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CONTAINS CONFIDENTIAL BUSINESS INFORMATION

GENERALLY RECOGNIZED AS SAFE (GRAS) NOTIFICATION FOR BASIC METHACRYLATE COPOLYMER

Date **June 2017**
Made by **Ramboll Environ**

Ramboll Environ
4350 North Fairfax Drive
Suite 300
Arlington, VA 22203
USA
T +1 703 516 2300
F +1 703 516 2345
wwww.ramboll-environ.com

RAMBOLL ENVIRON

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Part 1. Signed Statements and Certification

1.1 Statement of Submission

In accordance with 21 CFR § 170, subpart E, Ramboll Environ is pleased to present a "Generally Recognized as Safe" (GRAS) notice to the U.S. Food and Drug Administration (FDA) for Basic Methacrylate Copolymer.

At the request of the Massachusetts Institute of Technology (MIT), Ramboll Environ conducted a safety evaluation of Basic Methacrylate Copolymer, and has concluded, based on scientific procedures, that Basic Methacrylate Copolymer is GRAS for use as a protective coating for micronutrients.

Under the Federal Food, Drug, and Cosmetic Act as amended (the "Act"), GRAS substances are exempt from premarket approval by FDA. A substance is GRAS if experts qualified by scientific training and experience to evaluate the safety of substances added to food agree that the substance is safe and generally recognized as safe for its intended use (21 CFR §170.30).

1.2 Name and Address of Organization

This notice is filed by:

Ramboll Environ US Corporation
4350 North Fairfax Drive, Suite 300
Arlington, VA 22203.

1.3 Substance Name

The subject of this notice is Basic Methacrylate Copolymer.

1.4 Intended Conditions of Use

Basic Methacrylate Copolymer is intended for use as a protective coating for a variety of micronutrients to help prevent degradation under adverse transportation, storage, and use conditions. This technology enables a broad array of foods to be fortified that currently are not a good source of important nutrients.

1.5 Statutory Basis for Conclusions

This report has been prepared in accordance with the scientific requirements outlined in 21 CFR § 170.30(b): "General recognition of safety based upon scientific procedures shall require the same quantity and quality of scientific evidence as is required to obtain approval of a food additive regulation for the ingredient. General recognition of safety through scientific procedures shall ordinarily be based upon published studies which may be corroborated by unpublished studies and other data and information."

1.6 Exemption from Premarket Approval

The substance of this notification is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act, based on Ramboll Environ's conclusion that it is safe and GRAS under the conditions of its intended use.

1.7 Availability of Data and Information

Should the need arise for FDA to seek the data and information forming the basis of Ramboll Environ's conclusions, Ramboll Environ agrees to the following:

i. Upon FDA's request, to make the data and information available to FDA;

ii. Upon FDA's request, to allow the FDA to review and copy the data and information during customary business hours at the following address, where the data and information will be available to FDA:

Ramboll Environ
4350 North Fairfax Drive, Suite 300
Arlington, VA 22203
USA

iii. Upon FDA's request, to provide FDA with a complete copy of the data and information either in an electronic format that is accessible, or on paper.

1.8 Freedom of Information Act (FOIA) Exemption

Figures 1 and 2 are currently exempt from disclosure under the Freedom of Information Act (FOIA) 5 USC 552 because they are part of a manuscript that is currently under review for publication. Once the manuscript is accepted for publication, the figures (and the supporting manuscript) will be disclosable.

1.9 Certification

Ramboll Environ certifies, to the best of its knowledge, that this GRAS notice is a complete, representative, and balanced submission of information that includes both favorable and unfavorable findings, known to Ramboll Environ and pertinent to the evaluation of the safety and GRAS status of the use of this product.

1.10 Name(s) and Positions(s) of Signatories

Based on an evaluation of relevant data laid out within this report, Basic Methacrylate Copolymer is considered to be safe for its intended use and generally recognized as safe (GRAS) under the terms of 21 CFR § 170.30(b).

We conclude that other "experts qualified by scientific training and experience to evaluate the safety of food and food ingredients" would agree.

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Joseph V. Rodricks, PhD, DABT

Principal, Ramboll Environ US Corporation

4350 North Fairfax Drive, Suite 300, Arlington, VA 22203

(b) (6)

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Duncan Turnbull, DPhil, DABT

Senior Science Advisor, Ramboll Environ US Corporation

4350 North Fairfax Drive, Suite 300, Arlington, VA 22203

1.11 Additional Authorizations

This Part is not applicable to this notice.

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

2.1 Identity of Basic Methacrylate Copolymer

Basic Methacrylate Copolymer is manufactured by Evonik Röhm GmbH and is designated by the International Union of Pure and Applied Chemistry (IUPAC) as follows: *Poly[(dimethylaminoethyl methacrylate)-co-(methyl methacrylate)-co-(butyl methacrylate)].* The molar ratio is (0.24:0.34:0.25) and the weight ratio % is (49:25:26).

