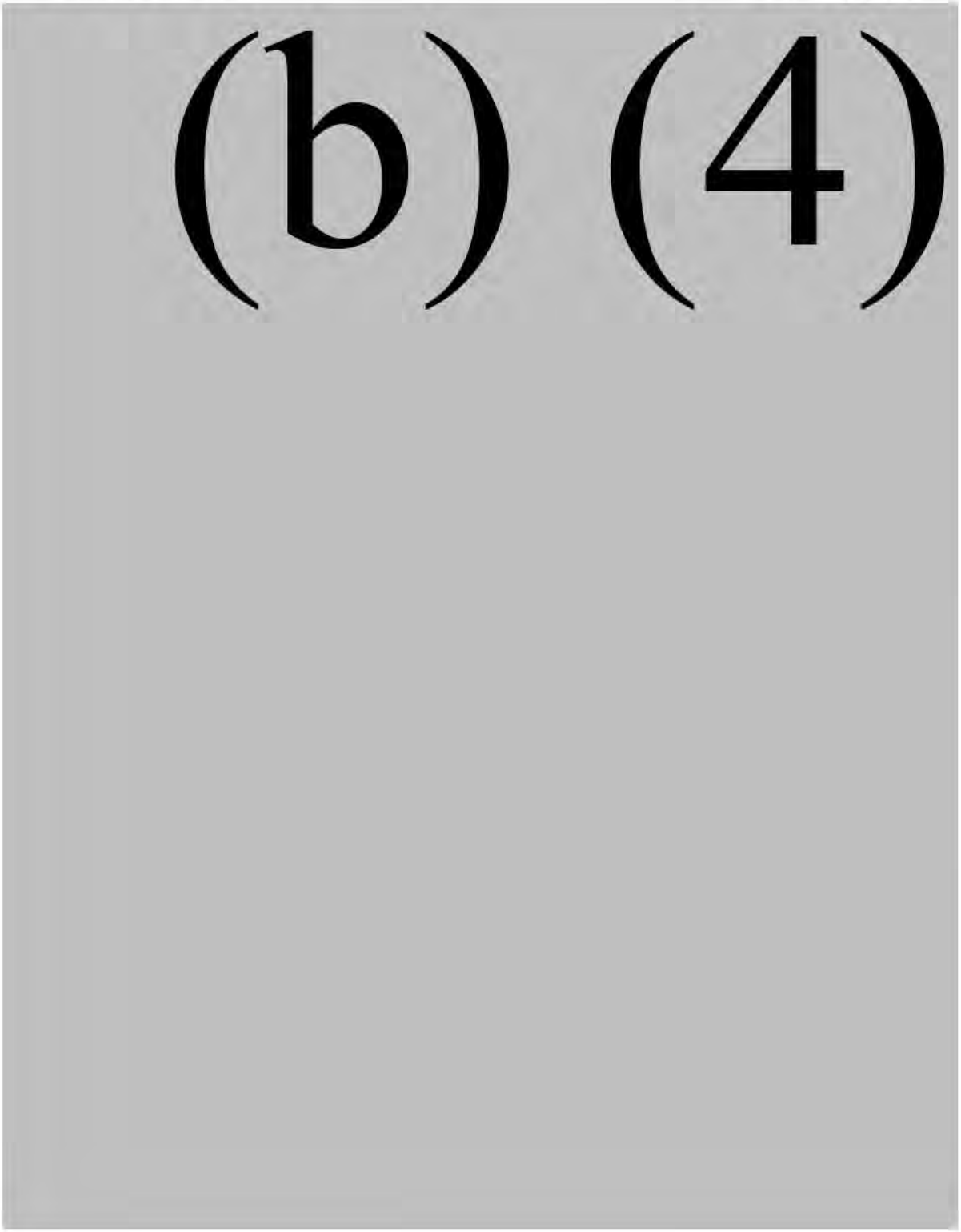
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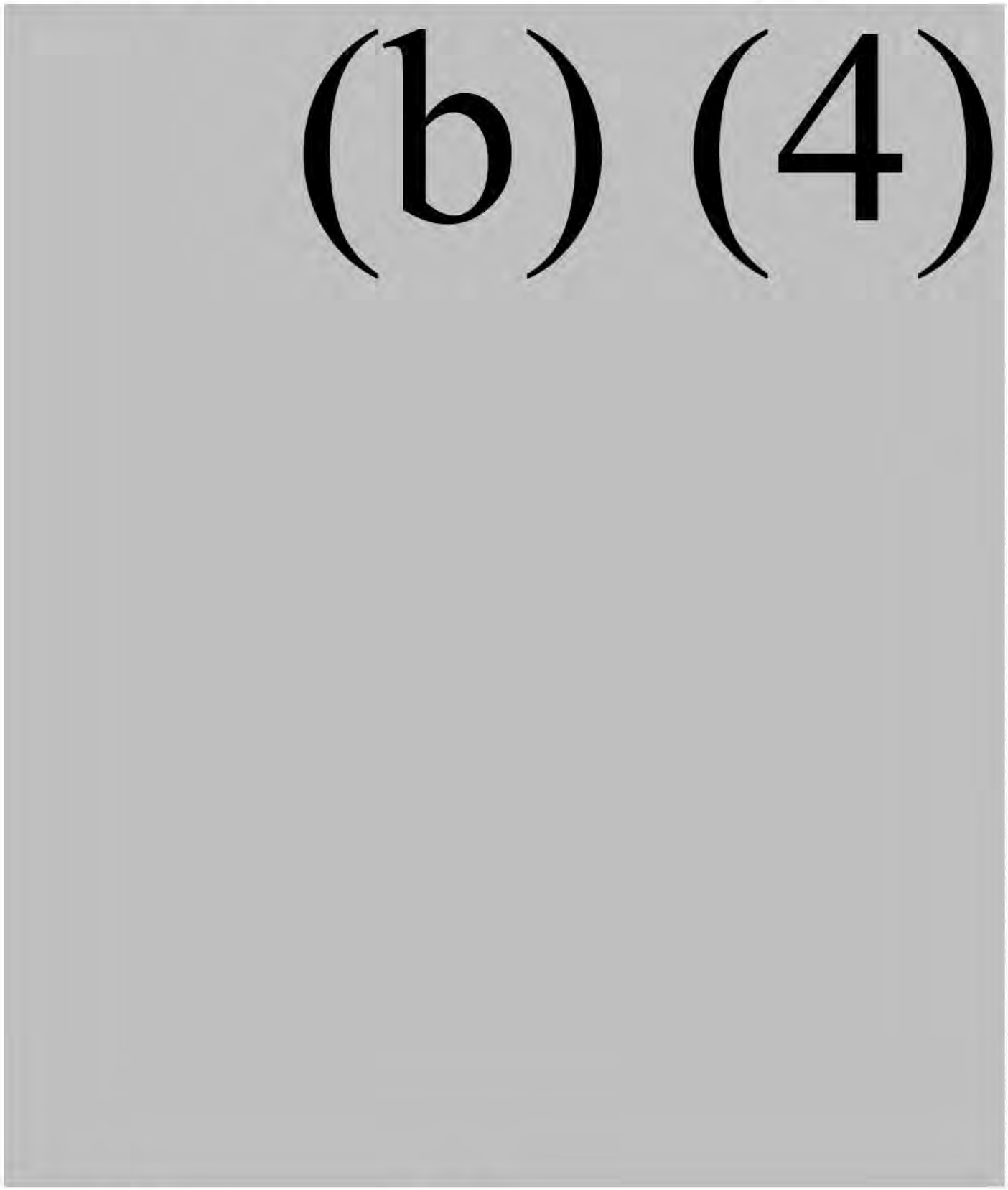
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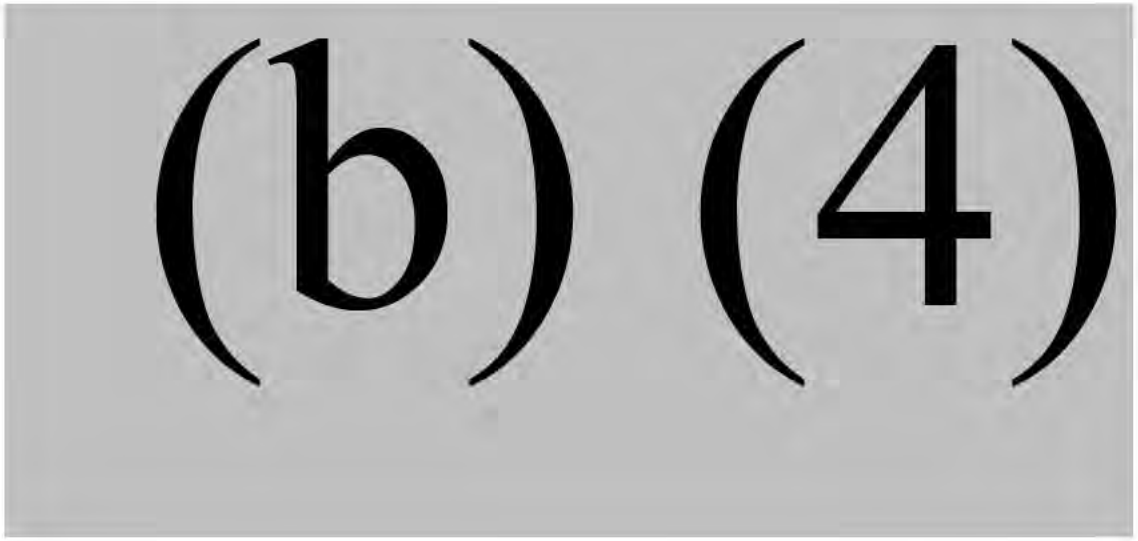
(b) (4)

Copy of Compositional Calculations Reported in GRAS Notice

		Batch Data: Lactylates					Mean in PURAMIX 100 (as Na Salt)	Mean in Aloapur, based on (b) (4) C12/C14 Lacylates	MW (as Na Salt)	Moles (as Na Salt)	Moles of Lactic Acid	Moles of C12 Fatty Acid	Moles of C14 Fatty Acid
Constituent	**Unit**	**1**	**2**	**3**	**4**	**5**	**wt%**	**wt%**					
lactate	[% (w/w)]	(b) (4)					8.98	3.23	(b) (4)				
lactoyl lactic acid	[% (w/w)]						2.78	1.00					
C12 fatty acid	[% (w/w)]						18.4	6.62					
C14 fatty acid	[% (w/w)]						8.1	2.92					
C12-1 lactylate	[% (w/w)]						33.4	12.02					
C14-1 lactylate	[% (w/w)]						14	5.04					
C12-2 lactylate	[% (w/w)]						6.24	2.25					
C14-2 lactylate	[% (w/w)]						2.22	0.80					
SUM							94.12	33.8832					

Mass of each component in 100 g of C12/C14 Lactylates	Lactic Acid	C12 Fatty Acid	C14 Fatty Acid	Sum	Remarks
Moles (Total)					
MW (anion)					
MW (Na salt form)					

(b) (4)

Based on intended use of C12/C14 Lactylates in Feed *Provided by ALOAPUR (either as ester or free fatty acid in sodium salt form)*				
		% in C12/C14 Lactylates	*g in 1.8 g (5 g ALOAPUR)*	*g in 0.7 g (2 g ALOAPUR)*
Lactic acid		(b) (4)		
Lauric acid				
Myristic acid				
	Lauric + Myristic SUM			

(b) (4)

2 Summary

The aim of the present study was to determine the metabolism and kinetics of disappearance of lauroyl lactylate (C12), myristoyl lactylate (C14) and palmitoyl lactylate (C16) from the digestive tract of broiler chickens. Five dietary treatments were evaluated. The birds (Ross 308 male chickens) in each experimental group received either one of five experimental diets which contained: 1. No test product, 2. A mixture of sodium lauroyl lactylates (C12) and sodium myristoyl lactylates (C14) (liquid) (Test Product name: PURAMIX 30L). 3. A mixture of sodium lauroyl lactylates (C12) and sodium myristoyl lactylates (C14) (Test Product name: PURAMIX 30S), 4. Sodium myristoyl lactylate (C14) (Test Product name: PURAMIX 14S), 5. Sodium palmitoyl lactylates (C16) (Test Product name: PURAMIX 16S). The intended dose levels of C12 and C14 lactylates in diets for treatments 2 and 3 were 900 and 375 mg/kg for C12 and C14 lactylates, respectively. The study was performed over a period of 27 d, during which the birds housed in metabolic cages and received the experimental diets ad libitum. Each treatment was evaluated in four replicates with 11 birds per cage at the start of the study.
The concentrations of lauroyl lactylate, myristoyl lactylate and palmitoyl lactylates in feed, in digesta obtained from various compartments of the digestive tract and in blood were determined on day 27 of the study for treatments 1 to 3 upon necropsy to determine the absorption of lauroyl lactylate (C12), myristoyl lactylate (C14) from the digestive tract and their appearance in the blood circulation. Feed intake, body weight gain and feed conversion ratio of the birds over the experimental period were measured for each of the dietary treatments.

There were no significant effects of the dietary treatments on feed intake, body weight gain and feed conversion ratio during the experimental period. The concentrations C12, C14 and C16 lactylates in digesta were generally low and/or below the lowest level of quantification in the samples of birds of treatment 1. In digesta samples of treatments 2 and 3 significant concentrations of C12 and C14 lactylates were found, the concentrations decreasing from the proximal part of the GI tract (crop and gizzard) towards the distal parts (ileum and colon). The calculated apparent absorption coefficients for C12 lactylates were lowest in the gizzard (7 and -5%, for treatment 2 and 3, respectively) and increased towards the distal parts of the GI tract to values of about 100%, suggesting complete apparent absorption or metabolism. There were no significant differences between treatments 2 and 3 with regard to the absorption coefficients for C12 and C14 lactylates within compartments of the GI tract. The apparent absorption coefficients for C14 lactylates were similar compared to those for the absorption of C12 lactylates. In both treatments 2 and 3 there was an almost complete apparent absorption of C12 and C14 lactylates from the distal small intestine onwards. The concentrations of C12 and C14 lactylates in blood plasma of birds of treatments 2 and 3 were low, but significantly higher compared to the values for birds of the control group ($P<0.05$).

It was concluded that C12 and C14 lactylates present in Puramix 30L and Puramix 30S, included in the diet at levels up to 732 and 301 mg/kg, respectively, are readily metabolized or absorbed in the proximal part of the small intestine (duodenum and first part of the small intestine) of broilers. It was concluded that the nature of the formulation (solid Puramix 30 S and liquid Puramix 30L) did not influence the apparent absorption or metabolism of C12 and C14 lactylates in the digestive tract. The excretion of intact C12 and C14 lactylates via the faecal route in the excreta is low (<3% of the dietary intake). The study did not reveal information on the exact nature and location of the metabolism of C12 and C14 lactylates (hydrolysis into lactic acid and their constituting fatty acids and subsequent use in anabolic metabolism or for oxidation) in the intestinal lumen or the intestinal mucosa. A very small proportion of the C12 and C14 lactylates present in Puramix 30L and Puramix 30S was recovered in intact form in the blood plasma pool of the broilers.

Key Study Information

The trial locations used are mentioned in the schedule below.

Trial locations		
Animal trials	(b) (4), (b)(6)	
Analysis of lactylates in diets, digesta and blood		
Analysis of nutrient composition of diets		
Analysis of chromium in diets and digesta samples		
Feed preparation site		

Test article

Generic Name of Active Ingredient:	Mix of sodium lauroyl lactylate (C12) and sodium myristoyl lactylate (C14) in monopropylene glycol, liquid
Product name	Puramix 30 L
Chemical name	Mix of sodium lauroyl lactylate (C12) and sodium myristoyl lactylate (C14) in monopropylene glycol
Source and Manufacturer:	Purac Biochem bv, P.O. Box 21, 4200 AA Gorinchem (The Netherlands)
Lot Number:	1008003354, 22 Augustus 2011
Expiration Date:	24 August2012
Storage Conditions:	Dry and cool (15-20°C)

Test article

Generic Name of Active Ingredient:	Mix of sodium lauroyl lactylate (C12) and sodium myristoyl lactylate (C14), solid
Product name	Puramix 30 S
Chemical name	Mix of sodium lauroyl lactylate (C12) and sodium myristoyl lactylate (C14)
Source and Manufacturer:	Purac Biochem bv, P.O. Box 21, 4200 AA Gorinchem (The Netherlands)
Lot Number:	S-P100-ABS, 16-18 Mei 2011
Expiration Date:	18 May 2012
Storage Conditions:	Dry and cool (15-20°C)

Test article

Generic Name of Active Ingredient:	Sodium myristoyl lactylate (C14)
Product name	Puramix 14 S
Chemical name	Sodium myristoyl lactylate
Source and Manufacturer:	Purac Biochem bv, P.O. Box 21, 4200 AA Gorinchem (The Netherlands)
Lot Number:	S-14-ABS, 16-18 Mei 2011
Expiration Date:	18 May 2012
Storage Conditions:	Dry and cool (15-20°C)

Test article

Generic Name of Active Ingredient:	Sodium palmitoyl lactylate (C16)
Product name	Puramix 16 S
Chemical name	Sodium palmitoyl lactylate
Source and Manufacturer:	Purac Biochem bv, P.O. Box 21, 4200 AA Gorinchem (The Netherlands)
Lot Number:	S-16-ABS, 16-18 Mei 2011
Expiration Date:	18 May 2012
Storage Conditions:	Dry and cool (15-20°C)

Statistical Analysis

- Mean body weight:
  The mean average body weight of the birds of a given treatment group was calculated using the individual body weight of the birds at a certain time point.
- Gross feed intake:
  [Weight of feed in bins at beginning of period + feed added during period – Feed remaining at end of period]
- Feed conversion ratio:
  [Gross feed intake for period / Total body weight gain for period]
- Mean daily feed intake per bird (g/d) over days 0-27 of the study.
- Mean daily body weight gain per bird (g/d) over days 0-27 of the study.
- Mean feed conversion ratio per pen over days 0-27 of the study.
- The concentrations of C12 lactylate, C14 lactylate and C16 lactylate in digesta from the crop, gizzard, duodenum, proximal small intestine, distal small intestine, ileum and colon (Treatments 1 to 3).
- The proportion (%) of C12 lactylate, C14 lactylate and C16 lactylate ingested via the diet disappeared up to various compartments of the digestive tract (crop, gizzard, duodenum, proximal small intestine, distal small intestine, ileum and colon) in treatments 1, 2 and 3 on day 27 of age (Treatments 1 to 3).
- The concentrations of C12 lactylate, C14 lactylate and C16 lactylate in blood serum of the birds in treatments 1, 2 and 3 on day 27 of age (Treatments 1 to 3).
- Apparent absorption coefficient (AC) of lactylates up to various compartments of the digestive tract (Treatments 1 to 3)

$$AC\,lactylate = \left[\, 1 - \frac{[Cr]_{diet} \times [lactylate]_{digesta}}{[Cr]_{digesta} \times [lactylate]_{diet}} \,\right] \times 100\ \%$$

in which $AC_{lactylate}$ is the apparent absorption coefficient of the lactylate (%), [Cr] is the concentration of Cr in the diet or digesta and [lactylate] is the concentration of the lactylate in the diet or in digesta.

7.8.4 Statistical Design and Analysis

Data on the concentrations of lactylates in digesta and blood serum, apparent absorption coefficients of lactylates and data on production performance of the birds (feed intake, body weight gain and feed conversion ratio) were analysed using analysis of variance with "treatment" as statistical fixed factor.

Effects were considered significant at $P < 0.05$. If the overall treatment is significant, contrasts between treatments were further considered using a Least Significant Difference (LSD) test (Snedecor en Cochran, 1980).

(b) (4) was used as statistical software.

Calculation of Plasma Pool

	Treatment 2	3	Ratio Trm 3/2
Feed intake, d 21-27 (g/d)	162	153	
Content C12 (mg/kg) in the diet	584	792	
Content C14 (mg/kg) in the diet	276	321	
Intake C12 (mg/d)	94	121	128
Intake C14 (mg/d)	45	49	110
LW d 27 (g)	1798	1708	
Blood (8% of LW) (g)	144	137	
Plasma in blood (%)	65	65	
Concentration C12 plasma (ng/ml)	308	389	126
Concentration C14 plasma (ng/ml)	80	83	104
C12 in plasma, mg	0.029	0.035	
C14 in plasma, mg	0.007	0.007	
Pool size in plasma, % of daily intake			
C12	0.030	0.029	
C14	0.017	0.015	

Results:

(b) (4)

(b) (4)

(b) (4)

Appendix. Ingredient and nutrient composition (g/kg, unless stated otherwise) of the experimental starter diets (day 0 till 7).

