Toxicological profile for

Lecithin

This ingredient has been assessed to determine potential human health effects for the consumer. It was considered not to increase the inherent toxicity of the product and thus is acceptable under conditions of intended use.

1. Name of substance and physico-chemical properties

1.1. IUPAC systematic name

CAS RN 8002-43-5: [(2R)-3-Hexadecanoyloxy-2-[(9E,12E)-octadeca-9,12-dienoyl]oxypropyl] 2-(trimethylazaniumyl)ethyl phosphate (PubChem a); [(2R)-3-Hexadecanoyloxy-2-octadeca-9,12-dienoyloxypropyl] 2-(trimethylazaniumyl)ethyl phosphate (PubChem b); (3-Hexadecanoyloxy-2-octadeca-9,12-dienoyloxypropyl) 2-(trimethylazaniumyl)ethyl phosphate (PubChem c); (2R)-2,3-Diacetoxypropyl [2-(trimethylammonio)ethyl]phosphonate (ChemSpider a); O-{[(2S)-2,3-Bis(stearoyloxy)propoxy](hydroxy)phosphoryl}-L-serine (ChemSpider b); 2,3-Bis(stearoyloxy)propyl [2-(trimethylammonio)ethyl]phosphonate (ChemSpider c), (2R)-3-[(9E,12Z)-9,12-Octadecadienoyloxy]-2-(palmitoyloxy)propyl 2-(trimethylammonio)ethyl phosphate (ChemSpider d); (2R)-2-[(9Z,12Z)-9,12-Octadecadienoyloxy]-3-(palmitoyloxy)propyl 2-(trimethylammonio)ethyl phosphate (ChemSpider e)

CAS RN 85711-58-6: No data available to us at this time.

1.2. Synonyms

CAS RN 8002-43-5: LECITHIN; 1,2-Diacyl-sn-glycero-3-phosphocholine; Soybean phospholipid; Soybean Lecithin; (3-hexadecanoyloxy-2-octadeca-9,12-dienoyloxypropyl) 2-(trimethylazaniumyl)ethyl phosphate; (R)-2-((9Z,12Z)-octadeca-9,12-dienoyloxy)-3-(palmitoyloxy)propyl 2-(trimethylammonio)ethyl phosphate; L-alpha-Lecithin; 1-hexadecanoyl-2-(9E,12E-octadecadienoyl)-sn-glycero-3-phosphocholine; 1,2-Diacyl-sn-glycero-3-phosphocholine; 2-Linoleoyl-1-palmitoyl-sn-glycero-3-phosphocholine; phosphatidylcholines; 3,5,8-Trioxa-4-phosphahexacosa-17,20-dien-1-aminium, 4-hydroxy-N,N,N-trimethyl-9-oxo-7-[[(1-oxohexadecyl)oxy]methyl]-, inner salt, 4-oxide, (R)-; L-a-Phosphatidylcholine; Phosphatidylcholines soya; Soybean phosphatidylcholine; 2-linoleoyl-1-palmitoyl-sn-glyc-ero-3-phosphocholine; 1-Hexadecanoyl-2-(cis-9,12-octadecadienoyl)-sn-glycero-3-phosphocholine (PubChem a-c).

CAS RN 85711-58-6: Lecithins, hydrolyzed; EINECS 288-318-8 (PubChem d).

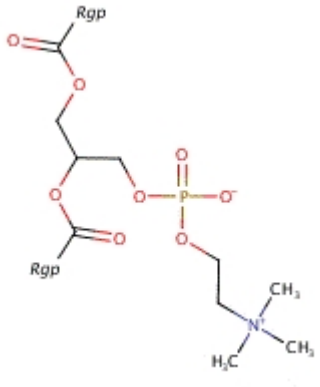
1.3. Molecular formula

CAS RN 8002-43-5: C42H80NO8P (PubChem a–c)

CAS RN 85711-58-6: No data available to us at this time.

1.4. Structural Formula

CAS RN 8002-43-5:



1.5. Molecular weight (g/mol)

CAS RN 8002-43-5: 758.1 (PubChem a–c)

1.6. CAS registration number

8002-43-5; 85711-58-6

1.7. Properties

1.7.1. Melting point

(°C): **CAS RN 8002-43-5:** 90.27 (estimated) (EPISuite, 2017); 236-237 (PubChem a-c)

1.7.2. Boiling point

(°C): **CAS RN 8002-43-5:** 816.3±75.0 at 760 mmHg (estimated) (ChemSpider b); 480 (estimated) (EPISuite, 2017)

1.7.3. Solubility

CAS RN 8002-43-5: 2.751 x 10^{-11} mg/L at 25°C (estimated) (EPISuite, 2017); Only partially soluble in water; readily hydrates to form emulsions (JECFA, 2007); Insoluble in water (PubChem a-c)

1.7.4. pKa

No data available to us at this time.

1.7.5. Flashpoint

(°C): **CAS RN 8002-43-5**: 447.5±37.1 (estimated) (ChemSpider b)

1.7.6. Flammability limits (vol/vol%)

No data available to us at this time.

1.7.7. (Auto)ignition temperature

(°C): No data available to us at this time.

1.7.8. Decomposition temperature

(°C): No data available to us at this time.

1.7.9. Stability

Incompatible materials: Strong oxidizing agents (PubChem a-c)

1.7.10. Vapor pressure

CAS RN 8002-43-5: 1.78 x 10^{-10} mmHg at 25°C (estimated) (EPISuite, 2017); 0.0±6.3 mmHg at 25°C (estimated) (ChemSpider b)

1.7.11. log Kow

CAS RN 8002-43-5: 13.41 (estimated) (EPISuite, 2017); -4.45, 10.90, 12.55 or 16.13 (estimated) (ChemSpider a-e); 12.9 (estimated) (PubChem a–c)

2. General information

2.1. Exposure

Lecithin (CAS RN 8002-43-5/8030-76-0) (soybean) is used as an antistatic, skin conditioning – emollient, surfactant - emulsifying and skin conditioning agent in cosmetics in the EU.

Phosphatidylcholine (CAS RN 8002-43-5) is used as a surfactant - emulsifying and skin conditioning agent in cosmetics in the EU.

Lysolecithin (CAS RN 85711-58-6) is used as a surfactant - emulsifying agent in cosmetics in the EU.

As taken from CosIng (undated).

Lecithin and Hydrogenated Lecithin are used in a large number of cosmetic formulations as skin conditioning agents-miscellaneous and as surfactant-emulsifying agents. Hydrogenated Lecithin is also used as a suspending agent-nonsurfactant. Historical data on concentration of use of Lecithin reveals that 0.1% to 1.0% is the concentration range most frequently seen, with concentrations up to 50% reported for two moisturizing products. A solution of 65% Lecithin is currently reported to be used at concentrations up to 3% in cosmetics (Fiume, 2001).

Lecithins (CAS RN 8002-43-5) are listed as fragrance ingredients on the US EPA InertFinder Database and by IFRA.

Lecithin (CAS RN 8002-43-5) is listed as an ingredient (at given concentrations, where specified) in auto, home maintenance (1.0-3.0%, includes an 'old' product), inside the home, personal care (1-3%), pesticide (0.1-7.5%) and pet care (>1%) products by the CPID.

"The aim was to describe the exposure to excipients among neonates hospitalised in the neonatal intensive care unit (NICU) of a public hospital in Brasilia, Brazil. This was a retrospective study based on medicines that were prescribed electronically to neonates (≤28 days) who were admitted to the NICU of a hospital in Brasilia between January 1 and March 31, 2012. Excipients were identified from the medicine package leaflets and were classified according to toxicity. Seventy-nine infants received a total of 1,303 prescriptions comprising 77 formulations and 70 active drugs. Eighty-six excipients were identified, of which, 9 were harmful excipients (HE) and 48 were potentially harmful excipients (PHE). Almost all the neonates (98.7 %) were exposed to at least one HE and PHE. Preterm neonates (n = 64; 1,502 neonate days) presented high risk of exposure to polysorbate 80 (3.26/100 neonate days), sodium hydroxide (3.39), PG (3.19) and propylparaben (3.06). Full-term neonates (n = 15; 289 neonate days) presented risks in relation to phenol (4.84), ethanol (3.8) and sodium citrate (3.46). CONCLUSION: Neonates in NICUs in Brazil are exposed to a wide variety of HE and PHE with unpredictable results. Safer alternatives are needed, as well as further studies on the subject." As taken from Souza A Jr et al. 2014. Eur. J. Pediatr. 173(7), 935-45. PubMed, 2015 available at http://www.ncbi.nlm.nih.gov/pubmed/?term=24500397

The Panel considered that the refined exposure assessment approach resulted in more realistic long-term exposure estimates compared to the maximum level exposure assessment scenario. From the refined estimated exposure scenario, in the brand-loyal scenario, mean exposure to lecithins (E 322) ranged from 7 mg/kg bw per day in adolescents to 82 mg/kg bw per day in children. The 95th percentile ranged from 15 mg/kg bw per day in adolescents to 187 mg/kg bw per day in children. In the non-brand-loyal scenario, mean exposure ranged from 3 mg/kg bw per day in adults/elderly to 22 mg/kg bw per day in toddlers. The 95th percentile ranged from 6 mg/kg bw per day in adults/elderly to 62 mg/kg bw per day in infants.

The Panel considered that dietary intakes of lecithins (E 322) from the regular diet could be estimated in average ranging from 4 to 71 mg/kg bw per day across all population age groups.

As taken from EFSA, 2017

“Dietary exposure assessment using food-consumption data and ingredient-use level is essential for assessing the safety of food ingredients. Dietary exposure estimates are compared with safe intake levels, such as the acceptable daily intake (ADI). The ADI is estimated by applying a safety factor to an experimentally determined no-observed-adverse-effect level of a test substance. Two food ingredients classified as emulsifiers, carboxymethylcellulose (CMC) and polysorbate 80 (P80), received attention recently due to their putative adverse effects on gut microbiota. Because no published dietary exposure estimates for commonly used emulsifiers exist for the US population, the current investigation focused on the estimation of dietary exposure to seven emulsifiers: CMC, P80, lecithin, mono- and diglycerides (MDGs), stearoyl lactylates, sucrose esters, and polyglycerol polyricinoleate. Using maximum-use levels obtained from publicly available sources, dietary exposures to these emulsifiers were estimated for the US population (aged 2 years and older) for two time periods (1999-2002 and 2003-10) using 1- and 2-day food-consumption data from the National Health and Nutrition Examination Survey, and 10-14-day food-consumption data from NPD Group, Inc.'s National Eating Trends - Nutrient Intake Database. Our analyses indicated that among the emulsifiers assessed, lecithin and MDGs have the highest mean exposures at about 60 and about 80 mg kg-1 bw day-1, respectively, whereas the exposure to CMC is half to one-third that of lecithin or MDGs; and the exposure to P80 is approximately half that of CMC. The review of available safety information such as ADIs established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), in light of our updated dietary exposure estimates for these seven emulsifiers, did not raise safety concerns at the current specified levels of use. Additionally, by examining two time periods (1999-2002, 2003-10), it was concluded that there is no evidence that exposure levels to emulsifiers have substantially increased.” As taken from Shah R et al. 2017. Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess. 34(6), 905-917. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28346062

Lecithin (CAS RN 8002-43-5) is used as an antioxidant synergist, coating agent, filler, gelling agent, oleaginous vehicle, preservative antioxidant, skin-conditioning agent, solubilizing agent, stabilizing agent, surfactant - emulsifying agent and thickening agent in oral and/or topical non-medicinal natural health products. It is also used as a homeopathic material (no CAS RN given) (Health Canada, 2021).

2.2. Combustion products

No data available to us at this time.

2.3. Ingredient(s) from which it originates

LECITHIN: Usually prepared from oil-bearing seeds used for food, especially soybeans; may also be prepared from animal sources; a complex mixture of acetone-insoluble phosphatides which consists chiefly of phosphatidylcholine, phosphatidyl- ethanolamine, and phosphatidyl-inositol, combined with various amounts of other substances such as triglycerides, fatty acids, and carbohydrates; refined grades may contain any of these components in varying proportions and combinations depending on the type of fractionation used; its oil-free forms, the preponderance of triglycerides and fatty acids is removed and the product contains 90% or more of phosphatides representing all or certain fractions of the total phosphatide complex. (JECFA, 2007)

LECITHIN, PARTIALLY HYDROLIZED: Prepared by partial hydrolysis of lecithin by the use of a suitable lipase. When the desired degree of hydrolysis is attained, the product is heated in order to inactivate the residual enzyme. (JECFA, 2007)

An ADI 'not limited' was established at the 17th JECFA (1973)

Lecithin is a naturally occurring mixture of the diglycerides of stearic, palmitic, and oleic acids, linked to the choline ester of phosphoric acid, commonly called phosphatidylcholine. Hydrogenated Lecithin is the product of controlled hydrogenation of Lecithin (Fiume Z, 2001).

CAS RN 8002-43-5: “Lecithin is a component of the cell membrane and commercially extracted from soybeans and egg yolk” (PubChem c).

Lecithin (CAS RNs 8002-43-5 / 8030-76-0 (soybean)) is the complex combination of diglycerides of fatty acids linked to the choline ester of phosphoric acid.

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As taken from CosIng (undated).

“Lecithins are mixtures or fractions of phosphatides obtained by physical procedures from animal or vegetable foodstuffs.”

As taken from EFSA, 2017

3. Status in legislation and other official guidance

Lecithin (CAS RN 8002-43-5) is included on the US FDA’s list of Substances Added to Food (formerly EAFUS) as an anticaking or free-flow agent, antioxidant, drying agent, emulsifier or emulsifier salt, humectant, lubricant or release agent, masticatory substance, nutrient supplement, surface-active agent and texturizer, is generally recognized as safe (GRAS) under 21 CFR section 184.1400 (Lecithin) and is also covered under 21 CFR sections 133.169 (Pasteurized process cheese); 133.173 (Pasteurized process cheese food); 133.179 (Pasteurized process cheese spread.); 136.110 (Bread, rolls, and buns); 169.140 (Mayonnaise); 169.150 (Salad dressing); 175.300 (Resinous and polymeric coatings) and 176.170 (Components of paper and paperboard in contact with aqueous and fatty foods) (FDA, 2024a).

This substance has been evaluated for acceptable daily intake by the Joint FAO/WHO Expert Committee on Food Additives (see Annex 1, Ref. No. 7) in 1963. Evaluation: Estimate of acceptable daily intake for man: NOT LIMITED.

As taken from JECFA, 1974

Lecithins (CAS RN 8002-43-5) are not registered under REACH (ECHA).

There is a REACH dossier on lecithins, hydrolyzed (CAS RN 85711-58-6) (ECHA).

Lecithin (CAS RN 8002-43-5) is listed on the US FDA’s database of Select Committee on GRAS Substances (SCOGS) and is “generally recognized as safe” (GRAS) (21 CFR 184.1400) (FDA, 1979).

Lecithins (CAS RNs 8002-43-5; 8030-76-0) are listed on the US EPA InertFinder Database as approved for food, non-food and fragrance use pesticide products. Lecithins (CAS RN 8002-43-5) are listed on the US EPA Toxic Substances Control Act (TSCA) inventory and also on the US EPA 2024 CDR list (Chemical Data Reporting Rule) and 2024 CDR Partial Exempt list.

Neither lecithins (CAS RN 8002-43-5) nor lecithins, hydrolyzed (CAS RN 85711-58-6) are classified for packaging and labelling under Regulation (EC) No. 1272/2008 (ECHA, 2024).

Lecithins (CAS RN 8002-43-5) are included on the US EPA Safer Chemical Ingredients List (US EPA, 2024).

Lecithins (E322) are authorised for use as food additives in the EU under legislation (EU) Nos 1129/2011 and, as a member of Group I, Additives, also under 438/2013, 2015/0647, 2015/1832 and 2018/1497.

As taken from European Commission, a, b.

“Lecithins (E 322) is an authorised food additive in the European Union (EU) according to Annex II and Annex III to Regulation (EC) No 1333/2008 on food additives, and have been previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1973 (JECFA, 1974a,b) and by the Scientific Committee on Food (SCF) in 1982 (SCF, 1982).”

“The EFSA Panel concluded that there was no need for a numerical ADI for lecithins (E 322) and that there was no safety concern for the general population from more than 1 year of age at the refined exposure assessment for the reported uses of lecithins (E 322) as a food additive. The Panel further concluded that there is no safety concern for the exposure to the choline from lecithins (E 322) as a food additive at use and use levels reported by industry. For infants (from 12 weeks up to 11 months of age), the Panel concluded that there was no safety concern at the refined exposure assessment for the reported uses of lecithins (E 322) as a food additive and for the choline from lecithins (E 322) as a food additive at use and use levels reported by industry. For infants and young children consuming foods for special medical purposes, the Panel concluded that there was no safety concern with respect to the refined exposure assessment for the reported uses of lecithins (E 322) as a food additive and for exposure to choline resulting from these uses of lecithins (E 322).”

“The Panel recommended that the maximum limits for the impurities of toxic elements (lead, mercury and arsenic) in the EU specification for lecithins (E 322) should be revised in order to ensure that lecithins (E 322) as a food additive will not be a significant source of exposure to those toxic elements in food. The Panel recommended that the limit for cadmium should be included in the specifications.”

“The Panel noted some case reports of hypersensitivity reactions associated with soya and egg lecithins (see Section 3.5.7). The Panel agree with the opinion from EFSA NDA Panel (2014) that this hypersensitivity is due to the residual proteins in lecithins (E 322) and therefore their content should be reduced as much as possible.”

As taken from EFSA, 2017

Lecithins (CAS RN 8002-43-5) “pose no unreasonable risk to human health based on Tier I assessment under the NICNAS IMAP assessment framework” and have been “identified as low concern to human health by application of expert validated rules under the NICNAS targeted tier I approach” (AICIS, 2017).

LECITHIN

General Information

Chemical Names:	COMPLEX MIXTURE OF ACETONE-INSOLUBLE PHOSPHATIDES INCLUDING: PHOSPHATIDYL-CHOLINE, PHOSPHATIDYL-ETHANOLAMINE AND PHOSPHATIDYL-INOSITOL, COMBINED WITH VARIOUS AMOUNTS OF OTHER SUBSTANCES SUCH AS TRIGLYCERIDES, FATTY ACIDS AND CARBOHYDRATES
CAS number:	8002-43-5
INS:	322(i)
Functional Class:	ood Additives NTIOXIDANT EMULSIFIER

Evaluations

Evaluation year:	**1973**
ADI:	NOT LIMITED
Meeting:	17
Specs Code:	R (1993)
Report:	NMRS 53/TRS 539-JECFA 17/20

Tox Monograph:	FAS 5/NMRS 53A-JECFA 17/234
Specification:	COMPENDIUM ADDENDUM 11/FNP 52 Add. 11/89 (METALS LIMITS) (2003). R; FAO JECFA Monographs 1 vol.2/259
Previous Years:	1993, COMPENDIUM ADDENDUM 2/FNP 52 Add.2/65. R. PRESENT SPECIFICATIONS APPLY TO BOTH BLEACHED AND UNBLEACHED LECITHINS 1990, COMPENDIUM/835 (FOR BOTH BLEACHED AND UNBLEACHED LECITHINS). R 1986, FNP 37-JECFA 30/65. R,T (APPLIES TO BOTH BLEACHED AND UNBLE

LECITHIN, PARTIALLY HYDROLYZED

CAS number:	8002-43-5 (LECITHIN)
INS:	322
Functional Class:	ood Additives NTIOXIDANT_SYNERGIST EMULSIFIER YNERGIST

Evaluation year:	**1990**
Meeting:	43
Specs Code:	S (1993)
Specification:	COMPENDIUM ADDENDUM 8/FNP 52 Add.8/203 (METALS LIMITS) (2000). R; FAO JECFA Monographs 1 vol.2/263
Previous Years:	1993, SPECIFICATIONS CONFIRMED. S 1990, COMPENDIUM/841 (FOR BOTH BLEACHED AND UNBLEACHED LECITHINS). R 1986, FNP 37-JECFA 30/69. N

As taken from WHO, 2022.

Lecithin (CAS RN 8002-43-5) is included on the US FDA's list of inactive ingredients for approved drug products. It is permitted for use as an ingredient in various products, at the following maximum potencies per unit dose and maximum daily exposure:

Inactive Ingredient	**Route**	**Dosage Form**	**CAS Number**	**UNII**	**Maximum Potency per unit dose**	**Maximum Daily Exposure (MDE)**
CAPRYLIC/CAPRIC TRIGLYCERIDE/LECITHIN/ALCOHOL	ORAL	CAPSULE		NA	NA	
LECITHIN	INTRAMUSCULAR	INJECTION	8002435	NA	0.6%w/v	
LECITHIN	ORAL	CAPSULE	8002435	NA	15mg	
LECITHIN	ORAL	CAPSULE,	80024	NA	NA	

		EXTENDED RELEASE	35			
LECITHIN	ORAL	CAPSULE, LIQUID FILLED	8002435	NA		3,900mg
LECITHIN	ORAL	POWDER, FOR SUSPENSION	8002435	NA	167mg/5 ml	
LECITHIN	ORAL	SUSPENSION	8002435	NA		150mg
LECITHIN	ORAL	TABLET	8002435	NA		40mg
LECITHIN	ORAL	TABLET, DELAYED RELEASE	8002435	NA		1mg
LECITHIN	ORAL	TABLET, EXTENDED RELEASE	8002435	NA		10mg
LECITHIN	ORAL	TABLET, FILM COATED	8002435	NA		2mg
LECITHIN	ORAL	TABLET, FILM COATED, EXTENDED RELEASE	8002435	NA	0.14mg	
LECITHIN	RECTAL	SUPPOSITORY	8002435	NA	6.5mg	
LECITHIN	TOPICAL	GEL	8002435	NA	1%w/w	
LECITHIN	TOPICAL	SOLUTION	8002435	NA	1.4%w/w	
LECITHIN	VAGINAL	CREAM	8002435	NA	1%w/w	

As taken from FDA, 2024b

Lecithin (CAS RN 8002-43-5) is classified as a natural health product (NHP) under Schedule 1, item 2 (an extract) of the Natural Health Products Regulations (Health Canada, 2021).

Lecithin (CAS RN 8002-43-5) is included on the US FDA's list: Inventory of Food Contact Substances Listed in 21 CFR and covered under sections 175.300 176.170, 184.1400 (Food additive and GRAS regulations (21 CFR Parts 170-186) and 133.169 , 133.173 , 133.179 , 136.110 , 169.115 , 169.140 , 169.150 Food labeling and standards regulations (21 CFR Parts 100-169).

As taken from FDA, 2024c.

4. Metabolism/Pharmacokinetics

4.1. Metabolism/metabolites

The major route of Lecithin (phosphatidylcholine) biosynthesis in mammalian cells is the cytidine diphosphate (CDP) choline pathway (George et al. 1991). Other routes of Lecithin synthesis include methylation of phosphatidylethanolamine, Ca(2+) dependent base exchange, and reacylation of lysophospholipid. Other references regarding Lecithin synthesis include Bjørnstad

and Bremer 1966; Price, Morris, and Hall 1989; Vance 1990; George et al. 1991; Tercé et al. 1991; Fisk and Kano-Sueoka 1992.

As taken from Fiume Z. Final Report on the Safety Assessment of Lecithin and Hydrogenated Lecithin. International Journal of Toxicology. 20 (Suppl. 1):21-45, 2001.

“Phosphatidylcholine and other phosphoglycerides are degraded through a series of so-called phospholipases to fatty acids, choline and glycerine metabolites to be in turn re-synthesised in the liver and other organs (Blumenthal et al., 2000).”

As taken from EMA, 2017

“Among lecithins, phosphatidylcholine is hydrolysed in choline in the cytidine-5-diphosphate-choline pathway in all cells of the body. The content of choline that can theoretically be released from phosphatidylcholine containing two linoleate groups is 13.2%.”