2.2 Common or Trade Name

Basic Methacrylate Copolymer.

2.3 CAS Registry Numbers

The CAS Registry Number of Basic Methacrylate Copolymer is 24938-16-7.

2.4 Characteristic Properties

As described by ECHA (2008), basic methacrylate copolymer is a fully polymerized copolymer derived from acrylic and methacrylic acid esters. The substance is described as a cationic copolymer based on dimethylaminoethyl methacrylate and neutral methacrylic esters (butyl methacrylate and methyl methacrylate).

2.5 Source and Description of Manufacture

Basic Methacrylate Copolymer is manufactured by Evonik Röhm GmbH and sold under the trade name, Eudragit®. It is currently approved as an inactive ingredient in pharmaceutical products (http://www.accessdata.fda.gov/scripts/cder/iig/getiigWEB.cfm) under the name "DIMETHYLAMINOETHYL METHACRYLATE - BUTYL METHACRYLATE - METHYL METHACRYLATE COPOLYMER" in 19 products, including at up to 214 mg in an orally disintegrating tablet. It is also approved in the EU as a food additive with the designation, E1205, for use as a glazing agent/coating agent in solid food supplements as defined in Article 2 of Directive 2002/46/EC of the European Parliament and of the Council (4) at a level of 100 000 mg/kg (http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32011R1129&from=EN).

2.5.1 Residual Impurities

Evonik has published data on levels of residual monomers and solvents in four lots of Eudragit® E PO Basic Methacrylate Copolymer, both as manufactured, and after 37-49 months storage at ambient temperature.[1] This information is reproduced in Table 1, and shows very low levels of residual impurities, with no increase in impurities with storage.

2.5.2 Stability

Evonik has published specification data for four lots of Eudragit® E PO Basic Methacrylate Copolymer, both as manufactured, and after 37-49 months storage at ambient temperature.[1] This information is reproduced in Table 2, and shows that the polymer is stable for at least 36 months.

[1] Evonik Nutrition & Care GmbH. 2016. EUDRAGIT® E PO. Technical Information -- Storage Stability. STAB.EPO/E. May.

Table 1. Residual Monomers and Solvents in New and Aged Eudragit® E PO Polymer

	Specification	Strictest Limits	Lot No. G090131009		Lot No. G090131011		Lot No. G100331064		Lot No. G120231021	
Age (Months)		Stable for ≥36 months	0 mo.	39mo.	0 mo.	39 mo.	0 mo.	49 mo.	0 mo.	37 mo.
Monomers (ppm)	Ph. Eur.,INFO 7.1	< 2,500	325	240	363	282	475	402	273	223
Butyl methacrylate (ppm)	JPE, INFO 7.1	< 500	92	70	103	79	99	72	77	65
Methyl methacrylate (ppm)	JPE, INFO 7.1	< 1,000	18	5	20	1	6	6	11	13
Dimethylaminoethyl methacrylate (ppm)	JPE, INFO 7.1	< 1,000	215	165	240	202	370	324	185	145
Residual solvents:	Ph. Eur.**, USP**									
Isopropyl alcohol (%)	INFO 7.1	< 0.5	0.03	0.03	0.02	0.03	< 0.01	< 0.01	0.01	0.02
Methyl alcohol (%)	INFO 7.1	< 0.1	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
n-Butyl alcohol (%)	INFO 7.1	< 0.5	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