	1	2	3	4	5
Wheat	250.0	250.0	250.0	250.0	250.0
Maize	285.6	285.6	285.6	285.6	285.6
Peas	50.0	50.0	50.0	50.0	50.0
Soybeans, toasted	50.0	50.0	50.0	50.0	50.0
Soybean meal	255.0	255.0	255.0	255.0	255.0
Rapeseed meal	30.0	30.0	30.0	30.0	30.0
Animal fat	30.0	30.0	30.0	30.0	30.0
Soya oil	13.0	13.0	13.0	13.0	13.0
Premix broilers[2,3]	5.0	5.0	5.0	5.0	5.0
Chalk	14.0	14.0	14.0	14.0	14.0
Monocalcium phosphate	9.0	9.0	9.0	9.0	9.0
NaCl	2.2	2.2	2.2	2.2	2.2
$NaHCO_3$	2.0	2.0	2.0	2.0	2.0
Phytase (b) (4)	0.1	0.1	0.1	0.1	0.1
L-lysine HCl	1.5	1.5	1.5	1.5	1.5
DL-methionine	2.4	2.4	2.4	2.4	2.4
L-threonine	0.6	0.6	0.6	0.6	0.6
Puramix 30L[1]		7.56			
Puramix 30S[1]			7.56		
Puramix C14S[1]				7.56	
Puramix C16S[1]					7.56
Dry matter	880	880	880	880	880
Crude protein	215	215	215	215	215
Crude fat	73	73	73	73	73
Ash	56	56	56	56	56
Crude fibre	31	31	31	31	31
ME broilers (kcal/kg)	2,850	2,850	2,850	2,850	2,850
Calcium	8.9	8.9	8.9	8.9	8.9
Phosphorus	5.7	5.7	5.7	5.7	5.7
Phosphorus, dig. (oP)	4.1	4.1	4.1	4.1	4.1
Ca/oP	2.2	2.2	2.2	2.2	2.2
Sodium	1.5	1.5	1.5	1.5	1.5
Potassium	9.5	9.5	9.5	9.5	9.5
Chloride	2.0	2.0	2.0	2.0	2.0
Base-excess (meq/kg)	251	251	251	251	251
Lysine	12.4	12.4	12.4	12.4	12.4
Methionine	5.5	5.5	5.5	5.5	5.5
Cystine	3.6	3.6	3.6	3.6	3.6

	1	2	3	4	5
Methionine + Cystine	9.1	9.1	9.1	9.1	9.1
Threonine	8.4	8.4	8.4	8.4	8.4
Tryptophan	2.5	2.5	2.5	2.5	2.5
Valine	9.9	9.9	9.9	9.9	9.9
Arginine	14.4	14.4	14.4	14.4	14.4
Dig. Lysine	10.7	10.7	10.7	10.7	10.7
Dig. Methionine	5.1	5.1	5.1	5.1	5.1
Dig. Cystine	2.9	2.9	2.9	2.9	2.9
Dig. Methionine + Cystine	8.0	8.0	8.0	8.0	8.0
Dig. Threonine	7.0	7.0	7.0	7.0	7.0
Dig. Tryptophan	2.2	2.2	2.2	2.2	2.2
Dig. Valine	8.4	8.4	8.4	8.4	8.4
Dig. Arginine	12.4	12.4	12.4	12.4	12.4

[1]The test products Puramix 30L, Puramix 30S, Puramix C14S and Puramix C16S were included on top of the formulation.
[2]A xylanase preparation was included in the form of (b) (4) at a level of 100 mg/kg.
[3] (b) (4) .

Appendix. Ingredient and nutrient composition (g/kg, unless stated otherwise) of the experimental grower diets (day 7 till day 28).

	1[2]	2[2]	3[2]	4[2]	5[2]
Wheat	350.0	350.0	350.0	350.0	350.0
Maize	156.1	156.1	156.1	156.1	156.1
Peas	100.0	100.0	100.0	100.0	100.0
Soybeans, toasted	100.0	100.0	100.0	100.0	100.0
Soybean meal	180.0	180.0	180.0	180.0	180.0
Rapeseed meal	30.0	30.0	30.0	30.0	30.0
Animal fat	40.0	40.0	40.0	40.0	40.0
Soya oil	15.0	15.0	15.0	15.0	15.0
Premix broilers[3,4]	5.0	5.0	5.0	5.0	5.0
Chalk	11.5	11.5	11.5	11.5	11.5
Monocalciumfosfaat	4.5	4.5	4.5	4.5	4.5
NaCl	2.2	2.2	2.2	2.2	2.2
$NaHCO_3$	1.7	1.7	1.7	1.7	1.7
Phytase (b) (4)	0.1	0.1	0.1	0.1	0.1
L-lysine HCl	1.2	1.2	1.2	1.2	1.2
DL-methionine	2.3	2.3	2.3	2.3	2.3
L-threonine	0.4	0.4	0.4	0.4	0.4
Puramix 30L[1]		7.56			
Puramix 30S[1]			7.56		
Puramix C14S[1]				7.56	
Puramix C16S[1]					7.56
Dry matter	881	881	881	881	881
Crude protein	208	208	208	208	208
Crude fat	90	90	90	90	90
Ash	48	48	48	48	48
Crude fibre	33	33	33	33	33
ME broilers (kcal/kg)	2,958	2,958	2,958	2,958	2,958
Calcium	7.1	7.1	7.1	7.1	7.1
Phosphorus	4.7	4.7	4.7	4.7	4.7
Phosphorus, dig. (oP)	3.2	3.2	3.2	3.2	3.2
Ca/oP	2.2	2.2	2.2	2.2	2.2
Sodium	1.4	1.4	1.4	1.4	1.4
Potassium	9.2	9.2	9.2	9.2	9.2
Chloride	1.9	1.9	1.9	1.9	1.9
Base-excess (meq/kg)	242	242	242	242	242
Lysine	11.8	11.8	11.8	11.8	11.8
Methionine	5.2	5.2	5.2	5.2	5.2
Cystine	3.5	3.5	3.5	3.5	3.5

	1[2]	2[2]	3[2]	4[2]	5[2]
Threonine	7.9	7.9	7.9	7.9	7.9
Tryptophan	2.4	2.4	2.4	2.4	2.4
Valine	9.5	9.5	9.5	9.5	9.5
Arginine	13.7	13.7	13.7	13.7	13.7
Dig. Lysine	10.2	10.2	10.2	10.2	10.2
Dig. Methionine	4.9	4.9	4.9	4.9	4.9
Dig. Cystine	2.8	2.8	2.8	2.8	2.8
Dig. Methionine + Cystine	7.7	7.7	7.7	7.7	7.7
Dig. Threonine	6.5	6.5	6.5	6.5	6.5
Dig. Tryptophan	2.1	2.1	2.1	2.1	2.1
Dig. Valine	8.0	8.0	8.0	8.0	8.0
Dig. Arginine	12.1	12.1	12.1	12.1	12.1

[1]The test products Puramix 30L, Puramix 30S, Puramix C14S and Puramix C16S were included o top of the formulation.
[2]0.25 g/kg Cr_2O_3 was included in the grower diets as digestibility marker.
[3]A xylanase preparation was included in the form of (b) (4) at a level of 100 mg/kg

(b) (4)

Appendix 17

Studies in Target Animals using Lactic Acid and Its Calcium Lactate

There are a number of studies in the published literature in which lactic acid was fed to poultry, swine and ruminants. A detailed evaluation of these studies was not considered necessary to establish the safety of the lactic acid component of C12/C14 lactylates but for completeness the studies are summarized below (Table A).

Table A: Studies in Target Animals using Lactic Acid Supplementation		
Reference	**Study Design**	**Key Results**
Poultry		
Abdel-Fattah *et al*., 2008 Objective: Thyroid activity, some blood constituents, organ morphology and performance of broiler chicks fed supplemental organic acids	Animals: 1-day old broiler chicks Treatment: Basal diet (rice/SBM starter ration, rice/corn/SBM finisher ration) with 0, 15,000, or 30,000 mg lactic acid/kg complete feed 2 feeding phases, starter (0-4 weeks) and finisher (5-6 weeks of age) Duration: 42 days (6 weeks)	Results: – ↑'d BWG and improved FCR after 6 weeks in animals fed diets including 15,000, or 30,000 mg lactic acid/kg complete feed vs. control – ↓'d FI after 6 weeks in animals fed diets including 15,000 mg lactic acid/kg complete feed vs. control – NSD in FI after 6 weeks in animals fed diets including 30,000 mg lactic acid/kg complete feed vs. control – Thyroid function or blood chemistry analysis reported no adverse effects by the inclusion of lactic acid in the diet vs. control Conclusions: Broiler chicks of dietary lactic acids had superior improvement in performance (BWG and FCR) as compared to those of unsupplemented diet. No remarkable differences were noted between the addition of 1.5 and 3% of lactic acid in most studied traits.
Adil *et al*., 2010 Objective: Effect of dietary supplementation of organic acids on performance, intestinal histomorphology and serum biochemistry in broiler chickens	Animals: 7-day old Cobb broiler chicks Treatment: Basal diet (corn/SBM/FM) with 0, 20,000 or 30,000 mg lactic acid/kg complete feed Duration: 35 days	Results: – ↑'d final BWG (both groups vs. control; 1602 and 1673 vs. 1525 g/bird) – NSD in FI among groups – Improved FCR (both groups vs. control; 1.85 and 1.84 vs. 2.02) – NSD in carcass characteristics – ↑'d villus height in the duodenum (high dose vs. control) and jejunum (both groups vs. control) – ↑'d serum calcium and phosphorus concentrations (both groups vs. control) – NSD on serum concentrations of glucose, cholesterol, and liver markers among groups (AST and ALT) Conclusions: Dietary supplementation of lactic acid improved the performance in terms of body weight and feed conversion ratio, and may be incorporated in the diets of broiler chicken as growth promoters

Table A: Studies in Target Animals using Lactic Acid Supplementation		
Reference	**Study Design**	**Key Results**
Cave, 1983 Objective: Effect of dietary propionic and lactic acids on feed intake by chicks	Animals: 21-day old female meat strain (Shaver Starbro) chicks Treatment: Basal diet (corn/wheat/SBM) with 0, 10,000 or 30,000 mg lactic acid/kg complete feed Duration: 8 days (Day 21-29)	Results: – NSD in BWG or FI among groups between animals fed diets containing lactic acid vs. control Conclusion: Adding lactic acid to Shaver Starbro diets did not significantly alter performance parameters after 8 days of supplemntation
Khalid *et al*., 2002 Objective: Effect of replacement of feed additive antibiotic with different levels of lactic acid on broiler performance	Animals: 1-day old broiler chicks Dietary Treatment: Basal diet (rice/SBM starter ration, rice/corn/SBM finisher ration) with 0, 10,000, 20,000 or 30,000 mg lactic acid/kg complete feed 2 feeding phases, starter (0-4 weeks) and finisher (5-6 weeks of age) Duration: 42 days (6 weeks)	Results: – NSD in BWG or FI among groups; over the 6 weeks – FCR not reported by author but calculated to be 1.80, 1.77, 1.76, and 1.71 for control, low-, mid-, and high-dose groups respectively Conclusion: Adding lactic acid to broiler chicken diets did not significantly alter performance parameters when considering the cumulative time period (0-6 weeks)
Swine		
Burnett & Hanna, 1963 [Abstract only] Objective: Evaluate the effect of dietary calcium lactate and lactic acid on faecal *Escherichia coli* counts in pigs	**Trial 1** Animals: Pigs with 32-36 kg IBW Treatment: Basal diet with 0 or 2% calcium lactate Duration: 4 weeks	Results: – NSD in BWG after 4 weeks between animals fed calcium lactate vs. control Conclusions: Lactic acid did not influence growth
	Trial 2 Animals: Pigs with 27-32 kg IBW	Results: – NSD in BWG in animals fed calcium lactate between weeks 5 to 8 vs. control

Table A: Studies in Target Animals using Lactic Acid Supplementation		
Reference	**Study Design**	**Key Results**
	Treatment: Basal diet with 0 or 2 g/lb lactic acid (from 5th to 8th weeks) Duration: 8 weeks	Conclusions: Lactic acid did not influence growth
Geary, 1999 [Abstract only] Objective: Evaluate the effect on weaner pig performance and diet microbiology of feeding a liquid diet acidified to pH 4 with either lactic acid or though fermentation with *Pediococcus acidilactici*	Animal: 24-day old weaned pigs with 7 kg IBW Treatment: Control diet acidified to pH 4 with lactic acid or control diet acidified to pH 4 by fermentation with *P. acidilactici* Duration: 28 days	Results: – NSD in growth parameters (BWG and FCR) between diets – Reducing pH (<4.0) in either of the liquid diets was effective in eliminating coliform bacteria Conclusions: Fermentation of liquid diets for newly weaned piglets could provide a more cost effective means of acidifying diets than the use of organic acids. Reducing the pH of the liquid diet to 4.00 by fermentation with *P.acidilactici* was a cost effective method of eliminating enteropathogens and spoilage organisms from the diet
Howard *et al*., 2003 [Abstract only] Objective: Evaluate the efficacy of lactic acid to prevent rapid salmonella infection in market weight swine	Animals: Pigs Treatment: Drinking water with 0% lactic acid + 0 g Tylan, 20 g Tylan/907 kg of feed, 0.44% lactic acid or 0.44% lactic acid + 20 g Tylan/907 kg of feed Duration: 7 days	Results: – NSD in *S typhimurium* prevalence from stomach fluid, ileal tissue, ileocecal lymph nodes, cecal contents, and distal colonic contents between animals treated with lactic acid vs. control Conclusions: Lactic acid was ineffective in reducing *Salmonella* prevalence; lack of lactic acid concentration differences in stomach fluid suggests orally consumed lactic acid was either rapidly absorbed or administered at a level that was inadequate to raise it above physiological values
Kershaw *et al*., 1966 [Abstract only]	Animals: 8 weeks old pigs Treatment:	Results: – ↑'d BWG and FCR after lactic acid and sodium acrylate vs. control

Table A: Studies in Target Animals using Lactic Acid Supplementation		
Reference	**Study Design**	**Key Results**
Objective: Lactic acid and sodium acrylate: effect on growth rate and bacterial flora in the intestines of weaned pigs.	Drinking water with 0, 1% lactic acid or 0.5% sodium acrylate Duration: 3 weeks	– A month after the start there was no difference between the groups – ↓'d *E. coli* in duodenum and jejunum and hemolytic *E coli* between animals fed lactic acid or sodium acrylate vs. control – NSD in *Lactobacillus* counts and pH between treated groups vs. control after treatment ceased Conclusions: Both additions improved daily gain and efficiency of feed conversion, lactic acid having most effect although this was not present after 1 month The additives eliminated *E. coli* in duodenum and jejunum and no hemolytic *E. coli* were found in all subsequent examinations
Schmidt & Kliesch, 1938 [Abstract only] Objective: Evaluate the effect of lactic acid supplements in pig fattening	Animals: Pigs with 37.4 kg IBW Treatment: Basal diet with 0 or 20 g lactic acid/head/day Duration: 77 days	Results: – ↑'d BWG in animals fed lactic acid vs. control – NSD in FI in animals fed lactic acid vs. control Conclusions: Liveweight increased but no advantage in food consumption had resulted from animals receiving lactic acid.
Svoboda *et al*., 2005 Objective: Evaluate the effect of voluntary consumption of Fe Lactate supplements on development of hematological indices of suckling piglets	Animals: Piglets Treatment: Group 1: received Fe lactate in a loose crystalline form, containing 142 g Fe^{2+}/kg Group 2: was given a finely granular mixture of Fe lactate (70%), milk protein lactoferrin (20%) and whey powder (10%) in the same period. Dietary Fe^{2+} content was 99.5 g Fe^{2+}/kg Group 3: was given 200 mg Fe^{3+} i.m. in the form of dextran on day 3 after birth	Results: – ↓'d Hemoglobin, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin and plasma Fe concentration after 7 days in animals from group 1, group 2, and group 4 vs. group 3 Conclusions: The timely supplementation of Fe in a suitable form is essential in order to utilise the high growth ability of piglets that is typical of the first weeks after birth. The results indicate that the consumption of Fe lactate supplements was negligible and did not prevent anaemia of piglets.

Table A: Studies in Target Animals using Lactic Acid Supplementation		
Reference	**Study Design**	**Key Results**
	Group 4: was given 200 mg Fe^{3+} i.m. in the form of dextran on day 21 after birth (Anaemic group) Duration: 42 days Sows were transferred into farrowing pens 14 days before parturition and piglets were monitored for 28 days	
Thaela *et al*., 1998 Objective: Evaluated the effect of lactic acid supplementation on pancreatic secretion in pigs after weaning	Animals: Crossbred piglets (Duroc x Danish Landrace x Yorkshire) Treatment: All the pigs received: Standard weaner diet (control) during period I Standard weaner diet supplemented with 2.5% lactic acid during period II Standard weaner diet (control) during period III Duration: 3 weeks	Results: – ↑'d pancreatic secretion in terms of volume and protein content in period II (P<0.05) vs. period I – Chymotrypsin and trypsin content of pancreatic juice was increased in period II vs period I but nit significantly – NSD in bicarbonate concentration in pancreatic secretion in period II vs. period I – NSD in period III in any of the measured parameters of pancreatic secretion vs. period II Conclusions: Lactic acid supplementation of feed for piglets after weaning stimulates pancreatic secretion. However, bicarbonate secretion was almost unaffected by lactic acid supplementation, which makes it unlikely that the stimulation of pancreatic secretion observed was via a decrease in dietary and gastric pH
Willamil *et al*., 2011 Objective: Evaluate the effect of a microencapsulated feed additive of lactic and formic acid on the prevalence of *Salmonella* in pigs arriving at the abattoir	**Trial 1** Animals: Finishing pigs (Landrace x Large White) with 100 kg IBW Treatment: 0% (control) or Non-protected lactic acid (NPB; 0.4% lactic acid + 0.4% formic acid) or protected blend diet (PB;0.14% lactic acid + 0.14%)	Results: - NSD in pH, total short chain fatty acids (SCFA), lactic/formic acid concentrations in any section of the gastrointestinal tract or in caecal lactic acid bacteria or enterobacteria between NPB diets or PB diets vs. control Conclusions: More studies will be needed to confirm the usefulness of protected acids to prevent *Salmonella* prevalence and shedding at the abattoir.

Table A: Studies in Target Animals using Lactic Acid Supplementation		
Reference	**Study Design**	**Key Results**
	Duration: 10 days	
	Trial 2 Animals: Finishing pigs (Landrace x Large White) with 100 kg IBW Treatment: 0% (control) or Non-protected lactic acid (NPB; 0.4% lactic acid + 0.4% formic acid) or protected blend diet (PB; 0.14% lactic acid + 0.14%) Duration: 35 days	Results: – NSD in pH, between NPB diets or PB diets vs. control – ↑'d lactic acid, formic acid and acetic in acid in the caecum digesta of animals fed NPB diets or PB diets (P<0.05) vs. control – ↓'d propionic acid and Branched chain fatty acids (BCFA) of animals fed NPB diets or PB diets (P<0.05) vs. control – ↑'d Enterobacteria counts in the caecum of animals fed control diets(P<0.05) vs. NPB diets or PB diets – NSD in lactobacilli counts were observed between treatments – ↑'d in *Salmonella* fecal shedding was found related to the stress previous to slaughter with the control group and PB, but not in the NPB group Conclusions: The results have revealed the ability of microencapsulated acids to significantly modify caecal fermentation. However, non-protected acids seem to be more effective in decreasing Salmonella seroprevalence in the herd probably due to a reinforcement of the gastric barrier
Zheng *et al*., 2019 [Abstract only] Objective: Dietary supplementation with lactic acid and glutamine improve intestinal digestive and regulate intestinal	Animals: 24-day old piglets (Duroc × Landrace × Yorkshire) with 7.24 kg IBW Treatment: Basal diet with 0%, 2 % lactic acid (LS), 1% glutamine (GS) or 2% lactic acid + 1% glutamine (LGS) Duration: 28 days	Results: – ↑'d ADG in animal fed diets LGS (P<0.05) vs. control – ↑'d apparent total tract digestibility of CP in animals fed diets LS, GS and LGS (P<0.05) vs. control – ↑'d serum growth hormone win animals fed diets LS and GS (P<0.05) vs. control – ↓'d index of diarrhea in animals fed diets LS and LGS (P<0.05) vs. control – ↓'d serum IGF-1 in animals fed diets LS, GS and LGS (P<0.05) vs. control

Table A: Studies in Target Animals using Lactic Acid Supplementation		
Reference	**Study Design**	**Key Results**
microflora in weaning piglets		Conclusions: Dietary addition of lactic acid and glutamine combination could improve growth performance through the promotion of the small intestinal development, increasing digestive, and regulating balances of microflora in piglets
Ruminants		
Morgan & L'Estrange [Abstract only] Objective: Voluntary feed intake and metabolism of sheep when lactic acid is administered in the feed or intraruminally	**Experiment 1** Animals: Sheep (4 sheep in a 4 × 4 Latin Square experiment) Treatment: Grass meal pellet with 0, 600, 800 or 1000 mmol lactic acid/kg DM (added to the feed or infused intraruminally) Duration: 6 day periods	Results: – NSD in DM intake when added to feed directly – ↓'d DM intake when infused intraruminally particularly at 1000 mmol lactic acid/kg DM vs. control Conclusions: Lactic acid supplementation had little effect on the pH and total volatile fatty acid concentration of rumen
	Experiment 2 Animals: Sheep (4 sheep in a 4 × 4 Latin Square experiment) Treatment: Grass meal pallet with 0, 900, 1200 or 1500 mmol/kg DM (added to the feed or infused intraruminally) Duration: 15 day periods	Results: – ↓'d DM intake in lactic acid treatment but not significantly vs. control Conclusions: Lactic acid supplementation had little effect on the pH and total volatile fatty acid concentration of rumen
	Experiment 3 Animals: Sheep Treatment: Infusions of lactic acid at a fixed daily rate of 40	Results: – ↓'d DM intake in lactic acid treatment but the effect was less consistent than in Experiment 1 Conclusions: Lactic acid supplementation had little effect on the pH and total volatile fatty acid concentration of rumen

Table A: Studies in Target Animals using Lactic Acid Supplementation		
Reference	**Study Design**	**Key Results**
	mmol per kg $W^{-0.75}$ per day or of an equal volume of distilled water while being offered grass meal pellets *ad libitum* Duration: 3 days	
Stehling, 1971 [Abstract only] Objective: Evaluated the effect of large doses of potassium and lactic acid on metabolism of Mg, Ca, P, K and Na and on apparent digestibility of nutrients in sheep	Animals: 1-year old male German Blackheaded Mutton sheep with 51.3 kg IBW Treatment: Basal meal pallet with 0%, 102 g 25.5% $KHCO_3$ or 250 ml 20% lactic acid solution Or, 102 g 25.5% $KHCO_3$ or 250 ml 20% lactic acid solution were introduced into the rumen directly Duration: 5-10 days	Results: – ↓'d excretion of P in animals fed diets supplemented with lactic acid vs. control – ↑'d retention of K in animals fed diets supplemented with lactic acid vs. control – ↓'d decreased apparent digestibility of Na in animals fed diets supplemented with lactic acid vs. control – NSD in apparent digestibility of Mg or Ca in animals fed diets supplemented with lactic acid vs. control Conclusions: The lactic acid reduced P in faeces, increased retention of K, decreased apparent digestibility of Na slightly and raised blood pCO_2 a little, but did not affect the metabolism of Mg or Ca
Aquaculture		
Xiao *et al*., 2009 [Abstract only] Objective: Evaluate the effects of lactic acid levels in feed on growth performance and digestibility of nutrients of fish	Animals: *Carassius auratus* with 8.15 g IBW (goldfish) Treatment: Basal diet with 0, 0.1, 0.15, 0.2, 0.25 or 0.3% lactic acid Duration: 50 days	Results: – ↑'d SGR and PER and improved FCR in animals fed diets containing 0.2% vs. control Conclusions: Based on broken line regression which was used to analyze the relationship among lactic acid levels and the specific growth ratio, the protein efficiency and the feed conversion efficiency, the optimal lactic acid levels in feed was 0.2 %
Cats		
Scherl *et al*., 2019 Objective:	**3-Month Feeding Study** Animals: 4-6 years old neutered male and spayed female cats	Conclusions: For both the 3-month and 1-year studies, all cats completed all assessments without any apparent adverse effects. Cats readily ate the

Table A: Studies in Target Animals using Lactic Acid Supplementation		
Reference	**Study Design**	**Key Results**
Two randomized trials demonstrate lactic acid supplementation in pet food inhibits dental plaque, calculus, and tooth stain in cats	Treatment: Pet food with 0 or 1.2% lactic acid Duration: 3 months	foods, maintained weight, and were overall healthy throughout the studies
	1-Year Feeding Study Animals: 4-6 years old neutered male and spayed female cats Treatment: Pet food with 0 or 1.2% lactic acid Duration: 1 year	

Abbreviation: ↑'d = increased; ↓'d = decreased; ADG = average daily gains; BCFA = branched cain fatty acid; BWG = bodyweight gain; DM = dry matter; *E. Coli* = *Escherichia Coli*; FCR = feed conversion ratio; FM = fish meal; FMF = fermented moist feed; IBW = initial body weight; IGF-1 = insulin like growth factor 1; I.M. = intramuscular injection; NDF neutral detergent fiber; NSD = No significant difference; PER = Protein Efficiency ratio; OM = organic matter; SBM = soybean meal; SFCA = short chain fatty acids; SGR = Specific growth rate; *S. Typhimurim* = *Salmonella Typhimurim* VFA = volatile fatty acid.