As taken from EFSA, 2017

4.2. Absorption, distribution and excretion

Five fasted human subjects, one male and four females, were given three capsules of radioactive Lecithin (1 g of Lecithin containing 50 µCi di-[l’-14C]linoleoyl-3-*sn*-glycerophosphocholine and 150 µCi 3H-polyenephosphatidylcholine) (Zierenberg and Grundy 1982). The subjects excreted 2 ± 0.7% and 4.5 ± 1.5% 3H and 14C, respectively, in the feces and 6 ± 0.8% and 1.2 ± 0.4%3H and 14C respectively, in the urine over 7 days. After a 2-hours lag time, radioactive lipids could be measured in the blood; the peak of 14C was reached between 4 and 12 hours and the peak of 3H was reached between 6 and 24 hours.

In rats and monkeys given Lecithin (soy phosphatidylcholine) orally, the major target organ was the liver, although significant amounts of radioactivity were detectable after 6 hours in striated muscle, depot fat, and the kidneys (Nattermann Phospholipid GmbH 1995). After repeated administration for 1 week, the organ distribution was similar, with additional small amounts of radioactivity in the lungs, testes, intestines, skin, thymus, and thyroid gland. Excretion via the feces was only 38%, and 15% of a single oral dose was exhaled. For both rats and monkeys, 7% to 18% of the dose was excreted in the urine in 5 days.

Fasted Wistar rats were given radioactive polyunsaturated Lecithin and absorption was monitored (LeKim and Betzing 1976). The absorption rate, as measured by disappearance from the gastrointestinal tract, was comparatively rapid in the first 6 to 8 hours and then became considerably slower. More than 90% of the radioactivity was absorbed from the intestinal tract within 24 hours of administration.

14C-Dilinoleoylphosphatidylcholine, with the radioactivity attached at the 1- or 2-position in the acyl moiety or at the choline moiety, and dilinoleoylphosphatide, with 3H in the acyl moiety and 14C in the choline moiety, were given orally to fasted male and female Wistar rats at a dose of 70 mg/kg (Fox, Betzing, and LeKin 1979). A low percentage of radioactivity was excreted in the feces (1.1% 3H and 14C after 24 hours; 8.2% and 32%, respectively, after 120 hours), indicating that Lecithin was almost completely absorbed from the intestinal lumen. A considerable amount of radioactivity was found in the intestinal wall 3, 6, and 8 hours after dosing. The amount of 3H and 14C in the urine was 2.0% and 1.6%, respectively, 24 hours after dosing and 15.6% and 6.4%, respectively, 120 hours after dosing. The amount in expired air (3H20 and 14CO2) was 1.0% and 16.6% 24 hours after dosing and 6.6% and 32.0% 120 hours after dosing, respectively. The amount recovered in the carcass was 58.8% and 51.3%, respectively, 120 hours after dosing.

Human beings, dogs, and rats were given radioactive 1,2-dilinoleoyl-sn-glycerol(3) phosphocholine (Nattermann Phospholipid GmbH 1995). More than 90% was absorbed from the intestinal lumen within 24 hours.

Normal human subjects consumed equimolar doses of (soy) Lecithin and urine was collected daily (Zeisel, Wishnok, and Blusztajn 1983). After ingestion of Lecithin, significantly greater amounts of dimethylamine and trimethylamine (TMA) were excreted as compared to control values obtained after consumption of a normal diet If the Lecithin was "cleaned" prior to dosing (in which the Lecithin contained only 4% TMA as compared to the non-cleaned compound), methylamine excretion did not increase to as great an extent.

As taken from Fiume Z. Final Report on the Safety Assessment of Lecithin and Hydrogenated Lecithin. International Journal of Toxicology. 20 (Suppl. 1):21-45, 2001.

“Several skin diseases such as psoriasis and atopic dermatitis are associated with the depletion or disturbance of stratum corneum (SC) lipids such as ceramides (CERs), free fatty acids and cholesterol. Studies suggested that replenishment of these lipids might help to treat diseased, affected or aged skin. With this premises in mind, there are some formulations in the market that contain SC lipids and currently, to facilitate permeation of the lipids deep into the SC, various CERs, and other SC lipid microemulsions (MEs) were developed and characterised using lecithin or TEGO® CARE PL 4 (TCPL4) as base surfactants. However, to date, there are no reports that involve the permeability of SC lipids into and across the SC, and therefore, the penetration of CER [NP] as a model ceramide from various formulations was investigated ex vivo using Franz diffusion cell. Besides, the toxicity of the MEs was assessed using hen's egg test chorioallantoic membrane (HET-CAM). The results of the study showed that CER [NP] could not permeate into deeper layers of the SC from a conventional hydrophilic cream. Unlike the cream, CER [NP] permeated into the deeper layers of the SC from both type of MEs, where permeation of the CER was more and into deeper layers from droplet type and lecithin-based MEs than bicontinuous (BC) type and TCPL4 based MEs, respectively. The CER also permeated into deeper layers from ME gels which was, however, shallow and to a lesser extent when compared with the MEs. The results of HET-CAM showed that both MEs are safe to be used topically, with lecithin-based MEs exhibiting better safety profiles than TCPL4 based MEs. Concluding, the study showed that the MEs are safe to be used on the skin for the controlled penetration of CER [NP] deep into the SC.” As taken from Sahle FF et al. 2014. Eur. J. Pharm. Biopharm. 86, 244-50. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23896195

AIMS: To describe the effect of two food emulsifiers, lecithin(E322) and citric acid esters of mono- and diglycerides of fatty acids (E472c), on the intestinalabsorptionof lipids. METHODS: The experiment was conducted on 24 male Wistar rats randomly assigned in three groups. For two groups of six rats, 30% of the lipid intake was replaced withlecithin(L) or citric acid ester of mono and diglycerides, (E); the remaining 12 rats were the control group (C). Diet and fecal fat analysis was used to determine the apparent lipidabsorption(ALA) and fatty acids. RESULTS: ALA was significantly lower in the group E than in the groups C and L ($p < 0.001$). ALA of long saturated chain fatty acids decreased while the length of the carbon chains increased, and this decrease was higher in the group E. CONCLUSION: E472c emulsifier decreased the intestinalabsorptionof lipids. As taken from Sadouki and Bouchoucha 2014. Int J Food Sci Nutr 65(6), 728-732. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/24655117

“Absorption

The absorption rate following oral administration within 24 h is higher than 90% in animals (Gundermann et al., 2011). The Commission E describes that phospholipids are degraded to lysophophatidylcholine in the intestine and absorbed primarily in this form (animal data). In the gut wall, the phospholipids are in part re-synthesised (Blumenthal et al., 2000).

Distribution

Phospholipids are primarily incorporated into the liver, with minor incorporation into other organs such as the gastrointestinal tract, spleen, lungs, muscles, kidneys and brain (Gundermann et al., 2011).

In plasma, phosphatidylcholine and other phosphoglycerides are tightly bound to lipoproteins or albumin, or to both (Blumenthal et al., 2000).

Elimination

Renal excretion after a single dose in the first eight days was 17.4% of the administered dose in rats and 17.7% in rhesus monkeys. The excretion in the faeces was low, with 3–8% of the dose excreted in the first 5–7 days in rats. Hence, a considerable part of the phospholipids are thought to be incorporated in the cell membrane of different cells (Gundermann et al., 2011)."

As taken from EMA, 2017

"Following oral administration, phosphatidylcholine is absorbed intact or as lysophosphatidylcholine or choline after intestinal hydrolysis. In humans, dietary lecithins are hydrolysed by phospholipases to liberate choline which is rapidly absorbed and appears in plasma predominantly as free choline."

As taken from EFSA, 2017

"BACKGROUND: Plasma concentrations of choline and its metabolites might serve as biomarkers for the health outcomes of several pathological states such as cardiovascular disease and cancer. However, information about the reliability of biomarkers of choline status is limited. We investigated biological variations in repeated measures of choline and metabolites in healthy adults to assess them as biomarkers. METHODS: Blood samples were collected after an overnight fast at three-time points 12 days apart from 40 adults (mean age, 33 y; male, n = 21). A subset (n = 19; [male, n = 8]) provided one additional sample after a breakfast meal. Plasma free choline, betaine and dimethylglycine were measured using liquid chromatography-tandem mass spectrometry, and plasma phosphatidylcholine, sphingomyelin and lysophosphatidylcholine were measured using high-performance liquid chromatography. RESULTS: The biological variations observed for choline and metabolites were ≤ 13% for adult fasting samples. This corresponded to intra-class correlations (ICC) that ranged from 0.593 to 0.770 for fasting values for choline and metabolites. A similar ICC range was also obtained between fasting and post-prandial states. Although most post-prandial concentrations of choline and metabolites were significantly higher ($P < .05$) than fasting, all fell within a calculated reference interval. The participants were correctly classified in tertiles for fasting and post-prandial states for choline (68%) and metabolites (range = 32% phosphatidylcholine and 79% for sphingomyelin). CONCLUSIONS: These findings indicate that biological variations of choline and metabolites are low in healthy adults and values from a single blood sample can be used as a biomarker. However, choosing phosphatidylcholine as a biomarker is less reliable." As taken from Wiedeman AM et al. 2018. Clin. Biochem. 60, 77-83. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30125545

4.3. Interactions

"Herbal medicine, especially traditional Chinese medicine and Ayurvedic medicine have played and still play an important role in fighting against various diseases. Emerging clinical studies regarding traditional Chinese medicine have provided convincing evidence for the first time to gain credibility and reputation outside China. Although synergistic therapeutic actions of herbal ingredients have been frequently reported, few reports have offered clear underlying mechanisms. This might be the main reason for the conflicting views with respect to the therapeutic efficacy of medicinal herbs. Therefore, this paper reviews the herb synergisms reported in the recent literature and discusses thoroughly the mechanisms underlying synergistic actions of herbal ingredients. The authors conducted an electronic literature search to detect articles published mainly in the last five years. Articles were included if they pertained to synergy research of medicines or the active compounds derived from them, included verification of synergy effects using modern analytical tools and molecular-biological methods. Results have revealed that the multi-component nature of medicinal herbs makes them particularly suitable for treating complex diseases and offers great potential for exhibiting synergistic actions. The mechanisms underlying synergistic therapeutic actions of herb

medicines are (1): different agents may regulate either the same or different target in various pathways, and therefore cooperate in an agonistic, synergistic way; (2): regulate the enzymes and transporters that are involved in hepatic and intestinal metabolism to improve oral drug bioavailability; (3): overcome the drug resistance mechanisms of microbial and cancer cells; and (4): eliminate the adverse effects and enhance pharmacological potency of agents by "processing" or by drug-drug interaction. The exploration of synergistic mechanisms of herbal ingredients will not only help researchers to discover new phytomedicines or drug combinations but also help to avoid the possible negative synergy. Further clinical research is required for verifying these reported drug combinations and discovered synergistic mechanisms." As taken from Yang Y et al. 2014. Fitoterapia 92, 133-47. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24177191

"Prolonged usage of nonsteroidal anti-inflammatory drugs (NSAIDs) causes gastrointestinal injury. Bile acids and phospholipids have been shown to exasperate and attenuate NSAIDs' toxicity, respectively. However, the molecular mechanisms underlying these effects remain undetermined. We have investigated the molecular interactions in various mixtures of indomethacin (Indo), a commonly used NSAID, and cholic acid (CA), a bile acid, in the presence and absence of palmitoyloleylphosphatidylcholine (POPC) lipids. We found that CA and Indo spontaneously form mixed micelles, with the hydrophobic face of CA and hydrophobic region of Indo forming the core. Increasing the Indo concentration resulted in more stable and larger aggregates that contain a progressively larger number of Indo molecules. More dynamic aggregates with a maximum size of 15 were obtained when the relative concentration of CA was higher. The mixture of CA, Indo, and POPC also led to ternary mixed micelles in which CA and Indo distribute almost uniformly on the surface such that intra-CA, intra-Indo, and CA/Indo interactions are minimized. A number of previous reports have shown that Indo perforates the cell membrane in the presence of bile acids (e.g., Petruzzelli et al., (2006) Dig. Dis. Sci., 51, 766-774). We propose that this may be related to the stable, highly charged, large CA/Indo binary micelles observed in our simulations. Similarly, the diminished ability of the CA/Indo mixture to aggregate in the presence of POPC may partly explain the lower toxicity of PC-conjugated NSAIDs." As taken from Prakash P and Gorfe AA. 2013. Biochemistry 52, 7461-9. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24066846

"Although cisplatin is widely used in the treatment of cancers, clinical use of cisplatin is limited due to its nephrotoxicity. Pathophysiological mechanism of cisplatin-induced renal toxicity is a complex process and has not been fully understand. Reactive oxygen species (ROS) and oxidative stress have been presumed to be involved in this damage process. Phosphatidylcholine (PC) has antioxidant effect and prevents oxidative stress. Therefore, the present study aimed to investigate potential protective effects of PC on cisplatin-induced renal damage in rat. We examined the protective effects of PC on cisplatin-induced renal damage by assessment of serum creatinine, BUN, lipid peroxidation, total glutathione, glutathione peroxidase activity, catalase activity, superoxide dismutase activity and histophathological changes. PC ameliorated cisplatin-induced increases in serum creatinine, urea and oxidative stress. PC also decreased tubular degeneration and hypertrophy of glomeruli. PC may have a protective effect against cisplatin-induced nephrotoxicity in rats via enhancing antioxidant enzyme activity." As taken from Lee HS et al. 2013. Food Chem. Toxicol. 58, 388-93. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23684996

"Docetaxel is a taxane chemotherapeutic agent used in the treatment of breast cancer, prostate cancer and gastric cancer, but several side effects such as peripheral neurotoxicity could occur. The present study was designed to investigate the therapeutic potential of phosphatidylcholine (PC) on docetaxel-induced peripheral neurotoxicity. Rats were randomly divided into three groups and treated for 4 weeks. Behavioral tests were conducted to measure the effects of PC on docetaxel-induced decreases in mechanical & thermal nociceptive threshold. Biochemical tests were conducted to measure the level of oxidative stress on sciatic nerve. Histopathological and immunohistochemical experiments were also conducted to assess neuronal damage and glial activation. PC treatment significantly attenuated docetaxel-induced changes in mechanical &

thermal nociceptive response latencies. PC decreased oxidative stress in sciatic nerve by increasing antioxidant levels (glutathione, glutathione peroxidase and superoxide dismutase activity). In immunohistochemical evaluation, PC treatment ameliorated docetaxel-induced neuronal damage and microglial activation in the sciatic nerve and spinal cord. Thus, PC showed protective effects against docetaxel-induced peripheral neurotoxicity. These effects may be attributed to its antioxidant properties and modulation of microglia." As taken from Kim ST et al. 2018. Drug Chem. Toxicol. 41(4), 476-485. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29210293

"This study investigated the ameliorative potential of exogenous phosphatidylcholine (PC) against aluminum-induced toxicity in male albino rats. Four groups of rats were used for this study (N = 8): group I served as the control, group II (PC treated) received L-α-phosphatidylcholine (egg yolk-derived) 100 mg/kg bwt/day orally, group III (aluminum treated) received aluminum chloride 100 mg/kg bwt/day orally, and group VI (aluminum + PC treated) received similar oral dose of aluminum and PC (100 mg/kg bwt/day). Treatment was continued for 8 weeks. Results revealed that aluminum chloride treatment leading to a significant elevation in serum aspartate aminotransferase, serum alanine aminotransferase, urea, creatinine, malondialdehyde, serum cytokines (tumor necrosis factor-α, interleukin-6), and brain content of acetylcholine, as well as a significant reduction in serum-reduced glutathione, serum testosterone, and brain content of acetylcholinesterase. Moreover, aluminum administration caused significant histopathological alteration in liver, kidney, brain, testes, and epididymis. Co-treatment with exogenous PC resulted in significant improvement in intensity of histopathologic lesions, serum parameters, testosterone level, proinflammatory cytokines, and oxidative/antioxidative status. However, it does not affect the brain content of acetylcholine and acetylcholinesterase. Conclusively, treatment with exogenous PC can retrieve the adverse effect of aluminum toxicities through its antioxidative and anti-inflammatory properties." As taken from Khafaga AF. 2017. Environ. Sci. Pollut. Res. Int. 24(18), 15589-15598. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28523611

"Gundermann et al. reviewed in 2011 that cytoprotective properties of lecithin have been corroborated in 25 in vitro studies and in 145 in vivo experiments in 8 different animal species. In these studies, lecithin has primarily been administrated to avoid hepatic toxicity induced by chemicals (e.g. carbon tetrachloride) or drugs (e.g. cyclosporine A) (Gundermann et al., 2011)."

"Lecithin had both preventive and curative effect on ethanol-induced alteration on liver weights in rats (Das and Vasudevan, 2006). Pretreatment with lecithin also had a positive impact with reduction of D-galactosamine induced hepatotoxicity in rats (Raj et al., 2011)."

As taken from EMA, 2017

"The potential ameliorative effects of L-α-phosphatidylcholine (PC) against mercuric chloride ($HgCl_2$)-induced hematological and hepato-renal damage were investigated. Rats were randomly allocated into four groups (n = 12): control, PC (100 mg/kg bwt, intragastrically every other day for 30 consecutive days), $HgCl_2$ (5 mg/kg bwt, intragastrically daily), and PC plus $HgCl_2$. Hematological and hepato-renal dysfunctions were evaluated biochemically and histopathologically. Hepatic and renal oxidative/antioxidative indices were evaluated. The expression of proinflammatory cytokines (tumor necrosis factor-α and interleukin-6) was also detected by ELISA. $HgCl_2$ significantly increased serum aminotransferases (ALT, AST), urea, and creatinine levels that are indicative of hepato-renal damage. $HgCl_2$ also induced a significant accumulation of malondialdehyde (+ 195%) with depletion of glutathione (- 43%) levels in the liver and renal tissues. The apparent hepato-renal oxidative damage was associated with obvious organ dysfunction that was confirmed by impairments in the liver and kidney histoarchitecture. Furthermore, $HgCl_2$ significantly attenuated the expression of proinflammatory cytokines named tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6). Conversely, PC treatment attenuated these effects, which improved the hematological and serum biochemical alternations, reduced the oxidative stress and proinflammatory cytokine levels, and ameliorated the intensity of the histopathological alterations in livers and kidneys of $HgCl_2$-treated rats. It could be concluded that PC displayed potential anti-

inflammatory and antioxidant activities against HgCl2-induced hepato-renal damage via suppression of proinflammatory cytokines and declining oxidative stress." As taken from Elblehi SS et al. 2019. Environ. Sci. Pollut. Res. Int. 26(9), 9333-9342. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30721437

5. Toxicity

5.1. Single dose toxicity

Record for 8002-43-5:

Organism	Test Type	Route	Reported Dose (Normalized Dose)	Effect	Source
rat	LD	oral	> 8mL/kg (8mL/kg)	Liver: Other Changes Liver: Fatty Liver Degeration	Laboratory Investigation. Vol. 47, Pg. 194, 1982.

As taken from RTECS, 2006

"Lipodissolve is a product that is composed of phosphatidylcholine and deoxycholate mixture, and other adjuvant. Lipodissolve injection seems to be performed in many countries for local fat reduction without any legal and scientific evidences of its safety and efficacy despite the US FDA warning.1 Herein, we report a case with agitation and metabolic acidosis following lipodissolve injection." As taken from Kyong YY and Choi KH. 2013. Clin. Toxicol. (Phila.) 51, 804-5. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23962098

Groups of five male and five female albino CD-1 outbred mice were dosed orally with 1, 2, 4, 8, or 16 g/kg Lecithin and observed for 14 days (Food and Drug Research Laboratories, Inc. [FDRL] 1973a). No animals died during the study; the oral LD50 for albino CD-I outbred mice was > 16 g/kg.

The oral LD50 for albino Wistar rats was determined according to the same procedure as above, using the same number of animals per group and the same dosages (FDRL 1973b). No animals died during the study and the oral LD50 for albino Wistar rats was >16mg/kg.

In a study using groups of five male and five female Dutch-Belted rabbits, following the same procedures as above, the animals were dosed with 3, 4, 8, 9, or 16 g/kg Lecithin (FDRL 1973c). The oral LD_{50} for Dutch-Belted rabbits was estimated to be 4.75 ± 0.64 g/kg.

The maximal nontoxic oral dose of Lecithin (purified soya phospholipids containing 75% to 98% phosphatidylcholine) for the mouse, rat, rabbit, and dog was 20, 20, 5, and 10 g/kg, respectively (Nattermann Phospholipid GmbH 1995).

The maximum nontoxic oral dose of Hydrogenated Lecithin (a fully saturated phospholipid containing 80% to 90% stearic acid) for the mouse and rat was 10 g/kg (Nattermann Phospholipid GmbH 1995).

Five male and five female Sprague-Dawley rats were dosed orally with 5 g/kg 1:2 w:v Hydrogenated Lecithin in deionized water and observed for 14 days (Leberco-Celsis Testing 1997a). All animals appeared normal, and none died. The oral LD50 of 1:2 w:v Hydrogenated Lecithin for Sprague-Dawley rats was >5g/kg.

As taken from Fiume Z. Final Report on the Safety Assessment of Lecithin and Hydrogenated Lecithin. International Journal of Toxicology. 20 (Suppl. 1):21-45, 2001.

“In the Commission E monograph on lecithin enriched extracts from soya bean, doses of phosphatidylcholine of up to 10 g/kg bw in mice and rats and 4.5 g/kg bw in rabbits given intravenous, intraperitoneally, and orally in a single dose are reported to be non-toxic (Blumenthal et al. 2000).”

As taken from EMA, 2017

“The acute toxicity of lecithins (E 322) in mice, rats and rabbits is low.”

As taken from EFSA, 2017

5.2. Repeated dose toxicity

Oral

The no-effect daily oral dose of Lecithin (soya phosphatidylcholine) was determined using rats over periods of 4, 6, or 12 weeks (Nattermann Phospholipid GmbH 1995). In these studies, the animals were dosed with ≤0.8, ≤1.35, or ≤2.8 g/kg, respectively. The no-effect daily oral dose for rats was greater than the maximum dose used in each study. The no-effect daily oral dose for dogs dosed for 6 weeks was 1.9 g/kg (details not provided).

Four dogs were fed 5 g/day (soybean) Lecithin for 25 to 60 days (Davis 1944). After a latent period of ≥5 days, the erythrocyte count was gradually reduced, with a maximal decrease of 15% to 20% occurring 12 to 25 days after dose initiation. Erythrocyte numbers returned to normal 11 to 20 days after discontinuation of dosing.

Groups of four male chimpanzees were fed a diet containing 37.6 g/day unsaturated Lecithin or 26.6 g/day Hydrogenated (saturated) Lecithin and one group was given basal diet for 1 month; the diets were adjusted so that each contained the same amount of protein, carbohydrates, and fat and the same number of calories (Rosseneu et al. 1979). Total lipoprotein concentration was similar for the group fed the diet containing Lecithin as compared to controls. Plasma very-low-density lipoproteins and low-density lipoprotein concentrations were "strongly" increased for the group fed the diet containing hydrogenated Lecithin.

A group of 15 male and 15 female rats was fed 6.0% (soya) Lecithin for 90 days and a control group was fed untreated feed (Gaunt, Grasso, and Gangolli 1967). The animals were housed five per cage. Body weights and feed consumption were determined weekly. Blood was collected from 10 animals/sex/group during week 6 and from all animals at study termination. No animals died during the study, and all animals appeared normal. Body weights of test animals were not significantly different from control values. Feed consumption was slightly but statistically insignificantly decreased for males and females of the test groups as compared to that by controls. At week 6, hemoglobin and hematocrit values were statistically significantly decreased for female animals of the test group; this was not observed at study termination or for males of the test group at either time period. Serum chemistry and urinalysis did not differ significantly from control values. Absolute spleen and kidney weights were significantly increased for females of the test group; no significant differences were observed in relative organ weights between the test and control group.