** Residual Solvents according to Ph. Eur. General Text 5.4., USP <467>

Table 2. General Specifications and Stability Data for Eudragit® E PO Polymer

	Specification	**Strictest Limits**	**Lot No. G090131009**		**Lot No. G090131011**		**Lot No. G100331064**		**Lot No. G120231021**	
Container			Cardboard box with inner PE bag		Cardboard box with inner PE bag		Cardboard box with inner PE bag		Cardboard box with inner PE bag	
Temperature		do not store above 25 °C	< 25 °C		< 25 °C		< 25 °C		< 25 °C	
Age (Months)		Stable for ≥36 months	0	39	0	39	0	49	0	37
Appearance	Ph. Eur., JPE, INFO 7.1	conforms	conforms	conforms	conforms	conforms	conforms	conforms	conforms	conforms
Dry substance (DS, %)	INFO 7.1	≥ 98.0	99.6	99.3	99.5	99.2	99.7	99.3	99.8	99.4
Loss on Drying (%)	Ph. Eur., JPE, INFO 7.1	≤ 2.0	0.4	0.7	0.5	0.8	0.3	0.8	0.2	0.6
Particle size (d50v/v, µm)	INFO 7.1	< 50	10	11	11	10	11	12	10	12
Alkali value (mg KOH/g DS)	INFO 7.1	162 – 198	174	175	173	174	175	174	176	174
Dimethylaminoethyl units, based on DS (%)	Ph. Eur., INFO 7.1	20.8 – 25.5	22.4	22.5	22.3	22.4	22.5	22.4	22.6	22.4
Assay (% Nitrogen)	JPE, INFO 7.1	4.0 – 6.0	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3
Viscosity (mPa·s)	Ph. Eur., INFO 7.1	3 – 6	4	5	4	5	5	5	4	5
Viscosity (mm^2/s)	JPE, INFO 7.1	2.5 – 5.5	3.4	3.4	3.4	3.3	3.3	3.3	3.3	3.3
Identity (IR spectrum of solid or film)	Ph. Eur., JPE, INFO 7.1	conforms	conforms	conforms	conforms	conforms	conforms	conforms	conforms	conforms
Absorbance at 420 nm	Ph. Eur., INFO 7.1	≤ 0.300	0.041	0.044	0.046	0.027	0.024	0.032	0.022	0.032

Part 3. Dietary Exposure

3.1 Background

Basic Methacrylate Copolymer will be used to coat various micronutrients to protect them from deterioration due to adverse transportation, storage, and use conditions (heat, humidity, and light exposure). The polymer coating is effective in maintaining the potency of the micronutrients under severe adverse conditions.

Eighty five percent of Americans don't consume FDA's RDI of the most important vitamins and minerals necessary for proper physical and mental development.[2] For instance, even though foods containing Vitamin A are commonly consumed by children in the US (i.e. fortified cereals and milk), 25% of children in the US do not get enough Vitamin A. Children who do not get enough micronutrients are at risk for compromised immune system, stunted physical growth, reduced mental capacity, chronic disease, and death. Loss in productivity and wages for the sick and higher assisted living costs are additional problems for adults. Many foods are difficult to fortify because they need to be cooked or boiled, which destroys the micronutrients (e.g., pasta, rice, bouillon). Increasing the variety of foods that can be fortified, increases the likelihood that all Americans will have affordable access to healthy food that they enjoy and want to eat. The use of Basic Methacrylate Copolymer in this way enables a broad array of foods to be fortified that currently are not a good source of important nutrients. The ability of Basic Methacrylate Copolymer to protect micronutrients from elevated temperature, light, and chemical reactions is illustrated in Section 6.2.

3.2 Current Uses of Basic Methacrylate Copolymer

Basic Methacrylate Copolymer has been used as an excipient in preparations for oral pharmaceuticals for many years since its introduction for this purpose in 1959. Basic Methacrylate Copolymer is used as a film coating material in order to achieve taste and odor masking as well as rapid disintegration in the gastrointestinal tract.

3.3 Estimated Daily Intake (EDI) from the Proposed Use

The intake of the polymer will vary depending upon the particular micronutrient and the fraction of the Recommended Daily Intake (RDA) or Adequate Intake (AI, where an RDA is not established) supplied. Table 3 illustrates the estimated intake of the polymer resulting from its use to coat a variety of micronutrients at 100%, 75%, and 50% of the respective RDA/AI for pregnant/lactating women (generally the highest requirement), based on Institute of Medicine (IOM) recommendations.[3]

As illustrated in Table 3, when used to coat all of the listed micronutrients supplied at 100% of the respective RDA/AI for pregnant/lactating women, the total intake of the polymer would be 2.3 g/day, with lower intakes with lower percentages of the RDA/AI, or where only some of the listed micronutrients are supplied. For example, if only vitamin A, folic acid, and iron were supplied, each at the RDA, the total daily intake of the polymer would be 0.79 g. As the technology improves, it may be possible to use lesser amounts of the polymer to coat micronutrients effectively, or to coat different substances. With any such changes in usage of the polymer, care will be taken to ensure an adequate

[2] https://www.theguardian.com/lifeandstyle/2015/feb/10/nutrition-hunger-food-children-vitamins-us

[3] Otten JJ, Hellwig JP, Meyers LD, eds. 2006. Dietary Reference Intakes: The Essential Guide to Nutrient Requirements. Institute of Medicine, National Academies Press, Washington, DC.

margin of exposure between the EDI of the polymer and the animal NOAELs, as discussed in Section 6.5, below.