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The following information was provided as feedback on the remaining questions from the CVM after evaluation of Animal GRAS Notice No. 62, Sodium Salts of Lauric and Myristic Acids (C12/C14 Lactylates).

Used Equipment

(b) (4)

4.3.1 – Update: Equipment and materials

Use the following or similar equipment:

(b) (4)

4.3.2 – Update: Settings of the GC equipment

Settings of the (b) (4)

(b) (4)

4.4.2 – Update: Typical retention times and chromatograms

Feedback CVM: The typical chromatogram provided (Section 4.4.2 on page 3 of Appendix 7C) is not legible. The notifier should provide a legible chromatogram in which every peak along with retention time is clearly identified.

(b) (4)

(b) (4)

Table 1: Typical retention times of the components, including 8 different lactylate standards.

(b) (4)

4.4.3. – Update: Preparation of the calibration standards

Feedback CVM: In Table 2 of Appendix 7C, the notifier provided a list of compounds used for calibration. For both the method procedure and the validation, the notifier should provide the procedure to prepare the calibration standards for each of these compounds at all calibration concentrations (including the concentrations in between lowest and highest).

Preparation of the stock solutions

(b) (4)

(b) (4)

Preparation of the standards

(b) (4)

Derivatization of the standards:

(b) (4)

Validation parameters

Feedback CVM: The provided summary tables of validation parameters (Tables 5, 6, and 7 of Appendix 7C) are not adequate to demonstrate method validation. In general, the method validation package should at least address the method precision, accuracy, specificity, linearity, range, limit of detection (LOD), limit of quantitation (LOQ), robustness, and system suitability. To address each method validation characteristic, the notifier should provide procedural information to describe how the validation characteristic is addressed and submit corresponding data and representative legible chromatogram(s). The notifier should provide applicable equations and example calculations used for the determination of the level of each of the analytes. The notifier may refer to Guidance for Industry (GFI) #64 – Validation of Analytical Procedures: Methodology, for the types of information that should be included for a comprehensive method validation package.

1. Specificity

The specificity is covered by the GC-chromatogram as presented in Figure 1.

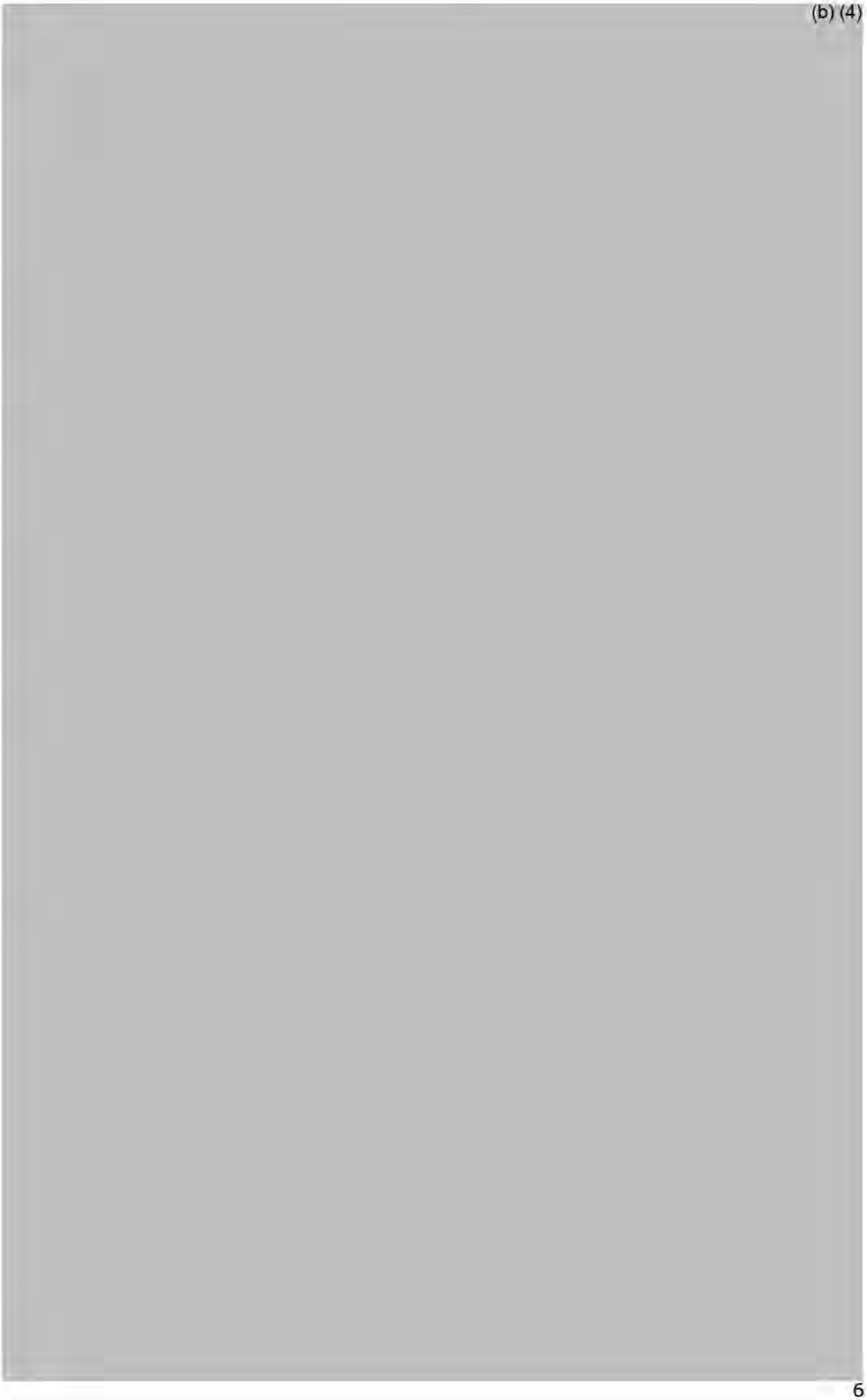
2. Linearity

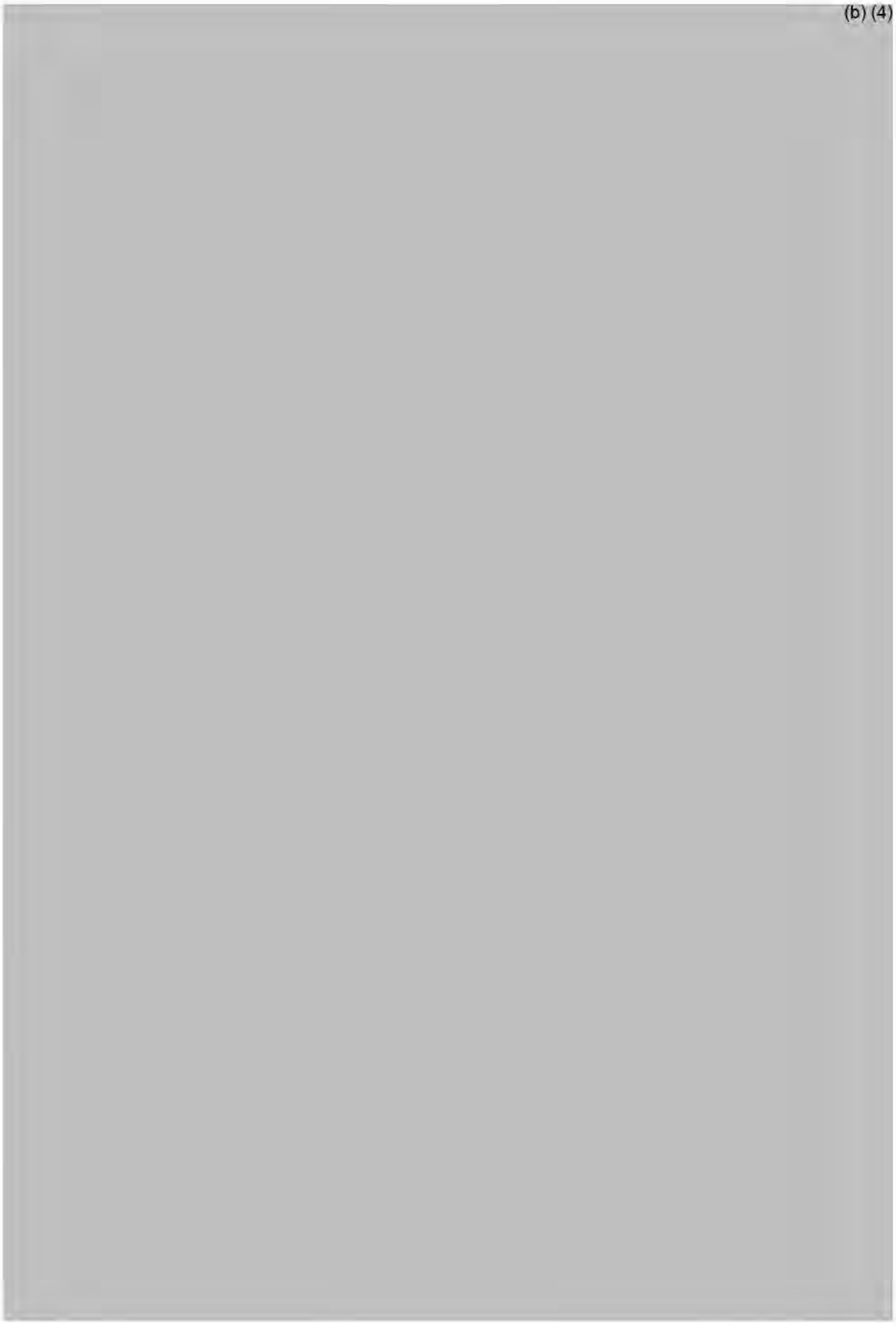
Typical calibration plots are presented in Figures 2 – 9.

(b) (4)

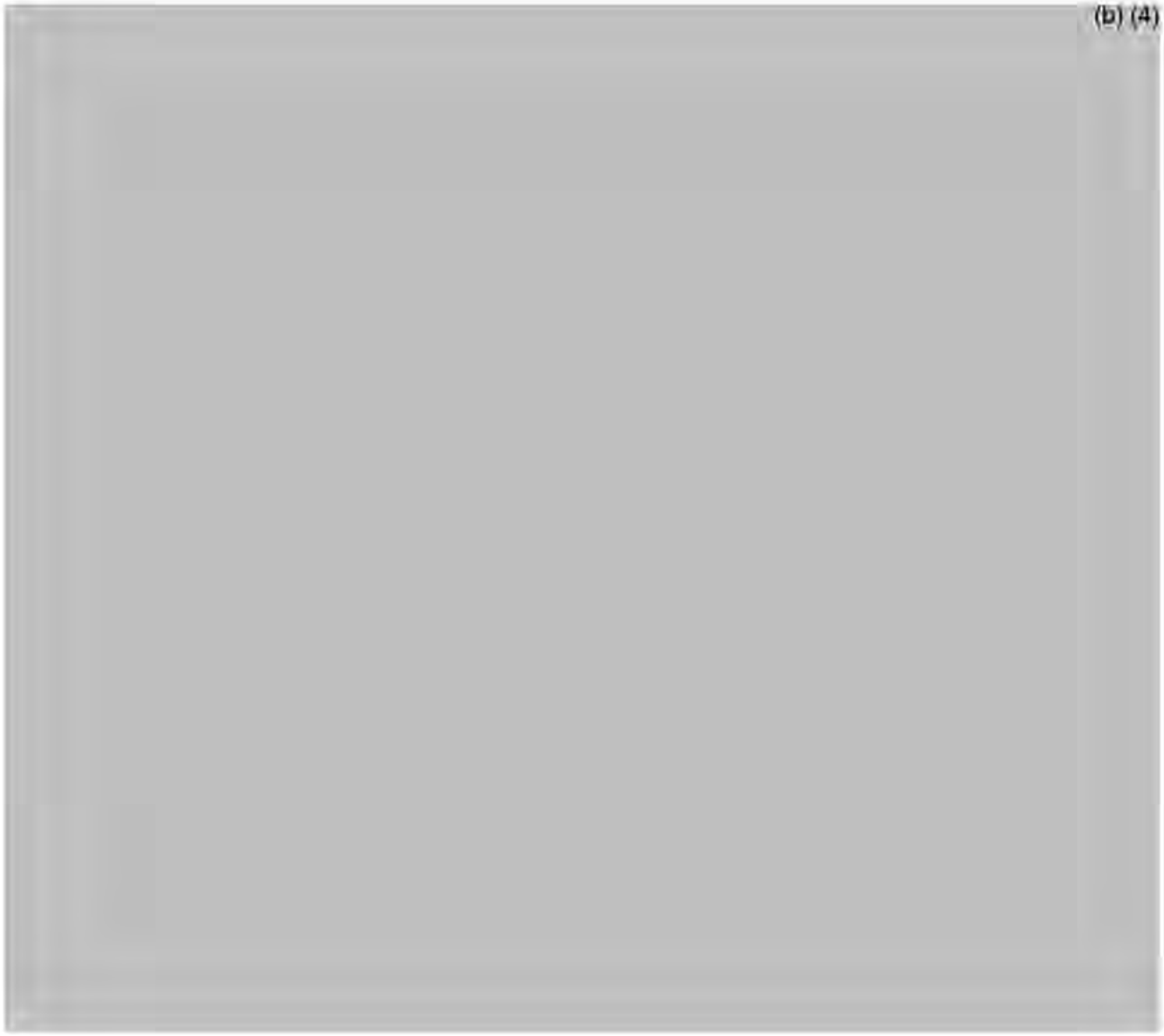
(b) (4)

(b) (4)




(b) (4)

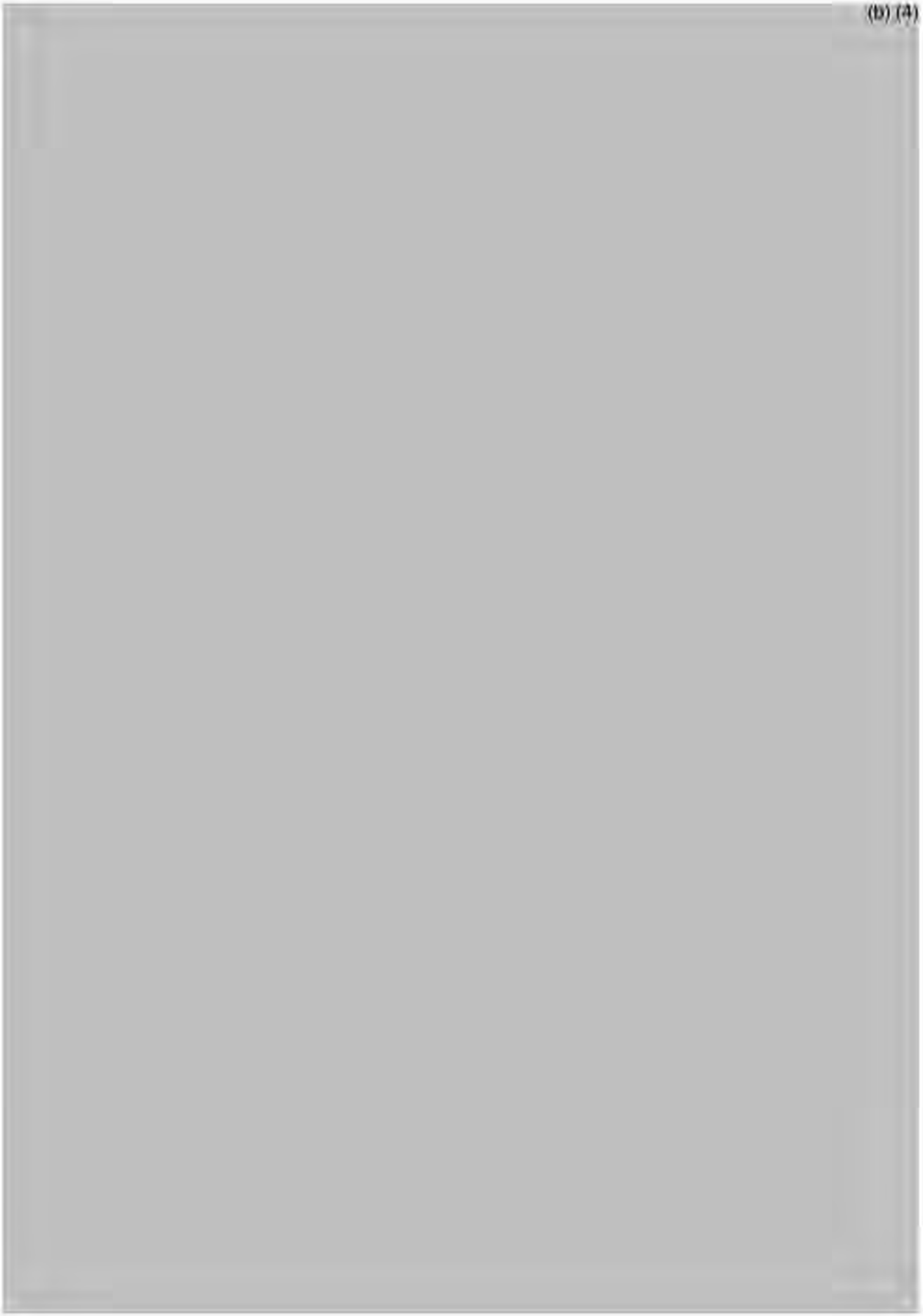
(b) (4)



(b) (4)


(b) (4)


(b) (4)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Analyserapport

(b) (4)

Purac Biochem BV (QC)
t.a.v. (b) (4)
Postbus 21
4200 AA GORINCHEM

Monsternummer	:	M22000310002
Klantnummer	:	D05421
Monsterontvangstdatum	:	05-01-2022
Digitale order ID	:	(b) (4)

Uw monsterkenmerken

Reden onderzoek	:	2668
Productnaam	:	2102002765/T=0
Omschrijving	:	Aloapur PM
Monsternamedatum	:	3-1-2022
Kostencode	:	4700013971

Analyseparameter	Analyseuitslag	Eenheid	Methode	Accr./cert.
Fluoride	(b) (4)	mg/kg	10012	Q G-B10
Arseen		mg/kg	10222	Q G-B11 QS
Cadmium		mg/kg	10222	Q G-B11 QS
Kwik		mg/kg	10222	Q G-B10 QS
Lood		mg/kg	10222	Q G-B11 QS
Aflatoxine B1 (LC-MS/MS)		mg/kg	10370	
Dioxine & Dioxine Like PCB's			10463	
WHO-TEQ (PCDD/F + DL-PCBs) incl. LOQ		ng TEQ/kg		Q G-B11 QS
Dioxine TEQ (WHO 2005) incl. LOQ		ng TEQ/kg		Q G-B11 QS
2,3,7,8-TCDD		ng/kg		Q G-B11 QS
1,2,3,7,8-PeCDD		ng/kg		Q G-B11 QS
1,2,3,4,7,8-HxCDD		ng/kg		Q G-B11 QS
1,2,3,6,7,8-HxCDD		ng/kg		Q G-B11 QS
1,2,3,7,8,9-HxCDD		ng/kg		Q G-B11 QS
1,2,3,4,6,7,8-HpCDD		ng/kg		Q G-B11 QS
OCDD		ng/kg		Q G-B11 QS
2,3,7,8-TCDF		ng/kg		Q G-B11 QS
1,2,3,7,8-PeCDF		ng/kg		Q G-B11 QS
2,3,4,7,8-PeCDF		ng/kg		Q G-B11 QS
1,2,3,4,7,8-HxCDF		ng/kg		Q G-B11 QS
1,2,3,6,7,8-HxCDF		ng/kg		Q G-B11 QS
2,3,4,6,7,8-HxCDF		ng/kg		Q G-B11 QS
1,2,3,7,8,9-HxCDF		ng/kg		Q G-B11 QS
1,2,3,4,6,7,8-HpCDF		ng/kg		Q G-B11 QS
1,2,3,4,7,8,9-HpCDF		ng/kg		Q G-B11 QS
OCDF		ng/kg		Q G-B11 QS
DL-PCB TEQ (WHO 2005) incl. LOQ		ng TEQ/kg		Q G-B11 QS
PCB-77		ng/kg		Q G-B11 QS
PCB-81		ng/kg		Q G-B11 QS
PCB-126		ng/kg		Q G-B11 QS

(b) (4)