The no-effect daily oral dose for rats dosed with Lecithin (soya phosphatidylcholine) for 24 weeks was >2.8 g/kg (Nattermann Phospholipid GmbH 1995) (details not provided).

A group of 48 male and 48 female SPF Wistar rats was fed 4% (soya) Lecithin for 2 years while a control group was fed commercial diet only (Brantom et al. 1973). Feed consumption and body weights were determined prior to dosing, at intervals up to week 95, at week 102, and at study termination. The mean Lecithin intake was 1470 and 2280 mg/kg/day for males and females, respectively. No significant differences were observed for mortality, feed consumption, or body weight between the treated and control groups, but it was noted that feed consumption and body weight were sometimes greater in the treated group as compared to controls. Hematology values of animals of the treated group were similar to those of control animals, as were organ weights and gross and microscopic alterations. Parathyroid gland hyperplasia was increased, particularly in the males; this increase was attributed to an increased phosphate intake. The incidence of tumor formation was similar for the treated and control groups. A slightly increased incidence of myocardial fibrosis was associated with parathyroid gland hyperplasia.

As taken from CIR, 2020

The no-effect daily oral dose for rats dosed with Lecithin (soya phosphatidylcholine) for 48 weeks was >2800 mg/kg; for dogs dosed for 52 weeks, the no-effect daily oral dose was >750 mg/kg. In neither case were study details provided (Nattermann Phospholipid GmbH 1995).

Record for CAS RN 8002-43-5:

Route/Organism	Dose	Effect	Reference
oral, woman	TDLo: 96 mg/kg/3D-I	BEHAVIORAL: Changes in psychophysiological tests	Psychopharmacology (Berlin). (Springer-Verlag New York, Inc., Service Center, 44 Hartz Way, Secaucus, NJ 07094) V.47- 1976-175,84,2004

As taken from RTECS, 2006.

Human data:

Administration to human subjects of lecithin in daily doses varying from 22 to 83 g for two to four months to improve working capacity was not accompanied by any untoward reactions (Atzler & Lehmann, 1937).

Lecithin in large amounts (25-40 g per day) given for some months will frequently lower the serum cholesterol level intolerance to this amount limits its use (Merrill, 1959).

Some crude phosphatides (e.g., cardiac extracts) containing 93% lecithin showed pharmacological effects when given parenterally (Kunze, 1941). It is not clear if the observed effects were due to unidentified by-products.

Comments: Although fewer toxicological studies have been conducted than would normally be required for substances used as food additives, it is considered that nutritional and clinical experience with lecithin is sufficiently extensive to compensate for the incompleteness of the experimental data.

Since many observations have been made in man it is not considered necessary to calculate the safe intake level from animal experiments.

As taken from JECFA, 1974

Dermal

A group of 15 Crl:COBS CD(SD)BR female rats received dermal applications of 5130 mg/kg (5.1 ml/kg) of a commercial tanning oil containing 3.0% Lecithin 65% once daily, 5 days per week, for a total of 68 doses; the dose was estimated to be a 50x multiple of normal human use (assuming 102.6 mg/kg as average daily human use) (CTFA 1980a). The test material was applied by gentle inunction to a shaved dorsal area of the back. A negative-control group was untreated. Pharmacologic and toxiological observations were made daily. Blood was drawn from 10 animals

per group during weeks 7 and 13 for blood chemistry and hematology analysis, and pooled urine samples were also collected at this time. All animals were necropsied at study termination.

All test animals survived until study termination. Significant differences in body weight between test and control animals were not observed. With the exception of "sporadic minimal skin irritation," adverse reactions to dosing were not observed. Statistically significant decreased serum glucose and increased blood urea nitrogen (BUN) and activities of serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), and serum alkaline phosphatase (SAP) determined at week 7 and decreased hemoglobin and serum glucose and increased white blood cell, BUN, SGPT and SGOT activities determined at week 13 were concluded to be toxicologically insignificant. No significant gross lesions were found at necropsy. Statistically significant increases in absolute liver weights, liver-to-body weight ratios, and absolute kidney weights were observed for the test animals as compared to the controls; these differences were also considered toxicologically insignificant At microscopic examination, grade 1 hyperkeratosis was found in three animals. The researchers concluded that "except for skin irritation, no significant systemic toxic effects were noted" for a tanning oil containing 3.0% Lecithin 65% and that "based on the exaggerated dose levels used in this study...dermal application is not likely to produce adverse effects under conditions of consumer use."

A group of 15 female Sprague-Dawley rats were dosed with 450 mg/kg (0.45 ml/kg) of a liquid foundation containing 0.3% Lecithin 65% 5 days per week for 66 days following the procedure described above; the dose was estimated to be a 100x multiple of normal human use (assuming 4.5 mg/kg as average daily human use) (CTFA 1982). The negative control group was untreated. One animals died during week 9; the death was not considered treatment-related. Toxicological effects were no observed. A statistically significant increase in serum glucose observed at week 13 was considered toxicologically insignificant. Dose-related lesions were not observed at necropsy. At microscopic examination, grade 1 hyperkeratosis was found in three test animals. The researchers concluded that a liquid foundation containing 0.3% Lecithin 65% "was well tolerated by the test animals" and that it was "considered to be Safe from the viewpoint of cumulative, systemic toxicity."

As taken from Fiume Z. Final Report on the Safety Assessment of Lecithin and Hydrogenated Lecithin. International Journal of Toxicology. 20 (Suppl. 1):21-45, 2001.

“BACKGROUND: Phosphatidylcholine and deoxycholate (PC-DC) injections are a popular nonsurgical method to eliminate unwanted fat. The safety and efficacy of this approach is uncertain. OBJECTIVE: The authors evaluate the effects of PC-DC treatments on body composition, adipocyte function, and mechanisms responsible for fat loss. METHODS: This randomized, open-label study enrolled 13 women with a body mass index (BMI) ≤30 kg/m(2) and lower abdominal subcutaneous fat suitable for small-volume liposuction. Patients were randomized by the final digit of their Social Security numbers and received between 2 and 4 PC-DC treatments, spaced 8 weeks apart. One side below the umbilicus was injected with PC-DC. The contralateral, control side received no treatment. Adipose tissue biopsies were performed on the treated side at baseline, 1 week after the first treatment, and 8 weeks after the final treatment. The primary outcome was change in adipose tissue thickness at baseline and 8 weeks after the final treatment. RESULTS: Seven women completed the study. Treatment with PC-DC significantly reduced the thickness of the anterior subcutaneous abdominal fat (P = .004). Adipose tissue showed rapid increases in crown-like structures, macrophage infiltration, and reduced expression of leptin, hormone-sensitive lipase, adipose tissue triglyceride lipase, and CD36. Plasma C-reactive protein, lipid profile, and plasma glucose concentrations were unchanged. CONCLUSIONS: PC-DC injections can effectively reduce abdominal fat volume and thickness by inducing adipocyte necrosis. These treatments do not appear to increase circulating markers of inflammation or affect glucose and lipid metabolism.” As taken from Reeds DN et al. 2013. Aesthet. Surg. J. 33, 400-8. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23439063

“A 90-day feeding study was performed to evaluate the safety of dietary soy lecithin transphosphatidylated phosphatidylserine [soybean-derived phosphatidylserine (SB-PS)], with or

without fish oil-derived long-chain polyunsaturated fatty acids (LC-PUFA) mixed or conjugated to the glyceride backbone. One-hundred-two male Wistar rats (wild type, pathogen free) were randomly assigned to 6 groups. The 5 groups consumed 100 mg chow containing each of the following components, respectively, incorporated in 1 ml of milk-based supplement matrix:

medium-chain triglycerides (MCT group)

fish oil diluted with MCT to yield 30% (w/w) of omega-3 long-chain polyunsaturated fatty acids [LC-PUFA] (omega-3 group)

soybean 78% powdered SB-PS (final concentration of 20% SB-PS (w/w)) emulsified with 13% phosphatidylcholine (PC), 2% phosphatidylethanolamine, 1% phosphatidylinositol, 4% phosphatidic acid and further diluted with MCT (SB-PS group)

fish oil mixed with soybean 78% powdered SB-PS and diluted with MCT to yield a final concentration of 20% SB-PS (w/w) and 30% (w/w) of omega-3 LC-PUFA (omega-PS group)

20% phosphatidylserine (w/w) consisting largely of molecular species of palmitic acid (16:0) and docosahexanoic acid [DHA] (22:6) or eicosapentanoic acid (20:5), resulting in 30% (w/w) of omega-3 LC-PUFA (PS-DHA group).

The control group consumed normal chow. Blood samples were drawn and hematological parameters evaluated. Signs of toxicity were not observed during the feeding period. At the end of the study, gross examination of organs was performed. The following mortalities were reported: 1 rat (control group), 2 rats (MCT group), 1 rat (omega-3 group), and 1 rat (PS-DHA group). Pathological examinations did not reveal a specific cause of death; however it was concluded that the deaths were not treatment-related. Hematological parameters were normal in all treatment groups. At gross pathological examination, there were mild signs of liver enlargement in 5 of 102 rats, and these were considered unrelated to treatment. Possible early signs of lung metastasis (pale color nodes and different tissue consistency) were observed in 4 of 102 rats, but these findings were considered typical and abundant in rats of this age (15 months old). It was noted that none of these pathological findings occurred in PS-fed rats. It was concluded that no adverse effects were associated with diets fed in this study".

As taken from CIR, 2020.

"Subchronic toxicity studies in rats and dogs did not report any adverse effect, even at the highest doses tested (3,750 mg essential phospholipid (EPL)/kg body weight (bw) per day, 1,000 mg soya phosphatidylinositol or EPL/kg bw per day in rats and dogs, respectively, and 5,460 mg lecithins/kg bw per day in rats)."

"Chronic toxicity studies in rats did not report any adverse effects, even at the highest dose tested (3,750 mg EPL/kg bw per day)."

As taken from EFSA, 2017

5.3. Reproduction toxicity

"Several skin diseases such as psoriasis and atopic dermatitis are associated with the depletion or disturbance of stratum corneum (SC) lipids such as ceramides (CERs), free fatty acids and cholesterol. Studies suggested that replenishment of these lipids might help to treat diseased, affected or aged skin. With this premises in mind, there are some formulations in the market that contain SC lipids and currently, to facilitate permeation of the lipids deep into the SC, various CERs, and other SC lipid microemulsions (MEs) were developed and characterised using lecithin or TEGO® CARE PL 4 (TCPL4) as base surfactants. However, to date, there are no reports that involve the permeability of SC lipids into and across the SC, and therefore, the penetration of CER [NP] as a model ceramide from various formulations was investigated ex vivo using Franz diffusion cell. Besides, the toxicity of the MEs was assessed using hen's egg test chorioallantoic membrane (HET-CAM). The results of the study showed that CER [NP] could not permeate into deeper layers

of the SC from a conventional hydrophilic cream. Unlike the cream, CER [NP] permeated into the deeper layers of the SC from both type of MEs, where permeation of the CER was more and into deeper layers from droplet type and lecithin-based MEs than bicontinuous (BC) type and TCPL4 based MEs, respectively. The CER also permeated into deeper layers from ME gels which was, however, shallow and to a lesser extent when compared with the MEs. The results of HET-CAM showed that both MEs are safe to be used topically, with lecithin-based MEs exhibiting better safety profiles than TCPL4 based MEs. Concluding, the study showed that the MEs are safe to be used on the skin for the controlled penetration of CER [NP] deep into the SC." As taken from Sahle FF et al. 2014. Eur. J. Pharm. Biopharm. 86, 244-50. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23896195

Because of the clinical and biochemical state of a 26 years old patient an erythroblastosis was expected. Continuous controls revealed that delivery in the 36th week of pregnancy was necessary. A therapeutic research with lecithin through the maternal circulation in this special case was carried out in order to prevent a respiratory distress syndrome of the premature infant. (Grosspietzsch R. et al., 1976)

In order to find possibilities to influence therapeutically the respiratory distress syndrome at the premature infant animal experiences were carried out with 14C-lecithine. High pregnant rabbits got 14C-lecithin by intraamnial application. Investigation of the distribution of lecithin in the amniotic fluid and in the fetal organism showed an accumulation of lecithine metabolites in lung and lever tissue (Oberheuser F. et al., 1977)

The use of soybean lecithin in a glycerol-based solution for slow freezing of in vitro matured, fertilized and cultured (IVMFC) bovine embryos was examined. Embryos were developed in vitro in INRA Menezo's B2 medium supplemented with 10% fetal calf serum (FCS) on Vero cells monolayers. Day 7 blastocysts were frozen in a two-step protocol consisting of exposure to 5% glycerol and 9% glycerol containing 0.2 M sucrose in F1 medium + 20% FCS. Soybean lecithin was either added or not to the freezing solutions at a final concentration of 0.1% (w/v). In Experiment 1, blastocysts were equilibrated in cryoprotectant solutions without cooling. Cryoprotectant was diluted from embryos with 0.5 M and 0.2 M sucrose. The percentages of fully expanded and hatched blastocysts treated with or without lecithin after 24 and 48 h in culture were not significantly different (100 versus 100% and 93.3 versus 100%, respectively). In Experiment 2, the in vitro survival of frozen-thawed IVMFC blastocysts was compared when cryoprotectant solutions were either supplemented or not with lecithin. No significant effect of lecithin was found on the ability of frozen-thawed blastocysts to re-expand after 48 h in culture (65.6 and 54.2%, respectively). However, the post-thaw hatching rate of embryos cryopreserved in the presence of 0.1% lecithin was significantly higher after 72h in culture (52 and 31.8%, respectively). In Experiment 3, the ability of frozen-thawed IVMFC blastocysts to establish pregnancy following single embryo transfer was determined. Transfers of 58 and 66 frozen-thawed embryos cryopreserved with or without lecithin resulted in 6 and 10 (10.3 and 15.1%, respectively) confirmed pregnancies at Day 60. Addition of lecithin to cryoprotectants did not improve the in vivo development rate of cryopreserved IVMFC bovine blastocysts. (Guyader-Joly C. et al., 1999)

Infertility is well-established harmful effect in chronic alcoholism and so far, there is no effective treatment for this condition. The study was conducted to determine the effects of lecithin, a known hepato-protective on ethanol induced testicular injuries in male albino rats of Wistar strain. Five groups (n=6) of animals were used. Group I served as control. Group II received daily 1.6 g ethanol/kg body weight/day for 4 weeks orally. Group III received 1.6 g ethanol + 500 mg lecithin/kg body weight/day for four weeks orally. Group IV received 1.6 g ethanol/kg body weight for/day 4 weeks and followed by 500 mg lecithin/kg body weight/ day for four weeks orally. Group V received 1.6 g ethanol/kg body weight/ day orally for 4 weeks, followed by 4 weeks abstinence. Twenty-four hours after the last treatment the rats were sacrificed using anesthetic ether. Testes were removed and used for the estimation of extent of lipid peroxidation and tissue levels of antioxidants and steroidogenic enzymes. Lecithin protected testes from ethanol induced oxidative stress. However,

the drug did not show any considerable effect on the activities of testicular delta5, 3beta-HSD and 17beta-HSD. In conclusion, ethanol induced oxidative stress can be reversed by treatment with lecithin. However the effect of lecithin on steroidogenesis was not promising (Maneesh M. et al., 2005).

A total of 28 adult V-line rabbits were fed ad libitum a control diet or a diet supplemented with 0.5%, 1.0% and 1.5% soybean lecithin (SL) for 12 weeks. Bucks that received 0.5%, 1.0% or 1.5% dietary SL had a higher ejaculate volume, mass motility, sperm concentration, total sperm output and total motile sperm. Dietary SL reduced the percentage of dead sperm and increased the normal sperm, and this concurred with an increase in blood testosterone concentration. Blood and seminal plasma total lipid, acid phosphatase and seminal plasma alkaline phosphatase were significantly increased because of inclusion of SL. Interestingly, SL reduced blood and seminal plasma thiobarbituric acid-reactive substances while increasing blood and seminal plasma glutathione content, glutathione S-transferase, glutathione peroxidase and superoxide dismutase activity. Conception rate and litter size at birth and weaning were also significantly improved. Practically, it could be suggested that SL is a suitable supplement for improving semen quality, antioxidant status, reproductive traits and the economic efficiency of V-line rabbit bucks and 1% is an adequate concentration (Attia Y.A. et al., 2012).

“The Panel considered that no adverse effects were observed in the developmental toxicity studies performed in mice, rat and rabbits up to the highest dose tested. However, the Panel noted that no reproductive toxicity studies were available. Several neurodevelopmental toxicity studies were conducted with lecithin. The Panel concluded that the relevance of the studies is limited but, at concentrations of 5% soya lecithin and higher in the diet during the gestation, lactation and the postweaning period, there were indications for alterations in the development of the brain.”

As taken from EFSA, 2017

Record for lecithin (CAS RN 8002-43-5):

Safety Evaluation

Quantitative Risk Type	Quantitative Risk Value	Product Use	Safety Evaluation Owner	POD Method	POD Value	POD Owner
Not calculated	Not calculated	Not specified	COSMOS TTC (NON-CANCER)	NOAEL	1400.0	COSMOS TTC (NON-CANCER)

Critical study: RAT (Reproductive/Developmental Toxicity) Oral – dietary exposure for 1 GEN

NOEL/LEL Owner	Original NOEL	Original LEL	Critical Sites	Critical Effects
PAFA	1400.0 mg/kg bw/day	Not established		• NO EFFECTS

Safety Evaluation Comments: no comments available.

Source Document: no source document available

As taken from the COSMOS database

5.4. Mutagenicity

The mutagenic potential of Lecithin was determined using Salmonella typhimurium strains TA1535, TA1537, and TA1538 and Saccharomyces cerevisiae D4 with and without metabolic activation (Litton Bionetics, Inc. 1975). Plate tests were performed using 0.02% Lecithin and S. typhimurium and suspension tests were performed using 0.01% to 0.04% and S. typhimurium strains and with 1.875% to 7.5% Lecithin and S. cerevisiae. The vehicle, dimethylsulfoxide, was used as the negative control. Positive controls were dimethylnitrosamine and 2-acetylaminofluorene with activation and ethylmethane sulfonate, 2-nitrofluorene, and quinacrine mustard without activation.

Lecithin was not mutagenic in either the plate or suspension assays with or without metabolic activation.

Lecithin (highly purified soya phosphatidylcholine) was not mutagenic in an Ames test using five strains of S. typhimurium, in three yeast strains and human embryonic epithelial cell line (EUE) cells, or in the mouse host-mediated and urinary assays in vivo; study details were not provided (Nattermann Phospholipid GmbH 1995).

The effect of Lecithin (egg phosphatidylcholine): cholesterol (4:1) liposomes on 3H-thymidine incorporation into L1210 cell DNA was examined (Campbell 1983). The liposomes did not seem to inhibit 3H-thymidine incorAporation.

An Ames test was performed using S. typhimurium strains TA1535, TA1537, TA1538, TA98, and TA100 with and without metabolic activation on an artificially decomposed mixed micelle solution that originally contained 169.3 mg Lecithin (described earlier in the "Short-Term Toxicity" section) (Teelmann et al. 1984). The concentrations tested were 0.1,1.0,10.0, and 500.0 µl/plate. The artificially decomposed mixed micelle solution was not mutagenic with or without metabolic activation.

The addition of Lecithin (phosphatidylcholine) to incubations of TA98 and 1,8-dinitropyrene reduced mutagenicity (Shah, Combes, and Rowland 1991). However, the reduction in mu-tagenicity was less than that seen with uninduced S9.

As taken from Fiume Z. Final Report on the Safety Assessment of Lecithin and Hydrogenated Lecithin. International Journal of Toxicology. 20 (Suppl. 1):21-45, 2001.

“The Panel considered the available genotoxicity data on lecithins (E 322) to be sufficient to conclude that there is no concern with respect to genotoxicity.”

As taken from EFSA, 2017

5.5. Cytotoxicity

This study was designed to investigate the cytotoxicity of bile salt-lecithin mixed micelles on the Caco-2 cell model. Cell viability and proliferation after mixed micelles treatments were evaluated with the MTT assay, and the integrity of Caco-2 cell monolayer was determined by quantitating the transepithelial electrical resistance and the flux of tracer, FITC-dextran 4400. The apoptosis induced by mixed micelles treatments was investigated with the annexin V/PI protocol. The particle size of mixed micelles was all smaller than 100 nm. The mixed micelles with lower than 0.2mM sodium deoxycholate (SDC) had no significant effects on cell viability and proliferation. When the level of SDC was higher than 0.4mM and the lecithin/SDC ratio was lower than 2:1, the mixed micelles caused significant changes in cell viability and proliferation. Furthermore, the mixed micelles affected tight junctions in a composition-dependent manner. Specifically, the tight junctions were transiently opened rather than damaged by the mixed micelles with SDC of between 0.2 and 0.6mM. The mixed micelles with more lecithin also induced less apoptosis. These results demonstrate that relatively higher concentrations of mixed micelles are toxic to Caco-2 cells, while phospholipids can attenuate the toxicity of the bile salts (Tan Y et al., 2013).

While studying the toxicity of hemoglobin solutions, Feola et al. (1989) found that phosphatidylethanolamine and phosphatidylserine from red blood cell membranes may be the active agents in the well recognized hemolyzed red blood cell toxicity. Phosphatidylethanolamine, phosphatidylserine, phosphatidylcholine (Lecithin), and phosphatidylinositol were tested by stimulation of murine peritoneal macrophages. At doses of 5 µg/ml only phosphatidylethanolamine and phosphatidylserine were toxic. At doses of 50 and 100 µg/ml, phosphatidylcholine (Lecithin) and phosphatidylinositol were also toxic, but less so compared to phosphatidylethanolamine and phosphatidylserine. The authors postulate that phosphatidylethanolamine and phosphatidylserine are responsible for the toxicity of hemolysed red blood cells.

Reybrouck (1978) tested a number of materials that are potentially used as inactivators of disinfectant activity in studies of disinfectant effectiveness (i.e., to eliminate residual activity in postexposure recovery medium). The results demonstrated that Lecithin, 0.3% and 2%, had a germicidal effect toward Staphalococus aureus and Pseudomonas aeroginosa and so would not be suitable for the intended purpose.

Postulating that certain fatty acids may be responsible for the antimycobacterial activity of immunologically stimulated macrophages, Kondo and Kanai (1978) developed a model system in which tubercle bacilli were exposed to liposomes prepared from Lecithin and cholesterol treated with phospbolipase A2. The authors demonstrated that myristic acid and linoleic acid from the liposomes had the greatest antimycobacterial activity.

As taken from Fiume Z. Final Report on the Safety Assessment of Lecithin and Hydrogenated Lecithin. International Journal of Toxicology. 20 (Suppl. 1):21-45, 2001.