Table 3. Estimated Daily Intake of Basic Methacrylate Copolymer from Proposed Uses

	Intake of Polymer (g) from Use on Percentage of RDA/AI		
Micronutrient	**100%**	**75%**	**50%**
VitA (Retinyl palmitate)	0.011	0.008	0.006
VitB9 (Folic acid)	0.333	0.250	0.167
VitB12 (Cobalamin)	0.002	0.001	0.001
VitD3	0.002	0.001	0.001
$FeSO_4$ (Iron)	0.445	0.334	0.223
$ZnSO_4$ (Zinc)	1.135	0.851	0.567
KIO_3 (Iodine)	0.012	0.009	0.006
VitB1 (Thiamin)	0.069	0.052	0.034
VitB2 (Riboflavin)	0.035	0.026	0.018
VitB5 (Pantothenic Acid)	0.228	0.171	0.114
VitB6 (Pyridoxine)	0.069	0.052	0.035
VitB7 (Biotin)	0.009	0.006	0.004
Total from all listed micronutrients:	2.349	1.762	1.174
Total, excluding zinc	1.2148	0.9111	0.6074

Part 4. Self-Limiting Levels of Use

Part 4 is not applicable to this GRAS notice.

Part 5. Experience Based on Common Use in Food Before 1958

The statutory basis for this notice is based upon scientific procedures (21 CFR § 170.30(b)). Hence Part 5 is not applicable to this GRAS notice.

Part 6. Narrative

6.1 Introduction

The available toxicological data on Basic Methacrylate Copolymer have been summarized in two published comprehensive reviews.[4] Ramboll Environ's evaluation of the safety of Basic Methacrylate Copolymer relied primarily on these reviews; a European Food Safety Authority (EFSA) scientific opinion on the proposed use of Basic Methacrylate Copolymer as a food additive (EFSA 2010), and a toxicological characterization of Basic Methacrylate Copolymer published by Eisele et al. (2011) for the purposes of a GRAS evaluation.

6.2 Functionality Information

As noted in Section 3.1, Basic Methacrylate Copolymer is intended for use to provide a protective coating on micronutrients supplied to individuals who might otherwise suffer from nutritional deficiencies because of the instability of some nutrients under adverse conditions of transportation, storage, and use, particularly in developing countries, but also in the United States. The ability of Basic Methacrylate Copolymer to provide this protection has been investigated, and results of these studies, currently under review for publication, are briefly summarized below. Full details of this work is included in the attached preprint (Xu et al. 2017), which must remain confidential and embargoed until it has been accepted for publication.

Briefly, eleven individual micronutrients [vitamin A (retinyl palmitate), vitamin B9 (folic acid), vitamin B12 (cobalamin), vitamin D3, $FeSO_4$ (iron), $ZnSO_4$ (zinc), KIO_3 (iodine), vitamin B1 (Thiamin), vitamin B2 (Riboflavin), vitamin B5 (Pantothenic Acid), vitamin B6 (Pyridoxine), and vitamin B7 (Biotin)], or a combination of four (vitamins A, D, B9, B12) were encapsulated using Basic Methacrylate Copolymer, with or without hyaluronic acid as an additional stabilizing excipient, producing particles approximately 200 µm in diameter. When encapsulated in this way, the micronutrients were not released into room temperature water, or into boiling water over two hours, but were released into simulated gastric fluid (SGF), largely within 30 minutes, as shown in Figure 1(a). Similarly with co-encapsulation of vitamins A, D, B9, B12, there was very little release of the vitamins into room temperature water, or into boiling water over two hours, but substantial release into SGF within 30 minutes, as shown in Figure 1(d, e, f, and g).

The release into SGF demonstrates that the micronutrients are bioavailable when ingested. This is confirmed *in vivo* in mice and humans (Xu et al. 2017).

[4] Eisele J, Haynes G, Rosamilia T. 2011. Characterisation and toxicological behaviour of basic methacrylate copolymer for GRAS evaluation. *Regul Toxicol Pharmacol*. 61(1):32-43; European Food Safety Authority (EFSA). 2010. Scientific Opinion on the use of Basic Methacrylate Copolymer as a food additive. *EFSA Journal* 8(2):1513.