Analyseparameter	Analyseuitslag	Eenheid	Methode	Accr./cert.
PCB-169	(b) (4)	ng/kg		Q G-B11 QS
PCB-105		ng/kg		Q G-B11 QS
PCB-114		ng/kg		Q G-B11 QS
PCB-118		ng/kg		Q G-B11 QS
PCB-123		ng/kg		Q G-B11 QS
PCB-156		ng/kg		Q G-B11 QS
PCB-157		ng/kg		Q G-B11 QS
PCB-167		ng/kg		Q G-B11 QS
PCB-189		ng/kg		Q G-B11 QS
NDL-PCB's (polychloorbifenylen)			10463	
Polychloorbifenyl-28		µg/kg		Q G-B11 QS
Polychloorbifenyl-52		µg/kg		Q G-B11 QS
Polychloorbifenyl-101		µg/kg		Q G-B11 QS
Polychloorbifenyl-138		µg/kg		Q G-B11 QS
Polychloorbifenyl-153		µg/kg		Q G-B11 QS
Polychloorbifenyl-180		µg/kg		Q G-B11 QS
NDL-PCB Totaal (upper bound)		µg/kg		Q G-B11 QS

Methode omschrijvingen

Methode/analyse	(b) (4)
10012	
10222	
10463	

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

(b) (4)

Analyserapport

(b) (4)

Purac Biochem BV (QC)
t.a.v. (b) (6)
Postbus 21
4200 AA GORINCHEM

Monsternummer	:	(b) (4)
Klantnummer	:	D05421
Monsterontvangstdatum	:	05-01-2022
Digitale order ID	:	(b) (4)

Uw monsterkenmerken

Reden onderzoek	:	2668
Productnaam	:	7500068230/T=0
Omschrijving	:	Aloapur PM
Monsternamedatum	:	3-1-2022
Kostencode	:	4700013971

Analyseparameter	Analyseuitslag	Eenheid	Methode	Accr./cert.
Fluoride	(b) (4)	mg/kg	10012	Q G-B10
Arseen		mg/kg	10222	Q G-B11 QS
Cadmium		mg/kg	10222	Q G-B11 QS
Kwik		mg/kg	10222	Q G-B10 QS
Lood		mg/kg	10222	Q G-B11 QS
Aflatoxine B1 (LC-MS/MS)		mg/kg	10370	
Dioxine & Dioxine Like PCB's			10463	
WHO-TEQ (PCDD/F + DL-PCBs) incl. LOQ		ng TEQ/kg		Q G-B11 QS
Dioxine TEQ (WHO 2005) incl. LOQ		ng TEQ/kg		Q G-B11 QS
2,3,7,8-TCDD		ng/kg		Q G-B11 QS
1,2,3,7,8-PeCDD		ng/kg		Q G-B11 QS
1,2,3,4,7,8-HxCDD		ng/kg		Q G-B11 QS
1,2,3,6,7,8-HxCDD		ng/kg		Q G-B11 QS
1,2,3,7,8,9-HxCDD		ng/kg		Q G-B11 QS
1,2,3,4,6,7,8-HpCDD		ng/kg		Q G-B11 QS
OCDD		ng/kg		Q G-B11 QS
2,3,7,8-TCDF		ng/kg		Q G-B11 QS
1,2,3,7,8-PeCDF		ng/kg		Q G-B11 QS
2,3,4,7,8-PeCDF		ng/kg		Q G-B11 QS
1,2,3,4,7,8-HxCDF		ng/kg		Q G-B11 QS
1,2,3,6,7,8-HxCDF		ng/kg		Q G-B11 QS
2,3,4,6,7,8-HxCDF		ng/kg		Q G-B11 QS
1,2,3,7,8,9-HxCDF		ng/kg		Q G-B11 QS
1,2,3,4,6,7,8-HpCDF		ng/kg		Q G-B11 QS
1,2,3,4,7,8,9-HpCDF		ng/kg		Q G-B11 QS
OCDF		ng/kg		Q G-B11 QS
DL-PCB TEQ (WHO 2005) incl. LOQ		ng TEQ/kg		Q G-B11 QS
PCB-77		ng/kg		Q G-B11 QS
PCB-81		ng/kg		Q G-B11 QS
PCB-126		ng/kg		Q G-B11 QS

(b) (4)

Analyseparameter	Analyseuitslag	Eenheid	Methode	Accr./cert.
PCB-169	(b) (4)	ng/kg		Q G-B11 QS
PCB-105		ng/kg		Q G-B11 QS
PCB-114		ng/kg		Q G-B11 QS
PCB-118		ng/kg		Q G-B11 QS
PCB-123		ng/kg		Q G-B11 QS
PCB-156		ng/kg		Q G-B11 QS
PCB-157		ng/kg		Q G-B11 QS
PCB-167		ng/kg		Q G-B11 QS
PCB-189		ng/kg		Q G-B11 QS
NDL-PCB's (polychloorbifenylen)			10463	
Polychloorbifenyl-28		µg/kg		Q G-B11 QS
Polychloorbifenyl-52		µg/kg		Q G-B11 QS
Polychloorbifenyl-101		µg/kg		Q G-B11 QS
Polychloorbifenyl-138		µg/kg		Q G-B11 QS
Polychloorbifenyl-153		µg/kg		Q G-B11 QS
Polychloorbifenyl-180		µg/kg		Q G-B11 QS
NDL-PCB Totaal (upper bound)		µg/kg		Q G-B11 QS

Methode omschrijvingen

Methode/analyse
10012
10222
10463

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

PART 6. §570.250. NARRATIVE

6.1 INFORMATION TO ESTABLISH SAFETY FOR THE TARGET ANIMALS

6.1.1 Introduction

C12/C14 lactylates is the product of esterification of lauric (C12) and myristic (C14) acids with lactic acid in the presence of sodium hydroxide. The fatty acid raw materials are obtained from food grade PKO and food-grade lactic acid. The resultant product comprises an equilibrium mixture of sodium lauroyl-1-lactylate (*ca*. 33%), sodium myristoyl-1-lactylate (*ca*. 14%), sodium laurate (C12 fatty acid; *ca*. 18%), sodium myristate (*ca*. 8%), sodium lactate (*ca*. 9%), sodium lauroyl-2-lactylate (*ca*. 6%), sodium myristoyl-2-lactylate (*ca*. 2%), sodium lactoyl lactate (*ca*. 3%) and minor amounts of oligomers of lactate (not quantified). Based on this typical composition, C12/C14 lactylates comprises around 42% lauric acid, 19% myristic acid and 28% lactic acid in the free fatty acid and esterified form. The combined content of lauric and myristic acids is in the region of 61%.

Calcium and sodium stearoyl-lactylates are authorized food additives with an established metabolic fate in animals. Published and pivotal *in vivo* and *in vitro* data indicate that calcium and sodium stearoyl-lactylates will be hydrolyzed *in vivo* by gastric lipases to the individual components, i.e., stearic acid, lactic acids and the respective cations (Philips *et al*., 1981). Gastric lipases are common to all animals and are expected to hydrolyze the structurally similar C12/C14 lactylates, differing from stearoyl-lactylates in comprising lauric (C12) and myristic (C14) acids rather than stearic (C18) acid. Moreover, the sodium and calcium salts will dissociate on ingestion by animals into the lactylates and their respective sodium and calcium ions. This effect is common to salts of carboxylic acids including organic acids and fatty acids (Section 6.1.2.1). Thus, data on calcium and sodium stearoyl-lactylates may reasonably be considered pivotal in the assessment of the metabolic fate of C12/C14 lactylates. Evidence for the appropriateness of the extrapolation and dissociation of C12/C14 lactylates into its component parts *in vivo*, is provided by the findings of an unpublished study conducted by Corbion at the University of Wageningen (Jansman and van Wikselaar, 2013) (Section 6.1.2.2). Therefore, the toxicological assessment of C12/C14 lactylates may be based on the individual components.

Medium- and long-chain saturated fatty acids such as lauric acid and myristic acid are normal components of vegetable and animal fats. They also have a history of use as additives in human food and as such have been the subject of evaluations by authoritative bodies as described in Section 6.1.3.1. Lauric and myristic acid from C12/C14 lactylates are expected to be metabolized by established fatty acid pathways in animals as outlined in Section 6.1.3.2. The long and established history of consumption as a component of the diet naturally and as a component of food, as well as their generally recognized metabolism to innocuous compounds is pivotal to the safety assessment of these components. The levels of lauric and myristic acid in common fats used in the diet of animals, and the potential exposure by animals to these fatty acids from the daily ration are considered in Sections 6.1.3.3 to 6.1.3.5. There are a number of studies in the published literature in which animals were fed diets containing lauric or myristic acids as the free fatty acids. These are briefly considered in Section 6.1.3.6. Additionally, a limited toxicological data set is available which is summarized in Section 6.1.3.7. The toxicological data is not pivotal to the safety assessment and provides only corroborating information for completeness.

Lactic acid and calcium lactate have a long and established history of use as a technological additive (preservative) in feed and food in the U.S., the EU and elsewhere. A summary of the scientific evaluations by authoritative bodies to support the use as additives in feed and food is provided in Section 6.1.4.1. Lactic acid is rapidly absorbed by animals and ultimately metabolized to carbon dioxide and water as outlined in Section 6.1.4.2 using publicly available information. Animals will be exposed to lactic acid from the normal diet, particularly through the use of Fermented Liquid feeds (FLF) for pigs and ensiled forages for ruminants. Background exposure by animals to lactic acid from the diet is estimated in Section 6.1.4.4. On the basis that exposure by animals to lactic acid from the background diet is lower for poultry, swine and ruminants, a summary of the available published literature in which animals were fed diets containing lactic acid are summarized in Section 6.1.4.5. Together, the published data, history of use and generally recognized metabolic fate provide pivotal evidence of the safety of this component for the target animals. Additionally, a limited set of toxicological data are available and outlined in Section 6.1.4.7. The toxicological data is not pivotal to the safety assessment and provides only corroborating information for completeness.

It is recognized that C12/C14 lactylates will also provide a source of sodium. The sodium content of C12/C14 lactylates is limited to not more than 8% by the product specifications for the ingredient (see Table 2.7). The impact of the sodium content of C12/C14 lactylates on the nutritional status of animals is considered in Section 6.1.5.

As mentioned in Part 2, sodium sulfate is added to the market formulation, ALOAPUR® PM as a processing aid at a level of less than 0.1% by weight. The safety of the sulfate component of C12/C14 lactylates for animals under the conditions of intended use is evaluated in Section 6.1.6.

The data summarized in Part 6 may be extrapolated to support the safety of all categories and species of animal on the basis that (a) the metabolic fate of C12/C14 lactylates is common in all animals; (b) lauric acid, myristic acid and lactic acid are metabolized by well-established pathways; and (c) there is a long and established history of safe consumption of these components from the background diet as well as from additive use (lactic acid).

6.1.2 Absorption, Distribution, Metabolism and Excretion (ADME) of Lactylates

Data are available in the published literature supporting the ADME of calcium and sodium stearoyl-lactylates. On ingestion by animals, both sodium and calcium lactylates will dissociate into their respective cations (sodium and calcium anions) and anions (lactylates). This dissociation is common to salts of fatty acids and there are numerous scientific opinions published by the European Food Safety Authority (EFSA) and Joint Food and Agricultural Organization (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) (e.g., JECFA 1974a and b; EFSA, 2013 and 2019) in which carboxylic acids and their sodium and calcium salts, including sodium and calcium lactylates, are evaluated as a group. This generally recognized approach for extrapolating data on calcium salts to support the safety of sodium salts of lactylates is applied herein.

Calcium and sodium stearoyl-lactylates are authorized food additives in the U.S. and EU in which safety is established by a body of published data. These pivotal data, along with a body of unpublished supporting information, have been summarized in scientific evaluations conducted by both JECFA

(1974a) and EFSA (2013). Calcium and sodium stearoyl-lactylates are the products of esterification of stearic and lactic acid and conversion to the respective salts. By comparison, C12/C14 lactylates is the product of esterification of lactic acid and myristic acid with lactic acid and formation of the sodium salts. Structurally, therefore, the lactylates are similar differing only in the identity of the fatty acids, i.e., steric acid (C18) vs. lauric (C14) and myristic (C16) acids. The identity of the fatty acid component is not expected to influence the overall metabolic fate or toxicological profile of the lactylates and as further justified below, data on calcium and sodium stearoyl-lactylates in the published domain may be considered pivotal data in the safety assessment of C12/C14 lactylates (see Section 6.1.2.1). Corroborating evidence that the metabolic fate of C12/C14 lactylates in the sodium salt form is comparable to that of calcium stearoyl-lactylate is provided by an unpublished study conducted by Corbion (see Section 6.1.2.2). This unpublished study is intended only to support the pivotal conclusion from the available data in calcium and sodium stearoyl-lactylates, that C12/C14 lactylates will by hydrolyzed *in vivo* into the component parts, i.e., fatty acids, lactic acid and sodium.

6.1.2.1 Studies using Sodium and Calcium Stearoyl-Lactylates

The ADME of calcium stearoyl-lactylate (CSL) was compared with that of sodium DL-lactate in a radiolabeled study conducted in mice and Guinea pigs by Phillips *et al*. (1981). Animals were placed within specialized metabolic cages for the evaluation of expired gases. A single oral dose of ^{14}C-CSL (aqueous solution) was administered by gavage at a concentration of either 90 or 900 mg/kg body weight to groups of 4 male Tuck TO mice or groups of 4 male Dunkin-Hartley guinea pigs. An additional group of 3 mice or 3 guinea pigs received DL-^{14}C-sodium lactate (^{14}C-lactate) by gavage at a concentration of 325 mg/kg body weight, providing the equivalent lactate concentration as 900 mg CSL/kg body weight. Radioactivity was determined in exhaled air, urine, feces, liver, kidneys, heart, lungs, spleen, testes and the GI tract for up to 48 hours after administration of ^{14}C-CSL.

The excretion of radioactivity from the mice and guinea pigs during the 48 hours following administration of the single oral dose of ^{14}C-CSL or ^{14}C-lactate is summarized in Table 6.1. Following administration of ^{14}C-CSL at 90 mg/kg body weight to mice and guinea pigs, more than 97% of the radioactivity was eliminated within 48 hours with the majority (*ca*. 77%) excreted as ^{14}C-carbon dioxide within 24 hours. The remaining radioactivity was mostly excreted in the urine within 24 hours (14.8% of the administered dose), with only low levels measured in the feces (2.7% of the administered dose) after 48 hours and urine (0.7% of the administered dose) in the 24-to-48-hour period. At the higher dose of administration of ^{14}C-CSL of 900 mg/kg body weight, the level of radioactivity at 7 hours excreted as ^{14}C-carbon dioxide was less than in animals administered the lower dose of 90 mg/kg body weight (57.5 vs. 69.7% of the administered dose) but the total excreted after 48 hours was comparable (82.6 vs. 80.2% of the administered dose). The percentage of radioactivity excreted in the urine and feces within 48 hours was also comparable (16.2 vs. 15.5% of the administered dose in urine and 2.1 vs. 2.7% in feces). The majority of the radioactivity following administration of 325 mg ^{14}C-lactate acid/kg body weight (equivalent to the high dose of ^{14}C-CSL) to mice was also excreted as ^{14}C-carbon dioxide within 24 hours (92.2% of the administered dose). Less of the radioactivity was excreted in the urine and feces (4.0 and 1.1% of the administered dose, respectively after 48 hours).

The excretion of radioactivity by guinea pigs was similar to that observed in mice. The rate and extent of conversion of ^{14}C-CSL to ^{14}C-carbon dioxide was comparable in guinea pigs and mice administered 90 or 900 mg/kg body weight (78.8 and 81.9% of the administered dose, respectively after 48 hours). However, the percentage of the dose excreted in the urine (10.0 and 9.1% of the administered dose, respectively for the 2 dose groups), and by all three routes was less over the 48-hour period.

In both mice and guinea pigs, the total residual activity and distribution of radioactivity in tissues was similar after administration of 90 or 900 mg/kg body weight of ^{14}C-CSL, or 325 mg/kg body weight of ^{14}C-lactate. The total radioactivity sampled in the tissues of guinea pigs (6.1, 6.7 and 10.2% of the administered doses, respectively for each dose group) was higher than in mice (1.8, 2.1 and 2.1% of the administered dose, respectively for each dose group) at 48 hours. In both mice and guinea pigs, the radioactivity in tissues was mainly in the liver and GI tract. Lactic acid was identified by thin layer chromatography (TLC) to be the metabolite of ^{14}C-CSL and ^{14}C-lactate in the urine of both mice and guinea pigs.

Table 6.1: Excretion of Radioactivity by Male Mice and Guinea-Pigs Given a Single Oral Dose of ^{14}C-labelled Calcium Stearoyl-Lactylate or DL-[U-^{14}C]Lactate (Phillips *et al.*, 1981)

Route of Excretion	Time from Dosing (Hours)	Radioactivity Excreted (% of Dose) after Single Oral Administration		
		^{14}C-CSL		^{14}C-Lactate
		90 mg/kg bw n=4	900 mg/kg bw n=4	325 mg/kg bw n=3
Mice				
Exhaled CO_2	0-24	76.8	79.8	89.3
	0-48	80.2	82.6	92.2
Urine	0-24	14.8	14.1	3.4
	0-48	15.5	16.2	4.0
Feces	0-24	2.4	1.8	0.9
	0-48	2.7	2.1	1.1
Organs[1]	At 48	1.8	2.1	2.1
Total recovery		100.2	103.0	99.4
Guinea Pigs				
Exhaled CO_2	0-24	75.3	78.6	81.6
	0-48	78.8	81.9	84.1
Urine	0-24	9.2	8.1	3.3
	0-48	10.0	9.1	3.7
Feces	0-24	3.0	2.3	1.6
	0-48	3.8	2.9	2.1
Organs[1]	At 48	6.1	6.7	10.2
Total recovery		98.7	100.6	100.1

Abbreviations: bw = body weight; CSL = calcium stearoyl-lactylate;
[1]Sum of organs evaluated (liver, kidneys, heart, lungs, spleen, testes and GI tract).

A series of *in vitro* studies were also conducted by Phillips *et al.* (1981) to investigate the hydrolysis of ^{14}C-CSL from liver and GI homogenates of the rat, mouse and guinea pig as well as GI homogenate from human duodenum. The appearance of lactate and disappearance of stearoyl-lactate during hydrolysis

of ^{14}C-CSL were monitored. The liver homogenates from all 3 species readily hydrolyzed ^{14}C-CSL with the highest rate exhibited by guinea pigs (24.7 µmol/g liver/hour) and the least in the mouse (7.5 µmol/g liver/hour). In liver homogenates 55% (rat liver), 45% (guinea pig liver), or 35% (mouse liver) of the initial radioactivity occurred in the form of ^{14}C-lactate within 60 minutes after initiation of incubation. The hydrolysis of ^{14}C-CSL by homogenates of the GI mucosa also occurred rapidly. The initial rate of hydrolysis by the rat and the guinea pig were significantly faster than that of mouse mucosa, with 40 % (guinea pig) and 35% (rat) of the initial radioactivity present in the form of lactate after 1 hour compared to 30% in the mouse. The rate of hydrolysis by human intestinal mucosa was slower in comparison to intestinal mucosa samples from experimental animals with 18% (human) of the initial activity appearing as lactate after an hour. Whole blood from rats and mice hydrolyzed the compound, but at a much slower rate than in the liver or intestinal mucosa homogenates. No significant hydrolysis of ^{14}C-CSL was detected using human blood.

The findings of these studies are corroborated by unpublished studies cited by JECFA (1974a). Experiments were conducted comparing the metabolism of mixed stearic (C18) acid and ^{14}C-lactic acid with calcium stearoyl-lactylate (^{14}C-CSL; lactic acid labeled) (Hodge, 1955 – unpublished study; no further details available). 58% of the administered dose of radioactivity was excreted from the mixture, and 60% from ^{14}C-CSL within 24 hours. No differences were reported in the distribution or excretion of the radioactivity between the two groups.

In another unpublished study cited by JECFA (1974a) in which rats were fed calcium stearoyl-lactylate, only traces of lactate were detected in the fecal fat, with good utilization of stearic acid and calcium by the animal (Hodge, 1961 – unpublished study; no further details available).

The results of supporting unpublished *in vitro* studies conducted using calcium or sodium stearoyl-lactylate were also summarized by JECFA (Hodge, 1961 – unpublished study; JECFA, 1974a). The hydrolysis of calcium stearoyl-lactylate by lipases was reported to occur readily to form stearic and lactic acid (no further details specified).

The available published *in vivo* and *in vitro* pivotal evidence, supported by unpublished studies, indicate that calcium stearoyl-lactylate is rapidly absorbed and utilized by animals. On the basis that lipases are present in all animals and are demonstrated to hydrolyze the ester linkage, it is assumed that the absorption and metabolism of the lauric acid, myristic acid and lactic acid components of calcium stearoyl-lactylates will follow established pathways for these free fatty acids and organic acid in target species (see Sections 6.1.3 and 6.1.4, respectively). Considering the structural similarities of stearoyl (C18)-lactylate and C12/C14 lactylates outlined above, it is reasonable to conclude that similar behavior will be displayed by C12/C14 lactylates under the intended conditions of use as a nutritional ingredient in feed at levels of up to 5 g/kg complete feed. Although the rate of hydrolysis of C12/C14 lactylates may differ between species, these differences are not anticipated to impact the metabolic fate in animals. Thus, as described in Section 6.1, it is reasonable to assess the safety of C12/C14 lactylates in terms of the individual components, lauric acid, myristic acid and lactic acid.

An unpublished study has been conducted by Corbion to evaluate the digestion and kinetics of disappearance of C12/C14 lactylates in broilers. These data support the extrapolation of metabolic data on calcium and sodium stearoyl-lactylates to C12/C14 lactylates based on structural similarities. The available unpublished data are outlined below and together provide evidence for the appropriateness of basing the safety assessment on the individual components of C12/C14 lactylates.

6.1.2.2 Study in Broilers using C12/C14 Lactylates

An unpublished study was conducted by (b) (4) in which the digestion and kinetics of disappearance of sodium lauroyl (C12)-lactylate, sodium myristoyl (C14)-lactylate and palmitoyl (C16)-lactylate was investigated following administration in the diet of broiler chickens. The C12/C14 lactylates test articles used in the study are liquid (Puramix 30L) and solid (Puramix 30S) formulations prepared by combining 38 to 42% C12/C14 lactylates with either monopropylene glycol or (b) (4) (b) (4) (natural clay/sediment from diatoms) as the diluent/carrier. The treatment levels in the study were 7.5 g/kg complete feed of Puramix 30L or 30S. A summary of the study is provided in Appendix 018.