“The reorganization of metabolic pathways in cancer facilitates the flux of carbon and reducing equivalents into anabolic pathways at the expense of oxidative phosphorylation. This provides rapidly dividing cells with the necessary precursors for membrane, protein and nucleic acid synthesis. A fundamental metabolic perturbation in cancer is the enhanced synthesis of fatty acids by channeling glucose and/or glutamine into cytosolic acetyl-CoA and upregulation of key biosynthetic genes. This lipogenic phenotype also extends to the production of complex lipids involved in membrane synthesis and lipid-based signaling. Cancer cells display sensitivity to ablation of fatty acid synthesis possibly as a result of diminished capacity to synthesize complex lipids involved in signaling or growth pathways. Evidence has accrued that phosphatidylcholine, the major phospholipid component of eukaryotic membranes, as well as choline metabolites derived from its synthesis and catabolism, contribute to both proliferative growth and programmed cell death. This review will detail our current understanding of how coordinated changes in substrate availability, gene expression and enzyme activity lead to altered phosphatidylcholine synthesis in cancer, and how these changes contribute directly or indirectly to malignant growth. Conversely, apoptosis targets key steps in phosphatidylcholine synthesis and degradation that are linked to disruption of cell cycle regulation, reinforcing the central role that phosphatidylcholine and its metabolites in determining cell fate.” As taken from Ridgway ND. 2013. Crit. Rev. Biochem. Mol. Biol. 48, 20-38. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23350810

“PURPOSE: Cationic agents have been reported to possess anti-neoplastic properties against various cancer cell types. However, their complexes with lipids appear to interact differently with different cancer cells. The purpose of this study was to (i) design and generate novel cationic lecithin nanoparticles, (ii) assess and understand the mechanism underlying their putative cytotoxicity and (iii) test their effect on cell cycle progression in various cancer-derived cell lines. In addition, we aimed to evaluate the in vivo potential of these newly developed nanoparticles in oral anti-cancer delivery. METHODS: Cationic lecithin nanoparticles were generated using a single step nanoprecipitation method and they were characterized for particle size, zeta potential, stability and in vitro release. Their cytotoxic potential was assessed using a sulforhodamine B assay, and their effect on cell cycle progression was evaluated using flow cytometry. The nanoparticle systems were also tested in vivo for their anti-tumorigenic potential. RESULTS: In contrast to cationic agents alone, the newly developed nanoformulations showed a specific toxicity against cancer cells. The mechanism of toxic cell death included apoptosis, S and G2/M cell cycle phase arrest, depending on the type of cationic agent and the cancer-derived cell line used. Both blank and drug-loaded systems exhibited significant anti-cancer activity, suggesting a synergistic anti-tumorigenic effect of the drug and its delivery system. CONCLUSIONS: Both in vitro and in vivo data indicate that cationic agents themselves exhibit broad anti-neoplastic activities. Complex formation of the cationic agents with phospholipids was found to provide specificity to the anti-cancer activity. These formulations thus possess potential for the design of effective anti-cancer delivery systems.” As taken from Dhawan VV et al. 2014. Cell. Oncol. (Dordr.) 37(5), 339-51. PubMed, 2015 available at http://www.ncbi.nlm.nih.gov/pubmed/25204961

“A phosphatidylcholine (PPC) formulation has been used to treat cellulite; however, its underlying mechanism of action remains unclear. In this study, we demonstrated that PPC induces lipolysis and apoptosis in adipocytes, and evaluated a possible tumor necrosis factor alpha (TNFα)-dependent pathway, whereby PPC exerts these effects. For in vitro study, fully differentiated 3T3-L1 cells, mouse adipocytes were treated with various concentrations of PPC and cell apoptosis and lipolysis were assayed. For in vivo experiments, mice fed on a high-fat diet for 8 weeks were injected twice to abdominal subcutaneous fat tissues of either vehicle or PPC. We found that PPC induced lipolysis and apoptosis dose-dependently in fully differentiated 3T3-L1 cells. In addition, PPC augmented both expression and release of TNFα in a dose-dependent fashion. Induction of TNFα by PPC was associated with the stimulation of nuclear factor kappa B (NFκB)-mediated transcriptional activity. Small interfering RNA (siRNA)-mediated suppression of NFκB abrogated the effect of PPC on TNFα secretion. Suppression of TNFα with specific siRNA abrogated the effects of PPC on lipolysis and apoptosis. Through in vivo experiments, we demonstrated that PPC injection not only stimulated the local lipolysis and apoptosis, resulting in weight loss, but also induced TNFα mRNA expression and neutrophil infiltration. Furthermore, PPC injection prevented lipogenesis and suppressed the mRNA -expression of adipokines (such as adiponectin and leptin), due to the down-sizing of adipocytes. In conclusion, we suggest that PPC induces lipolysis and apoptosis in adipocytes through TNFα-dependent pathways.” As taken from Jung TW et al. 2018. Pharmacology 101(3-4), 111-119. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29186713

“Phosphatidylcholine (PPC) formula has been therapeutically used to reduce areas of localized fat. However, no single research has been carried out on its effect on a variety of cells in adipose and muscle tissues. Herein, the current study aimed to explore the activity of PPC on different cells in adipose and muscle tissues and to investigate the molecular mechanisms contributing to the effects of PPC on lipolysis and apoptosis. mRNA expression levels of various genes were measured by quantitative real-time PCR. Protein expression levels were observed through Western blotting and cell viability was measured by MTT assay. Lipolysis and caspase 3 activity assay were performed using commercial kits. PPC induces lipolysis and apoptosis in adipocytes (3T3-L1), but not in the other tested cells, including skeletal muscle cells (C2C12 myocytes), endothelial cells (HUVEC), and fibroblasts (BJ). The possible role of TNFα and IL-1β-mediated pathways on the effects of PPC was also revealed. We confirmed that treatment with PPC caused lipolysis and apoptosis in a dose-dependent manner (only in 3T3-L1 adipocytes). The effect of PPC observed in 3T3-L1 adipocytes was not evident in C2C12 myocytes, HUVEC, and fibroblasts. PPC also increased TNFα and IL-1β expression and release in 3T3-L1 adipocytes in a dose-dependent fashion, but not in C2C12 myocytes, HUVEC, and BJ. Suppression of TNFα or IL-1β reversed PPC-induced lipolysis and apoptosis in 3T3-L1 adipocytes, suggesting that PPC could promote adipocyte-specific lipolysis and apoptosis through TNFα and IL-1β-mediated signaling. We conclude that the specific activity of PPC on adipocyte in adipose without other tissue damages can be an effective approach for melting lipid.” As taken from Jung TW et al. 2019. PLoS One 14(4), e0214760. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30958839

5.6. Carcinogenicity

TM strain mice were fed 5 to 10 mg Lecithin mixed with sugar (for palatability) and a second group was fed Lecithin and 4 to 5 mg cholesterol (Szepsenwol 1969). The mice were bred and their offspring dosed following the same procedures; dosing continued until all mice became moribund or had died. A control group was given laboratory feed ad libitum. The total number of mice fed Lecithin, Lecithin and cholesterol, or control feed was 166, 212, and 360, respectively. The brains of the animals that were killed upon the initiation of weight loss were examined; the brains of the animals that died at night were not examined because they could not be removed undamaged by the time the dead animal was found. No complete necropsy results were reported. Brain nerve cell tumors (2-5 mm) were found in 18 of 73 examined animals fed Lecithin and in 27 of 88 examined

animals fed Lecithin and cholesterol, whereas no brain nerve cell tumors were found in 188 control animals.

As taken from Fiume Z. Final Report on the Safety Assessment of Lecithin and Hydrogenated Lecithin. International Journal of Toxicology. 20 (Suppl. 1):21-45, 2001.

“BACKGROUND: Intakes of choline and betaine have been inversely related to the risk of various neoplasms, but scant data exist on nasopharyngeal carcinoma (NPC). We examined the association between consumption of choline and betaine and risk of NPC. METHODS: We conducted a case-control study with 600 incident NPC patients and 600 controls 1 : 1 matched by age, sex and household type in Guangdong, China. Dietary intake was assessed by a food frequency questionnaire through face-to-face interview. RESULTS: Intakes of total choline, betaine and choline+betaine were inversely related to NPC after adjustment for various lifestyle and dietary factors (all P-trend <0.001). Adjusted odds ratios (95% CI) for quartile 4 (vs quartile 1) were 0.42 (0.29, 0.61) for total choline, 0.50 (0.35, 0.72) for betaine and 0.44 (0.30, 0.64) for betaine+total choline. Regarding various sources of choline, lower NPC risk was associated with greater intakes of choline from phosphatidylcholine, free choline, glycerophosphocholine and phosphocholine, but not sphingomyelin. CONCLUSION: These findings are consistent with a beneficial effect of choline and betaine intakes on carcinogenesis.” As taken from Zeng FF et al. 2014. Br. J. Cancer. 110, 808-16. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24169354

Substance Name: SOYBEAN LECITHIN
CAS Registry Number: 8002-43-5
Data Type: Tumor Inhibition

Studies Data:

Tumor Inhibition Studies:

Species:	Mouse
Number of Animals Tested:	(? ,41)/ (? ,40)
Strain/Sex:	Nigp(S)/Male
Dose (Inhibitor):	0; 25 mg/ml in Drinking Water for 6 Wk (Study Duration: 9 Wk)
Route (Inhibitor):	Oral
Carcinogen:	Benzo [A] Pyrene; 50-32-8
Route (Carcinogen):	Subcutaneous
Dose (Carcinogen):	0.5 Mg Injected In the Scapular Region Of Newborn Mice Once
Promoter:	None Used
Target Tissue: Type Of Lesion: Lung:	Adenoma
Endpoint (Incidence):	37%, 25%, 32%, Not Calculated
Endpoint (Multiplicity):	1.02, 0.43, 58%, Not Calculated
Reference:	[Yun,TK, Kim,SH And Lee,Y; Trial Of A New Medium-Term Model Using Benzo(A)Pyrene Induced Lung Tumor In Newborn Mice; Anticancer Res. 15(3): 839-845, 1995]
Species:	Mouse
Number of Animals Tested:	(? ,39)/ (? ,40)
Strain/Sex:	Nigp(S)/Female
Dose (Inhibitor):	0; 25 mg/ml in Drinking Water for 6 Wk (Study Duration: 9 Wk)
Route (Inhibitor):	Oral

Carcinogen:	Benzo [A] Pyrene; 50-32-8
Route (Carcinogen):	Subcutaneous
Dose (Carcinogen):	0.5 Mg Injected In the Scapular Region Of Newborn Mice Once
Promoter:	None Used
Target Tissue:	
Type Of Lesion:	Lung: Adenoma
Endpoint (Incidence):	56%, 33%, 41%, Not Calculated
Endpoint (Multiplicity):	1.38, 0.50, 64%, Not Calculated
Reference:	[YUN,TK, KIM,SH AND LEE,YS; Trial Of A New Medium-Term Model Using Benzo(A)Pyrene Induced Lung Tumor In Newborn Mice; Anticancer Res. 15(3): 839-845, 1995]

As taken from CCRIS, 2008.

“No carcinogenic effects were reported in rats, even at the highest dose tested (1,470 and 2,280 mg soya lecithin/kg bw per day in males and females, respectively) for 2 years.”

As taken from EFSA, 2017

“The dietary intakes of choline and betaine have been related to the mortality of some neoplasms, but their effects on hepatocellular carcinoma (HCC) mortality are still unknown. We examined the associations between dietary choline, five choline-containing compounds, different choline forms, betaine intake and HCC mortality. In total, 905 newly diagnosed HCC patients were enrolled in the Guangdong Liver Cancer Cohort study. Dietary intake was assessed by a valid food frequency questionnaire. Liver cancer-specific mortality (LCSM) and all-cause mortality (ACM) were calculated. Hazard ratios (HRs) and 95% confidence intervals (CIs) were computed by Cox proportional hazards models. It was found that a higher total choline intake was associated with lower ACM, Q4 vs. Q1: HR = 0.72, 95% CI: 0.53-0.97, Ptrend = 0.012 in the fully adjusted model. The associations between total choline intake and LCSM were not significant. Similar associations were found between water-soluble choline intake and HCC mortality, where the fully adjusted HR for ACM was 0.72, 95% CI: 0.53-0.98, Ptrend = 0.017. However, null associations were found between neither phosphatidylcholine (the most abundant lipid-soluble choline) nor total lipid-soluble choline intake and HCC mortality. These results implied that the favorable associations between the total choline intake and ACM were more attributed to water-soluble choline. Furthermore, no significant associations were observed between betaine intake and HCC mortality. Future human intervention trials regarding choline supplementation and liver disease recovery should take the forms into consideration rather than just the total amount alone.” As taken from Liu ZY et al. 2020. Food Funct. 11(9), 7866-7877. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/32812611/

5.7. Irritation/immunotoxicity

“Several skin diseases such as psoriasis and atopic dermatitis are associated with the depletion or disturbance of stratum corneum (SC) lipids such as ceramides (CERs), free fatty acids and cholesterol. Studies suggested that replenishment of these lipids might help to treat diseased, affected or aged skin. With this premises in mind, there are some formulations in the market that contain SC lipids and currently, to facilitate permeation of the lipids deep into the SC, various CERs, and other SC lipid microemulsions (MEs) were developed and characterised using lecithin or TEGO® CARE PL 4 (TCPL4) as base surfactants. However, to date, there are no reports that involve the permeability of SC lipids into and across the SC, and therefore, the penetration of CER [NP] as a model ceramide from various formulations was investigated ex vivo using Franz diffusion cell. Besides, the toxicity of the MEs was assessed using hen's egg test chorioallantoic membrane (HET-CAM). The results of the study showed that CER [NP] could not permeate into deeper layers of the SC from a conventional hydrophilic cream. Unlike the cream, CER [NP] permeated into the

deeper layers of the SC from both type of MEs, where permeation of the CER was more and into deeper layers from droplet type and lecithin-based MEs than bicontinuous (BC) type and TCPL4 based MEs, respectively. The CER also permeated into deeper layers from ME gels which was, however, shallow and to a lesser extent when compared with the MEs. The results of HET-CAM showed that both MEs are safe to be used topically, with lecithin-based MEs exhibiting better safety profiles than TCPL4 based MEs. Concluding, the study showed that the MEs are safe to be used on the skin for the controlled penetration of CER [NP] deep into the SC." As taken from Sahle FF et al. 2014. Eur. J. Pharm. Biopharm. 86, 244-50. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23896195

Dermal Irritation

The primary skin irritation of Lecithin or lecithin-containing products was determined using rabbits n a number of single insult occlusive patch tests. All products were tested at 100% concentration.

The skin irritation potential of a soap containing 0.83% Lecithin powder, tested at 0.5%, was determined in a guinea pig immersion study (CTFA 1983a). The immersion score was 9.5, indicating that "the product is practically nonirrtating."

The skin irritation potential of Hydrogenated Lecithin was determined using six New Zealand white rabbits (Leberco-Celsis Testing 1997b). An occlusive patch containing 0.5 g Hydrogenated Lecithin was applied for 24 hours to an intact and an abraded site on the clipped skin on the mid-dorsal area of the trunk of each animal. The test sites were scored on a scale of 0 to 4 immediately and 48 hours after patch removal. Application of Hydrogenated Lecithin resulted in very slight erythema and no edema, and flaking skin was observed at one site. The primary irritation score was 0.21/8, and Hydrogenated Lecithin was not a primary dermal irritant.

Two single insult (24 hours) occlusive patch tests were performed using 20 subjects to determine the irritation potential of a tanning oil containing 3.0% Lecithin 65%; the lotion was applied undiluted (CTFA 1978b). A suntan oil and a "tanning blend" were used as reference controls in the studies, respectively. Significant differences in irritancy between the test material and the control were not observed, and the primary irritation index (PII) of the tanning oil was 0.00 in both studies.

A single-insult (24 hours) occlusive patch test was performed using 18 subjects to determine the irritancy potential of a soap containing 0.83% Lecithin powder, the soap was tested as a 0.5% aqueous solution (CTFA 1983b). A soap was used as a reference control. Significant differences in irritancy between the test material and the control were not observed, and the PII for the test soap was 0.25.

A 4-day cumulative irritancy assay of a foundation containing 0.3% Lecithin 65% was completed using 17 female subjects (CTFA 1981b). Occlusive patches containing 0.10 ml of undiluted test material were applied to the upper back of each subject for 24 hours on 4 consecutive days. An irritating positive control was used. The test sites were scored on a scale of 0 to 4 immediately after patch removal and 5 hours after removal of the fourth patch, and the PII was calculated after the 5-hour scoring. If a score of ≥2 was observed at any time during the study, patching was discontinued and that score was entered for all subsequent scorings. Thirteen of the subjects reacted, with the greatest score being a score of 2 (moderate erythema) for one subject The majority of the scores, seven subjects, was a ± reaction (barely perceptible erythema). The PII of a foundation containing 0.3% Lecithin 65% was 0.65.

A 4-day minicumulative assay of an eyeliner containing 3.0% Lecithin 65% was performed using 20 subjects according to the methods described previously, with the exception that the sites were only scored 5 hours after the fourth application (CTFA 1987). However, if a score ≥2 was noted at any time during the testing, patching was discontinued. Five of the subjects reacted with scores of ± , and the PII was 0.13.

A 21 -day patch test was completed using 11 subjects, 2 males and 9 females, to determine the cumulative irritation potential of a tanning oil containing 3.0% Lecithin 65% (Hill Top Research, Inc.

1978). Vaseline Intensive Care baby oil and concentrate from a purchased deodorant were used as reference materials. Approximately 0 3 ml of each test material was applied for 23 hours to the backs of each subject under an occlusive patch, after which time the patches were removed and the sites washed. The sites were scored 1 hour after patch removal, and the same sites were used daily. A tanning oil containing 3.0% Lecithin 65% was nonirritating.

A double-blind 4-week clinical use study was performed using groups of approximately 38 female subjects to determine the irritation potential of a tanning oil containing 3.0% Lecithin 65% (CTFA 1978c). Control products were also used. Significant clinical or subjective irritation was not observed.

Application of Lecithin (soya phosphatidylcholine) as liposomes at a dose of 3 mg/cm^2 under an occlusive chamber for 48 hours was not irritating and 24- to 48-hour patch testing with Hydrogenated Lecithin (soy phosphatidylcholine) did not result in an irritant effect. In neither case were study derails provided (Nattermann Phospholipid GmbH 1995).

As taken from Fiume Z. Final Report on the Safety Assessment of Lecithin and Hydrogenated Lecithin. International Journal of Toxicology. 20 (Suppl. 1):21-45, 2001.

“In single-insult occlusive patch tests (rabbits), Lecithin 65% (solution of 65% Lecithin) was minimally irritating, products containing 3% Lecithin 65% were practically non- to mildly irritating, and a product containing 2.25% Lecithin 65% was non-irritating to the skin of rabbits. In a guinea pig immersion study, 0.5% of a soap containing 0.83% Lecithin powder was practically nonirritating. Hydrogenated Lecithin was not a primary dermal irritant in rabbits.

In clinical irritation studies, cosmetic formulations containing 0.3% or 3% Lecithin 65% (solution of 65% Lecithin), a soap containing 0.83% Lecithin powder (tested at 0.5%), and Lecithin liposomes were generally nonirritating. Barely perceptible erythema was the most severe reaction observed.”

“Additionally, a tanning oil containing 3% Lecithin 65%, a mascara containing 0.1% Lecithin 65%, and a foundation containing 0.3% Lecithin 65% were non-sensitizing.”

As taken from CIR, 2020.

Ocular Irritation

The ocular irritation of Lecithin or Lecithin-containing products was determined using rabbits in a number of Draize tests. All products were tested at 100% concentration, except where noted. The eyes were not rinsed after instillation.

Fifty microliters of an (egg) Lecithin liposome preparation and a positively charged (egg) Lecithin liposome preparation containing stearylamine was placed in the left conjunctival sac of five male albino rabbits once every 15 minutes for 2 hours for a total of nine applications in a Draize test (Taniguchi et al. 1988). The control group of five rabbits had saline applied to the right conjunctival sac. The maximum mean total score did not exceed "practically nonirritating" and immediately decreased to "nonirritating." Slight hyperemia was observed in the conjunctiva of the right eye. No lesions were found at microscopic examination.

A rabbit blinking test was performed according to the methods of Tanaka et al. (1985) using groups of six male albino rabbits to determine the ocular irritation potential of neutral and positively charged (egg) Lecithin liposome preparations (Taniguchi et al. 1988). Fifty microliters of each test preparation was placed into the conjunctival sac of one eye of each rabbit and 50 µl of saline was placed in the conjunctival sac of the other eyes. The number of blinks of each eye was counted for 5 minutes following instillation. This procedure was repeated six times at 1-hours intervals, alternating the eye in which the test article was placed. Instillation of the neutral liposome preparation did not produce a statistically significant change in the number of blinks, but instillation of the positively charged liposome preparation significantly increased the number of blinks; the blinking count excluding that of both eyes together was also significantly increased.

The ocular potential of Hydrogenated Lecithin was determined by instilling 0.07 g of the test material into the conjunctival sac of one eye of six albino rabbits; the contralateral eyes were untreated and served as controls (Leberco-Celsis Testing 1997c). The eyes were scored 24, 48, and 72 hours postinstillation. Hydrogenated Lecithin produced minimal conjunctival irritation, and all signs of irritation were cleared by day 2. Hydrogenated Lecithin was not a primary ocular irritant.

As taken from Fiume Z. Final Report on the Safety Assessment of Lecithin and Hydrogenated Lecithin. International Journal of Toxicology. 20 (Suppl. 1):21-45, 2001.

Sensitization

A Draize-Shelanski repeat-insult patch test (RIPT) was completed using 99 subjects to determine the sensitization potential of a tanning oil containing 3.0% Lecithin 65% (Research Testing Laboratories, Inc. 1978). The test material was applied under an occlusive patch three times weekly for a total of 10 applications using a "quadrant approach," that is, the first quadrant received patches 1, 4, 7, and 10 the second quadrant received patches 2, 5, and 8, and the third quadrant received patches 3, 6, and 9 (the length of patch duration was not stated). The test sites were scored according to the International Research Contact Dermatitis Group at 48 or 72 hours. Following a 10-day non-treatment period, a challenge patch was applied to a previously untested site on the fourth quadrant of the back; this site was evaluated after 48 and 96 hours.

One subject developed a "1+" reaction (a weak, non-vesicular, reaction) upon application of the seventh patch, and this reaction continued through the 96-hour challenge reading. This subject had 1 + reactions at 24 hours upon retesting with both undiluted test material and a 1:3 dilution of the test material; open tests with the product were negative when it was applied three times a day for 5 days. The researchers concluded that this subject had a "low level of sensitization," but it was "not of clinical significance" because it was a low level of reaction and the open tests were negative. Two subjects had 1+ reactions with the fourth patch only; these were not considered significant. The researchers conclude that a tanning oil containing 3.0% Lecithin 65% "did not demonstrate any irritation or sensitization."

A modified Draize assay was performed to determine the sensitization potential of 15% Hydrogenated Lecithin in petrolatum and was completed with 110 of 120 initial subjects (International Research Services, Inc. [IRSI] 1997a). During induction, 0.025 g of the test material was applied to the scapular area of the back under occlusive patches. A total of 10 applications were made. Forty-eight hours after patch application (72 hours on weekends), the patches were removed and the test sites were rinsed and evaluated. New patches were then applied. Twelve days after removal of the last patch, a challenge patch with the same dose used during induction was applied to a previously untested site. The challenge patch was removed 48 hours after application, and the site was evaluated 48 and 96 hours after application.

During the induction phase of the study, two 1+ reactions (erythema throughout the entire patch area) were observed in one subject. At the 48- and 96-hour challenge readings, one other subjects had 1+reactions. The researchers concluded that "no evidence of sensitization" to 15% Hydrogenated Lecithin in petrolatum was observed.

A maximization study was completed using 25 subjects, 10 males and 15 females, to determine the contact-sensitization potential of a mascara containing 0.1 % Lecithin 65% (Ivy Research Laboratories, Inc. 1982). Five 48-hour occlusive patches containing 0.3 g of the test material were applied to the volar aspect of the forearm following application of sodium lauryl sulfate (SLS). Following a 10-day non-treatment period, a 48-hour occlusive challenge patch was applied to a previously untreated site, and observations were made immediately and 24 hours after patch removal. Reactions were not observed at the sites of induction or challenge patches, and the researchers concluded that it was "unlikely that (a mascara containing 0.1% Lecithin 65%) would present a danger ocontact-sensitization during normal, intended use."