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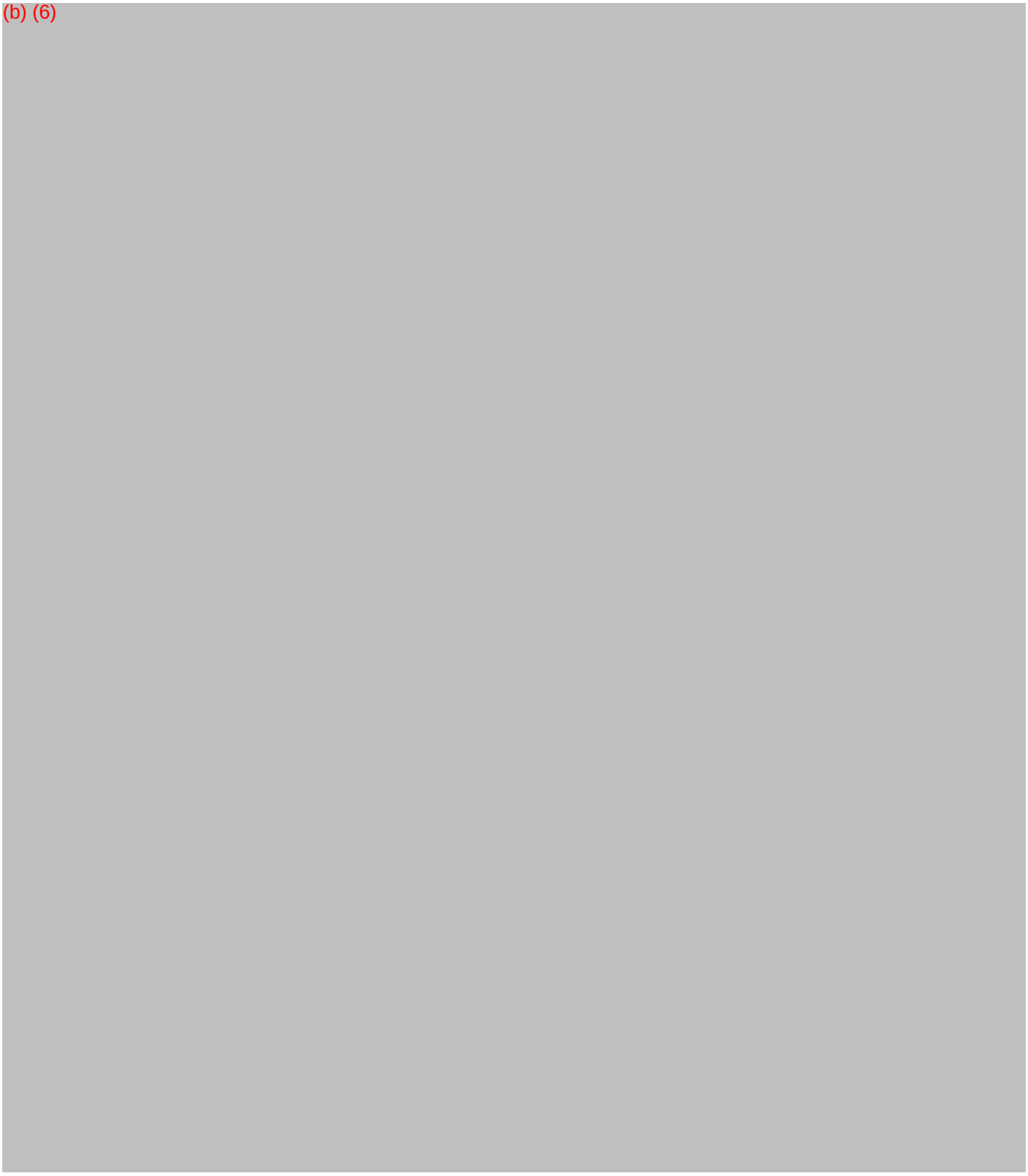