Two hundred and twenty, 1-day old healthy male Ross 308 broilers were randomly assigned to one of 20 cages containing 11 birds per cage. There were 4 cages per treatment. The birds were fed diets containing no lactylates (control; treatment 1), 7.5 g C12/C14 lactylates in liquid form (Puramix 30L[1]; treatment 2), 7.5 g C12/C14 lactylates in solid form (Puramix 30S[2]; treatment 3), 7.5 g C14 lactylate in solid form (Puramix 14S; treatment 4) or 7.5 g C16 lactylate in solid form (Puramix 16S; treatment 5) per kg complete feed for 27 days. On the basis that treatments 4 and 5 were not part of the digestion and kinetics assessment in the study, no further information on these groups is provided herein. Birds received a starter diet from day 0 to day 7, and a grower diet from day 7 to day 27. Chromium oxide was included in the feed as a digestibility marker. Feed and water were available *ad libitum* for the duration of the study. The calculated and analyzed concentrations of the C12, C14 and C16 components in the treatment diets are provided in Table 6.2.

Table 6.2: Calculated and Analyzed Concentrations of Lactylates in the Treatment Diets

Component	Analyzed Concentrations[1]				
	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
	Control (0 g/kg Complete Feed)	7.5 g Puramix 30L/kg Complete Feed (C12/C14 Lactylates)	7.5 g Puramix 30S/kg Complete Feed (C12/C14 Lactylates)	7.5 g Puramix 14S/kg Complete Feed (C14 Lactylates)	7.5 g Puramix 16S/kg Complete Feed (C16 Lactylates)
C12 lactylate (mg/kg complete feed)	<0.5	584	792	24	2
	(0)	(900)	(900)	(0)	(0)
C14 lactylate	<0.5	276	321	992	49
	(0)	(375)	(375)	(1,275)	(0)

[1] Puramix 30L: (38 to 42%; mean 40%) C12/C14 lactylates diluted using monopropylene glycol as a liquid (58 to 62%)

[2] Puramix 30S: (38 to 42%; mean 40%) C12/C14 lactylates on a solid carrier (58 to 62%) (natural clay/sediment from diatoms) – (b) (4)

Table 6.2: Calculated and Analyzed Concentrations of Lactylates in the Treatment Diets					
Component	**Analyzed Concentrations[1]**				
	Treatment 1	**Treatment 2**	**Treatment 3**	**Treatment 4**	**Treatment 5**
	Control (0 g/kg Complete Feed)	**7.5 g Puramix 30L/kg Complete Feed (C12/C14 Lactylates)**	**7.5 g Puramix 30S/kg Complete Feed (C12/C14 Lactylates)**	**7.5 g Puramix 14S/kg Complete Feed (C14 Lactylates)**	**7.5 g Puramix 16S/kg Complete Feed (C16 Lactylates)**
(mg/kg complete feed)					
C16 lactylate	<0.5	<0.5	<0.5	<0.5	1,073
(mg/kg complete feed)	(0)	(0)	(0)	(0)	(1,313)

[1]Values in brackets () are calculated values from feed preparation.

The animals were housed in metabolic cages in an environmentally controlled room for the duration of the study. Behavior and physical condition of the birds was inspected at least once per day over the 27-day feeding period. Mortality was recorded as well as the body weight of dead birds. Individual body weights of birds were measured at day 0, 7 and 27. Feed consumption was measured per pen for the periods 0 to 7, 7 to 14 and 21 to 27 days. Blood samples were taken for analysis of C12, C14 and C16 component concentrations from 12 birds per treatment (3 birds/cage) at day 27. The same birds were euthanized by lethal injection on day 27 for necropsy. Upon necropsy, samples of liver, skin and breast meat tissues were taken as well as digesta samples from the crop, gizzard, duodenum, proximal and distal small intestine, ileum and colon. For treatments 1 to 3 only, the concentrations of C12, C14 and C16 components in blood plasma and the digesta from the various compartments of the GI tract were determined. The apparent absorption coefficient (AC) of lactylates from the different compartments of the digestive tract was calculated for treatments 1 to 3 in order to evaluate the apparent quantitative absorption/metabolism of C12/C14 lactylates from the feed.

During the study, 7 birds died or were removed from the study due to adverse health conditions. None of the mortalities were associated with treatment although, the reason of death for 5 of the birds was unclear.

Feed intake, body weight gain (BWG) and feed conversion ratio (FCR) of the birds over the duration of the study (0 to 27 days) is reported in Table 6.3. There was no effect of treatment on feed intake, BWG or FCR among treatments (P>0.05).

Table 6.3: Effect of Lactylates Supplementation on Performance of Broilers							
Parameter	**Performance[1]**					**P-value**	**LSD**
	Treatment 1	**Treatment 2**	**Treatment 3**	**Treatment 4**	**Treatment 5**		
	Control (0 g/kg Complete Feed)	**7.5 g Puramix 30L/kg Complete Feed (C12/C14 Lactylates)**	**7.5 g Puramix 30S/kg Complete Feed (C12/C14 Lactylates)**	**7.5 g Puramix 14S/kg Complete Feed (C14 Lactylates)**	**7.5 g Puramix 16S/kg Complete Feed (C16 Lactylates)**		
FI (g/day)	87.1	88.1	84.6	87.7	88.1	0.22	3.9

BWG (g/day)	1,704	1,752	1,661	1,706	1,742	0.51	119
FCR	1.381	1.369	1.375	1.391	1.367	0.82	0.04

Abbreviations: BWG = body weight gain; FCR = feed conversion ratio; FI = feed intake; LSD = least significant difference;
[1]Significance determined at P<0.05.

The concentrations of the C12, C14 and C16 lactylates components in the plasma of birds in treatments 1 to 3 on day 27 of the study are reported in Table 6.4. The plasma concentrations of the C12 lactylates component was lower (P<0.05) in birds fed diets containing 7.5 g/kg complete feed of Puramix 30L compared to Puramix 30S (treatments 2 and 3; 308 vs. 389 ng/mL). The plasma concentration of C14 lactylates component was comparable (P>0.05) between birds fed diets containing 7.5 g/kg complete feed of Puramix 30S and 30L (treatments 2 and 3; 80 vs. 83 ng/mL).

Table 6.4: Effect of Lactylates Supplementation on Plasma Fatty Acids of Broilers

Lactylate Component	Plasma Fatty Acid Levels			P-value	LSD
	Treatments 1	Treatment 2	Treatment 3		
	Control (0 g/kg Complete Feed)	7.5 g Puramix 30L/kg Complete Feed (C12/C14 Lactylates)	7.5 g Puramix 30S/kg Complete Feed (C12/C14 Lactylates)		
C12 lactylate (ng/mL)	<50[a]	308[b]	389[c]	<0.001	71
C14 lactylate (ng/mL)	<50[a]	80[b]	83[b]	<0.001	14
C16 lactylate (ng/mL)	<50[a]	<50	<50	-	-

Abbreviations: LSD = least significant difference;
[abc]Values with different superscripts within a row differ significantly at P<0.05.

The concentrations of C12, C14 and C16 lactylate components in the digesta of different compartments of the digestive tract are presented in Table 6.5 on an as-is basis. In digesta samples from treatments 2 and 3, the concentrations of C12 and C14 lactylate components were reported to be highest in the crop at 111 and 161 mg/kg for C12 lactylate, respectively and 56 and 71 mg/kg for C14 lactylate, respectively. The concentrations of C12 and C14 lactylates components decreased progressively through the compartments of the digestive tract and were ≤3 mg/kg in the ileum and colon for birds from treatments 2 and 3. The digesta concentrations are reported on an as-is basis and will have been influenced by the digestion and/or absorption of the C12 and C14 lactylate components, as well as changes in DM content.

Table 6.5: Concentration of Lactylates in the Digesta of Broilers on Day 27						
Lactylate Component	Compartment	Digesta Concentrations			P-value[1]	LSD[1]
		Treatment 1	Treatment 2	Treatment 3		
		Control (0 g/kg Complete Feed)	7.5 g Puramix 30L/kg Complete Feed (C12/C14 Lactylates)	7.5 g Puramix 30S/kg Complete Feed (C12/C14 Lactylates)		
C12 lactylate (mg/kg as-is)	Crop	<0.1[a]	111[b]	161[b]	<0.01	63
	Gizzard	<0.1[a]	98[b]	152[c]	<0.001	44
	Duodenum	<0.1[a]	29[b]	43[b]	<0.05	25
	Proximate SI	<0.1[a]	24[b]	29[b]	<0.05	27
	Distal SI	<0.5[a]	3[b]	2[b]	<0.05	1.9
	Ileum	<0.5[a]	1[b]	2[b]	<0.05	1.4
	Colon	<0.5[a]	3[c]	1[b]	<0.01	1.2
C14 lactylate (mg/kg as-is)	Crop	<0.2[a]	56[b]	71[b]	<0.01	28
	Gizzard	<0.2[a]	45[b]	62[b]	<0.001	19
	Duodenum	<0.5[a]	14[b]	17[b]	<0.05	11
	Proximate SI	<0.5[a]	12[c]	7[b]	<0.01	4.5
	Distal SI	<0.5[a]	4[b]	3[b]	<0.05	2.4
	Ileum	<0.5[a]	3[b]	2[b]	<0.05	1.9
	Colon	<0.5[a]	3[b]	2[b]	<0.05	1.8
C16 lactylate (mg/kg as-is)	Crop	1.0[b]	0.1[a]	0.3[a]	<0.01	0.2
	Gizzard	0.7[b]	0.2[a]	0.3[a]	<0.05	0.3
	Duodenum	0.1	<0.15	<0.15	0.42	0.1
	Proximate SI	<0.15	<0.15	<0.15	-	-
	Distal SI	<0.15	<0.15	<0.15	-	-
	Ileum	<0.15	<0.15	<0.15	-	-
	Colon	<0.15	<0.15	<0.15	-	-

Abbreviations: LSD = least significant difference; SI = small intestine;
[abc] Values with different superscripts within a row differ significantly at P<0.05;
[2]P-Value and LSD for the overall effect of experimental treatment; in the statistical analysis of data values < lowest level of quantification (LLOQ) were set at 50% of the LLOQ value.

The dry matter (DM) contents of the digesta in the crop ranged from 292 to 356 g/kg among treatments (not shown). Lower DM contents ranging from 123 to 215 g/kg were reported for the gizzard, duodenum, proximal and distal small intestine, ileum and colon. Within compartments of the GI tract, there were no differences in DM content between treatments.

The apparent digestibility of DM and ACs of C12 and C14 lactylates components from the digesta of different compartments of the digestive tract in birds fed treatment diets 1 to 3 are reported in Table 6.6. The ACs for C12 lactylate component were lowest in the gizzard (7 and -5%, respectively for treatments 2 and 3) and increased to around 100% in the distal small intestine, ileum and colon for treatments 2 and 3. The calculated ACs for C12 lactylate component were higher in the crop (35 and 47%, respectively for treatments 2 and 3) compared to the gizzard (7 and -5%, respectively for treatments 2 and 3). Likewise, the ACs for C14 lactylate component were lowest in the gizzard (13 and -4%, respectively for treatments 2 and 3) and increased to around 100% in the distal small intestine,

ileum and colon for treatments 2 and 3. The calculated ACs for C14 lactylate component were higher in the crop (32 and 43%, respectively for treatments 2 and 3) compared to the gizzard (13 and -4%, respectively for treatments 2 and 3). These findings indicate complete apparent absorption of C12 and C14 lactylates components from the GI tract. The study investigators suggested that the higher observed ACs in the crop compared to the gizzard may be due to non-synchronized passage of C12/C14 lactylates and the digestibility marker (chromium oxide) through the crop, resulting in unrealistic ACs for this compartment. There were no significant differences in ACs for C12 or C14 lactylates components within compartments of the GI tract between treatments 2 and 3.

Table 6.6: Apparent Digestibility of Dry Matter and Apparent Absorption Coefficient (AC) of Lactylates from the Digesta of Broilers on Day 27

Lactylate Component	Compartment	Apparent Digestibility of DM and AC[1]			P-value	LSD
		Treatment 1	Treatment 2	Treatment 3		
		Control (0 g/kg Complete Feed)	7.5 g Puramix 30L/kg Complete Feed (C12/C14 Lactylates)	7.5 g Puramix 30S/kg Complete Feed (C12/C14 Lactylates)		
Dry Matter (Apparent digestibility, %)	Crop	7	0	2	0.30	11
	Gizzard	-12	-14	-16	0.94	25
	Duodenum	-13	-19	-1	0.17	40
	Proximate SI	53	51	52	0.53	4.5
	Distal SI	70	67	70	0.45	4.9
	Ileum	71	72	68	0.35	6.1
	Colon	70	69	67	0.43	7.2
C12 (Apparent absorption coefficient, %)	Crop	-	35	47	0.41	39
	Gizzard	-	7	-5	0.34	35
	Duodenum	-	(80)[2]	(33)[2]	-	-
	Proximate SI	-	90	91	0.80	8.7
	Distal SI	-	99	100	0.14	0.8
	Ileum	-	100	100	0.44	0.5
	Colon	-	99	101	0.27	1.7
C14 (Apparent absorption coefficient, %)	Crop	-	32	43	0.53	48
	Gizzard	-	13	-4	0.27	40
	Duodenum	-	(54)[2]	(65)[2]	-	-
	Proximate SI	-	90	95	0.05	4.4
	Distal SI	-	98	99	0.26	2.3
	Ileum	-	99	99	0.76	1.3
	Colon	-	98	97	0.74	4.4

Abbreviations: AC = absorption coefficient; LSD = least significant difference; SI = small intestine;
[1]The apparent absorption of C16 lactylates could not be determined because of low concentrations of C16 lactylates in the experimental diets;
[2]Estimates with a low accuracy as only based on n=2, as some pooled digesta samples were too small in size to perform analysis of the content of chromium oxide as digestibility marker.

Overall, there was no effect of dietary inclusion of C12/C14 lactylates, C14 lactylate or C16 lactylate on performance. However, the study was not designed as a performance trial and the animals were housed in cages rather than in floor pens.

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Analyses of blood samples of the birds at day 27 revealed elevated concentrations of C12 lactylate and C14 lactylate components from both formulations (Puramix 30L and Puramix 30S) when compared to birds receiving the control diet. The authors of the study used data obtained from the birds in treatments 2 and 3 (Puramix 30L and Puramix 30S) to evaluate the pool size of C12 lactylate and C14 lactylates components in blood plasma. These calculations indicated that the pool size of C12 and C14 lactylates components was less than 0.05% of the mean daily dietary intake of these components.

The concentrations of C12 lactylate and C14 lactylate components were observed to decrease from the proximal to the distal part of the GI tract in treatments in which C12/C14 lactylates were added to the diet as Puramix 30L or Puramix 30S at 7.5 g/kg complete feed. The decrease in concentration of these lactylates in various parts of the GI tract is related to both the intestinal absorption and to changes in the DM intake, endogenous water secretion and intestinal water absorption. The lactylates appear to be absorbed or digested only to a limited extent prior to the small intestine. A significant portion of C12/C14 lactylates appears to be metabolized in the duodenum and proximal part of the small intestine, with the mean apparent ACs for the C12 lactylate component determined to be 90 and 95% for the liquid (Puramix 30L) and solid (Puramix 30S) formulations. These values increased further in the distal parts of the GI tract. Comparable results were obtained for the C14 lactylate component.

Taken together, the authors of the study concluded that C12/C14 lactylates included in the diet at dietary levels of 7.5 g Puramix 30L or Puramix 30S/kg complete feed, equivalent to 3 g C12/C14 lactylates/kg complete feed are extensively and readily metabolized (absorbed and/or digested) from the proximal part of the small intestine. Only a small portion of C12/C14 lactylates consumed in the diet was recovered intact in the blood plasma of broilers.

C12/C14 lactylates are intended for use in animal feed at levels typically in the range of 0.7 g/kg complete feed and not exceeding 1.8 g/kg complete feed. The amount of C12/C14 lactylates added to the feed of the broiler study (3 g C12/C14 lactylates/kg complete feed) exceeds the maximum anticipated use in practice. Thus, the findings with respect to the fate of the lauric and myristic acid components of C12/C14 lactylates have relevance to commercial conditions of use. Moreover, it is reasonable to assume that on the basis of the possession of gastric lipases, animals will process lactic acid esters of fatty acids by similar mechanisms and that the findings in broilers can be extrapolated to all target species.

6.1.3 Information to Establish the Safety of Lauric and Myristic Acids for the Target Animal

6.1.3.1 Natural Occurrence in the Diet and Overview of Previous Safety Evaluations for Use in Food

Lauric and myristic acids are fatty acids which are widely consumed as part of the normal background diet of animals and humans. Their natural occurrence in the diet and well-established metabolic fate therefore, can be used as a basis for the safety determination as described below. The validity of this approach has been demonstrated in previous evaluations of lauric and myristic acids as additives in food. Lauric and myristic acids along with other structurally-related saturated straight-chain fatty acids have a long and established history of use as food additives and flavorings. The safety of the fatty acids for these human food uses has been evaluated by EFSA and JECFA, and the most recent evaluations are summarized in Table 6.7.

Table 6.7: Summary of Previous Scientific Evaluations of Lauric and Myristic Acids		
Reference	**Scope of Evaluation**	**Summary**
EFSA, 2017a	Re-evaluation of fatty acids (E 570) as food additives [caprylic (C8), capric (C10), lauric (C12), myristic (C14), palmitic (C16), stearic (C18) and oleic (C18:1) acids]	No safety concern at current usage levels
JECFA, 1998	Safety evaluation of saturated aliphatic, acyclic linear primary alcohols, aldehydes and acids for use as flavoring substances in food	No safety concern at current level of usage as a flavoring agent

Abbreviations: EFSA = European Food Safety Authority; JECFA = Joint FAO/WHO Expert Committee on Food Additives.

The dietary exposure by humans to these saturated straight-chain fatty acids from their use as technological additives or flavorings is low compared to that from the normal diet. On this basis, safety for the use as a technological additive or flavoring in food was largely based on the low anticipated contribution to total intakes together with the known rapid and extensive metabolism via normally fatty acid and tricarboxylic acid pathways. Although toxicological data were not critical to the determination of safety, the body of available data were reviewed by EFSA and JECFA as part of their assessments.

A similar approach can be taken in evaluating the safety of the lauric and myristic acid components of C12/C14 lactylates for animals. The metabolic fate of lauric and myristic acid by animals is evaluated in Section 6.3.2. These fatty acids are present in feed products marketed in the U.S. under the definitions laid down in Section 33 of Chapter 6 of the AAFCO OP, and in particular, as the free fatty acids under the definition for Hydrolyzed Fat or Oil, Feed Grade (33.3; AAFCO, 2023). The levels of lauric and myristic acids in fats with a history of use in animal feed is considered in Section 6.1.3.3. The anticipated intakes of lauric and myristic acids from the diet are compared with those from C12/C14 lactylates under the conditions of intended use in Section 6.1.3.4. Taken together, these data demonstrate that the lauric and myristic acid components of C12/C14 lactylates will not pose a safety concern to animals under the intended conditions of use. Although studies in target animals are not considered necessary to establish the safety of lauric and myristic acids for animals, there are a number of studies in the published literature in which these fatty acids were fed to poultry and livestock. These studies are briefly described for completeness in Section 6.1.3.6. In addition, the body of toxicological information evaluated by EFSA and JECFA, together with a more recent short-term feeding study in rats using lauric acid is outlined in Section 6.1.3.7. These studies provide corroborative evidence of the safety of the lauric and myristic acid components of C12/C14 lactylates under the conditions of intended use.

6.1.3.2 ADME of Lauric and Myristic Acids

An overview of general fat digestion and absorption by mammals is provided below [e.g., as described in CIR (1987), Xenoulis and Steiner (2010), EFSA (2013 and 2017a) and Adeva-Andany *et al.* (2019) considering dogs and humans]. The stomach is the major site of fat emulsification, mechanically breaking down larger aggregates which are virtually insoluble in the aqueous environment into smaller fragments. Fatty acid esters will generally be hydrolyzed by lipases secreted by the dorsal surface of the tongue or gastric lipases secreted by the stomach. Gastric lipases hydrolyze short-, medium- and longer-chain fatty acids into free fatty acids initiating lipid absorption. Medium-chain fatty acids such as lauric acid are absorbed via the stomach wall into the portal vein for transport to the liver for hepatic

metabolism. Longer-chain fatty acids such as myristic acid and any remaining esters are transported into the duodenum as droplets and the latter further hydrolyzed by the action of pancreatic lipases. Smaller lipids droplets are exposed to bile in the duodenum where they combine to form micelles (4-8 nm in diameter). These micelles (water soluble aggregates) are comprised of mixed lipids and bile acids which allows for absorption in the proximal small intestine. Fatty acids can enter the enterocytes of the small intestine by simple diffusion across the epithelial cell membrane or via a transporter protein. In the intestinal cell, the fatty acids are transported as triglycerides in chylomicrons and very low-density lipoproteins via the lymphatic system and enter systemic circulation. Ultimately, the fatty acids are metabolized by normal fatty acid and tricarboxylic acid pathways in animals to form carbon dioxide which is excreted via exhalation.

The digestion of fat in ruminants is markedly different to that in non-ruminants (Harrison and Leat, 1972; Nafikov and Beitz, 2007; Brzozowska and Oprzadek, 2016). Whereas in non-ruminants, little digestion of fat will occur before the digesta reaches the small intestine where, as described above, lipid solubilization occurs by the action of pancreatic lipases and bile acids. By comparison in ruminants, dietary lipids are generally modified in the forestomach by the microbial population of the rumen, acting to hydrolyze esters and hydrogenate unsaturated fatty acids. Saturated free fatty acids pass into the small intestine where absorption occurs.

Studies using Lauric Acid

Fasted and refed rats were administered 0.5 mL rat serum containing 0.1 µeq 1-^{14}C-labeled lauric acid in combination with 0.1 µeq 9,10-^{3}H-labeled palmitic acid by intravenous injection (Göransson, 1965; cited in EFSA, 2017a). The disappearance of the two labels was measured 1 to 5 minutes after injection. In both fasted and refed rats, lauric acid was observed to disappear from blood at a faster rate than palmitic acid. Analysis of tissues indicated that lauric acid was more rapidly oxidized than palmitic acid.