Two cases of baker's asthma related to occupational soybean lecithin (8002435) exposure were examined. The first case was a nonsmoking 26 year old male who had been employed in an industrial bakery for 5 years where he added microdoses of alpha-amylase (9000902), citric-acid (77929), and soybean lecithin to the flour. He was nonatopic, developed rhinorrhea, cough with sputum, and wheezing usually within 15 minutes after beginning work, and also experienced nocturnal asthma attacks. Symptomatic treatment was not effective. He thought that soybean lecithin might be responsible for his symptoms because rhinitis appeared shortly after his first contact with it. He avoided contact with lecithin and his symptoms resolved within 3 months without treatment. The second case was a 29 year old nonsmoking male with a history of rhinitis and asthma, who had worked in the same bakery, a small family business, since was he was 18 years old. His symptoms began as rhinitis, but progressed to asthma during his work, especially when he handled flour to which soybean lecithin had been added. After a few months of symptomatic treatment, he changed his job. His symptoms cleared within 2 months. The two patients and three nonatopic volunteers and three asthmatic patients (referents) underwent skin prick testing with common bakery inhalant allergens and soybean dust, flour, pulp, and lecithin, serum radioallergosorbent (RAST) evaluations with soybean flour, and bronchial challenge with soybean lecithin. Skin prick testing with common bakery allergens produced negative results in the 26 year old patient. The 29 year old patient reacted to wheat flour and bakery dust. Soybean lecithin, dust, flour, and pulp produced positive responses in both patients. The RAST indicated the presence of soybean flour specific immunoglobulin-E (IgE) antibodies in both patients. The 29 year old patient also had wheat specific IgE antibodies. A significant immediate bronchial response was induced by a 10(-3) dilution of soybean lecithin in both patients. No positive skin prick or bronchial challenge responses were induced in the controls. The authors conclude that soybean lecithin, a known sensitizer to bakers, can provoke asthma attacks in bakers. Soybean lecithin should be used as a test allergen when evaluating cases of baker's asthma (Lavaud F et al., 1994).

“The antigenicity of soy Lecithin was studied using 30 soybean-sensitive patients and 22 controls.19 One control group (11 subjects) consisted of nonatopic individuals, and the other control group (11 subjects) consisted of allergic patients with negative IgE to soybean (radioallergosorbent test [RAST] score ¼ 0). The IgE- and IgG4-binding activities of the soy Lecithin proteins were evaluated by immunoblotting with sera obtained from the patients, 7 of whom had a positive challenge test. In 100 grams of sample, the soy Lecithin contained 2.8 mg of proteins. The proteins present in soy Lecithin were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis. For the soy Lecithin, the detection rate of only one protein (molecular weight: 31 kDa) by the serum IgE of patients was statistically significantly different when compared to serum from the 2 control groups combined (detection rates: 40% [patient sera] and 4.5% [control sera]). Proteins in the molecular weight range of 58 to 67 kDa were rarely bound to serum IgE. Only one of the patients with a positive challenge test had IgE antibodies to soy Lecithin proteins. The presence of IgG4- binding proteins in soy Lecithin was described as rare. It was concluded that the proteins present in soy Lecithin have little antigenicity with respect to soybean allergy.”

“A 3-year-old boy with a history of asthma and peanut allergy was treated for asthma that developed after an upper respiratory tract infection.72 He developed respiratory distress and generalized urticaria within an hour after receiving the second of 2 inhalations of an ipratropium bromide inhaler. All signs regressed within 48 hours of withdrawal of the drug. Soy Lecithin, an excipient in the metered dose inhaler, was strongly suspected of causing the adverse events.”

As taken from CIR, 2020

“Receptors of the advanced glycation products (RAGE) are activated to promote cell death and contributes to chronic diseases such as diabetes and inflammation. Advanced glycation end products (AGEs), which interact with RAGE are complex compounds synthesized during diabetes development and are presumed to play a significant role in pathogenesis of diabetes. Phosphatidylcholine (PC), a polyunsaturated fatty acid found in egg yolk, mustard, and soybean, is thought to exert anti-inflammatory activity. We investigated the effects of PC on AGEs-induced

hepatic and renal cell injury. Materials and Methods: In this study, we evaluated cytokine and NF-κB/MAPK signal pathway activity in AGEs induced human liver (HepG2) cells and human kidney (HK2) cells with and without PC treatment. Results: PC reduced RAGE expression and attenuated levels of inflammatory cytokines and NF-kB/MAPK signaling. Moreover, cells treated with PC exhibited a significant reduction in cytotoxicity, oxidative stress, and inflammatory factor levels. Conclusions: These findings suggest that PC could be an effective functional material for hepatic and renal injury involving with oxidative stress caused by AGEs during diabetic conditions." As taken from Choi J et al. (2022). Available at https://pubmed.ncbi.nlm.nih.gov/36363476/

5.8. All other relevant types of toxicity

Lecithins are "known not to cause problems in carcinogenicity, endocrine disruption, reproductive/developmental toxicity, AChE inhibition and neurotoxicity.

As taken from BPDB, 2022

6. Functional effects on

6.1. Broncho/pulmonary system

After exposing mice to aerosolized lecithin 4hr/day for 2 days, lungs showed focal endothelial cell swelling and interstitial edema. Raises surface tension of lung by interacting with the surfactant lining (HSDB, 2016).

As reported by Smolinske (1992) paradoxical bronchospasm was reported in 23 of 1450 (1.6%) asthmatics treated with a metered-dose inhaler containing (soy) Lecithin. In a follow-up study, a 4.4% incidence of immediate bronchoconstriction was reported and was ascribed to one or more of the excipients; however, the researchers did not determine which of the excipients was responsible (Yarbrough, Mansfield, and Ting 1985). After reformulation of a metered-dose inhaler to contain (soy) Lecithin, escalating reports of adverse reactions within 1 month of reformulation resulted in withdrawal of the new formulation (Smolinske 1992)

As taken from Fiume Z. Final Report on the Safety Assessment of Lecithin and Hydrogenated Lecithin. International Journal of Toxicology. 20 (Suppl. 1):21-45, 2001.

6.2. Cardiovascular system

Rapid infusion into cats of a 1.2% egg-yolk phosphatide emulsion containing 5% glucose (1 ml/kg/min) had no effect on the respiratory and circulatory systems; rapid infusion of soybean phosphatides caused a fall in blood pressure with apnoea (Schuberth & Wretlind, 1961).

As taken from JECFA, 1974

In Rhesus monkeys, lecithin supplementation to food reduced plasma cholesterol (Wong et al., 1980)."

"Reduction of LDL cholesterol as well as an increase in the level of HDL cholesterol was observed in rats feed a hypercholesterolemic diet in combination with lecithin (2.5 or 0.7%) (Jimenez et al., 1990)."

As taken from EMA, 2017

Lecithin's major property of regulating cholesterol levels is a pivotal aspect of its role in promoting cardiovascular health. It achieves this regulation through its capacity to reduce excess low-density lipoprotein (LDL), often dubbed as "bad cholesterol." High levels of LDL are associated with an increased risk of atherosclerosis and heart disease. Simultaneously, lecithin facilitates the synthesis of high-density lipoprotein (HDL), recognized as the "beneficial cholesterol". HDL contributes

substantially to the removal of excess cholesterol from the blood circulation, transporting it to the liver for excretion, thus contributing to a healthier cardiovascular profile. An increased presence of HDL is linked to a reduced risk of cardiovascular diseases. Studies, such as the one conducted by Brunet and associates in 2003, have shown that diets rich in lecithin stimulate the secretion of bile acids by enhancing the formation of mixed micelles, which facilitate the solubilization and excretion of cholesterol. This mechanism involves elevated levels of phospholipids and cholesterol compared to diets lacking lecithin. This, in turn, underscores lecithin's significance in maintaining a balanced cholesterol profile and supporting heart health. As taken from Comfort Onaolapo M et. Al. 2024. Egypt Heart J. Available at: https://pubmed.ncbi.nlm.nih.gov/39001966/

In the present study, we investigated the effects of LPC70 on HS-induced hypertension and cognitive impairment using behavioral and biochemical analyses. The association between HS intake and hypertension has been well-documented in both human and animal studies (Devarajan et al., 2015; Liu, 2009; Takase et al., 2015). Herein, we used 2% NaCl as the HS solution to induce hypertension in mice. Mice that ingested the HS solution showed elevated SBP with no changes in body weight or heart rate. Interestingly, LPC70 prevented HS-induced hypertension. Since there was no difference in the serum and urine levels of Na+ and Cl− between the HSW and HSW + LPC70 groups, the anti-hypertensive effect of LPC70 appears unrelated to electrolyte homeostasis. (…) Our results suggest an anti-inflammatory effect of LPC70, as it prevented the increase in iNOS expression in the small intestine induced by HS intake. Although relatively small amounts of NO produced by endothelial NOS (eNOS) play a crucial role in cardiovascular homeostasis, excessive NO production resulting from enhanced iNOS activity may adversely affect the cardiovascular system and potentially contribute to hypertension (Oliveira-Paula et al., 2014). Accordingly, suppression of iNOS expression by LPC70 may play an important role in preventing HS-induced hypertension. As taken from Kubota H et. al. 2024. Neurochem Int. Available at: https://pubmed.ncbi.nlm.nih.gov/39271020/

6.3. Nervous system

To investigate the effect of LPC70 on cognitive impairment induced by HS, we performed behavioral tests. Our previous study showed that HS intake impairs object recognition memory, but not short-term or spatial learning memory (Kubota et al., 2023). Therefore, to determine the effect of LPC70 on cognitive impairment, we focused on object recognition memory. Interestingly, our results showed that LPC70 prevented impairment of object recognition memory. Since there were no differences in locomotor activity or exploratory behavior, the preventive effect of LPC70 was not due to alterations in motor function or exploratory motivation. (…) Furthermore, HS intake causes neuroinflammation by promoting M1 microglial polarization, potentially leading to tau hyperphosphorylation (Chen and Yu, 2023; Hu et al., 2020; Zhang et al., 2020). Tauopathy, which is characterized by abnormal tau deposition and hyperphosphorylation, is a hallmark of the pathology of AD (Brunden et al., 2009). Surprisingly, our results showed that LPC70 effectively prevented HS-induced tau hyperphosphorylation in the PFC, suggesting a potential therapeutic strategy against AD-related pathologies. The association between vascular dysfunction, including hypertension and tau pathology has been supported by various animal studies. For instance, hypertension induced by the two-kidney, one-clip method accelerated tau pathology in 3xTg-AD mice (Shih et al., 2018). Similarly, our previous study showed that the anti-hypertensive drug losartan blocks hypertension as well as cognitive impairment and tau hyperphosphorylation induced by HS intake (Kubota et al., 2023). The dysfunction of eNOS due to intestinal inflammation can activate the calpain/cyclin-dependent kinase 5 (CDK5)/p25 pathway, leading to tau hyperphosphorylation (Faraco et al., 2019). Thus, the preventive effect of LPC70 on tau hyperphosphorylation may result from the neutralization of HS-induced hypertension and intestinal inflammation. Moreover, since lysolecithin easily crosses the blood-brain barrier and serves as a precursor to acetylcholine (Roy et al., 2022; Semba, 2020), LPC70 may have direct effects on cognitive function and tau phosphorylation in the brain. This possibility is supported by the evidence that choline-containing phospholipids exhibit anti-inflammatory effects by suppressing M1 microglial activation in animal models of cerebral

disease (Kim et al., 2018; Roy et al., 2022; Tokes et al., 2011).The collective results suggest that the protective mechanism of LPC70 against tau hyperphosphorylation may involve both peripheral and brain lesions induced by HS intake. As taken from Kubota H et. al. 2024. Neurochem Int. Available at: https://pubmed.ncbi.nlm.nih.gov/39271020/ *6.4. Other organ systems, dependent on the properties of the substance*

6.4. Other organ systems, dependent on the properties of the substance

6.4. Other organ systems, dependent on the properties of the substance

Egg-yolk soybean and hydrogenated soybean phosphatides are used for the preparation of fat emulsions for parenteral nutrition. The newer fat emulsions prepared using well-purified phosphatide preparations show a small incidence of side-effects in animals and man. Lecithin can be considered a non-toxic substance, even when given parenterally.

As taken from JECFA, 1974

“Although cisplatin is widely used in the treatment of cancers, clinical use of cisplatin is limited due to its nephrotoxicity. Pathophysiological mechanism of cisplatin-induced renal toxicity is a complex process and has not been fully understand. Reactive oxygen species (ROS) and oxidative stress have been presumed to be involved in this damage process. Phosphatidylcholine (PC) has antioxidant effect and prevents oxidative stress. Therefore, the present study aimed to investigate potential protective effects of PC on cisplatin-induced renal damage in rat. We examined the protective effects of PC on cisplatin-induced renal damage by assessment of serum creatinine, BUN, lipid peroxidation, total glutathione, glutathione peroxidase activity, catalase activity, superoxide dismutase activity and histophathological changes. PC ameliorated cisplatin-induced increases in serum creatinine, urea and oxidative stress. PC also decreased tubular degeneration and hypertrophy of glomeruli. PC may have a protective effect against cisplatin-induced nephrotoxicity in rats via enhancing antioxidant enzyme activity.” As taken from Lee HS et al. 2013. Food Chem. Toxicol. 58, 388-93. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23684996

7. Addiction

JTI is not aware of any information that demonstrates that this ingredient has any addictive effect.

8. Burnt ingredient toxicity

Tobacco smoke condensates from cigarettes containing lecithin hydrolyzed and an additive free, reference cigarettes were tested in a battery of in vitro and/or in vivo test(s). Within the sensitivity and specificity of the bioassay(s) the activity of the condensate was not changed by the addition of lecithin hydrolyzed (85711-58-6). Table below provides tested level(s) and specific endpoint(s).

Endpoint	Tested level (ppm)	Reference
Smoke chemistry	980	JTI KB Study Report(s)
In vitro genotoxicity	1500	JTI KB Study Report(s)
In vitro cytotoxicity	1500	JTI KB Study Report(s)
Inhalation study	1500	JTI KB Study Report(s)

9. Heated/vapor emissions toxicity

Aerosol from heated tobacco stick(s) containing Lecithins was tested in aerosol chemistry and a battery of in vitro test(s). Under the test conditions and within the sensitivity and specificity of the bioassay(s), the activity of the total particulate matter (TPM) and/or gas vapor phase (GVP) were

not increased by the addition of this ingredient when compared to TPM and/or GVP from reference combustible cigarettes. The table below provides the highest tested level(s) and specific endpoint(s):

Endpoint	Tested level (mg/stick)	Reference
Aerosol chemistry	0.110	Labstat International Inc. (2020a) Labstat International Inc. (2021a) Labstat International Inc. (2023a) JTI Heated Tobacco Stick Study Report(s)
In vitro genotoxicity	0.110	Labstat International Inc. (2020b) Labstat International Inc. (2021b) Labstat International Inc. (2023b) JTI Heated Tobacco Stick Study Report(s)
In vitro cytotoxicity	0.110	Labstat International Inc. (2020b) Labstat International Inc. (2021b) Labstat International Inc. (2023b) JTI Heated Tobacco Stick Study Report(s)

10. Ecotoxicity

10.1. Environmental fate

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that lecithins (CAS RN 8002-43-5) are not persistent in the environment.

Data accessed June 2015 on the OECD website

EPISuite provides the following data for CAS RN 8002-43-5

Henrys Law Constant (25 deg C) [HENRYWIN v3.20]:

Bond Method :	2.54E-022 atm-m3/mole (2.57E-017 Pa-m3/mole)
Group Method:	Incomplete
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:	HLC: 6.795E+000 atm-m3/mole (6.885E+005 Pa-m3/mole) VP: 1.78E-010 mm Hg (source: MPBPVP) WS: 2.75E-011 mg/L (source: WSKOWWIN)

Log Octanol-Air Partition Coefficient (25 deg C) [KOAWIN v1.10]:

Log Kow used:	13.41 (KowWin est)
Log Kaw used:	-19.984 (HenryWin est)
Log Koa (KOAWIN v1.10 estimate):	33.394
Log Koa (experimental database):	None

Probability of Rapid Biodegradation (BIOWIN v4.10):

Biowin1 (Linear Model): Biowin2 (Non-Linear Model) : Biowin3 (Ultimate Survey Model): Biowin4 (Primary Survey Model) : Biowin5 (MITI Linear Model) : Biowin6 (MITI Non-Linear Model): Biowin7 (Anaerobic Linear Model):	0.9328 0.9713 2.3125 (weeks-months) 3.6923 (days-weeks) 0.4498 0.0637 -0.2916
Ready Biodegradability Prediction:	NO

Hydrocarbon Biodegradation (BioHCwin v1.01):

Structure incompatible with current estimation method!

Sorption to aerosols (25 Dec C)[AEROWIN v1.00]:

Vapor pressure (liquid/subcooled):	1.01E-007 Pa (7.58E-010 mm Hg)
Log Koa (Koawin est):	33.394
Kp (particle/gas partition coef. (m3/ug)): Mackay model: Octanol/air (Koa) model:	 29.7 6.08E+020

Fraction sorbed to airborne particulates (phi):

Junge-Pankow model:	0.999
Mackay model:	1
Octanol/air (Koa) model:	1

Atmospheric Oxidation (25 deg C) [AopWin v1.92]:

Hydroxyl Radicals Reaction:

OVERALL OH Rate Constant =	324.8577 E-12 cm3/molecule-sec [Cis-isomer]
OVERALL OH Rate Constant =	355.2577 E-12 cm3/molecule-sec [Trans-isomer]
Half-Life =	23.706 Min (12-hr day; 1.5E6 OH/cm3) [Cis-isomer]
Half-Life =	21.678 Min (12-hr day; 1.5E6 OH/cm3) [Trans-isomer]

Ozone Reaction:

OVERALL Ozone Rate Constant =	52.000000 E-17 cm3/molecule-sec [Cis-]
OVERALL Ozone Rate Constant =	80.000000 E-17 cm3/molecule-sec [Trans-]
Half-Life =	31.735 Min (at 7E11 mol/cm3) [Cis-isomer]
Half-Life =	20.628 Min (at 7E11 mol/cm3) [Trans-isomer]
Reaction With Nitrate Radicals May Be Important!	
Fraction sorbed to airborne particulates (phi): 0.999 (Junge-Pankow, Mackay avg) 1 (Koa method) Note: the sorbed fraction may be resistant to atmospheric oxidation	

Soil Adsorption Coefficient (KOCWIN v2.00):

Koc :	2.943E+009 L/kg (MCI method)
Log Koc:	9.469 (MCI method)
Koc :	1.689E+008 L/kg (Kow method)
Log Koc:	8.228 (Kow method)

Aqueous Base/Acid-Catalyzed Hydrolysis (25 deg C) [HYDROWIN v2.00]:

Total Kb for pH > 8 at 25 deg C:	8.345E-002 L/mol-sec
Kb Half-Life at pH 8:	96.126 days
Kb Half-Life at pH 7:	2.632 years

(Total Kb applies only to esters, carbmates, alkyl halides)

Volatilization from Water:

Henry LC: 2.54E-022 atm-m3/mole (estimated by Bond SAR Method)

Half-Life from Model River:	6.512E+018 hours (2.713E+017 days)

Half-Life from Model Lake:	7.104E+019 hours (2.96E+018 days)

Removal In Wastewater Treatment:

Total removal:	94.04 percent
Total biodegradation:	0.78 percent
Total sludge adsorption:	93.26 percent
Total to Air:	0.00 percent

(using 10000 hr Bio P,A,S)

Level III Fugacity Model:

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	2.11e-012	0.317	1000
Water	10.9	900	1000
Soil	87.6	1.8e+003	1000
Sediment	1.49	8.1e+003	0

Persistence Time: 1.88e+003 hr

10.2. Aquatic toxicity

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that lecithins (CAS RN 8002-43-5) are of uncertain inherent toxicity to aquatic organisms.

Data accessed June 2015 on the OECD websiteECOSAR version 1.11 provides the following aquatic toxicity data for CAS RN 8002-43-5:

Values used to Generate ECOSAR Profile

Log Kow: 13.405 (EPISuite Kowwin v1.68 Estimate)

Wat Sol: 2.751E-011 (mg/L, EPISuite WSKowwin v1.43 Estimate)

ECOSAR v1.11 Class-specific Estimations
Esters

ALERT: The chemical you are assessing has structural features associated with known surfactant classes. If the chemical has surfactant properties, the user may consider evaluation under Surfactants-Cationic and Surfactants-Anionic Within the Special_Classes-Surfactants arm of ECOSAR (Menu Bar/Special Classes

ECOSAR Class	**Organism**	**Duration**	**End Pt**	**Predicted mg/L (ppm)**
Esters :	Fish	96-hr	LC50	2.33e-005 *
Esters :	Daphnid	48-hr	LC50	1.43e-005 *
Esters :	Green Algae	96-hr	EC50	9.98e-007 *
Esters :	Fish		ChV	1.6e-007 *
Esters :	Daphnid		ChV	3.68e-007 *
Esters :	Green Algae		ChV	1.04e-005 *
Esters :	Fish (SW)	96-hr	LC50	1.76e-005 *
Esters :	Mysid	96-hr	LC50	6.29e-008 *
Esters :	Fish (SW)		ChV	1.97e-005 *
Esters :	Mysid (SW)		ChV	3.72e-016
Neutral Organic SAR :	Fish	96-hr	LC50	3.75e-008 *

(Baseline Toxicity) :	Daphnid	48-hr	LC50	6.1e-008 *	
	Green Algae	96-hr	EC50	3.53e-006 *	
	Fish		ChV	1.27e-008 *	
	Daphnid		ChV	1.11e-007 *	
	Green Algae		ChV	9.66e-006 *	

Note: * = asterisk designates: Chemical may not be soluble enough to measure this predicted effect. If the effect level exceeds the water solubility by 10X, typically no effects at saturation (NES) are reported.

Class Specific LogKow Cut-Offs
If the log Kow of the chemical is greater than the endpoint specific cut-offs presented below, then no effects at saturation are expected for those endpoints.

Esters:

Maximum LogKow: 5.0 (Fish 96-hr LC50; Daphnid LC50, Mysid LC50)

Maximum LogKow: 6.4 (Green Algae EC50)

Maximum LogKow: 8.0 (ChV)

Baseline Toxicity SAR Limitations:

Maximum LogKow: 5.0 (Fish 96-hr LC50; Daphnid LC50)

ECOSAR Class: Surfactants, Anionic

Organism	Duration	End Pt	Predicted mg/L (ppm)
Fish	96-hr	LC50	9.31e+018
Daphnid	48-hr	LC50	9.31e+018
Green Algae	96-hr	EC50	1.84e+017
Fish	28-day	NEC	1.43e+018
Daphnid	21-day	NEC	1.43e+018
Green Algae	21-day	NEC	1.31e+017

ECOSAR Class: Surfactants, Cationic (C >= 16)

Organism	Duration	End Pt	Predicted mg/L (ppm)
Daphnid	acute	LC50	117.490
Fish	acute	LC50	2.69e+005

10.3. Sediment toxicity

No data available to us at this time.

10.4. Terrestrial toxicity

Record for lecithins (CAS RN 8002-43-5):

Spec. Sci. Name Spec. Common Name	Resp. Type Exp. Dur. (Days)	Media Type Test Loc.	Exp. Site Chem. Anal.	Dose# Res. Sample Unit	Endpoint BAF/BCF	Effect Effect Meas.	Signif. Sig. Level	Dose Dose Stat. Meth.
Algae, Moss, Fungi								
Oidium sp.		MIX	SP	2	LOEL	POP	ASIG	A 516.0

								ai g/L
Powdery Mildew	7	LAB	U			CNTL	0.05	
Oidium sp.		MIX	SP	2	LOEL	POP	ASIG	A 516.0 ai g/L
Powdery Mildew	6	LAB	U			CNTL	0.05	
Oidium sp.		MIX	SP	2	LOEL	POP	ASIG	F 2.0 %
Powdery Mildew	27	LAB	U			CNTL	0.05	
Oidium sp.		MIX	SP	2	LOEL	POP	ASIG	A 516.0 ai g/L
Powdery Mildew	27	LAB	U			CNTL	0.05	
Oidium sp.		MIX	SP	2	NOEL	POP	ANOSIG	A 516.0 ai g/L
Powdery Mildew	49	LAB	U			CNTL	0.05	

As taken from the US EPA ECOTOX database, accessed June 2015.