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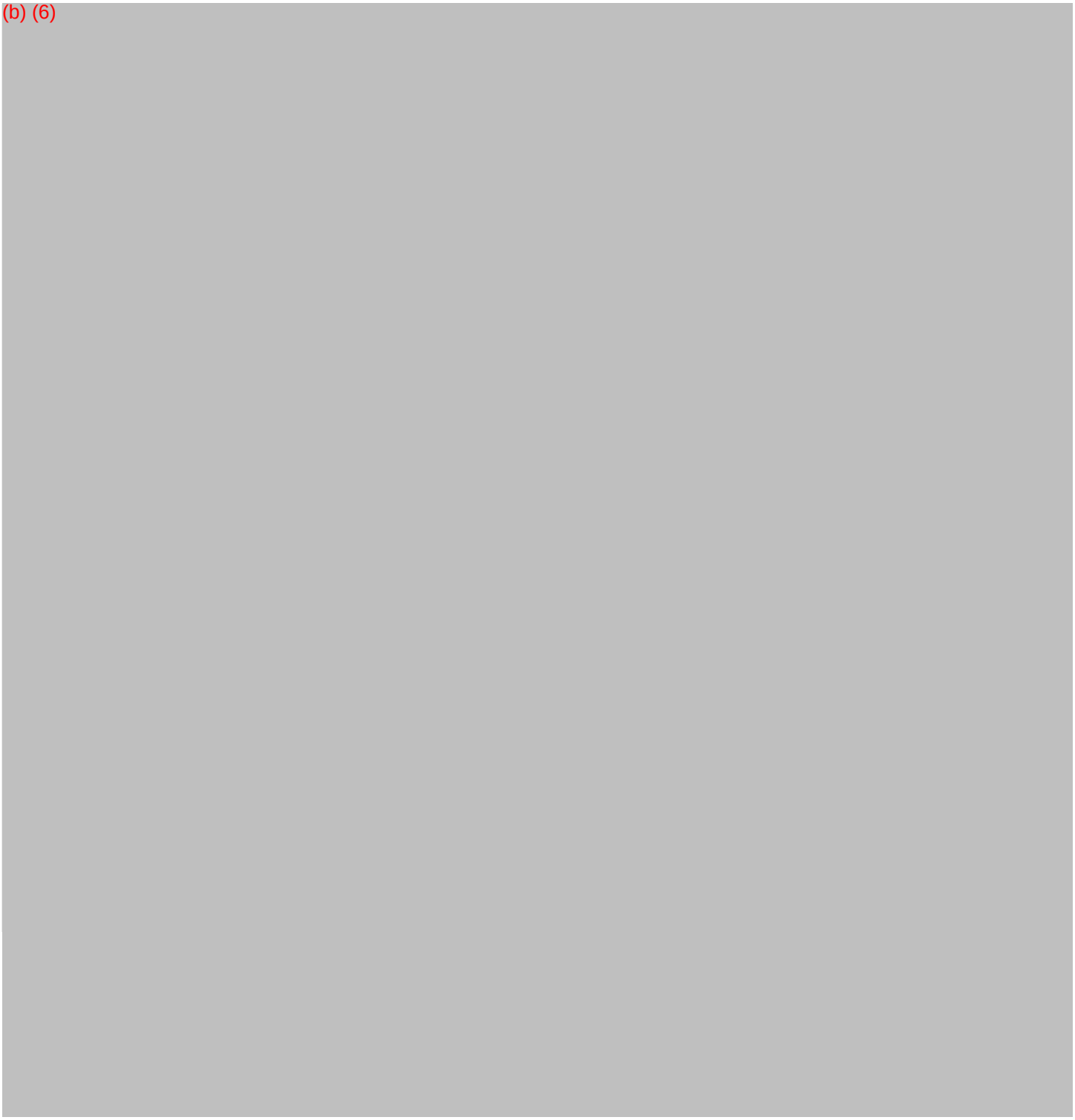

6.3 Animal Toxicology Data

6.3.1 Absorption, Distribution, Metabolism, and Excretion

The toxicokinetics of Basic Methacrylate Copolymer were evaluated in a study conducted by Daniel and Cooper (1979, as cited in Eisele et al. 2011 and EFSA 2010). In the first phase of the study, absorption was examined using five male rats with food and water available ad libitum. 40 mg of 14C-labelled aminoalkyl methacrylate copolymer E (Basic Methacrylate Copolymer) was administered as a single dose by gastric intubation at a volume of 1 mL/animal with a body weight range of 240-280 g. Urine and feces were collected from each animal at 24 hour intervals for five days prior to dosing, and five days after dosing. The animals were killed seven days after dosing. In a second phase of the study, nine male rats from the same source, and within the same body weight range, fasted overnight and were administered a single oral dose. The rats were killed in groups of three on day 1, 3 and 14 after dosing.

After each set of animals were killed, the kidneys, liver, mesenteric lymph nodes, spleen, small intestine, large intestine, and cardiac blood were removed to assess radioactivity. The major route of excretion was through the feces, with a mean total of 93.3% of the dose eliminated via this route, mostly within 48 hours following dosing. When feces of untreated animals were spiked with 14C-labelled product, similar values were also observed. Excretion in urine was low (0.013% of the dose being excreted over the five day collection period). Eisele et al. (2011) concluded that because radioactivity levels were increased relative to controls (though they were still close to background levels), minor absorption may be occurring at less than 0.02% of the administered dose. Levels of radioactivity in tissues and blood were similar to untreated controls, with no significant amount of any absorbed materials retained.

6.3.2 Acute Toxicity

Based on three older acute toxicity studies involving a single oral dose in rats, an LD_{50} of >3,000 mg/kg was identified (Leuschner 1970; Kinkel 1966; Kaneto 1970; all as cited in Eisele et al. 2011).

6.3.3 Short-term and Sub-Chronic Oral Toxicity

One sub-acute, and one sub-chronic study examined the potential toxicological effects of Basic Methacrylate Copolymer in beagle dogs and rats, respectively. Between the two studies, no treatment-related trends or effects of any biological significance were reported following a variety of analyses at different dose levels. The no observed adverse effect levels (NOAEL) observed in dogs and rats were 750 and 2,000 mg/kg/day, respectively, the highest dose levels investigated in each study. Study details and results are presented in Table 4.