In a study by Rioux *et al*. (2003), 1-^{14}C-labeled lauric acid was rapidly taken up by cultured rat hepatocytes and the initial radioactivity was cleared from the medium after 4 hours of incubation. Incorporation of the radioactivity into cellular lipids was low due to the high β-oxidation of lauric acid in hepatocytes and it was preferentially incorporated into triglycerides. It was also rapidly converted into palmitic acid by two successive elongations. Labeling studies with 11,12-^{3}H-lautic acid showed incorporation of radioactivity in several proteins as well as labeling of its elongation product, myristic acid.

The metabolic pathways of lauric acid and other fatty acids in liver microsomes of various mammalian species was also investigated by Adas *et al*. (1999). Cytochrome P450 2E1 was reported to catalyze the hydroxylation of lauric acid across all species.

Studies using Myristic Acid

Fasted and refed rats were administered 0.5 mL rat serum containing 0.1 µeq 1-^{14}C-labeled myristic acid in combination with 0.1 µeq 9,10-^{3}H-labeled palmitic acid by intravenous injection (Göransson, 1965). The disappearance of the two labels was measured 1 to 5 minutes after injection. In both fasted and refed rats, myristic acid was observed to disappear from blood at a faster rate than palmitic acid. Analysis of tissues indicated that myristic acid was more rapidly oxidized than palmitic acid.

The metabolism of myristic and palmitic acids was compared in cultured rat hepatocytes using 1-^{14}C-labeled fatty acids (Rioux *et al.*, 2000). Myristic acid was reported to be taken up more rapidly than palmitic acid (87 vs. 68% after 4 hours) and incorporation into cellular lipids was around 33% of initial radioactivity. After 12 hours of incubation, the radioactivity of cellular triglycerides, cellular phospholipids and secreted triglycerides was significantly lower for myristic than palmitic acid. Greater oxidation of myristic acid occurred than of palmitic acid with 15% of the initial radioactivity incorporated in β-oxidation products after 4 hours. Myristic acid also was elongated to palmitic acid to a greater degree than palmitic acid to stearic acid (12% vs. 5% of the initial radioactivity). The combination of elongation and β-oxidation of myristic acid resulted in the more rapid disappearance of myristic acid in cultured hepatocytes than palmitic acid.

Other Biological Considerations

Free fatty acids are recognized to specifically bind to G-protein coupled receptors (GPCR) (FFAR1-4, GPR84) which are widely expressed on a variety of cells and regulate both metabolic and immunological processes (Alvarez-Curto and Milligan, 2016). In this respect, free fatty acids can be critical signaling molecules and an imbalance of fatty acids ingested may have consequences for the animal. As mentioned in Sections 2.6, 3.1 and 3.2, C12/C14 lactylates are intended for use as part of nutritionally balanced diets alongside other fat sources such as grains, oilseed meals, SO and FO. Thus, no adverse effects as a result of fatty acid imbalances are expected under the intended conditions of use of C12/C14 lactylates in animal feed at practical levels of 0.7 g/kg complete feed and not exceeding 1.8 g/kg complete feed.

6.1.3.3 Comparison of the Composition of C12/C14 Lactylates and Other Sources of Dietary Fat

As mentioned above, C12/C14 lactylates comprises around 42% lauric acid and 19% myristic acid in the free fatty acid and lactic acid ester form. Animal fats, fish oil and vegetable oils are all commonly used as fat sources in practical animal diets in the U.S. Although lauric acid is only found in vegetable fats, myristic acid occurs widely in vegetable and animal fats (Woodgate and van der Veen, 2014; Merriman *et al.*, 2016). The fatty acid profiles of fish oil (FO), soy oil (SO), coconut (or copra) oil (CO), palm kernel oil (PKO)[3], palm oil (PO) and tallow which have a history of use by the U.S. feed industry are presented in Table 6.8. These fats were chosen for comparison to include those rich in lauric and myristic acids such as CO and PKO, but which have a limited history of use in the U.S., as well as those with a more established history of use such as SO, FO and tallow, but lower contents of lauric and myristic acids. Coconut oil and PKO contain *ca.* 41% and 49% lauric acid, respectively, which is similar to the level found in C12/C14 lactylates of *ca.* 42%. Fish oil, CO and PKO all contain significant levels of myristic acid at *ca.* 10, 17 and 16%, respectively, which are slightly lower than the levels reported in C12/C14 lactylates of *ca.* 19%. Consistent with animal fats generally, tallow is a source of myristic acid (*ca.* 3%) but contains only negligible levels of lauric acid (*ca.* 0.2%).

Table 6.8: Fatty Acid Profiles of Fats used in Animal Feed		
Fatty Acid	**Unit**	**Typical Values (% Fatty Acids)[1]**

[3] Palm kernel oil is less widely used in the U.S. than other fats and oils listed in Table 6.8 but is available as a product of the palm oil industry and is included for completeness as a myristic acid-rich oil.

		CO	FO	PKO	PO	SO	Tallow
C6 to C10 fatty acids	%	15.5	-	8.2	-	-	-
Lauric acid (C12:0)	***%***	***40.7***	***0.15***	***48.7***	***0.16***	***<0.1***	***0.2***
Myristic acid (C14:0)	***%***	***17.1***	***9.9***	***15.6***	***0.95***	***0.2***	***3.2***
Palmitic acid (C16:0)	%	9.2	20.9	7.5	40.3	10.7	26.3
Palmitoleic acid (C16:1, n-7)	%	-	12.5	-	0.1	0.1	3.8
Stearic acid (C18:0)	%	2.9	3.4	1.8	4.1	3.6	21.2
Oleic acid (C18:1, n-9)	%	6.9	13.0	14.8	36.7	21.9	38.5
Linoleic acid (C18:2, n-6)	%	1.7	1.1	2.5	9.3	51.3	2.8
alpha-Linolenic acid (ALA; 18:3, n-3)	%	0.1	0.8	-	0.2	6.9	0.7
Arachidic acid (C20:0)	%	0.1	0.4	-	0.3	0.3	1.1
Eicosenoic acid (C20:1)	%	-	1.9	-	0.1	0.2	0.3
Arachidonic acid (C20:4)	%	-	0.6	-	-	-	-
Eicosapentaenoic acid (EPA; C20:5, n-3)	%	-	12.2	-	-	-	-
Behenic acid (C22:0)	%	-	0.2	-	-	0.4	0.1
Erucic (C22:1)	%	-	0.7	-	-	0.1	0.2
Docosapentanoic (C22:5)	%	-	1.7	-	-	-	-
Docosahexaenoic (DHA; C22:6, n-3)	%	-	7.9	-	-	-	-
Lignoceric acid (C24:0)	%	-	0.2	-	-	0.2	0.05

Abbreviations: CO = coconut (copra) oil; FO = fish oil; PKO = palm kernel oil; PO = palm oil; SO = soy oil;
[1]Fatty acid profiles taken from the INRAE-CIRAD-AFZ feed tables (2022) except for palm kernel oil which was taken from Pantzaris and Ahmad (2001).

6.1.3.4 Background Exposure to Lauric and Myristic Acid from Other Fats in the Diet

As mentioned in Section 6.1.3.3, CO in particular is a significant source of lauric and myristic acids containing in the region of 41 and 17% of these fatty acids, respectively, and has a history of use in animal feed as a partial replacement for other fats such as FO, SO and canola oil. The amount of CO included in the diet of animals will vary depending on the species but may range from 0.5 to 2% of the diet of poultry, swine, dairy cows or fish (e.g., FAO, 1983; Jørgensen *et al*., 2000; Faciola and Broderick, 2014; Wang *et al*., 2015; Braundmeier-Fleming *et al*., 2020; Rolinec *et al*., 2020). At these inclusion levels of CO in the feed of 5 or 20 g/kg complete feed as-fed, the lauric acid content will be in the region of 0.2 to 0.8% (2 or 8 g/kg complete feed), and the myristic acid content in the region of 0.09 to 0.3% (0.9 to 3 g/kg complete feed). By comparison, under the conditions of intended use of C12/C14 lactylates in feed of typically 0.7 g/kg as-fed, animals will be provided with 0.3 and 0.1 g/kg complete feed of lauric and myristic acids, respectively (see Table 3.1). At the maximum intended use level of 1.8 g C12/C14 lactylates/kg complete feed, the lauric and myristic acid contents will be 0.8 and 0.3 g/kg as-fed, respectively (see Table 3.1). Thus, background exposure to lauric and myristic acids from the use of CO as a fat at levels of 0.5 to 2% of the diet, are higher (2.5- to 10-fold for lauric acid; 3- to 10-fold for myristic acid) higher than from C12/C14 lactylates at the maximum intended use level.

Additionally, medium-chain fatty acids derived from coconut oil have a history of use in the pet food industry (Beynen, 2019; Petco Store, 2020). Coconut oil and its derivative, medium-chain triglycerides (MCT) oil comprising primarily C8 (caprylic) and C10 (capric) fatty acids, are considered to have potential nutritional benefits to dogs (e.g., Pan *et al*., 2010; Hall and Jewell, 2012). Assuming a dog is provided with a treat or supplement containing 2 g of CO, the animal will be exposed to around 0.8 g of lauric

acid/day. By comparison, if a 15 kg dog consumes 250 g of a dry dog food containing 1.8 g C12/C14 lactylates/kg complete feed as-fed, daily exposure to lauric acid will be in the region of 0.2 g/day which is around 4 times lower than the estimated intake from 2 g of CO.

It is recognized that animal fats such as tallow or white grease are more commonly used in feed than CO. As mentioned in Section 6.1.3.3, tallow for example, contains around 3.2% myristic acid but only 0.2% lauric acid. Tallow can be incorporated into the diet of animals at higher levels in practice than CO, and may potentially represent around 8% of the feed (e.g., Reis de Souza *et al*., 1995; O'Neill *et al*., 1998; Tancharoenrat and Ravindran, 2014; Merriman *et al*., 2016). Incorporation of around 8% of tallow in the feed of animals will provide in the region of 0.02% lauric acid and 0.3% myristic acid (0.2 and 3.0 g/kg complete feed). Thus, the potential exposure to lauric acid from the use of tallow under typical conditions of inclusion in feed is lower than from the maximum intended use level of C12/C14 lactylates (0.2 vs. 0.8 g/kg complete feed) but exposure to myristic acid is higher (3.0 vs. 0.3 g/kg complete feed; 10-fold higher). Thus, the exposure by animals to myristic acid from the use of tallow in animal feed further corroborates the safety of this component of C12/C14 lactylates at the proposed inclusion levels. Black soldier fly larvae (BSFL) oil was also evaluated by the U.S. FDA in order to establish an AAFCO ingredient definition for use as a fat source in the diet of finfish, poultry, swine and adult dogs (AAFCO, 2023). The fatty acid profile of BSFL can be influenced to some extent by the larval feedstock but generally contains significant (*ca*. 30%) levels of lauric acid with lower amounts of myristic acid (*ca*. 5%) (Mai *et al*., 2019). Thus, BSFL oil represents another form of lauric and myristic acids which is not considered to pose a safety concern as a component of animal diets and has recently entered the U.S. market.

6.1.3.5 Background Exposure to Lauric and Myristic Acid from Oilseed Meals in the Diet

By-products of the CO and PO industries, in particular CM and PKM are also sources of lauric and myristic acids with a history of use as ingredients in animal feed, in particular in countries with extensive coconut and palm oil industries. The use of these oilseed meals is less common in the U.S., CM is a recognized feed ingredient and is defined in the AAFCO OP (see below; AAFCO, 2022) and is routinely included in U.S. relevant feed composition tables (e.g., United States-Canadian Tables of Feed Composition; NRC, 1982). Further details of the lauric and myristic acid contents of CM and PKM are provided below and the exposure by different species and categories of animals estimated under practical conditions of use. Although these ingredients have only a limited use in commercial feed in the U.S., these data are considered to provide corroborative evidence generally for the history of consumption of lauric and myristic acid-containing ingredients by a range of species as part of the normal diet.

Composition of CM and Scope of Use

Coconut meal is a by-product of the extraction of oil from dried coconut kernels (copra) and has a history of use as an ingredient in feed in the U.S. It is recognized as an economical feed which can partially replace other protein sources in the diet of animals (e.g., O'Doherty and McKeon., 2000; Kim *et al*., 2001; Stein *et al*., 2015) with AAFCO ingredient definitions established for coconut meal, mechanically extracted (71.60), and coconut meal, solvent extracted (71.61) (AAFCO, 2022). Coconut meal obtained by mechanical extraction for example, is reported to have a typical fat content of 9% of

which lauric and myristic acids represent 45 and 19%, respectively (INRAE-CIRAD-AFZ feed tables, 2022). On an ingredient basis, this equates to levels of lauric and myristic acid in CM of around 4 and 2%, respectively. The practical use of CM in animal feed includes poultry and swine feeds as detailed in the following section (Ravindran and Blair, 1992; Stein *et al*., 2015; Arbor Acres, 2017).

Estimated Exposure by Poultry and Swine to Lauric and Myristic Acids from CM

The use of CM as a nutrient source in poultry feed is limited by the amino acid profile and relatively high dietary fiber content although these effects can be overcome by dietary supplementation with lysine and methionine, and the use of enzymes (e.g., mannanases) to help degrade the fiber component (Khanongnuch *et al*., 2006; Sundu *et al*., 2008 and 2009; Diarra *et al*., 2014). However, use in layers, which have lower nutrient requirements than growing birds, is reported with optimal levels of use typically reported to be in the region of 10% (e.g., Moorthy and Viswanathan, 2006 and 2010; Sundu *et al*., 2009).

In a review of feeding studies evaluating the use of CM in swine feeds, Stein *et al*. (2015) concluded that on the basis of the available body of information, the optimum level of inclusion of CM is less than 15% in the diet of weaned piglets and less than 25% in the diets for grower-finisher pigs.

Assuming that CM is included in the diet of layers and pigs at a level of 10% which is within these recommended ranges of use of this ingredient in poultry and swine feeds, the complete feed will contain lauric and myristic acid at levels of 0.4 and 0.2%[4], respectively (or 4 and 2 g/kg complete feed as-fed). By comparison, under the maximum conditions of intended use of C12/C14 lactylates in feed of up to 1.8 g/kg as-fed, layers and swine will be provided with lauric and myristic acid at levels of 0.8 and 0.3 g/kg as-fed, respectively (see Table 3.1). Thus, the background exposure by layers and swine to lauric and myristic acids from the use of CM as a nutrient source at dietary levels of 10% is approximately 5- and 7-fold higher, respectively than from the maximum intended use of C12/C14 lactylates.

Composition of PKM and Scope of Use

Palm kernel meal is a by-product of the PO industry with an extensive history of use as a feed ingredient in Southeast Asian countries such as Malaysia, and also in parts of Africa (Perez *et al*., 2000; Alimon, 2004; FAO, 2012). PKM obtained by mechanical extraction only and referred to as PKM expeller, has a reported fat content of between 5 and 20% of which lauric acid represents around 48% and myristic acid around 16%. On an ingredient basis, PKM expeller containing 8% fat for example, will contain in the region of 4 and 1% lauric and myristic acids, respectively. It displays a chemical composition similar to that of coconut meal or corn gluten meal and on account of its fiber content (13-20% DM) and amino acid profile, is generally considered suitable for use in combination with other protein sources in ruminant diets (FAO, 2012; Stein *et al*., 2015). The practical use of PKM in cattle diets is considered below.

Estimated Exposure by Ruminants to Lauric and Myristic Acids from PKM

[4] Calculation: 10 x [0.09 x 0.45 (C12)] or [0.09 x 0.19 (C14)] = %C12 or C14 fatty acid in the complete feed

In Malaysia for example, PKM is widely used at levels of up to 80% of the total mixed ration (TMR) for feedlot cattle and buffaloes. Inclusion levels in dairy cattle rations are generally in the range of 30 to 40% and in sheep rations around 30% (Alimon, 2004; FAO, 2012). Assuming that feedlot cattle are provided a TMR containing 80% PKM expeller which has a fat content of 8%, the estimated dietary levels of lauric and myristic acids are 3 and 1%[5] (or 30 and 10 g/kg feed as-fed), respectively. Similarly, the lauric and myristic acid levels in a TMR for dairy cattle containing 35% PKM expeller of which 8% is fat, are estimated to be 1 and 0.4%[6] (or 10 and 4 g/kg feed as-fed), respectively. By comparison, under the maximum intended use level of C12/C14 lactylates in feed of 1.8 g/kg as-fed, cattle will be provided with lauric and myristic acid at levels of 0.8 and 0.3 g/kg as-fed, respectively (see Table 3.1). Background exposure to lauric and myristic acids from ingredient use of PKM expeller is approximately 38 and 33 times greater, respectively than from C12/C14 lactylates by feedlot cattle and approximately 12.5 and 13 times greater, respectively by dairy cattle at the maximum intended use level.

Estimated Exposure by Fish to Lauric and Myristic Acids from PKM

The amino acid contents and relatively high fiber contents of PKM and CM limit their use in fish feed. However, some use of PKM in fish is reported with levels typically not exceeding 10 to 20% (Ng *et al*., 2002; FAO, 2012; Park *et al*., 2016).

Assuming that fish are provided diets containing 10% PKM expeller, the estimated dietary levels of lauric and myristic acids are 0.4 and 0.1%[7], respectively (4 and 1 g/kg complete feed as-fed). By comparison, under the maximum intended use level of C12/C14 lactylates in feed of 1.8 g/kg as-fed, fish will be provided with lauric and myristic acid at levels of 0.8 and 0.3 g/kg as-fed, respectively (see Table 3.1). Background exposure to lauric and myristic acids from use of PKM expeller at 10% in the diet is 5- and 3-fold higher, respectively than from C12/C14 lactylates at the maximum intended use level in fish feed.

Summary and Conclusions

The exposure by poultry, swine, ruminants and aquaculture to lauric and myristic acids from the use of CO, CM and PKM as nutrient sources in feed under commercial feeding practices was estimated to be significantly greater than from C12/C14 lactylates under the intended conditions of use. CO and MCTs derived from CO were also reported to have a history of use in cat and dog food at levels higher than from the intended use of C12/C14 lactylates. Further evidence of the acceptability of lauric and/or myristic acid as components of the diet of animals is provided by the common use of tallow in feed in the U.S., as well as the development of an AAFCO ingredient definition for BSFL oil. Therefore, the safety of lauric and myristic acids in C12/C14 lactylates can be established from the higher background intakes of these fatty acids from the normal diet. As mentioned in Section 2.6, C12/C14 lactylates are not intended for use in feeds which contain other significant sources of lauric or myristic acids.

6.1.3.6 Studies in Target Animals using Lauric and Myristic Acids

[5] Calculation: 80 x [0.08 x 0.48 (C12)] or [0.08 x 0.16 (C14)] = %C12 or C14 fatty acid in the TMR
[6] Calculation: 35 x [0.08 x 0.48 (C12)] or [0.08 x 0.16 (C14)] = %C12 or C14 fatty acid in the TMR
[7] Calculation: 10 x [0.08 x 0.48 (C12)] or [0.08 x 0.16 (C14)] = %C12 or C14 fatty acid in the complete feed

Numerous studies are available in the published literature in which poultry, swine, ruminants or aquaculture were fed diets containing CO or PKO at levels comparable to, or higher than, the anticipated exposure to lauric and myristic acids under the conditions of intended use of C12/C14 lactylates. These studies support the background intakes of these fatty acids from the normal diet as described above.

In addition, studies were identified in the published literature in which lauric acid was fed to broilers (Londok *et al.*, 2018; Londok and Rompis, 2019) and lauric or myristic acid was fed to ruminants (Machmuller, 2006; Odongo *et al.*, 2007; Hristov *et al.*, 2009; Klop *et al.*, 2017). The dietary levels varied but were in the region of 0.1 to 0.3 g/kg complete feed in poultry and 65 g/kg DM in cattle. These studies were not designed as safety studies but were performed in healthy animals and included some limited parameters related to tolerability, such as performance. No adverse effects were reported in the studies under conditions comparable to the intended use of lauric and myristic acids in feed from C12/C14 lactylates of up to 0.8 and 0.3 g/kg complete feed, respectively. Therefore, the findings of the study provide corroborative evidence of the safety of the fatty acid components of C12/C14 lactylates to animals under the conditions of intended use.

6.1.3.7 Toxicological Information on Lauric and Myristic Acids

Summaries of the toxicological data on lauric and myristic acids evaluated by EFSA (2017a) and JECFA (1998) are provided in Tables 6.9 and 6.10, respectively. In addition, an acute toxicity study in rats using lauric acid was identified in the published literature and is included in Table 6.9 (Khan *et al.* 2020). An overview of the studies, primarily taken from the evaluations by EFSA and JECFA is provided below.

Acute Oral Toxicity Studies

In a modified acute toxicity in mice administered lauric acid for 3 days, the median lethal dose (LD_{50}) was reported to be >1,238 mg/kg body weight/day (Schafer and Bowles, 1985). Male Albino rats were administered increasing doses of lauric and myristic acid of up to 10,000 mg/kg body weight/day by gavage in another acute oral toxicity study ((b) (4) 1974). A dose of 10,000 mg/kg body weight/day of lauric acid resulted in one death. Congested lungs and kidneys and advanced autolytic changes were reported at necropsy. No deaths and no significant gross lesions were reported at necropsy on dosing with myristic acid. More recently, Khan *et al.* (2020) administered 2,000 mg/kg body weight of lauric acid orally to female SD rats. There were no mortalities or signs of toxicity after 15 days.

Subchronic Toxicity Studies

Male Osborne-Mendel Albino rats (5 rats/group; 40 to 50 kg body weight) were fed lauric acid in the diet at levels of 0 or 10% (equivalent to 0 and 9,000 mg/kg body weight/day) for 18 weeks (Fitzhugh *et al.*, 1960). Body weight, physical condition, behavior, appearance and mortality were monitored during the study. At the end of the study, blood samples were taken for hematological analysis, and necropsy and gross pathology was performed. No adverse findings were reported.

Stomach Irritation Studies

In a 150-day feeding study, Albino rats (10 rats/group; mixed sex and strain) were supplied with a rice diet containing 10% lauric acid (equivalent to 9,000 mg/kg body weight/day) (Mori, 1953). Interim sacrifices were conducted during the study and stomachs were examined for gross lesions. No remarkable changes were reported in the forestomach or the glandular stomach.