“A novel nanocarrier based on solid lipid nanoparticles (SLNs) was developed for insulin delivery using a novel double emulsion method. Physical stability of particles was assessed by size analysis using dynamic light scattering (DLS), matrix crystallinity by differential scanning calorimetry (DSC) and toxicity analysis by Drosophila melanogaster testing. Insulin-SLNs were composed of Softisan®100 1.25% wt, Lutrol®F68 1% wt, soybean lecithin 0.125% wt, and loaded with 0.73-0.58 mg/mL peptide. Placebo-SLNs (insulin-free) also contained 0.025% wt Tween®80. Mean particle sizes of placebo-SLN and insulin-SLN were 958 ± 9.5 and 978 ± 8.3 nm, respectively. The polydispersity index (PI) was 0.28 ± 0.018 and 0.29 ± 0.013, respectively. Polarized light microscopy analysis depicted no aggregation of developed particles. DSC analysis allowed characterizing SLN with 43-51% matrix crystallinity. Using Drosophila melanogaster test, no toxicity was reported for SLN and for the bulk lipid. This study shows that SLNs are promising and helpful to overcome conventional insulin therapy, in particular for their lack of toxicity for oral delivery.” As taken from Fangueiro JF et al. 2013. Pharm. Dev. Technol. 18, 545-9. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/21711084

“It was previously reported that the amounts of lysophosphatidylcholines (lysoPCs), which are naturally occurring bioactive lipid molecules, significantly increase following pathogen inoculation, as determined using ultraperformance liquid chromatography-quadrupole-time of flight/mass spectrometry analyses. Here, real-time quantitative RT-PCR was performed for the phospholipase A2 (PLA2) genes, Nt1PLA2 and Nt2PLA2, which are responsible for LysoPCs generation. The transcription level of Nt2PLA2 in pathogen-infected tobacco plants transiently peaked at 1h and 36 h, whereas induction of Nt1PLA2 transcription peaked at 36 h. A prominent biphasic ROS accumulation in lysoPC (C18:1(9Z))-treated tobacco leaves was also observed. Transcription of NtRbohD, a gene member of NADPH oxidase, showed biphasic kinetics upon lysoPC 18:1 treatment, as evidenced by an early transient peak in phase I at 1h and a massive peak in phase II at 12h. Each increase in NtACS2 and NtACS4 transcription, gene members of the ACC synthase family, was followed by biphasic peaks of ethylene production after lysoPC 18:1 treatment. This suggested that lysoPC (C18:1)-induced ethylene production was regulated at the transcriptional level of time-dependent gene members. LysoPC 18:1 treatment also rapidly induced cell damage. LysoPC 18:1-induced cell death was almost completely abrogated in ROS generation-impaired transgenic plants (rbohD-as and rbohF-as), ethylene production-impaired transgenic plants (CAS-AS and CAO-AS), and ethylene signaling-impaired transgenic plants (Ein3-AS), respectively. Taken together, pathogen-induced lysoPCs enhance pathogen susceptibility accompanied by ROS and ethylene biosynthesis, resulting in chlorophyll degradation and cell death. Expression of PR genes

(PR1-a, PR-3, and PR-4b) and LOX3 was strongly induced in lysoPC 18:1-treated leaves, indicating the involvement of lysoPC 18:1 in the defense response. However, lysoPC 18:1 treatment eventually resulted in cell death, as evidenced by metacaspase gene expression. Therefore, a hypothesis is proposed that the antipathogenic potential of lysoPC 18:1 is dependent on how quickly it is removed from cells for avoidance of lysoPC toxicity." As taken from Wi SJ et al. 2014. Phytochemistry 104, 48-59. PubMed, 2015 available at http://www.ncbi.nlm.nih.gov/pubmed/24837357

ECOSAR version 1.11 provides the following terrestrial toxicity data for CAS RN 8002-43-5:

Values used to Generate ECOSAR Profile

Log Kow: 13.405 (EPISuite Kowwin v1.68 Estimate)

Wat Sol: 2.751E-011 (mg/L, EPISuite WSKowwin v1.43 Estimate)

ECOSAR v1.11 Class-specific Estimations
Esters

ALERT: The chemical you are assessing has structural features associated with known surfactant classes. If the chemical has surfactant properties, the user may consider evaluation under Surfactants-Cationic and Surfactants-Anionic Within the Special_Classes-Surfactants arm of ECOSAR (Menu Bar/Special Classes

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)
Esters :	Earthworm	14-day	LC50	2.189 *

Note: * = asterisk designates: Chemical may not be soluble enough to measure this predicted effect. If the effect level exceeds the water solubility by 10X, typically no effects at saturation (NES) are reported.

Class Specific LogKow Cut-Offs
If the log Kow of the chemical is greater than the endpoint specific cut-offs presented below, then no effects at saturation are expected for those endpoints.

Esters:
Maximum LogKow: 6.0 (Earthworm LC50)

ECOSAR Class: Surfactants, Anionic and Surfactants, Cationic (C >= 16)

No entry for earthworm.

10.5. All other relevant types of ecotoxicity

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that lecithins (CAS RN 8002-43-5) are not bioaccumulative in the environment.

Data accessed June 2015 on the OECD websiteEPISuite provides the following data on CAS RN 8002-43-5:

Bioaccumulation Estimates (BCFBAF v3.01):

Log BCF from regression-based method:	1.850 (BCF = 70.79 L/kg wet-wt)
Log Biotransformation Half-life (HL):	1.6099 days (HL = 40.73 days)
Log BCF Arnot-Gobas method (upper trophic):	-0.048 (BCF = 0.8959)
Log BAF Arnot-Gobas method (upper trophic):	0.606 (BAF = 4.036)
log Kow used:	13.41 (estimated)

11. References

- AICIS (2017). Australian Government Department of Health. Australian Industrial Chemicals Introduction Scheme. Inventory Multi-Tiered Assessment and Prioritisation (IMAP) Tier I. Health record for lecithins (CAS RN 8002-43-5). Dated 17 December 2017. Available at:Attia Y.A. et al.(2012). Semen quality, testosterone, seminal plasma biochemical and antioxidant profiles of rabbit bucks fed diets supplemented with different concentrations of soybean lecithin. Animal; 6(5):824-33 available at https://services.industrialchemicals.gov.au/search-assessments/
- BPDB (2022). Biopesticide Database Last updated 28 July 2022. Available at: http://sitem.herts.ac.uk/aeru/bpdb/atoz.htm
- CCRIS (2008). Record for soybean lecithin. CCRIS Number: 7265. Last updated 15 January 2008 (no longer being updated as of 2011).Available at https://www.toxinfo.io/
- ChemSpider (a).Record for (2R)-2,3-diacetoxypropyl [2-(trimethylammonio)ethyl]phosphonate (CAS RN 8002-43-5).Undated, Available at https://www.chemspider.com/Chemical-Structure.21241544.html
- ChemSpider (b). Record for O-{[(2S)-2,3-bis(stearoyloxy)propoxy](hydroxy)phosphoryl}-L-serine (CAS RN 8002-43-5).Undated, Available at https://www.chemspider.com/Chemical-Structure.26563843.html
- ChemSpider (c). Record for 2,3-bis(stearoyloxy)propyl [2-(trimethylammonio)ethyl]phosphonate (CAS RN 8002-43-5).Undated, Available at https://www.chemspider.com/Chemical-Structure.24767653.html
- ChemSpider (d). Record for (2R)-3-[(9E,12Z)-9,12-octadecadienoyloxy]-2-(palmitoyloxy)propyl 2-(trimethylammonio)ethyl phosphate (CAS RN 8002-43-5).Undated, Available at https://www.chemspider.com/Chemical-Structure.50644834.html
- ChemSpider (e). Record for PC (CAS RN 8002-43-5). Undated, Available at https://www.chemspider.com/Chemical-Structure.4450224.html
- Choi, J. et al (2022). Protective Effects of Phosphatidylcholine against Hepatic and Renal Cell Injury from Advanced Glycation End Products.Medicina (Kaunas, Lithuania), 58(11), 1519. Available at https://pubmed.ncbi.nlm.nih.gov/36363476/
- CIR (2020). Safety assessment of lecithin and other phosphoglycerides as used in cosmetics. Available at: https://cir-reports.cir-safety.org/
- Comfort Onaolapo M et. Al. Lecithin and cardiovascular health: a comprehensive review. Egypt Heart J. 2024 Jul 13;76(1):92. doi: 10.1186/s43044-024-00523-0. Available at: https://pubmed.ncbi.nlm.nih.gov/39001966/
- CosIng. Cosmetic substances and ingredients database. Records for lecithin (CAS RN 8002-43-5/8030-76-0 (soybean)), phosphatidylcholine (CAS RN 8002-43-5) and lysolecithin (CAS RN 85711-58-6) Undated, Available at https://ec.europa.eu/growth/tools-databases/cosing/
- COSMOS Database. Integrated In Silico Models for the Prediction of Human Repeated Dose Toxicity of COSMetics to Optimise Safety. Record for lecithin (CAS RN 8002-43-5). Available at: https://ng.cosmosdb.eu/
- CPID (undated). Consumer Product Information Database. Record for lecithin (CAS RN 8002-43-5).Available at https://www.whatsinproducts.com/
- Dhawan VV et al. (2014). Apoptosis induction and anti-cancer activity of LeciPlex formulations. Cell. Oncol. (Dordr.) 37(5), 339-51. PubMed, 2015 available at http://www.ncbi.nlm.nih.gov/pubmed/25204961
- ECHA (2024). European Chemicals Agency. Classification and Labelling (C&L) Inventory database. Last updated 16 September 2024. Available at: https://echa.europa.eu/information-on-chemicals/cl-inventory-database
- ECHA. European Chemicals Agency. Information on Chemicals. Available at: https://echa.europa.eu/information-on-chemicals/registered-substances
- ECOSAR. Record for lecithins (CAS RN 8002-43-5). Accessed June 2015. Available to download, through EPISuite, at http://www.epa.gov/opptintr/exposure/pubs/episuitedl.htm

- EFSA (2017). European Food Safety Authority. Panel on Food Additives and Nutrient Sources added to Food (ANS). Re-evaluation of lecithins (E 322) as a food additive. EFSA J. 15, 4742. DOI: 10.2903/j.efsa.2017.4742. Available at https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2017.4742
- Elblehi SS et al. (2019). L-α-Phosphatidylcholine attenuates mercury-induced hepato-renal damage through suppressing oxidative stress and inflammation.Environ. Sci. Pollut. Res. Int. 26(9), 9333-9342. DOI: 10.1007/s11356-019-04395-9.PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30721437
- EMA (2017). Committee on Herbal Medicinal Products (HMPC), European Medicines Agency. European Union herbal monograph on Glycine max (L.) Merr., lecithinum. Final. 31 January 2017 EMA/HMPC/220599/2016. Available at http://www.ema.europa.eu/
- EPA ECOTOX Database. Record for lecithins (CAS RN 8002-43-5). Accessed June 2015.
- EPISuite (2017). Record for lecithins (CAS RN 8002-43-5). EPISuite version 4.11. Last updated June 2017. EPISuite is available to download at https://www.epa.gov/tsca-screening-tools/download-epi-suitetm-estimation-program-interface-v411
- EPISuite. Record for lecithins (CAS RN 8002-43-5). Accessed June 2015. EPISuite is available to download at http://www.epa.gov/opptintr/exposure/pubs/episuitedl.htm
- European Commission (a). Food Additives Database. Record for lecithins (E322). . Available at https://ec.europa.eu/food/food-feed-portal/screen/food-additives/search
- European Commission (b). Food Additives Database. Record for Group I, Additives.Available at https://ec.europa.eu/food/food-feed-portal/screen/food-additives/search
- Fangueiro JF et al. (2013). A novel lipid nanocarrier for insulin delivery: production, characterization and toxicity testing. Pharm. Dev. Technol. 18, 545-9. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/21711084
- FDA (1979). US Food and Drug Administration. Database of Select Committee on GRAS Substance (SCOGS). Lecithin. Report no. 106. Available at https://www.cfsanappsexternal.fda.gov/scripts/fdcc/?set=SCOGS
- FDA (2024a). US Food and Drug Administration. Substances Added to Food (formerly EAFUS). Last updated 2 July 2024. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/?set=FoodSubstances
- FDA (2024b). US Food and Drug Administration. Inactive Ingredient Database. Data through 3 July 2024. Available at https://www.accessdata.fda.gov/scripts/cder/iig/index.cfm
- FDA (2024c). The list of indirect additives used in food contact substances. Last updated 25 April 2024. Available at: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/?set=IndirectAdditives
- Fiume Z (2001). Final report on the safety assessment of Lecithin and Hydrogenated Lecithin.Int J Toxicol.2001; 20 Suppl 1:21-45.
- Grosspietzsch R. et al., (1976) [Case study of 14C-labelled lecithin placental transfer]. Fortschritte der Medizin; 94(27):1465-1467 [article in German] available at: http://europepmc.org/abstract/MED/989479
- Guyader-Joly C. et al., (1999).Effect of lecithin on in vitro and in vivo survival of in vitro produced bovine blastocysts after cryopreservation. Theriogenology; 52(7):1193-202. DOI: 10.1016/S0093-691X(99)00211-3.PubMed, available at https://pubmed.ncbi.nlm.nih.gov/10735097/
- Health Canada (2021). Drugs and Health Products. Natural Health Products Ingredients Database. Record for lecithin (CAS RN 8002-43-5), HPUS_lecithin and EHP_lecithin (no CAS RNs given). Last updated 1 July 2021. Available at: https://webprod.hc-sc.gc.ca/nhpid-bdipsn/
- HSDB (2016). Record for lecithins. Hazardous Substances Databank Number: 1903. Last Revision Date: 25 October 2016. Available at https://www.toxinfo.io/

- IFRA (undated). International Fragrance Association. IFRA Transparency List. Available at https://ifrafragrance.org/priorities/ingredients/ifra-transparency-list
- JECFA (1974). Joint FAO/WHO Expert Committee on Food Additives. Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers and thickening agents. WHO FOOD ADDITIVES SERIES NO. 5; Geneva, 25; World Health Organization Geneva. Available at http://www.inchem.org/documents/jecfa/jecmono/v05je42.htm
- JECFA (2007). Joint FAO/WHO Expert Committee on Food Additives. Online Edition: "Combined Compendium of Food Additive Specifications" Monograph 4 for Lecithin and Lecithin, Partially Hydrolyzed. Available at http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/
- JTI Heated Tobacco Stick Study Report(s)
- JTI KB Study Report(s).
- Jung TW et al. (2018). Phosphatidylcholine Causes Lipolysis and Apoptosis in Adipocytes through the Tumor Necrosis Factor Alpha-Dependent Pathway. Pharmacology 101(3-4), 111-119. DOI: 10.1159/000481571. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29186713
- Jung TW et al. (2019). Phosphatidylcholine causes adipocyte-specific lipolysis and apoptosis in adipose and muscle tissues. PLoS One 14(4), e0214760. DOI: 10.1371/journal.pone.0214760. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30958839
- Khafaga AF (2017). Exogenous phosphatidylcholine supplementation retrieve aluminum-induced toxicity in male albino rats.Environ. Sci. Pollut. Res. Int. 24(18), 15589-15598. DOI: 10.1007/s11356-017-9151-x.PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28523611
- Kim ST et al. (2018). Phosphatidylcholine attenuated docetaxel-induced peripheral neurotoxicity in rats. Drug Chem. Toxicol. 41(4), 476-485. DOI: 10.1080/01480545.2017.1390580. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29210293
- Kubota H et. Al. Soy lysolecithin prevents hypertension and cognitive impairment induced in mice by high salt intake by inhibiting intestinal inflammation. Neurochem Int. 2024 Sep 12:105858. doi: 10.1016/j.neuint.2024.105858. Available at: https://pubmed.ncbi.nlm.nih.gov/39271020/
- Kyong YY and Choi KH (2013). Woman with hemorrhagic spots on her abdomen. Clin. Toxicol. (Phila.) 51, 804-5. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23962098
- Labstat International Inc. (2020a) Characterization of Heat-not-Burn Emissions.Analytical Test Report(s).
- Labstat International Inc. (2020b) Determination of Mutagenic Response (Ames), Cytotoxic Response (NRU) and Genotoxic Response (ivMN) of Mainstream Aerosol Total Particulate Matter (TPM) and Mainstream Gas Vapor Phase (GVP) of Heat-not-burn Products.Biological Activity Test Report(s).
- Labstat International Inc. (2021a). Characterization of Heat-not-Burn Emissions.Analytical Test Report(s).
- Labstat International Inc. (2021b). Determination of Mutagenic Response (Ames), Cytotoxic Response (NRU) and Genotoxic Response (ivMN) of Mainstream Aerosol Total Particulate Matter (TPM) and Mainstream Gas Vapor Phase (GVP) of Heat-not-burn Products.Biological Activity Test Report(s).
- LabstatInternational Inc. (2023a). Characterization of Heat-not-Burn Emissions. Analytical Test Report(s).
- LabstatInternational Inc. (2023b). Determination of Mutagenic Response (Ames), Cytotoxic Response (NRU) and Genotoxic Response (ivMN) of Mainstream Aerosol Total Particulate

Matter (TPM) and Mainstream Gas Vapor Phase (GVP) of Heat-not-burn Products. Biological Activity Test Report(s).
- Lavaud F et al., (1994). Baker's Asthma Related to Soybean Lecithin Exposure.Allergy, Vol. 49, No. 3, pages 159-162.
- Lee HS et al. (2013). Protective role of phosphatidylcholine against cisplatin-induced renal toxicity and oxidative stress in rats. Food Chem. Toxicol. 58, 388-93. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23684996
- Liu ZY et al. (2020). Dietary choline, rather than betaine intake, is associated with hepatocellular carcinoma mortality. Food Funct. 11(9), 7866-7877. DOI: 10.1039/d0fo01890b. PubMed, 2021 available at https://pubmed.ncbi.nlm.nih.gov/32812611/
- Maneesh M. et al. (2005). Effect of exogenous lecithin on ethanol-induced testicular injuries in Wistar rats. Indian J Physiol Pharmacol; 49(3):297-304. As taken from http://www.ncbi.nlm.nih.gov/pubmed/16440847
- Oberheuser F. et al. (1977). [Intraamniotic application of lecithin in pregnant rabbits (author's transl)]. Zeitschrift fur Geburtshilfe und Perinatologie; 181(1):59-63. [Article in German] Available at: http://europepmc.org/abstract/MED/585273
- OECD. Organisation for Economic Cooperation and Development. The Global Portal to Information on Chemical Substances (eChemPortal). Lecithins (CAS RN 8002-43-5). Accessed June 2015. Available at: http://webnet.oecd.org/CCRWeb/Search.aspx
- Prakash P and Gorfe AA (2013). Phosphatidylcholine attenuates aggregation of nonsteroidal anti-inflammatory drugs with bile acid. Biochemistry 52, 7461-9. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24066846
- PubChem (a) (2023). Record for 1,2-diacyl-sn-glycero-3-phosphocholine (CAS RN 8002-43-5). Created 12 July 2007. Last modified 23 September 2023. Available at https://pubchem.ncbi.nlm.nih.gov/compound/16213884
- PubChem (b) (2023). Record for (R)-2-((9Z,12Z)-octadeca-9,12-dienoyloxy)-3-(palmitoyloxy)propyl 2-(trimethylammonio)ethyl phosphate (CAS RN 8002-43-5). Created 1 November 2017. Last modified 23 September 2023. Available at https://pubchem.ncbi.nlm.nih.gov/compound/131706484
- PubChem (c) (2023). Record for (3-hexadecanoyloxy-2-octadeca-9,12-dienoyloxypropyl) 2-(trimethylazaniumyl)ethyl phosphate (CAS RN 8002-43-5). Created 8 August 2005. Last modified 23 September 2023. Available at https://pubchem.ncbi.nlm.nih.gov/compound/122218
- PubChem (d) (2023). Record for Lecithins, hydrolyzed (CAS 85711-58-6).Created 21 March 2012. Modified 3 March 2023. Available at https://pubchem.ncbi.nlm.nih.gov/substance/135352609
- Reeds DN et al. (2013). Metabolic and structural effects of phosphatidylcholine and deoxycholate injections on subcutaneous fat: a randomized, controlled trial. Aesthet. Surg. J. 33, 400-8. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23439063
- Ridgway ND (2013). The role of phosphatidylcholine and choline metabolites to cell proliferation and survival. Crit. Rev. Biochem. Mol. Biol. 48, 20-38. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23350810
- RTECS (2006). Registry of Toxic Effects of Chemical Substances. Record for lecithins (CAS RN 8002-43-5).Last updated August 2006.
- Sadouki M and Bouchoucha M (2014). Changes of lipid and fatty acid absorption induced by high dose of citric acid ester and lecithin emulsifiers. Int J Food Sci Nutr 65(6), 728-732. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/24655117
- Sahle FF et al. (2014). Controlled penetration of ceramides into and across the stratum corneum using various types of microemulsions and formulation associated toxicity studies. Eur. J. Pharm. Biopharm. 86, 244-50. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23896195

- Shah R et al. (2017). Dietary exposures for the safety assessment of seven emulsifiers commonly added to foods in the United States and implications for safety. Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess. 34(6), 905-917. DOI: 10.1080/19440049.2017.1311420. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28346062
- Souza A Jr et al.(2014). Toxic excipients in medications for neonates in Brazil. Eur. J. Pediatr. 173(7), 935-45. PubMed, 2015 available at http://www.ncbi.nlm.nih.gov/pubmed/?term=24500397
- Tan Y et al., (2013). Lecithin in mixed micelles attenuates the cytotoxicity of bile salts in Caco-2 cells.Toxicol In Vitro.2013, Mar; 27(2):714-20.
- US EPA (2024). Safer Chemical Ingredients List. Last updated 22 May 2024. Available at https://www.epa.gov/saferchoice/safer-ingredients
- US EPA 2024 CDR (Chemical Data Reporting Rule) Partial Exempt list. Available at https://sor.epa.gov/sor_internet/registry/substreg/searchandretrieve/searchbylist/search.do
- US EPA 2024 CDR list (Chemical Data Reporting Rule). Available at https://sor.epa.gov/sor_internet/registry/substreg/searchandretrieve/searchbylist/search.do
- US EPA InertFinder Database (2024). Last updated 29 February 2024. Available at https://iaspub.epa.gov/apex/pesticides/f?p=INERTFINDER:1:0::NO:1
- US EPA TSCA inventory. Available at https://sor.epa.gov/sor_internet/registry/substreg/searchandretrieve/searchbylist/search.do
- WHO (2022). World Health Organization. Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Records for Lecithin and Lecithin, partially hydrolyzed (both CAS RN 8002-43-5). Includes all updates up to the 95th JECFA meeting (June 2022). Available at http://apps.who.int/food-additives-contaminants-jecfa-database/search.aspx
- Wi SJ et al. (2014). Lysophosphatidylcholine enhances susceptibility in signaling pathway against pathogen infection through biphasic production of reactive oxygen species and ethylene in tobacco plants. Phytochemistry 104, 48-59. PubMed, 2015 available at http://www.ncbi.nlm.nih.gov/pubmed/24837357
- Wiedeman AM et al. (2018). Variations in plasma choline and metabolite concentrations in healthy adults. Clin. Biochem. 60, 77-83. DOI: 10.1016/j.clinbiochem.2018.08.002. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30125545
- Yang Y et al. (2014). Synergy effects of herb extracts: pharmacokinetics and pharmacodynamic basis. Fitoterapia 92, 133-47. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24177191
- Zeng FF et al. (2014). Choline and betaine intakes are associated with reduced risk of nasopharyngeal carcinoma in adults: a case-control study. Br. J. Cancer 110, 808-16. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24169354

12. Other information

- Benton D Donohoe RT (2004). The influence on cognition of the interactions between lecithin, carnitine and carbohydrate.Psychopharmacology 175, 84.
- EFSA (2011). Consolidated list of Article 13 health claims. List of references received by EFSA. Part1m 2 and 4. IDs 1-1000, 1001-2000, 3001-4705. 5 April 2011.
- Gaby AR (2009). Nutritional approaches to prevention and treatment of gallstones.Alternative Medicine Review 14, 258.
- Nordic Working Group on Food Toxicology and Risk Assessment (NNT) (2002). Food additives in Europe 2000 - Status of safety assessments of food additives presently permitted in the EU.Nordic Food Additive Database.TEMANORD NUMBER 2002:560