Table 4. Summary of Short-term and Sub-chronic Oral Toxicity Studies on Basic Methacrylate Copolymer

Study (as cited in Eisele et al. 2011)	Design	Results
Bien 2003	Sub-acute: 28 days duration Beagle dogs Test substance: gelatin capsules (5.5 g/capsule) Treatment: 3 groups; each 3 males, 3 females, received oral gelatin capsules at 100, 300, 750 mg/kg bw daily Control: 1 group; 3 males, 3 females, received empty capsules daily	• No mortality observed in any of the groups • No abnormal clinical findings observed • Body weights changed slightly in all groups during study, but were within normal range. Appeared to be a trend in treated animals of both sexes, with statistically significant (SS) decreased weight in males in the 750 mg/kg/day group. This was matched with SS reduction in food consumption in the same group. Authors noted this may have been due to physicochemical properties of the copolymer rather than a toxicological effect. • No influence of treatment on hematological or urine clinical chemistry parameters considered to be of biological significance. Some SS differences observed for various parameters, both pre-dose and after 28 days, with no treatment-related trend. • Any observed organ weight differences were only slight in degree with no dose relationship apparent, and were considered to be of no toxicological relevance. • No alterations observed following macroscopic exam of the liver, and liver weight was within normal range. Microscopic findings were mild in degree, and characterized as common in young lab dogs. • The NOAEL was 750 mg/kg/day
Leuschner 1973	Sub-chronic: 26 weeks duration Sprague-Dawley rats Test substance: lacquer (film) of the copolymer sprayed onto powdered diet Treatment: 2 groups; 20 males, 20 females, received 500 and 2,000 mg/kg bw daily Control: 1 group; 20 males, 20 females, received diet coated with water (and dried) Clinical signs assessed daily, with weekly thorough exam. Blood and urine samples were taken from 10 rats/sex/group during weeks 6, 13, 18, 26	• No mortality observed in any of the groups • No clinical signs or different behavior were noted • Consistency and production of feces was normal in all animals • Sense of hearing was unaffected by treatment • Changes in body weight were unremarkable, though food consumption trended downward. No differences apparent between treated and control groups, and findings were within expected range. • Hematology and blood chemistry investigations revealed no significant treatment-related findings. The only notable finding occurred during week 13 when a statistically increased (relative to controls) total protein level was noted in the female 500 mg/kg/day group. This change was considered to be of no biological or toxicological significance. • No treatment-related effects observed in parameters assessed during urinalysis. Slight changes that did occur were regarded as being spontaneous, including traces of protein and individual leukocytes in the sediment. • There were no organ weight changes attributable to treatment, and no treatment-related findings (findings that were noted were regarded as a reflection of spontaneous pathology). • Microscopic examination of organs and tissues revealed no treatment-related findings, aside from some effects attributed to spontaneous pathology. • The NOAEL was 2,000 mg/kg/day

6.3.4 Chronic Toxicity

No data were available on the chronic toxicity of Basic Methacrylate Copolymer, however, the EFSA (2010) Panel concluded that such data was not required due to the high-molecular-weight of the substance, and its lack of absorption.

6.3.5 Genotoxicity

Two *in vitro* tests have been conducted to evaluate potential genotoxicity of Basic Methacrylate Copolymer (Miltenburger 1985; Wollny 2000, as cited by Eisele et al. 2011 and EFSA 2010). A single *in vivo* micronucleus assay was conducted by Cinelli (2000, as cited by Eisele et al. 2011 and EFSA 2010) to examine the potential genotoxicity of Basic Methacrylate Copolymer. Based on the results of these three studies, the EFSA (2010) Panel concluded that "basic methacrylate copolymer does not raise concern with respect to genotoxicity." Study details are presented in Table 5.