Genotoxicity Studies

The mutagenicity of lauric acid was evaluated in a reverse mutation assay using *Salmonella typhimurium* strains TA1535, TA1537, TA97, TA98 and TA100 up to a maximum concentration of 666 µg/plate in the presence and absence of metabolic activation (Zeiger *et al.*, 1988). The study complied with OECD TG 471 with the exception that tester strains *S. typhimurium* TA102 and *E. coli* WP2uvrA bearing AT mutation were not included. The same assay was also used to evaluate the mutagenicity of myristic acid up to a maximum concentration of 3,333 µg/plate in the presence and absence of metabolic activation. No mutagenicity was observed in either study.

A bacterial reverse mutation assay was conducted on myristic acid using *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 at concentrations of up to 10,000 µg/plate (Heck *et al.*, 1989). The findings of the study were negative. Myristic acid was also not observed to induce gene mutations in the mouse lymphoma assay (L5178Y TK+/- cells) at concentrations of up to 62 µg/mL without metabolic activation, or 125 µg/mL with metabolic activation, or at 300 µg/mL in an unscheduled DNA synthesis (UDS) assay using hepatocytes obtained from male Fisher rats. The reporting of the study was noted to be limited (EFSA, 2017a).

Table 6.9: Summary of Toxicity Studies using Lauric Acid

Study	Details	Result	Reference
Acute toxicity study	Mouse, oral	LD_{50} = 1,238 mg/kg bw	Schafer & Bowles, 1985 [Cited in JECFA, 1998]
Acute toxicity study	Rat, gavage	LD_{50} > 10,000 mg/kg bw	CIR, 1987
Acute toxicity study (OECD TG 423)	Female SD rat, oral	No mortality 15 days after a single dose of 2,000 mg/kg bw	Khan *et al.*, 2020
18-week toxicity study	Male rat, diet	NOEL >6,000 mg/kg bw per day (reported by JECFA, 1998) NOEL >9,000 mg/kg bw per day (reported by EFSA, 2017a)	Fitzhugh *et al.*, 1960 [Cited in EFSA, 2017a]
150-day toxicity study	Albino rat, both sexes, diet	No remarkable changes detected at 9,000 mg/kg bw per day	Mori, 1953 [Cited in JECFA, 1998]
Modified Ames test (pre-incubation method)	*S. typhimurium* TA98, TA100, TA1535, TA97 and TA1537	Negative[1] at up to 666 µg/plate	Zieger *et al.*, 1988 [Cited in JECFA, 1998]
Genotoxicity study	*Saccharomyces cerevisiae* D6	Aneuploidy at concentrations from 10 to 200 µg/ml,	Parry *et al.*, 1981 [Cited in EFSA, 2017a]

Abbreviations: bw = body weight; LD_{50} = median lethal dose; NOEL = no observed effect level; OECD = Organisation for Economic Co-operation and Development;
[1]Both with and without metabolic activation.

Table 6.10: Summary of Toxicity Studies using Myristic Acid			
Study	**Details**	**Result**	**Reference**
Acute toxicity study	Rat, oral	LD_{50} > 5,000 mg/kg bw	Moreno, 1977 [Cited in JECFA, 1998]
Acute toxicity study	Rat, gavage	LD_{50} > 10,000 mg/kg bw	CIR, 1987
Cell mutagenesis assay	Mouse lymphoma L5178Y TK +/-	Negative at 62.5 µg/ml[1] and 125 µg/ml[2]	Heck *et al.*, 1989 [Cited in JECFA, 1998]
Ames test (plate incorporation assay)	*S. typhimurium* TA98, TA100, TA1535, TA1537 and TA1538	Negative at 10 mg/plate[3]	Heck *et al.*, 1989 [Cited in JECFA, 1998]
Modified Ames test (pre-incubation method)	*S. typhimurium* TA97, TA98, TA100, TA1535 and TA1537	Negative at up to 3,333 µg/plate[3]	Zieger *et al.*, 1988 [Cited in JECFA, 1998]
UDS assay	Rat hepatocytes *in vitro*	Negative at 300 µg/ml	Heck *et al.*, 1989 [Cited in JECFA, 1998]

Abbreviations: bw = body weight; LD_{50} = median lethal dose; UDS = unscheduled DNA synthesis;
[1]Without metabolic activation;
[2]With metabolic activation;
[3]Both with and without metabolic activation.

Summary and Conclusions

The results of the available toxicity studies support the safety of lauric and myristic acids as components of C12/C14 lactylates under the intended conditions of use. Overall, lauric and myristic acid display low acute oral toxicity. Although irritation of the GI tract is unlikely on the basis that lauric and myristic acid in the free fatty acid and ester form in C12/C14 lactylates will only be present at relatively low levels in the feed (up to 1.8 g/kg complete feed of C12/C14 lactylates providing 0.8 and 0.3 mg lauric and myristic acids/kg complete feed), no irritation of the forestomach of rats was reported in animals fed diets containing 10% lauric acid. Only limited genotoxicity data are available and the studies conducted do not meet regulatory or OECD guidelines. However, no evidence of mutagenicity was observed in bacterial reverse mutation assays using lauric acid or myristic aid. The negative findings of UDS assay on myristic acid cannot be fully relied on due to limitations in the experimental design. No adverse findings were reported in rats fed 10% lauric acid in the diet for 18 weeks.

6.1.4 Information to Establish the Safety of Lactic Acid for the Target Animals

6.1.4.1 An Overview of Previous Safety Evaluations for Use in Feed and Food

Lactic acid and calcium lactate were recently re-evaluated by EFSA for use as preservatives in animal feed in the EU (EFSA, 2015, 2017b and 2019). A summary of these evaluations is provided in Table 6.11. In the original evaluation conducted in 2015, EFSA evaluated the safety of lactic acid and its calcium salt for ruminants and swine on the basis of the natural presence of the acid in the diet. On the basis that no adverse effects were observed in ruminants exposed to >100,000 mg lactic acid/kg DM, and swine exposed to 125,000 mg lactic acid/kg DM, no safety concerns were anticipated from preservative use at levels of up to 50,000 mg lactic acid/kg complete feed. However, in the absence of significant

background consumption of lactic acid by poultry similar conclusions could not be drawn for this species. Furthermore, although published data in broilers and layers were reviewed, the studies were not designed for regulatory purposes and were considered insufficient by EFSA for the determination of a safe level of use of lactic acid in poultry. A lack of data in pre-ruminants also resulted in no conclusions being drawn for this category of animal.

Following publication of the initial opinion by EFSA, the applicant submitted additional published information in 2017 in an effort to address the gaps in the available data on poultry and pre-ruminants (EFSA, 2017b). EFSA again considered the information provided insufficient, and the applicant conducted a tolerance trial in broilers which was evaluated in an opinion published in 2019. On the basis of the 42-day feeding trial in broilers fed lactic acid (comprised of 90% L-lactic acid and 10% D-lactic acid) and D-lactic acid, EFSA concluded that no safety concerns were anticipated when lactic acid or calcium lactate were included in the diet of poultry at levels of up to 20,000 mg/kg complete feed (the applicant proposed use level). EFSA extrapolated the results of the study in broilers to support the safety of lactic acid and calcium lactate at levels of 20,000 mg/kg complete feed for all animals except swine and ruminants for which higher levels were acceptable, and pre-ruminants for which there continued to be a lack of data.

Table 6.11: Summary of Previous Scientific Evaluations of Lactic Acid for Feed Use

Reference	Scope of the Evaluation	Summary
EFSA, 2015	Safety and efficacy of lactic acid and calcium lactate for use as technological additives for all animal species	The maximum levels of 50,000 mg lactic acid/kg complete feed and 30,000 mg calcium lactate/kg complete feed are considered safe for functional ruminants and pigs No conclusions on the safety for pre-ruminants or poultry could be drawn
EFSA, 2017b	Safety and efficacy of lactic acid and calcium lactate for use as technological additives for poultry and pre-ruminants	No safe concentration of lactic acid and calcium lactate in complete feed for chickens could be identified. No conclusions on the safety for lactic acid in milk replacer for pre-ruminants is possible. In the absence of data in poultry and pre-ruminants safety for all species cannot be established (i.e., only pigs and functional ruminants as detailed in EFSA, 2015)
EFSA, 2019	Safety and efficacy of lactic acid and calcium lactate for use as technological additives for poultry and all animal species except pre-ruminants	The maximum levels of 20,000 mg lactic acid/kg complete feed and 24,000 mg calcium lactate/kg complete feed are considered safe for all animals except swine, ruminants and pre-ruminants. Swine and ruminants can safely consume lactic acid and calcium lactate at higher levels (EFSA, 2015). No safe level is established for pre-ruminants.

Abbreviations: ADI = acceptable daily intake; EFSA = European Food Safety Authority.

A similar approach can be taken in evaluating the safety of the lactic acid component of C12/C14 lactylates under the intended conditions of use of not more than 1.8 g/kg complete feed, equivalent to 0.5 g lactic acid/kg complete feed. The metabolic fate of lactic acid in animals is considered in Section

6.1.4.2. Exposure by animals to lactic acid from C12/C14 lactylates is compared with that from the existing use as a preservative in feed in Section 6.1.4.3. In Section 6.1.4.4, the anticipated levels of intake of lactic acid from its natural presence in the diet are estimated. The results of the unpublished study in broilers which formed the basis for positive opinion issued by EFSA in 2019 on the safety of lactic acid for poultry are summarized in Section 6.1.4.5. In the same section, a brief outline of other published feeding trials in animals using lactic acid is provided.

Additionally, lactic acid and its ammonium, calcium, sodium and potassium salts have a long and established history of use as technological additives and flavorings in human food. The safety of lactic acid and its salts has been evaluated by the Scientific Committee on Food (SCF), EFSA and JECFA and a summary of these evaluations is provided in Table 6.12. The dietary exposure by humans to lactic acid from the regular diet of humans was determined to be significantly higher than that from use as a flavoring or food additive in the evaluations conducted by the SCF (1991) and JECFA (1974b). Although toxicological data were not considered critical to the safety determination on this basis, some limited toxicity studies were reviewed by the experts. The body of available toxicological information is summarized in Section 6.1.4.7.

Table 6.12: Summary of Previous Scientific Evaluations of Lactic Acid for Food Use

Reference	Scope of Evaluation	Purpose	Summary
SCF, 1991	Dicarboxylic acids and their salts	Food additive	ADI of "not specified" was established Only L-lactic acid should be used in infant foods
EFSA, 2009	Aliphatic acyclic diols, triols and related substances	Flavoring substance	Insufficient information were available for EFSA to draw a conclusion
JECFA, 1974b	Lactic acid and its ammonium, calcium, potassium and sodium salts	Food additive	ADI of "not limited" was established Neither D-lactic acid nor DL-lactic acid should be used in infant foods

Abbreviations: ADI = acceptable daily intake; EFSA = European Food Safety Authority; JECFA = Joint FAO/WHO Expert Committee on Food Additives; SCF = Scientific Committee on Food.

6.1.4.2 ADME of Lactic Acid

Studies to evaluate the metabolic fate of lactic acid are available in the published literature and the majority have been evaluated by JECFA (1974b). A summary of these studies is provided below.

Lactic acid is an endogenous carboxylic acid and normal intermediary of carbohydrate and amino acid metabolism. Lactate produced by mammalian cells is L-lactate and this is the main isomer in blood whereas D-Lactate is normally present in very low concentrations (Ling *et al.*, 2012). L-Lactate is rapidly metabolized to pyruvate by L-lactate dehydrogenase, whereas D-lactate is converted to pyruvate by D-α-hydroxy acid dehydrogenase which metabolizes D-lactate at about one-fifth the rate that L-lactate dehydrogenase metabolizes L-lactate (Ewaschuk *et al.*, 2005). Absorbed lactic acid will ultimately be oxidized in animals to carbon dioxide and water.

Groups of rats (no further detail stated) were administered 1,700 mg/kg of L-, D-, or DL-lactate orally or by subcutaneous injection (Cori and Cori, 1929). The largest rise in liver glycogen was displayed following administration of L-lactate, with 40 to 95% of the absorbed dose being converted within 3 hours. Almost no glycogen was formed from the D-isomer. The highest blood lactate levels were observed following administration of D-lactate with 30% of the amount absorbed excreted into the urine. No L-lactate was identified in the urine. The authors concluded that both D- and L-isomers were absorbed at the same rate from the intestine, but D-lactate was utilized four times more slowly.

In another study, groups of male and female rats (6/sex/group) received sodium DL-lactate in the diet at a concentration of 215 mg/kg body weight (Cori, 1930). The absorption of sodium DL-lactate from the intestine was determined 1, 2, 3 and 4 hours after ingestion. The rate of absorption decreased with time and was roughly proportional to the amount of lactate present in the gut. The rate of absorption in some animals was limited by slow evacuation of the stomach.

Rabbits with blood levels of lactate of 200 or 250 mg/L, displayed excitation, dypnoea and tachycardia (Collazo *et al*., 1933; cited in JECFA 1974b – limited details available). In another oral study in rabbits, animals were administered 600 to 1,600 mg/kg bodyweight of DL-lactate (Fürth and Engel, 1930). Most animals were reported to die within three days. The urinary excretion of lactate varied from 0.26 to 0.31% and was not affected by alkalosis.

Although mammalian tissues only produce L-lactate, studies in tissues of the brain, heart, kidney and liver from ducks and rats indicate that both L- and D-isomers can be oxidized (Brin, 1965). Measurements of oxygen consumption and carbohydrate synthesis in rat liver showed that L-lactate was utilized almost entirely but virtually no D-isomer was used. Small but measurable amounts of D-lactate were utilized by rat kidney tissue, but none was used by the grey brain matter. L-Lactate was observed to stimulate oxygen consumption and carbon dioxide consumption in all rat tissues, whereas D-lactate only slightly stimulated respiration of the liver and heart tissue. The results of studies in duck tissue were similar, with L-lactate found to be oxidized three to five times more rapidly than D-lactate in heart and liver slices, and ten to twenty times more rapidly by brain tissue. D-Lactate was utilized to the same extent as the L-isomer in the duck and rat heart slices but to a lesser extent by the brain and liver tissues.

The lactic acid raw material used in the manufacture of C12/C14 lactylates is 98% L-isomer, with only minor levels of the D-isomer. Consequently, lactic acid from C12/C14 lactylates should be readily absorbed and utilized by animals.

6.1.4.3 History of Use of Lactic Acid in Feed

Lactic acid and calcium lactate are listed in 21 CFR §582.1061 and 21 CFR §582.1207 as GRAS for use as general purpose food additives in the feed and drinking water of animals when used in accordance with good manufacturing or feeding practice (U.S. FDA, 2021). No use levels of lactic acid or its calcium salt are specified in the U.S. beyond the minimum required to achieve the intended effect in accordance with good feeding or manufacturing practices. However lactic acid and its calcium salt are also authorized for use as preservatives in water and feed for animals in the EU (EC, 2022), and in the recent evaluations of these ingredients in feed in the EU outlined above (see Section 6.1.4.1), the applicant

indicated that the maximum use level of lactic acid in feed for pigs and ruminants but excluding pre-ruminants, was 50 g/kg complete feed. For all other species except pre-ruminants, a maximum use level of 20 g/kg complete feed was proposed (EFSA, 2019). EFSA concluded that based on the available data, the levels of use proposed by the applicant do not pose a safety concern for animals, and therefore, it is reasonable to conclude that the amount of lactic acid in feed can be in the region of 50 g (pigs and ruminants) and 20 g (poultry and other animals)/kg complete feed in the EU. On the basis that lactic acid is a preservative and the amount added to feed will be that required to achieve the desired effect, it is also likely that the use levels in feed in the U.S. can reach similar amounts (FEFANA, 2014; (b) (4) Intelligence, 2022).

By comparison, under the intended conditions of use of C12/C14 lactylates in feed proposed by Corbion of not more than 1.8 g/kg complete feed, animals will be exposed to 0.5 g lactic acid/kg complete feed. Animals consuming C12/C14 lactylate will therefore, be exposed to lactic acid levels which are 100-fold less than the level of 50 g lactic acid/kg complete feed reported in the EU as representing the practical use of the organic acid as a preservative for pigs and ruminants. Similarly, animals consuming 1.8 g C12/C14 lactylates/kg complete feed will be exposed to levels of lactic acid 40 times lower than from the use of the organic acid as a preservative at 20 g/kg complete feed for poultry and other non-ruminant and non-swine species. Thus, evidence of the safety of the lactic acid component of C12/C14 lactylates is provided by the significantly higher levels of existing use of the acid and its calcium salt as a preservative in animal feed.

6.1.4.4 Background Exposure to Lactic Acid from the Diet

Swine

Fermented liquid feeds (FLFs), prepared by mixing dry feed with water and storing under conditions which allow lactic acid bacteria (LAB) and yeasts in the feed ingredients to act and reach steady state, have a history of use in animal feed, particularly swine feed, in the U.S. (EFSA, 2006; Plumed-Ferrer and von Wright, 2009; Missotten *et al*., 2015). The fermentation may be natural or controlled and the objective is to generate a feed with low pH which helps retard the growth of pathogenic microorganisms in feed and also can have a positive impact on the digestive health of the animal. The lactic acid content is generally recognized to be critical to the effectiveness of the FLF, with reports of desirable levels being in the range of 100 or 150 mmol/L primarily as L-lactic acid (EFSA, 2006; Missotten *et al*., 2015). Assuming that a weaned piglet of 20 kg consumes 4 L of FLF containing 150 mmol/L of lactic acid, the estimated exposure to the organic acid is 27 to 54 g/day (EFSA, 2006). By comparison, a piglet of 20 kg consuming 1 kg complete feed/day (EFSA, 2017c) containing C12/C14 lactylates at the maximum use level of 1.8 g/kg complete feed will be exposed to 0.5 g of lactic acid, which is >50-fold lower than from FLF. Thus, pigs can consume levels of lactic acid from the normal diet which are significantly higher than from the intended use of C12/C14 lactylates in feed.

Ruminants

Lactic acid bacteria present naturally in crops are responsible for the fermentation of water-soluble carbohydrates and the production of lactic acid during the ensiling process. Likewise, LAB can be utilized as additives to enhance the preservation and quality of the silage (Charmley, 2001; Muck *et al*., 2018).

Lactic acid levels in corn silage for example, were reported to vary from 6.35 to 7.72% DM either untreated or treated with one of a number of *Lactobacillus* species (Ranjit and Kung, 2000). The ratio of L- and D-lactic acid was approximately 1:1 across all of the silages tested. Other studies indicate that silages which have undergone extensive homolactic fermentation can contain lactic acid at levels of 10 or 15% DM (McDonald *et al*., 1991; Charmley, 2001). The relative amounts of L- and D-lactic acid vary depending on the inoculant used, with up to 90% D-lactic acid produced by LAB isolates obtained from forages and crops by Cai *et al*. (1998). Assuming that silage represents around 35% DM of the TMR for cattle (NRC, 2001), animals can be exposed to levels of lactic acid of 2.5 to 5.3% (25 to 30 g/kg DM) from its natural presence in the feed. By comparison, ruminants are estimated to be exposed to 0.05% (or 0.5 g/kg DM) of lactic acid from the maximum intended use of C12/C14 lactylates in the diet of 1.8 g/kg complete feed, which is >50-fold lower. Thus, ruminants can consume levels of lactic acid from the normal diet which are significantly higher than from the intended use of C12/C14 lactylates in feed.

Summary and Conclusions

Swine and ruminants can be exposed to lactic acid from its natural presence in FLF or ensiled forages at significantly higher levels than anticipated from the use of C12/C14 lactylates as a source of lauric and myristic acids in animal feed. The safety of the lactic acid component of C12/C14 lactylates for swine and ruminants can be established on the basis of the significantly higher background exposure from the normal diet.

6.1.4.5 Studies in the Target Animals

Other Target Animal Studies using Lactic acid

There are a number of studies in the published literature in which lactic acid was fed to poultry, swine and ruminants. A detailed evaluation of these studies was not considered necessary to establish the safety of the lactic acid component of C12/C14 lactylates on the basis of the information on background intakes outlined above. However, for completeness, a summary of the feeding studies identified in the literature are provided in Appendix 019 an the overall findings were briefly as follows:

- Broilers were fed diets of up to 30,000 mg/kg complete feed in the diet for 35 to 42 days with no adverse effects reported on performance or routine blood parameters. Under the conditions of intended use of C12/C14 lactylates in feed, broilers will be exposed to up to 1.8 g C12/C14 lactylates/kg complete feed equivalent to 0.5 g (or 500 mg) lactic acid/kg complete feed. Therefore, a margin of safety of 60 can be established from the results of these tolerance studies.
- Cats tolerated lactic acid when fed for one year at a level of 1.2% (12,000 mg/kg complete feed) in the diet. Under the conditions of intended use of C12/C14 lactylates in feed, broilers will be exposed to up to 1.8 g C12/C14 lactylates/kg complete feed equivalent to 0.5 g (or 500 mg) lactic acid/kg complete feed. Therefore, a margin of safety of 24 can be established from the results of these tolerance studies.
- One study in goldfish was identified in which lactic acid appeared to be well-tolerated at 0.2% in the feed.

- Swine were fed diets containing lactic acid at varying levels of lactic acid, including 1 or 2% in the diet with no reports of adverse effects. The studies were not designed to support safety but provide evidence of the ability of pigs to tolerate lactic acid.
- Only short-term studies were identified in ruminants which were not considered relevant to safety.

Taken together, these data along with the estimates of background exposure to lactic acid from the diet, indicate that the consumption of lactic acid as a component of C12/C14 lactylates under the conditions of intended use will not pose a safety concern.

Study in Broilers using Lactic Acid

An unpublished study was summarized by EFSA in its most recent opinion on lactic acid and calcium lactate (EFSA, 2019). The study was conducted using lactic acid comprising 90% L-lactic acid and 10% D-lactic acid. The lactic acid component of C12/C14 lactylates comprises 98% of the L-isomer with only low levels of the D-isomer. Thus, absorption and metabolism of lactic acid should be sufficiently similar for the results of the broiler study to be extrapolated to the acid component of C12/C14 lactylates.