- Pestel S et al. (2006). Effect of commonly used vehicles on gastrointestinal, renal, and liver function in rats.Journal of Pharmacological and Toxicological Methods 54, 200.
- Andrioli G et al. (1999). Differential effects of dietary supplementation with fish oil or soy lecithin on human platelet adhesion.Thrombosis and Haemostasis 82, 1522.
- Anon (1974). Lecithin-its composition, properties and use in cosmetic formulations.Cosmet. Perfum.89, 31.
- Anon (1993). Smart chocolate to feed the mind.New Scientist 138, 1867 13.
- Anon (2002). Phosphatidylcholine monograph. Alternative Medicine Review 7, 150.
- Armentia A (1994). Allergens associated with baker's asthma.Allergy 49, 906.
- Awazuhara H et al. (1998). Antigenicity of the proteins in soy lecithin and soy oil in soybean allergy.Clinical and Experimental Allergy 28, 1559.
- Azuma K et al. (2002). Combination of lipids and emulsifiers enhances the absorption of orally administered quercetin in rats.Journal of Agricultural and Food Chemistry 50, 1706.
- Bain MA et al. (2005). Trimethylamine: metabolic, pharmacokinetic and safety aspects.Current Drug Metabolism 6, 227.
- Baker Z et al. (1941). Inhibition by phospholipids of the action of synthetic detergents on bacteria.Journal of Experimental Medicine 74, 621.
- Barbeau A (1978). Lecithin in neurologic disorders. New England Journal of Medicine 299, 200.
- Barbeau A (1980). Lecithin in Parkinson's disease. Journal of Neural Transmission Suppl.16 187.
- Bell JM Lundberg PK (1985). Effects of a commercial soy lecithin preparation on development of sensorimotor behavior and brain biochemistry in the rat.Developmental Psychobiology 18, 59.
- Bell JM Slotkin TA (1985). Perinatal dietary supplementation with a commercial soy lecithin preparation: effects on behavior and brain biochemistry in the developing rat.Developmental Psychobiology 18, 383.
- Blumenthal M et al. (2000). Herbal medicine. Expanded Commission E Monographs. American Botanical Council
- Buang Y et al. (2005). Dietary phosphatidylcholine alleviates fatty liver induced by orotic acid.Nutrition 21, 867.
- Buchman AL et al. (2000). The effect of lecithin supplementation on plasma choline concentrations during a marathon.Journal of the American College of Nutrition 19, 768.
- Campbell PI (1963). Toxicity of some charged lipids used in liposome preparations.Cytobios 37, 21.
- Canty DJ (2001). Lecithin and choline: new roles for old nutrients. Handbook of Nutraceuticals and Functional Foods. Wildman R.E.C. (Ed.).CRC Press.
- Canty DJ et al. (1994). Lecithin and choline in human health and disease.Nutrition Research 52, 327.
- Carlson SE et al. (1996). Visual acuity and fatty acid status of term infants fed human milk and formulas with and without docosahexaenoate and arachidonate from egg yolk lecithin.Pediatric Research 39, 882.
- Cockburn F (2003). Role of infant dietary long-chain polyunsaturated fatty acids, liposoluble vitamins, cholesterol and lecithin on psychomotor development.Acta Paediatrica 442, 19.
- Colgan M Colgan L (1984). Do nutrient supplements and dietary changes affect learning and emotional reactions of children with learning difficulties?A controlled series of 16 cases.Nutrition and Health 3, 69.
- Commission (2005). Provisional list of monomers and additives notified to European Commission as substances which may be used in the manufacture of plastics or coatings intended to come into contact with foodstuffs. Synoptic Document (updated to June 2005). Additives (pages 114-297)

- Consumer Association (1993). Smart drugs. Which?Way to Health 22.Feb-00.
- Cosmetic Ingredient Review Expert Panel (2001). Final report on the safety assessment of lecithin and hydrogenated lecithin.International Journal of Toxicology 20, Suppl.1 21.
- Deshmukh DS et al. (1981). Toxicity and neuronal transport of stable liposomes and phospholipid in the nervous system.Neurochemical Research 6, 143.
- EFEMA (1999). EFEMA index of food emulsifiers. European Food Emulsifier Manufacturers' Association.3rd Edition.
- Epstein SS et al. (1967). Toxicity of antioxidants to Tetrahymena pyriformis.Journal of Protozoology 14, 238.
- Fagan DG et al. (1977). Acute pulmonary toxicity of a commercial fluorocarbon-lipid aerosol.Histopathology 1, 209.
- FDA (2008). Agency Response Letter: GRAS Notice No.GRN 000226. Internet Jan-03
- Fukunaga K et al. (2008). Marine phosphatidylcholine suppresses 1,2-dimethylhydrazine-induced colon carcinogenesis in rats by inducing apoptosis.Nutrition Research 28, 635.
- Ghyczy M Boros M (2001). Electrophilic methyl groups present in the diet ameliorate pathological states induced by reductive and oxidative stress: a hypothesis.British Journal of Nutrition 85, 409.
- Growdon JH et al. (1984). Plasma choline responses to lecithin-enriched soup.Psychopharmacology Bulletin 20, 603.
- Growdon JH et al. (1986). Piracetam combined with lecithin in the treatment of Alzheimer's disease.Neurobiology of Aging 7, 269.
- Guin JD (2005). Use of consumer product ingredients for patch testing.Dermatitis 16, 71.
- Haller WT Stocker RK (2003). Toxicity of 19 adjuvants to juvenile Lepomis macrochirus (bluegill sunfish).Environmental Toxicology and Chemistry 22, 615.
- Hollander D Morgan D (1980). Effect of plant sterols, fatty acids and lecithin on cholesterol absorption in vivo in the rat.Lipids 15, 395.
- Holmes-McNary MQ et al. (1996). Choline and choline esters in human and rat milk and in infant formulas.American Journal of Clinical Nutrition 64, 572.
- Houba R et al. (1998). Occupational respiratory allergy in bakery workers: a review of the literature.American Journal of Industrial Medicine 34, 529.
- Hung M-C et al.(2001). Learning behaviour and cerebral protein kinase C, antioxidant status, lipid composition in senescence-accelerated mouse: influence of a phosphatidylcholine - vitamin B12 diet.British Journal of Nutrition 86, 163.
- Hurley HJ Shelley WB (1959). The colloidal state as a stimulus for non-allergic epithelioid granulomas: experimental studies in man with pure sodium stearate and palmitate.Journal of Investigative Dermatology 33, 203.
- Imaizumi K et al. (1988). Lipid and eicosanoid levels in rats fed gamma-linolenic acid as triglyceride or phospholipid of the mold origin.Nutrition Reports International 38, 767.
- Ishinaga M et al. (2005). Cholesterol intake is associated with lecithin intake in Japanese people.Journal of Nutrition 135, 1451.
- Jackson IV et al. (1979). Treatment of tardive dyskinesia with lecithin.American Journal of Psychiatry 11, 1458.
- JECFA (1964). Specifications for the identity and purity of food additives and their toxicological evaluation: emulsifiers, stabilizers, bleaching and maturing agents. Seventh Report of the FAO/WHO Expert Committee on Food Additives.281
- JECFA (1974). Toxicological evaluation of certain food additives with a review of general principles and of specifications. Seventeenth Report of the Joint FAO/WHO Expert Committee on Food Additives.539
- JECFA (1974). Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers and thickening agents. Seventeenth report of the Joint FAO/WHO Expert Committee on Food Additives.WHO Food Additive Series 5

- JECFA (1987). Evaluation of certain food additives and contaminants. 30th Report of the Joint FAO/WHO Expert Committee on Food Additives.751
- JECFA (1991). Evaluation of certain food additives and contaminants. Thirty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives.806
- JECFA (1992). Compendium of food additive specifications: Volume 2. Combined specifications from the first to the thirty-seventh meeting 1956-1990.FAO Food and Nutrition Paper 52
- JECFA (1993). Compendium of food additive specifications. Addendum 2. Joint FAO/WHO Expert Committee on Food Additives 41st Session, Geneva, 9-18 February 1993.Rome, pp.v + 138.FAO Food and Nutrition Paper 52 Add.2
- JECFA (1993). Evaluation of certain food additives and contaminants. Forty-first report of the Joint FAO/WHO Expert Committee on Food Additives.Technical Report Series, World Health Organization 837 47.
- Jimenez MA et al. (1990). Evidence that polyunsaturated lecithin induces a reduction in plasma cholesterol level and favorable changes in lipoprotein composition in hypercholesterolemic rats.Journal of Nutrition 120, 659.
- Khan ZU Misra UK (1988). Endoplasmic reticulum phospholipid and mixed function oxidase system on proteins feeding.Nutrition Research 8, 163.
- Kidd PM (1996). Phosphatidylcholine: a superior protectant against liver damage.Alternative Medicine Review 1, 258.
- Knuiman JT et al. (1989). Lecithin intake and serum cholesterol.American Journal of Clinical Nutrition 49, 266.
- Lavand F et al. (1994). Baker's asthma related to soybean lecithin exposure.Allergy 49, 159.
- Lavaud F et al. (1994). Baker's asthma related to soybean lecithin exposure.Allergy 49, 159.
- LeBlanc M-J et al.(1998). The role of dietary choline in the beneficial effects of lecithin on the secretion of biliary lipids in rats.Biochimica et Biophysica ACTA 1393, 223.
- Lim S-Y Suzuki H (2000).Effect of dietary docosahexaenoic acid and phosphatidylcholine on maze behavior and fatty acid composition of plasma and brain lipids in mice.International Journal for Vitamin and Nutrition Research 70, 251.
- MAFF (1992). Food Advisory Committee report on the review of the Emulsifiers and Stabilisers in Food Regulations.FAC Report FdAC/REP/11
- MAFF (1992). Food Advisory Committee report on the review of the use of additives in foods specially prepared for infants and young children.FAC Report FdAC/REP/12
- MAFF HSE (1992). HSE routine review of non-agricultural pesticides.Pesticides Register 4, 2 1.
- Mason P (2007). Dietary Supplements. Third edition.Pharmaceutical Press ISBN 978 0 85369 653 7
- Ministry of Health (1988). Order of 28 July 1988 establishing purity criteria for lecithins, calcium citrates and phosphoric acid used as additives in the manufacture of foodstuffs; and propylene glycol used as an antioxidant diluent.Boletin Oficial del Estado 328, 25157.
- Minnunni M et al. (1992). Natural antioxidants as inhibitors of oxygen species induced mutagenicity.Mutation Research 269, 193.
- Muehlmann LA et al. (2010). Dietary supplementation with soybean lecithin increases pulmonary PAF bioactivity in asthmatic rats.Journal of Nutritional Biochemistry 21, 532.(10.1016/j.jnutbio.2009.03.001).
- Neggers YH Stitt KR (1986). Effects of high lead intake in children.Journal of the American Dietetic Association 86, 938.
- Nicolosi RJ et al. (2001). Dietary effects on cardiovascular disease risk factors beyond saturated fatty acids and cholesterol.Journal of the American College of Nutrition 20, 421S.
- Nishimukai M et al. (2003). Enteral administration of soyabean lecithin enhanced lymphatic absorption of triacylglycerol in rats.British Journal of Nutrition 90, 565.

- Nishiuchi Y (1976). Toxicity of agrochemicals to some freshwater organisms.XXXV. Aquiculture 24, 25.
- Nishiyama T et al. (1994). Formation and inhibition of genotoxic glyoxal and malonaldehyde from phospholipids and fish liver oil upon lipid peroxidation.Journal of Agricultural and Food Chemistry 42, 1728.
- NTIS (1974). Scientific literature reviews on generally recognized as safe (GRAS) food ingredients- Lecithins.NTIS Paper PB241970.
- NTIS (1977). Review of health aspects of lecithin as food ingredients.NTIS PB275751.
- Nuzzo F et al. (1985). Liposomes induce chromosome aberrations in human cultured cells.Experimental Cell Research 157, 397.
- O'Brian BC Andrews VG (1993). Influence of dietary egg and soybean phospholipids and triacylglycerols on human serum lipoproteins.Lipids 28, 7.
- O'Brien BC Corrigan SM (1988). Influence of dietary soybean and egg lecithins on lipids responses in cholesterol-fed guinea pigs.Lipids 23, 647.
- Olthof MR et al. (2005). Choline supplemented as phosphatidylcholine decreases fasting and postmethionine-loading plasma homocysteine concentrations in healthy men.American Journal of Clinical Nutrition 82, 111.
- Ostlund RE et al. (1999). Sitostanol administered in lecithin micelles potently reduces cholesterol absorption in humans.American Journal of Clinical Nutrition 70, 826.
- Palm M et al. (1999). Food allergy to egg and soy lecithins.Allergy 54, 1116.
- Pattle RE Burgess F (1961). The lung lining film in some pathological conditions.Journal of Pathology and Bacteriology 82, 315.
- Polichetti E et al. (1996). Cholesterol-lowering effect of soyabean lecithin in normolipidaemic rats by stimulation of biliary lipid secretion.British Journal of Nutrition 75, 471.
- Polichetti E et al. (1998). Stimulation of the apo AI-high density lipoprotein system by dietary soyabean lecithin in humans.Journal of Nutritional Biochemistry 9, 659.
- Polichetti E et al. (2000). Dietary polyenylphosphatidylcholine decreases cholesterolemia in hypercholesterolemic rabbits: role of the hepato-biliary axis.Life Sciences 67, 2563.
- Quarterman J Tam K (1987). The effect of lead and phospholipids on serum cholesterol.Proceedings of the Nutrition Society 46, 115A.
- Randels PM et al. (1984). Lithium and lecithin treatment in Alzheimer's disease: a pilot study.Hillside Journal of Clinical Psychiatry 6, 139.
- Reifenrath RRO (1984). Antitussive and antiasthmatic compositions containing micelles of cholesterol and lecithin.Chemical Abstracts 100, 365.
- Renaud C et al. (1996). Allergy to soy lecithin in a child. Journal of Pediatric Gastroenterology and Nutrition 22, 328.
- Ros E (2000). Intestinal absorption of triglyceride and cholesterol.Dietary and pharmacological inhibition to reduce cardiovascular risk.Atherosclerosis 151, 357.
- Sasaki J-i et al.(1987).Macrophage activating property of bean-lecithin.Nutrition Research 7, 865.
- SCF (1978). Emulsifiers, stabilizers, thickeners and gelling agents. Reports of the Scientific Commission for Food. Seventh series. Commission of the European Communities.
- SCF (1995). First report on certain additives used in the manufacture of plastic materials intended to come into contact with foodstuffs. Reports of the Scientific Committee for Food (33rd series). European Commission, Luxembourg.
- SCF (1995). Minutes of the 97th meeting of the Scientific Committee for Food held on 1-2 June 1995 in Brussels.III/5373/95-EN, pp.11.
- SCF (1996). Opinion on additives in nutrient preparations foruse in infant formulae, follow-on formulae and weaning foods.Reports of the Scientific Committee for Food (40th series).
- SCF (1997). Minutes of the 106th meeting of the Scientific Committee for Food held on 20-21 March 1997 in Brussels.III/5157/97-EN, Brussels, pp.11.

- Semplicini A et al. (1991). Effects of linoleic acid supplementation on blood pressure and kinetics of red cell sodium transport: the Piove de sacco study.Journal of Hypertension 9, S310.
- Simonsson P et al. (1982). Postprandial effects of dietary phosphatidylcholine on plasma lipoproteins in man.American Journal of Clinical Nutrition 35, 36.
- Spilburg CA et al. (2003). Fat-free foods supplemented with soy stanol-lecithin powder reduce cholesterol absorption and LDL cholesterol.Journal of the American Dietetic Association 103, 577.
- Steering Group on Chemical Aspects of Food Surveillance (1993). Dietary intake of food additives in the UK: initial surveillance.Food Surveillance Paper 37
- Takechi M et al. (1992). Time course and inhibition of saponin-induced hemolysis.Planta Medica 58, 128.
- Taylor SL Hefle SL (2001). Ingredient and labeling issues associated with allergenic foods.Allergy 56, Suppl.67 64.
- Teelmann K et al. (1984). Preclinical safety evaluation of intravenously administered mixed micelles.Arzneimittel-Forschung 34, 1517.
- Teshima S-I et al. (1984). Hypocholesterolemic effects of eicosapentaenoic acid, phospholipids, and phytosterols in rats.Memoirs of the Faculty of Fisheries, Kagoshima University 33, 79.
- van Aerts LAGJM (2010). Adipocytolyse door subcutane injectie van Lipostabil. [Adipocytolysis by subcutaneous Lipostabil injection.] RIVM Briefrapport 360007001/2010
- van Ee JH (2009). Soy constituents: modes of action in low-density lipoprotein management.Nutrition Reviews 67, 4 222.
- Verrett MJ et al. (1980). Toxicity and teratogenicity of food additive chemicals in the developing chicken embryo.Toxicology and Applied Pharmacology 56, 265.
- Volavka J et al. (1983). Effects of lecithin on EEG and blood levels of choline.Biological Psychiatry 18, 1175.
- Wade MJ (1977). Review of the health aspects of lecithin as food ingredients.NTIS FASEB PB275751, FDABF7823.
- Wenk GL (1992). Dietary factors that influence the neural substrates of memory. The Vulnerable Brain and Environmental Risks Vol.1 Eds., Isaacson R.L. & Jenson K.F. Chapter 3.
- West I Maibach HI (1995). Contact urticaria syndrome from multiple cosmetic components.Contact Dermatitis 32, 121.
- Wilbur R et al. (1982). Lecithin for tardive dyskinesia.American Journal of Psychiatry 139, 1375.
- Wilson TA et al. (1998). Soy lecithin reduces plasma lipoprotein cholesterol and early atherogenesis in hypercholesterolemic monkeys and hamsters: beyond linoleate.Atherosclerosis 140, 147.
- Wu P-F et al. (1998). Nitro-polycyclic aromatic hydrocarbon contents of fumes from heated cooking oils and prevention of mutagenicity by catechin.Mutation Research 403, 29.
- Wurtman RJ et al. (1977). Lecithin consumption raises serum-free-choline levels.Lancet ii 68.
- Yamaguchi T (1985). Formation of pyrolyzate mutagens from phospholipids.Mutation Research 147, 277.
- Yamamoto M et al. (1988). Effects of food components and additives on the formation of nitrosamides.Food Additives and Contaminants 5, 289.
- Yamori Y (1989). Experimental evidence for dietary prevention of cardiovascular diseases.Clinical and Experimental Pharmacology and Physiology 16, 303.
- Zeisel SH Blusztajn JK (1994). Choline and human nutrition.Annual Review of Nutrition 14, 269.

- Zhang AQ et al. (1999). Dietary precursors of trimethylamine in man: a pilot study.Food and Chemical Toxicology 37, 515.

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Re-evaluation of lecithins (E 322) as a food additive

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Abstract

The present opinion deals with the re-evaluation of lecithins (E 322) when used as a food additive. Lecithins (E 322) is an authorised food additive in the EU according to Annex II and Annex III to Regulation (EC) No 1333/2008 on food additives, and have been previously evaluated by JECFA in 1973 and by the SCF in 1982. Among lecithins, phosphatidylcholine is hydrolysed in choline in the cytidine-5-diphosphate-choline pathway in all cells of the body. Following the conceptual framework for the risk assessment of certain food additives re-evaluated under Commission Regulation (EU) No 257/2010, the Panel concluded that there was no need for a numerical ADI for lecithins (E 322) and that there was no safety concern for the general population from more than 1 year of age at the refined exposure assessment for the reported uses of lecithins (E 322) as a food additive. The Panel further concluded that there is no safety concern for the exposure to the choline from lecithins (E 322) as a food additive at use and use levels reported by industry. For infants (from 12 weeks up to 11 months of age), the Panel concluded that there was no safety concern at the refined exposure assessment for the reported uses of lecithins (E 322) as a food additive and for the choline from lecithins (E 322) as a food additive at use and use levels reported by industry. For infants and young children consuming foods for special medical purposes, the Panel concluded that there was no safety concern with respect to the refined exposure assessment for the reported uses of lecithins (E 322) as a food additive and for exposure to choline resulting from these uses of lecithins (E 322).

Keywords: Lecithins, E 322, CAS No 8002-43-5 (lecithins), CAS No 85711-58-6 (hydrolysed lecithins), phosphatidylcholine, choline

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Summary

The present opinion deals with the re-evaluation of lecithins (E 322) when used as a food additive.

Lecithins are mixtures or fractions of phosphatides obtained by physical procedures from animal or vegetable foodstuffs. Lecithins (E 322) is an authorised food additive in the European Union (EU) according to Annex II and Annex III to Regulation (EC) No 1333/2008 on food additives, and have been previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1973 (JECFA, 1974a,b) and by the Scientific Committee on Food (SCF) in 1982 (SCF, 1982).

The Panel noted that the composition of the preparations used in the various studies was different. However, because all the constituents were qualitatively similar, the Panel considered the studies relevant for the risk assessment of lecithins (E 322).

Among lecithins, phosphatidylcholine is hydrolysed in choline in the cytidine-5-diphosphate-choline pathway in all cells of the body. The content of choline that can theoretically be released from phosphatidylcholine containing two linoleate groups is 13.2%. For choline, the EFSA NDA Panel (2016) prepared a scientific opinion on dietary reference values (DRVs) in 2016 in which it was concluded that average requirements (ARs) and population reference intakes (PRIs) for choline could not be derived for adults, infants (aged 7–11 months) and children, and therefore defined adequate intakes (AIs) for total choline (free and bound). For infants during the first 6 months of life, the amount of total choline provided in human milk was considered adequate.

Following oral administration, phosphatidylcholine is absorbed intact or as lysophosphatidylcholine or choline after intestinal hydrolysis. In humans, dietary lecithins are hydrolysed by phospholipases to liberate choline which is rapidly absorbed and appears in plasma predominantly as free choline.

The acute toxicity of lecithins (E 322) in mice, rats and rabbits is low.

Subchronic toxicity studies in rats and dogs did not report any adverse effect, even at the highest doses tested (3,750 mg essential phospholipid (EPL)/kg body weight (bw) per day, 1,000 mg soya phosphatidylinositol or EPL/kg bw per day in rats and dogs, respectively, and 5,460 mg lecithins/kg bw per day in rats).

The Panel considered the available genotoxicity data on lecithins (E 322) to be sufficient to conclude that there is no concern with respect to genotoxicity.

Chronic toxicity studies in rats did not report any adverse effects, even at the highest dose tested (3,750 mg EPL/kg bw per day). No carcinogenic effects were reported in rats, even at the highest dose tested (1,470 and 2,280 mg soya lecithin/kg bw per day in males and females, respectively) for 2 years.

The Panel considered that no adverse effects were observed in the developmental toxicity studies performed in mice, rat and rabbits up to the highest dose tested. However, the Panel noted that no reproductive toxicity studies were available. Several neurodevelopmental toxicity studies were conducted with lecithin. The Panel concluded that the relevance of the studies is limited but, at concentrations of 5% soya lecithin and higher in the diet during the gestation, lactation and the post-weaning period, there were indications for alterations in the development of the brain.

The Panel noted that, in Annex II of Regulation (EC) No 1333/2008, the use levels of lecithins (E 322) in food for infants under the age of 12 weeks are included in categories 13.1.1, 13.1.5.1 and 13.1.5.2. The Panel considered that these uses would require a specific risk assessment; therefore, the current re-evaluation of lecithins (E 322) as a food additive is not considered to be applicable for infants under the age of 12 weeks. Concerning uses of lecithins in food for infants and young children, the Panel concurs with the SCF (1998) and SCF (2003).The Panel noted that it is prudent to keep the number of additives used in foods for infants and young children to the minimum necessary.