Table 5. Summary of Genotoxicity Studies on Basic Methacrylate Copolymer

Study (as cited in Eisele et al. 2011 and EFSA 2010)	Design	Results
Miltenburger 1985	*In vitro* Ames assay using four strains of *Salmonella typhimurium*: TA98, TA100, TA1535, TA1537. Doses: 0, 10, 33, 100, 333, 1000, 5000 µg/plate of Basic Methacrylate Copolymer with and without metabolic activation (S9 mix), 3 plates for each concentration were scored for mutant colonies.	• Precipitation occurred at doses above 5000 µg/plate • Background growth was slightly reduced in strain TA98 with copolymer concentrations of 5000 µg/plate • A reproducible mutagenic effect of the test substance was not observed in any of the bacterial strains, with or without the S9 mix. • Reversion rates were in the range of negative controls, and were considered spontaneous.
Wollny 2000	*In vitro* Mouse Lymphoma L5178Y cell mutation test (gene mutation assay) Experiment 1:four hour treatment period at doses of 2.0, 3.9, 7.8, 15.6, 31.3, 62.5 µg/mL of Basic Methacrylate Copolymer with and without metabolic activation (S9 mix) Experiment 2: 24 hour treatment period in the absence of S9 at doses of 1.9, 3.9, 7.8, 15.5, 31.0 µg/mL	• Precipitation of the test substance was visible at 62.5 µg/mL, but could also be observed with a microscope at concentrations as low as 7.8 µg/mL • In experiment 1, toxic effects were observed in the absence of S9 at doses of 62.5 µg/mL and above, and at doses of 31.3 µg/mL and above in the presence of S9. • In experiment 2, toxic effects were observed at doses of 31.3 µg/mL and above. • Cell growth at the lowest concentration was approximately within the range of the negative control. • No substantial and reproducible dose-dependent increase in mutant colony numbers was observed in both experiments, and the ratio of small vs. large colonies was not shifted up to the maximum concentrations tested. • Reference mutagens used as positive controls showed an apparent increase in induced mutant colonies and an increase of the relative quantity of small vs. large colonies.
Cinelli 2000	*In vivo* Bone marrow micronucleus assay in Swiss CD-1 mice (5/sex/dose) Doses (intraperitoneal): 250, 500, 1000 mg/kg bw and 500, 1000, 2000 mg/kg bw of Basic Methacrylate Copolymer for males and females, respectively. A negative control group of 5 animals per sex received 10 ml/kg 0.9% NaCl. A positive control was treated with 2 mg/kg Mitomycin-C.	• No increase in incidence of micronucleated polychromatic erythrocytes (PCE) from mouse bone marrow over the control was observed at any dose level and at any sampling period (24 or 48 hours). • A statistically significant increase of the incidence of micronucleated PCEs was demonstrated with the positive control.

6.3.6 Reproductive and Developmental Toxicity

A single study was available that examined a potential effect of Basic Methacrylate Copolymer on embryo-fetal toxicity (Leuschner 1968, as cited in Eisele et al. 2011 and EFSA 2010). Two groups of Wistar rats were evaluated (20 in the test group, and 20 in the control group). The test group received approximately 1,000 mg/kg bw/day in the form of a lacquer coating solution on feed from day 6 to 16 of gestation. There was no evidence of a treatment effect on maternal animals. All animals were pregnant with live fetuses at termination. Food consumption was similar in both treatment and control groups, and changes in body weight throughout the study were considered normal. No maternal necroscopy findings were considered to be related to the treatment. There were no effects on fetal survival based on similar pre- and post-implantation losses, and litter sizes. Fetal and placental weights were unaffected by treatment, as was the incidence and types of major and minor fetal abnormalities. No statistically significant differences between the treatment and control groups were observed for any of the parameters examined. Therefore, the results of this study indicate a maternal and fetal NOAEL of 1,000 mg/kg bw/day.

6.4 Consideration of Maximum Safe Exposure Level

No adverse effects of any kind have been seen in any study with Basic Methacrylate Copolymer. This is not unexpected given the high molecular weight of the polymer and consequent low oral absorption, and the low level of residual monomers and other low molecular weight species. In its assessment of copolymer, EFSA (2010) refrained from deriving an acceptable daily intake (ADI), but rather compared the NOAEL in the six-month rat feeding study (2,000 mg/kg/day) with estimates of exposure from proposed uses. That is also the approach taken in this assessment.

6.5 Margin of Exposure (MOE)

As illustrated in Table 3, above, the total EDI of Basic Methacrylate Copolymer would be 2.3 g/day if used in all listed micronutrients, and each was supplied at 100% of its RDA or AI, or 0.79 g/day if only Vitamin A, folic acid, and iron were supplied each at 100% of its RDA, with lower intakes if the micronutrients were supplied at lower percentages of their RDAs. While the intake would be quite high if used on all micronutrients listed, a more likely exposure would involve just Vitamin A, folic acid, and iron. For a 70 kg person, this intake would provide an MOE of 177, indicating that such a usage would present no health risk. While the MOE would be smaller with more micronutrients encapsulated, even with all of the listed micronutrients at 100% of their RDA/AI, the MOE would still be more than 60-fold, which is adequate for a polymer like this that shows no adverse effects at any dose tested. Furthermore, as the technology improves, it may be possible to use lesser amounts of the polymer to coat micronutrients effectively. With any such changes in usage of the polymer, care will be taken to ensure an adequate margin of exposure between the EDI of the polymer and the animal NOAELs.

6.6 Conclusions

The proposed use of Basic Methacrylate Copolymer as a protective coating on micronutrients is safe and GRAS. Ramboll Environ has reviewed the available data and information related to Basic Methacrylate Copolymer, and is not aware of any data and information that are, or may appear to be, inconsistent with Ramboll Environ's conclusion of its GRAS status.

Part 7. List of Supporting Data and Information

Eisele J, Haynes G, Rosamilia T. 2011. Characterisation and toxicological behaviour of basic methacrylate copolymer for GRAS evaluation. *Regul Toxicol Pharmacol*. 61(1):32-43.

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