In the study, 1,320 one-day old Ross 308 broilers were allocated to 60 floor pens each containing 22 chickens such that each treatment was replicated 10 times. The animals were fed corn/wheat/SBM-based diets containing 0 (control, Treatment 1), 10,000 (Treatment 2), 20,000 (Treatment 3) or 30,000 (Treatment 4) mg lactic acid/kg complete feed for 42 days. Two phase mash feeds were provided, a starter feed (days 1 to 21) and grower feed (days 22 to 42). General health and mortality were monitored daily. Growth, body weight, feed intake and FCR were evaluated at days 1, 21 and 42 of the study. At day 42, 20 chickens per treatment (2 birds/pen) were slaughtered and subject to necropsy. Organ weights and gross histopathology of tissues was performed. Blood samples were collected from the same birds and were analyzed for routine clinical chemistry and hematology parameters. Tibia bones also were collected from 20 birds per treatment (2 birds/pen) and analyzed for calcium and phosphorus. Feces excreted were collected over a 3-day period (days 40 to 42) and analyzed for DM content, calcium and phosphorus.

The health of the animals was considered normal throughout the study, and no adverse events were noted. Total mortality/cull ratio after 42 days was 66/1,320 birds (5%). There was no effect of lactic acid treatment on average daily feed intake (ADFI), average daily gain (ADG) or FCR. All blood parameters were within normal ranges/historic controls for the weight and age of the birds studied. Relative to the control group (T1), there were no significant differences in hematology parameters, metabolites or blood enzymes of electrolytes analyzed among treatments with the exception of hematocrit. A significantly elevated percent hematocrit (HCT) level was reported in birds fed diets containing 20,000 mg lactic acid/kg complete feed (Treatment 2) compared to the control birds (Treatment 1) but the same effect was not observed in birds fed 30,000 mg lactic acid/kg complete feed (Treatment 3). Thus, the effect was not considered to be related to treatment. No gross findings were observed in any organs and tissues examined. Tibia ash, Ca and P levels, as well as Ca and P digestibility was not affected by lactic acid treatment. Under the experimental conditions of the study, the authors concluded that the findings of the study indicate lactic acid is well-tolerated by broilers at levels of up to 30,000 mg lactic acid/kg complete feed.

Under the conditions of intended use of C12/C14 lactylates in feed, broilers will be exposed to up to 1.8 g C12/C14 lactylates/kg complete feed equivalent to 0.5 g (or 500 mg) lactic acid/kg complete feed. Therefore, a margin of safety of 60 can be established from the results of the tolerance study. The findings of the study provide corroborating evidence of the safety of the lactic acid component of C12/C14 lactylates for poultry under the conditions of intended use.

6.1.4.6 Safety for Pre-Ruminants

In the original EFSA opinion on lactic acid and calcium lactate for use in animals, it was concluded that: *"In the absence of data, no conclusions on the safety of lactic acid in pre-ruminants can be drawn."* Furthermore, it was recognized that: *"When the rate of D-lactate production exceeds the body's capacity for its metabolism and excretion, D-lactate accumulates in the blood causing metabolic acidosis. D-lactate acidosis is well known and described in veterinary medicine as a consequence of grain overfeeding in adult ruminants and in neonatal animals with diarrhea (Ewaschuk et al., 2005)."*

D-lactic acidosis is recognized as a contributory factor to acidemia in calves diagnosed as ruminal drinkers (Ewaschuk *et al.* 2005). Excessive consumption or a malfunctioning of the esophageal groove leads to the accumulation of milk in the rumen resulting in fermentation of lactose and D-lactic acidosis. Likewise, D-lactic acid production is a recognized secondary complication in diarrheic calves, representing around 64% of the total increase in organic acids.

C12/C14 lactylates is not intended for use in milk replacer and therefore, the concerns raised by EFSA largely do not apply to this assessment. However, for young ruminants transitioning to feed, exposure to C12/C14 lactylates may be up to 1.8 g/kg complete feed, equating to 0.5 g lactic acid/kg complete feed. As mentioned above, only 2% of the lactic acid component will be in the form of D-lactate and therefore, under practical conditions of use of C12/C14 lactylates, D-lactic acidosis is not expected to pose a safety concern for non-functional ruminants.

6.1.4.7 Toxicological Information on Lactic Acid and Its Salts

The acute toxicity of lactic acid has been assessed in several animal studies. The LD_{50} values determined in these studies ranged from 1,810 to 4,875 mg/kg body weight and are summarized in Table 6.13. Rats have been reported to survive subcutaneous doses of 2,000 to 4,000 mg lactic acid/kw body weight, but mice were killed by subcutaneous doses in the same range (Fürth and Engel, 1930; Cited in JECFA, 1974b).

Table 6.13: Acute LD_{50} Values Determined for Lactic Acid

Test Compound	Animal Model	Route	LD_{50} (mg/kg BW)	Reference
Sodium lactate	Rat	Intraperitoneal	2,000	Rhône-Poulenc, 1965 [Cited in JECFA, 1974b]
Lactic acid	Rat	Oral	3,730	Smyth *et al.*, 1941 [Cited in JECFA, 1974b]
Lactic acid	Guinea pig	Oral	1,810	Smyth *et al.*, 1941 [Cited in JECFA, 1974b]
Lactic acid	Mouse	Oral	4,875	Fitzhugh, 1945 [Cited in JECFA, 1974b]

Abbreviations: LD_{50} = median lethal dose.

Repeat dose studies indicate that short-term oral administration of lactic acid and its salts were not associated with adverse effects in rats and dogs at doses up to 2,000 or 1,600 mg/kg body weight/day, respectively. A short-term (14 to 16 day) feeding study in rats, in which animals received daily doses of 1,000 and 2,000 mg/kg body weight sodium lactate (as lactic acid) reported no accumulation effects (Fürth and Engel, 1930; cited in JECFA, 1974b). In another short-term study in dogs, 2 dogs received 600 to 1,600 mg 42 times during a two and half month period and no ill effects were reported (Faust, 1910; cited in JECFA, 1974b). Feeding 10% lactic acid to birds (species and duration not stated) was attributed to the development of polyneuritic crises resembling B1 deficiency on diets rich in carbohydrates, proteins or fats. No long term studies were available.

In summary, only limited toxicological data are available on lactic acid but relatively high levels of administration appear to be tolerated by animals. Overall, the findings from the toxicity studies support the safety of lactic acid as a component of C12/C14 lactylates under the intended conditions of use.

6.1.5 Information to Establish the Safety of the Sodium Component of C12/C14 Lactylates for the Target Animals

It is recognized that C12/C14 lactylates will also provide a source of sodium. The sodium content of C12/C14 lactylates is limited to not more than 8% by the product specifications for the ingredient (see Table 2.8). Under the conditions of intended use, the maximum C12/C14 lactylates content of complete feed is 1.8 g/kg, equating to a maximum of 0.14 g sodium/kg. The requirements of animals for sodium (or sodium chloride) varies with species but typically falls within the range of 1 to 2 g sodium/kg complete feed (Berger, 2006). Thus, the sodium component of C12/C14 lactylates will contribute around 7 to 14% to the sodium requirements of animals and will be taken into account by nutritionists in formulating the feed of animals. Overall, it may be concluded that the sodium content of C12/C14 lactylate will not pose a safety concern to target animals under the conditions of intended use.

6.1.6 Information to Establish the Safety of the Sulfate Component of C12/C14 Lactylates for the Target Animals

As mentioned in Part 2, sodium sulfate is added to the market formulation, ALOAPUR® PM as a processing aid at a level of less than (b). The total sulfate content will be a combination of the contribution from the added sodium sulfate and the diatomaceous earth and mean values of sulfate and sulfur from 3 representative lots of ALOAPUR® PM were determined analytically to be 4,967 and 1,667 mg/kg, respectively.

Dietary sulfates are well absorbed by animals and ultimately excreted in the urine (Magee *et al.*, 2004; NRC, 2005). The NRC has established MTLs for sulfur in the complete feed of 0.4% DM for poultry and swine, 0.3% DM for cattle and sheep fed high concentrate diets and 0.5% DM for cattle and sheep fed high-forage diets (NRC, 2005). The MTL of sulfur in feed for horses is derived from interspecies extrapolation to be 0.5% DM and too little is known of sulfur toxicity in fish to calculate a limit (NRC, 2005). Notably, non-ruminants are far less likely to develop sulfur toxicosis than ruminants (NRC, 2005).

In non-ruminant and non-pseudo-ruminant feeds containing ALOAPUR® PM at the maximum level (5 g/kg as-fed), the sulfur content is estimated to be around 9.5 mg/kg (*ca*. 0.001%) DM[8] which is negligible compared to the MTLs of sulfur in poultry and swine feeds of 0.4% DM.

Likewise, 5 g ALOAPUR® PM/kg in the TMR (45% DM) of ruminants contains around 17.4 mg/kg DM (0.002%) DM[9] of sulfur which makes only a minor contribution to the MTLs of 0.3 to 0.5% DM for sulfur in cattle and sheep feeds.

It is also reported that 0.3% DM of sulfate in the diet of cattle can result in reduced feed intake (Knight, 1985). By comparison, the sulfate content of the TMR from the addition of 5 g/kg of ALOAPUR® PM containing 4,967 mg of sulfate will be in the region of 14 mg/kg as-fed or 31 mg/kg DM basis[10] (i.e., around 100 times lower than 0.3% DM). Thus, the sulfate content of ALOAPUR® PM is not expected to pose a safety concern to target animals under the conditions of intended use.

6.2 INFORMATION TO ESTABLISH HUMAN FOOD SAFETY

As discussed in Part 3 of the GRAS Notice (Section 3.2), the use of C12/C14 lactylates as a source of lauric acid and myristic acid in the diet of animals should not lead to the deposition of substances not normally present, or at levels not usually observed, in the edible tissues of animals. Thus, no human food safety concerns are anticipated under the conditions of intended use of C12/C14 lactylates in the diets of food-producing animals at levels of up to 1.8 g/kg complete feed as-fed, corresponding to 5 g/kg complete feed of ALOAPUR® PM.

6.3 SUMMARY AND BASIS FOR GRAS CONCLUSION

Corbion intends to market C12/C14 lactylates for use as a source of lauric and myristic acids for animals at levels not exceeding 1.8 g/kg complete feed.

C12/C14 lactylates (b) (4) which is marketed under the trade name ALOAPUR® PM. The use of the diatomaceous carrier allows C12/C14 lactylates to be supplied in a homogenous powdered form which is readily mixed with finished feeds. The manufacturing process is conducted in accordance with cGMP, and a Hazard Analysis Critical Control Point plan is in place. The manufacturer will comply with the requirements for importing feed into the U.S. laid down by the Food Safety Modernization Act (FSMA) including the foreign supplier verification program (FSVP).

[8] Calculation: mean sulfur content of 1,667 mg/kg in ALOAPUR® PM is equivalent to 8.3 mg/kg complete feed (12% moisture) or 9.5 mg/kg complete feed on a DM basis.
[9] Calculation: mean sulfur content of 1,667 mg/kg in ALOAPUR® PM is equivalent to 8.3 mg/kg complete feed or 17.4 mg/kg DM assuming a 45% DM content of the TMR.
[10] Calculation: mean sulfate content of 4,967 mg/kg in ALOAPUR® PM is equivalent to 14 mg/kg complete feed or 31 mg assuming a 45% DM content of the TMR.

Appropriate feed grade specifications have been established for C12/C14 lactylates which include measurement of the AV and EV which confirm the mixture is an equilibrium mixture of free fatty acids and lactic acid esters, as well as criteria to control levels of heavy metal contaminants. Conformance with the product specifications was demonstrated by analytical data on 5 commercial lots of C12/C14 lactylates. Additional analytical data on 5 commercial lots of C12/C14 lactylates verified that no levels of dioxins or PCBs were present above detection limits.

Additionally, analytical data were provided on representative commercial lots of the market formulation, ALOAPUR® PM, demonstrating that a consistent product is manufactured which conforms with proposed compositional and contaminant specifications. Sodium sulfate is used as a processing aid in ALOAPUR® PM and the level is limited to less than (b) (4)

A shelf-life of 24 months is proposed for C12/C14 lactylates in the form of ALOAPUR® PM when stored unopened in the original packaging under cool and dry conditions. Stability data were provided for one representative commercial lot of ALOAPUR® PM to verify the shelf-life of C12/C14 lactylates under real-time conditions (25°C, 60% RH). A further 2 commercial lots of ALOAPUR® PM stored under warehouse conditions were shown to conform with product specifications after 5 to 6 years of storage. An accelerated shelf-life study was also conducted in which 3 representative lots of ALOAPUR® PM were stored for 6 months at 40°C and 75% RH. These studies confirm that the during prolonged storage, a small amount of hydrolysis of the ester bond to form the free fatty acids can occur. These findings are not considered to impact the utility or safety of the product on the basis that hydrolysis of the esters occurs rapidly on ingestion of C12/C14 lactylates to yield free fatty acids and lactic acid which are absorbed and utilized by the animal.

Studies in feed demonstrate that C12/C14 lactylates can be mixed homogeneously into feed and exhibits acceptable stability to the pelleting process. Feed containing C12/C14 lactylates also displays acceptable stability when stored under ambient conditions for 3 months.

C12/C14 lactylates comprises an equilibrium mixture of sodium lauroyl-1-lactylate (*ca*. 33%), sodium myristoyl-1-lactylate (*ca*. 14%), sodium laurate (C12 fatty acid; *ca*. 18%), sodium myristate (*ca*. 8%), sodium lactate (*ca*. 9%), sodium lauroyl-2-lactylate (*ca*. 6%), sodium myristoyl-2-lactylate (*ca*. 2%), sodium lactoyl lactate (*ca*. 3%) and minor amounts of oligomers of lactate (not quantified). In this respect, animals will be provided with lauric (C12) and myristic (C14) acids as well as lactic acid in the free acid (or sodium salt) and esterified form. Based on this typical composition, C12/C14 lactylates is calculated to comprise around 42% lauric acid, 19% myristic acid and 28% lactic acid. The total fatty acids content is therefore, in the region of 61% (i.e., lauric acid + myristic acid). (b) (4)

C12/C14 lactylates is intended for use as a source of lauric and myristic acids in feed at levels in the region of 0.7 g/kg complete feed and not exceeding 1.8 g/kg complete feed. The equivalent levels of ALOAPUR® PM (the market formulation) are 2 g and 5 g/kg complete feed, respectively. At the maximum intended level of 1.8 g C12/C14 lactylates/kg complete feed, animals are estimated to be provided with 0.8 g lauric acid, 0.3 g myristic acid and 0.5 g lactic acid/kg complete feed. The combined

lauric acid and myristic acid intake from C12/C14 lactylates is estimated to be around 1.1 g/kg complete feed. The target animals are all major and minor food-producing animals as well as companion animals.

Medium- and long-chain saturated fatty acids have recognized nutritional value for animals although no specific dietary requirements are set. C12/C14 lactylates are intended to provide a supplemental source of these fatty acids, contributing to the overall fatty acid profile of the complete feed. The fatty acid-containing ingredient will be included in the diet alongside other common fats and fat-containing nutrient sources. The fatty acid and energy requirements of the animal will largely be met by these other nutrient sources, and no detrimental impact on the nutritional quality of the feed is anticipated under the conditions of intended use of C12/C14 lactylates in feed.

The lactic acid esters of lauric and myristic acid will be enzymatically hydrolyzed to lauric acid, myristic acid and lactic acid in the GI tract of animals. Evidence for the hydrolysis of C12/C14 lactylates by gastric lipases is primarily provided by findings of a series of published *in vitro* studies (Phillips *et al*., 1981) using a structurally related substance, calcium stearoyl-lactylate. Unpublished *in vitro* studies provide corroborative evidence of hydrolysis by gastric lipases (Hodge, 1961). Lactic acid esters of lauric acid, myristic acid and stearic acid are expected to be hydrolyzed in an analogous manner in the GI tract of animals and it is reasonable to extrapolate available ADME data on calcium stearoyl-lactylate to C12/C14 lactylates. Direct evidence for the validity of this extrapolation is provided by an unpublished study in broilers conducted by Corbion demonstrating the absorption or digestion of C12/C14 lactylates from the GI tract. Moreover, sodium and calcium salts of lactylates, analogous to other carboxylic acids and their salts, including fatty acids and lactic acid, will dissociate in vivo to the respective anions and cations, supporting the use of data in calcium salts to support sodium salts of lactylates.

The metabolism of the structurally related lactylate, calcium stearoyl (C18) lactylate also has been studied in the series of published ^{14}C-labeling experiments in rats, mice and guinea pigs conducted by Phillips *et al*., 1981. The findings of these studies indicate that lactylates are rapidly absorbed from the GI tract as their component parts and extensively metabolized by animals. Consistent with hydrolysis of lactylates to their constituents, the metabolism of a mixture of stearic and lactic acids was shown to be comparable to that of calcium stearoyl-lactylate in an unpublished radiolabeled study in rats (Hodge, 1955).

Together, the published data, supported by further body of unpublished information, demonstrate that C12/C14 lactylates will be hydrolyzed by gastric lipases in animals to lauric acid, myristic acid and lactic acid. Thus, the toxicological assessment of C12/C14 lactylates can be based on publicly available information on the individual components.

Medium- and long-chain saturated fatty acids such as lauric acid and myristic acid are well-documented in the published literature as normal components of vegetable and animal fats. No differences are anticipated in the metabolism of lauric and myristic acids as components of C12/C14 lactylates and as constituents of biological fats forming part of the normal diet. In general, fat products marketed in the U.S. under Section 33 of the AAFCO OP will potentially provide natural sources of these fatty acids in the animal feed. An assessment was conducted to estimate the potential exposure by animals to lauric and myristic acids from CO, CM and PKM at typical levels of inclusion in the diet of animals. These ingredients were identified to be particularly rich in lauric and myristic acids and although they are not

the most common nutrient sources used in feed in the U.S., they have some recognized history of use and are widely utilized in other parts of the world. Overall, it was estimated that lauric and myristic acid from products of the coconut and palm kernel industries are present at significantly higher dietary levels in feed than will be provided by C12/C14 lactylates. Thus, the safety of the lauric and myristic acid components of C12/14 lactylates can be established from their natural presence in the background diet of animals.

Additionally, lauric and myristic acid have a long and established history of use as additives in food and as such have been the subject of evaluations by EFSA (2017a) and JECFA (1998). As part of these evaluations, EFSA and JECFA have reviewed a body of limited toxicological information on lauric and myristic acid, including the results of acute oral toxicity studies, *in vitro* mutagenicity assays and an 18-week feeding study in rats. Overall, the studies provide supporting evidence for the safety of the lauric and myristic acid components of C12/C14 lactylates under the conditions of intended use.

Lactic acid and calcium lactate have a long and established history of safe use as a technological additive (preservative) in feed and food in the U.S., the EU and elsewhere. The acid is rapidly absorbed by animals and ultimately metabolized to carbon dioxide and water. Animals will be exposed to lactic acid from the normal diet, particularly through the use of FLF for pigs and ensiled forages for ruminants. The estimated levels of exposure by swine and ruminants to lactic acid from these feeds is significantly higher than from the conditions of intended use of C12/C14 lactylates. Likewise, the use of lactic acid as a preservative in feed is estimated to be higher than the exposure by animals from the intended use of C12/C14 lactylates. Recognizing that exposure by poultry to lactic acid from the background diet to poultry is lower than for swine and ruminants, additional evidence of safety for these species are provided by a body of published data, as well as one unpublished study reported by EFSA (2019), in which broilers tolerated levels of 30,000 mg/kg of lactic acid for 35 to 42 days. A margin of safety of 60 can be calculated from the study compared to the intended use of lactic acid as a component of C12/C14 lactylates in feed at levels of 1.8 g/kg complete feed, equivalent to 0.5 g lactic acid/kg complete feed. Thus, the findings of these studies support the extrapolation of data on the safety of lactic acid to all target animals, including avian species. Only limited toxicological information is available on lactic acid and its salts, including acute oral toxicity studies and a number of feeding studies in rodents and dogs. The results of these studies also provide supporting evidence for the safety the intended use of C12/C14 lactylates in feed.

It is recognized that C12/C14 lactylates also contains up to 8% sodium. Under the conditions of intended use, animals will be exposed to not more than 1.8 g C12/C14 lactylates/kg complete feed, equating to 0.14 g sodium/kg complete feed. The requirements of animals for sodium varies with species but is reported by the NRC to typically fall within the range of 1 to 2 g sodium/kg complete feed. Thus, the sodium component of C12/C14 lactylates will contribute around 7 to 14% of the dietary requirements for this mineral and is not therefore, expected to pose a safety concern to the target animals. Additionally, ALOAPUR® PM contains (b) (4) sodium sulfate and the contribution of this component to the MTL of sulfur in the feed of animals is negligible. Moreover, the amount of sulfate present in the feed from the intended use of C12/C14 lactylates falls 100 times below the level of 0.3% known to pose tolerability concerns to ruminants.

The data outlined above are considered sufficient to demonstrate the safety of C12/C14 lactylates for the intended use in animal feed. Moreover, sufficient data are available in the public domain to draw key conclusions on the safety of C12/C14 lactylates and these data, together with corroborative unpublished data, allow extrapolation to all categories and species of animal on the basis that (a) the metabolic fate of C12/C14 lactylates is common in all animals; (b) lauric acid, myristic acid and lactic acid are metabolized by well-established pathways; and (c) there is a long and established history of safe consumption of these components from the background diet as well as from additive use (lactic acid).

Considering that C12/C14 lactylates will be hydrolyzed into lauric acid, myristic acid and lactic acid which will be metabolized by established fatty acid and tricarboxylic acid pathways in animals, no deposition of new substances, or existing substances at higher levels normally observed, is anticipated in the edible tissues under the conditions of intended use. Thus, no human food safety concerns are anticipated under the conditions of intended use of C12/C14 lactylates in the diets of food-producing animals at levels of up to 1.8 g/kg complete feed as-fed.

Following a critical evaluation of the data and information summarized above, it can be concluded that C12/C14 lactylates produced by Corbion using suitable food-grade materials in accordance with cGMP and meeting appropriate feed grade specifications, is safe and suitable for the intended use as a source of lauric and myristic acids in animal feed. It is further concluded that Corbion's C12/C14 lactylates is generally recognized as safe (GRAS) for use in feed based on scientific procedures.