The Panel considered that the refined exposure assessment approach resulted in more realistic long-term exposure estimates compared to the *maximum level exposure assessment scenario*. From the *refined estimated exposure scenario*, in the *brand-loyal scenario*, mean exposure to lecithins (E 322) ranged from 7 mg/kg bw per day in adolescents to 82 mg/kg bw per day in children. The 95th percentile ranged from 15 mg/kg bw per day in adolescents to 187 mg/kg bw per day in children. In the *non-brand-loyal scenario*, mean exposure ranged from 3 mg/kg bw per day in adults/elderly to 22 mg/kg bw per day in toddlers. The 95th percentile ranged from 6 mg/kg bw per day in adults/elderly to 62 mg/kg bw per day in infants.

The Panel considered that dietary intakes of lecithins (E 322) from the regular diet could be estimated in average ranging from 4 to 71 mg/kg bw per day across all population age groups.

Lecithins (E 322) is used in a wide range of foods, and it is therefore not expected that brand-loyalty will result in higher exposure in general population, except in specific populations consuming foods for special medical purposes and in infants and young children consuming infant and/or

follow-on formulae. The Panel therefore selected the brand-loyal refined scenario as the most relevant exposure scenario for this additive in these specific situations when justified.

I. General population

a) Above 1 year of age

Following the conceptual framework for the risk assessment of certain food additives re-evaluated under Commission Regulation (EU) No 257/2010 (EFSA, 2014), and given that:

- adequate exposure data were available and the highest relevant exposure estimate calculated in the refined exposure assessment scenario based on the reported data from food industry was for toddlers (12–35 months) up to 175 mg lecithins/kg bw per day at the 95th percentile (brand-loyal scenario),
- exposure via natural occurrence as reported by JECFA provided a daily mean intake of several grams of lecithin (approximately 1–5 g corresponding to 14–71 mg/kg bw for a 70-kg adult population),
- lecithins are natural constituents of all cells in the human body and also are natural components of the diet,
- toxicity database for lecithins was overall sufficient but not adequate regarding the endpoint of neurobehavioural developmental effects,
- there was no concern with respect to genotoxicity,
- no adverse effects were reported in chronic and carcinogenicity study in rats at the highest dose tested of 3,750 mg lecithins/kg bw per day,

the Panel concluded that there was no need for a numerical acceptable daily intake (ADI) for lecithins (E 322) and that there was no safety concern for the general population from more than 1 year of age at the refined exposure assessment for the reported uses of lecithins (E 322) as a food additive.

Moreover, taking into consideration that:

- hydrolysed lecithins and choline are produced in the gut as a result of normal digestion of lecithins. Choline is rapidly absorbed and appears in plasma predominantly as free choline,
- choline is a precursor of the neurotransmitter acetylcholine,
- the content of choline, that can theoretically be released from phosphatidylcholine containing two linoleate groups, is up to 13.2%, and the measured content of choline from commercial lecithins (E 322) up to 3.4%,
- 13.2% release would result in exposure up to 23 mg choline/kg bw per day at the 95th percentile intake of lecithins in toddlers (brand loyal scenario),
- total choline intake considering regular diet (estimated in average ranging from 4 to 18 mg/kg bw per day) across all population age groups and choline intake resulting from lecithins (E 322) used as a food additive are below the upper intake level (UL) for choline defined by the IOM (1998),

the Panel concluded that there is no safety concern for the exposure to the choline from lecithins (E 322) as a food additive at use and use levels reported by industry.

b) Infants (from 12 weeks up to 11 months of age)

Taking further into consideration that:

- adequate exposure estimates calculated in the refined exposure assessment scenario based on the reported data from food industry for infants (12 weeks to 11 months) was up to 163 mg/kg bw per day at the 95th percentile (brand-loyal scenario),
- 13.2% release would result in exposure up to 22 mg choline/kg bw per day at the 95th percentile dietary exposure of lecithins (E 322) in infants (brand loyal scenario),
- total choline intake considering regular diet in the same population group (estimated in average ranging from 9 to 16 mg/kg bw per day), and choline intake resulting from lecithins used as a food additive were in the same order as the adequate intake levels (AI) (EFSA NDA, 2016),

the Panel concluded that there was no safety concern at the refined exposure assessment for the reported uses of lecithins (E 322) as a food additive and for the choline from lecithins (E 322) as a food additive at use and use levels reported by industry.

II. Infants and young children consuming foods for special medical purposes

Taking further into consideration that:

- with respect to the exposure estimates calculated based on the reported data from food industry for infants (12 weeks to 11 months) and young children, the highest exposure was 232 mg lecithins/kg bw per day for toddlers (12–35 months) at the 95th percentile (brand-loyal scenario),
- 13.2% release would result in exposure up to 31 mg choline/kg bw per day at the 95th percentile dietary exposure of lecithins (E 322) in toddlers (brand loyal scenario),
- total choline intake considering regular diet in the same population group (estimated on average as ranging from 13–18 mg/kg bw per day), and choline intake resulting from lecithins used as a food additive, are in the same order as the adequate intake levels (AI) (EFSA NDA, 2016),

the Panel concluded that there was no safety concern with respect to the refined exposure assessment for the reported uses of lecithins (E 322) as a food additive and for exposure to choline resulting from these uses of lecithins (E 322).

The Panel recommended that the maximum limits for the impurities of toxic elements (lead, mercury and arsenic) in the EU specification for lecithins (E 322) should be revised in order to ensure that lecithins (E 322) as a food additive will not be a significant source of exposure to those toxic elements in food. The Panel recommended that the limit for cadmium should be included in the specifications.

The Panel noted some case reports of hypersensitivity reactions associated with soya and egg lecithins (see Section 3.5.7). The Panel agree with the opinion from EFSA NDA Panel (2014) that this hypersensitivity is due to the residual proteins in lecithins (E 322) and therefore their content should be reduced as much as possible.

Regarding the results of the inadequate neurobehavioural studies, to clarify the relevance of the data, a study with lecithins (E 322) in compliance with the current OECD TG 426 would be warranted.

In case the food additive lecithins (E 322) is used in infant formulae and follow-on formulae supplemented with choline or choline salts (see Section 1.2), the Panel recommended that the intake of choline from all sources including the use of the food additive lecithins (E 322) via infant formulae (category 13.1.1), follow-on formulae (category 13.1.2) or other food should be in the order of the AIs defined by the EFSA NDA Panel (2016).

The Panel noted discrepancies between the data reported from industry and the Mintel database, where lecithins (E 322) is labelled in more products than in food categories for which data were reported from industry. Therefore, the Panel recommended collection of data of usage and use levels of lecithins (E 322) in order to perform a more realistic exposure assessment. Moreover, there are several authorised uses that are not supported by data submitted by industry nor by the Mintel database.

Table of contents

1. Introduction

The present opinion deals with the re-evaluation of lecithins (E 322) when used as a food additive.

1.1. Background and Terms of Reference as provided by the European Commission

1.1.1. Background as provided by the European Commission

Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives requires that food additives are subject to a safety evaluation by the European Food Safety Authority (EFSA) before they are permitted for use in the European Union. In addition, it is foreseen that food additives must be kept under continuous observation and must be re-evaluated by EFSA.

For this purpose, a programme for the re-evaluation of food additives that were already permitted in the European Union before 20 January 2009 has been set up under the Regulation (EU) No 257/2010[1]. This Regulation also foresees that food additives are re-evaluated whenever necessary in light of changing conditions of use and new scientific information. For efficiency and practical purposes, the re-evaluation should, as far as possible, be conducted by group of food additives according to the main functional class to which they belong.

The order of priorities for the re-evaluation of the currently approved food additives should be set on the basis of the following criteria: the time since the last evaluation of a food additive by the Scientific Committee on Food (SCF) or by EFSA, the availability of new scientific evidence, the extent of use of a food additive in food and the human exposure to the food additive taking also into account the outcome of the Report from the Commission on Dietary Food Additive Intake in the EU[2] of 2001. The report 'Food additives in Europe 2000'[3] submitted by the Nordic Council of Ministers to the Commission, provides additional information for the prioritisation of additives for re-evaluation. As colours were among the first additives to be evaluated, these food additives should be re-evaluated with a highest priority.

In 2003, the Commission already requested EFSA to start a systematic re-evaluation of authorised food additives. However, as a result of adoption of Regulation (EU) 257/2010 the 2003 Terms of References are replaced by those below.

1.1.2. Terms of Reference as provided by the European Commission

The Commission asks the European Food Safety Authority to re-evaluate the safety of food additives already permitted in the Union before 2009 and to issue scientific opinions on these additives, taking especially into account the priorities, procedures and deadlines that are enshrined in the Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with the Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives.

1.1.3. Interpretation of Terms of Reference

The Panel on Food Additives and Nutrient Sources added to Food (ANS) described its risk assessment paradigm in its Guidance for submission for food additive evaluations in 2012 (EFSA ANS Panel, 2012). This Guidance states that, in carrying out its risk assessments, the Panel sought to define a health-based guidance value, such as an acceptable daily intake (ADI) (IPCS, 2004), applicable to the general population. According to the definition above, the ADI as established for the general population does not apply to infants below 12 weeks of age (JECFA, 1978; SCF, 1998). In this context, the re-evaluation of the use of food additives, such as thickening agents and certain emulsifiers, in food for infants below 12 weeks represents a special case for which specific recommendations were given by the Joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) (JECFA, 1972, 1978) and by the SCF (SCF, 1996, 1998). The Panel endorsed these recommendations.

[1] Commission Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives. OJ L 80, 26.3.2010, pp. 19–27.
[2] COM(2001) 542 final.
[3] Food Additives in Europe 2000, Status of safety assessments of food additives presently permitted in the EU, Nordic Council of Ministers, TemaNord 2002, p. 560.

In the current EU legislation (Regulation (EC) No 1333/2008[1] use levels of additives in food for infants under the age of 12 weeks in categories 13.1.1 and 13.1.5.1 (Annex II) and uses of food additives in nutrient preparations for use in food for infants under the age of 12 weeks and maximum levels for the carry-over from these uses (Annex III, Part 5, section B) are included. The Panel considers that these uses would require a specific risk assessment in line with the recommendations given by JECFA and SCF and endorsed by the Panel in its current Guidance for submission for food additives evaluations (EFSA ANS Panel, 2012). Therefore a risk assessment as for the general population is not considered to be applicable for infants under the age of 12 weeks and will be performed separately.

This re-evaluation refers exclusively to the uses of lecithins (E 322) as a food additive in food, including food supplements, and does not include a safety assessment of other uses of lecithins.

1.2. Information on existing evaluations and authorisations

Lecithins

Lecithins (E 322) is an authorised food additive in the European Union (EU) according to Annex II and Annex III to Regulation (EC) No 1333/2008 on food additives and specific purity criteria on lecithins (E 322) have been defined in the Commission Regulation (EU) No 231/2012.

In the EU, lecithins (E 322) has been evaluated by the SCF in 1981 (SCF, 1982), who discussed hydrolysed lecithins and their comparability to lecithins stating that, in the final conclusion, 'hydrolysed lecithin is produced in the gut as a result of normal digestion. There appears to be no specific toxicological effect in rats due to feeding of hydrolysed lecithins. This substance can therefore be regarded metabolically and toxicologically as an alternative to lecithin'.

Referring to older neurobehavioural studies, the SCF considered in 1997 that 'the issue of lecithins and choline in infant formulae should be considered further. However, in the context of carry-over levels of only 0.5 mg/kg, the use of lecithins in nutrient preparations for infant formulae is acceptable and not likely to be of concern' (SCF, 1997). The SCF further outlined, in 1997, 'In an earlier report (SCF, 1983) the Committee considered lecithins as acceptable technological additives at levels up to 5 g/L. However, the Directive on Additives Other Than Colours and Sweeteners[4] lists the maximum level as 1 g/L. This reduction in the maximum level was agreed during the negotiations on the draft Directive in response to a report (UK Ministry of Agriculture Fisheries and Food, 1992) which recommended that the maximum level of lecithins in infant formulae should be restricted to that of human milk (1 g/L). This recommendation was based on studies which claimed neurobehavioural effects in the offspring of rats fed high doses of lecithin. Although these studies were of poor quality, the report noted that large increases in plasma choline could affect neurotransmission in the brain and that particular caution was needed in the infant since the brain was still actively developing'.

Lecithins (E 322) was evaluated by JECFA in 1974 (JECFA, 1974a,b). For lecithin (JECFA, 1974a), JECFA did not specify a numerical ADI (ADI 'not limited').

In 2014, the EFSA Panel on Dietetic Products, Nutrition and Allergies (EFSA NDA Panel, 2014) prepared a scientific opinion on the evaluation of allergenic foods and food ingredients for labelling purposes where the allergenicity of egg and soya lecithins were considered. The possibility of residual allergenicity in food products manufactured using egg lecithin has been reported in a double-blind placebo-controlled food challenge (DBPCFC). Heat denaturation and other food-processing treatments do not reliably reduce the allergenicity of egg. Minimum eliciting doses (MEDs) of ingested egg proteins reported to trigger objective reactions in clinical studies range from few micrograms to milligrams.

The prevalence of clinically confirmed soya allergy in unselected populations in Europe appears to be low, although available studies are scarce. The sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) protein pattern of the standard soya lecithin is very similar to that of soya flour. The lowest MED reported in soya-allergic patients undergoing DBPCFC was 0.2 mg of soya protein, although the majority of patients only reacted to higher doses (EFSA NDA Panel, 2014).

Soybeans and eggs and products thereof (including lecithins) are listed in the Annex II of the Regulation 1169/2011 as substances or products causing allergies or intolerances which indication as allergens is mandatory food information.

[4] European Parliament and Council Directive N.95/2/EC on 20 February 1995 on Food Additives Other Than Colours and Sweeteners, OJ L 61, 18.3.1995, p. 1.

Lecithins are currently authorised in the EU as feed additives (as emulsifying agents) for an unlimited period for all species or categories of animals (Commission Directive of 12 April 1991 amending the Annexes to Council Directive 70/524/EEC concerning additives in feedingstuffs (91/248/EEC)).[5]

In 2016, the EFSA Panel on Additives and Products or Substances used in Animal Feed (EFSA FEEDAP Panel, 2016) prepared a scientific opinion on safety and efficacy of lecithins for all animal species. The FEEDAP Panel considered that lecithins are safe for all target species, and that setting a maximum content for lecithins is not considered necessary.

According to the information provided by the European Medicines Agency (EMA), lecithins are used as an excipient in a large number of 'centrally authorized medical products' as well as in 'nationally authorized medical products'. The Committee on Herbal Medicinal Products (HMPC) of the EMA published a draft monograph accepting the traditional medicinal use of soya bean lecithin (deoiled, enriched phospholipids from soya bean).

Choline

In humans, dietary lecithins are known to be hydrolysed and liberate choline (see Section 3.5).

The EFSA NDA Panel (2016) prepared a scientific opinion on dietary reference values (DRVs) for choline. In this opinion, the NDA Panel considered dietary choline including choline compounds (e.g. glycerophosphocholine, phosphocholine, phosphatidylcholine, sphingomyelin). The NDA Panel considered that none of the biomarkers of choline intake or status was suitable for deriving DRVs for choline. With respect to choline intake and possible health consequences, the NDA Panel concluded that there is a lack of data on choline intake in infants in the second half year of life and on associations between choline intake and health outcomes in children that could be used to set requirement for choline in these age groups. Overall, the NDA Panel concluded that average requirements (ARs) and population reference intakes (PRIs) for choline could not be derived for adults, infants and children, and therefore defined adequate intakes (AIs):

- For all adults, the Panel set an AI at 400 mg/day based on the average observed choline intake in healthy populations in the EU and in consideration of the amounts of choline needed to replete about 70% of depleted subjects who showed signs of organ dysfunction in a depletion/repletion study.
- Considering that there is no evidence for an insufficient choline intake of fully breast-fed infants during the first 6 months of life, the amount of choline provided in human milk was considered to be adequate. Considering a choline concentration of 145 mg/L (average of two studies on full-term infants) and assuming a mean milk transfer of 0.8 L/day during the first 6 months of lactation in exclusively breastfeeding women, the estimated choline intake of fully breast-fed infants during the first 6 months of life would be 116 mg/day, rounded up to 120 mg/day.
- For all infants aged 7–11 months, the NDA Panel derived an AI of 160 mg/day and, for children aged 1–17 years, AIs range from 140 mg/day (1–3 years) to 400 mg/day (15–17 years).
- For pregnant women, the NDA Panel derived an AI of 480 mg/day, calculated by extrapolation from the AI for non-pregnant women and the mean gestational increase in body weight.

For lactating women, the amount of choline secreted per day in human milk during the first 6 months of exclusive breastfeeding (120 mg/day) was added to the AI for non-lactating women, and an AI of 520 mg/day is set. With regard to excessive intake of choline, the NDA Panel referenced on the setting of tolerable upper intake levels (ULs) for choline by the US Institute of Medicine (IOM, 1998) and noted that no UL was established by IOM for infants (EFSA NDA Panel, 2016).

In 1998, the Food and Nutrition Board of the IOM established ULs for choline (Table 1) (IOM, 1998). The recommendation for adults was based on a single case report of hypotension, several other studies involving cholinergic effects and secondarily, on preventing the fishy body odour due to increased excretion of trimethylamine. For infants, the UL was judged not determinable because of a lack of data concerning adverse effects in this age group and concern about the infant's ability to handle excess amounts. According to IOM, 'the only source of intake of choline for infants should be from food or formula to prevent high levels of intake'. The UL of 3.5 g/day for adults was adjusted for children and adolescents on the basis of relative body weight.

[5] Reg (EC) No 1831/2003. European Union Register of Feed Additives. Edition 254. Appendixes 3e, 4 – 23.03.2017 European Union legislation on feed additives: http://ec.europa.eu/food/safety/animal-feed/feed-additives/index_en.htm

Table 1: Tolerable upper intake level (UL) for choline (IOM, 1998)

Age group	UL (mg/day)
Infants 0–12 months	Not possible to establish; source of intake should be food and formula only
Children 1–8 years	1,000
Children 9–13 years	2,000
Adolescents 14–18 years*	3,000
Adults 19 years and older*	3,500

*: Including pregnancy and lactation.

The IOM noted that individuals with trimethylaminuria, renal or liver disease, depression or Parkinson's disease might be at increased risk of adverse effects with choline intakes at the UL (IOM, 1998).

Choline, choline chloride, choline citrate, choline bitartrate are listed in Annex III of Commission Directive 2006/141/EC on infant formulae and follow-on formulae and amending Directive 1999/21/EC of 22 December 2006 and may be used in the manufacture of infant formulae and follow-on formulae.

2. Data and methodologies

2.1. Data

The ANS Panel was not provided with a newly submitted dossier. EFSA launched public calls for data[6,7,8] and, if relevant, contacted other risk assessment bodies to collect relevant information from interested parties.

The Panel based its assessment on information submitted to EFSA following the public calls for data, information from previous evaluations and additional available literature up to the last Working Group meeting before the adoption of the opinion.[9] Attempts were made at retrieving relevant original study reports on which previous evaluations or reviews were based; however, these were not always were these available to the Panel.

The EFSA Comprehensive European Food Consumption Database (Comprehensive Database)[10] was used to estimate the dietary exposure.

The Mintel's Global New Products Database (GNPD) is an online resource listing food products and compulsory ingredient information that should be included in labelling. This database was used to verify the use of lecithins (E 322) in food products.

2.2. Methodologies

This opinion was formulated following the principles described in the EFSA Guidance on transparency in the scientific aspects of risk assessment (EFSA Scientific Committee, 2009) and following the relevant existing Guidances from the EFSA Scientific Committee.

The ANS Panel assessed the safety of lecithins (E 322) as a food additive in line with the principles laid down in Regulation (EU) 257/2010 and in the relevant guidance documents: Guidance on submission for food additive evaluations by the SCF (2001) and taking into consideration the Guidance for submission for food additive evaluations in 2012 (EFSA ANS Panel, 2012).

When the test substance was administered in the feed or in the drinking water, but doses were not explicitly reported as mg/kg bw per day based on actual feed or water consumption, the daily intake was calculated by the Panel using the relevant default values as indicated in the EFSA Scientific Committee Guidance document (EFSA Scientific Committee, 2012) for studies in rodents or, in the case of other animal species, by JECFA (2000). In these cases, the daily intake is expressed as equivalent. When, in human studies in adults (aged above 18 years), the dose of the test substance administered

[6] Call for scientific data on food additives permitted in the EU and belonging to the functional classes of emulsifiers, stabilisers and gelling agents. Published: 23 May 2010. Available online: http://www.efsa.europa.eu/en/dataclosed/call/ans091123

[7] Call for food additives usage level and/or concentration data in food and beverages intended for human consumption – Extended deadline: 30 September 2014. Available online: http://www.efsa.europa.eu/sites/default/files/consultation/140310.pdf

[8] Call for data on lecithins (E 322) permitted as food additives in the EU – Extended Deadline: 31 December 2015. Available online: http://www.efsa.europa.eu/it/data/call/150608

[9] 23 November 2016.

[10] Available online: http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb.htm

was reported in mg/person per day, the dose in mg/kg bw per day was calculated by the Panel using a body weight of 70 kg as default for the adult population as described in the EFSA Scientific Committee Guidance document (EFSA Scientific Committee, 2012).

Dietary exposure to lecithins (E 322) from its use as a food additive was estimated combining food consumption data available within the EFSA Comprehensive European Food Consumption Database with the maximum levels according to Annex II to Regulation (EC) No 1333/2008[11] and/or reported use levels and analytical data submitted to EFSA following a call for data. Different scenarios were used to calculate exposure (see Section 3.3.1). Uncertainties on the exposure assessment were identified and discussed.

In the context of this re-evaluation, the Panel followed the conceptual framework for the risk assessment of certain food additives re-evaluated under Commission Regulation (EC) No 257/2010 (EFSA ANS Panel, 2014).

3. Assessment

3.1. Technical data

3.1.1. Identity of the substance

According to Commission Regulation (EU) No 231/2012[12], the lecithins (E 322) is identified as mixtures or fractions of phosphatides obtained by physical procedures from animal or vegetable foodstuffs. They also include the corresponding hydrolysed products. Although Commission Regulation No 231/2012 includes both types of lecithins (non-hydrolysed and hydrolysed) under the same food additive (E 322), JECFA differentiates between them and treats them as different food additives (INS 322i and INS 322ii) with distinct specifications (see Section 3.1.2).

In the CAS Registry Numbers database, different CAS numbers are listed for specific lecithins.[13] The general CAS number for lecithins is 8002-43-5. The CAS number for hydrolysed lecithins is 85711-58-6. However, depending on the source of the lecithins, different CAS numbers have been assigned. For example, the soya bean lecithins have the CAS number 8030-76-0, and the egg phospholipids have the CAS number 93685-90-6. The European Inventory of Existing Commercial Chemical Substances (EINECS) number for lecithins, described as the complex combination of diglycerides of fatty acids linked to the choline ester of phosphoric acid, is 232-307-2. This is also the EINECS number given in the Commission Regulation No 231/2012, even though, under this number, the EINECS database does not refer to hydrolysed lecithins. For hydrolysed lecithins, the EINECS number is 288-318-8. The EINECS number to soya bean lecithins is 310-129-7 and, for egg yolk, lecithins is 297-639-2.

According to Commission Regulation No 231/2012, lecithins appear as a brown liquid or viscous semiliquid or powder. Hydrolysed lecithins are light brown to brown viscous liquid or paste.

Synonyms for lecithins are phosphatides or phospholipids. For hydrolysed lecithins, the synonyms are lysolecithins or lysophospholipids (Tanno, 2012; SciFinder, 2013).

The main source of lecithins is soya bean oil. Other plant sources include oil from cottonseeds, corn, sunflower seeds and rapeseed, together with animal sources such as egg yolk and bovine brain (Wendel, 1995; Tanno, 2012). The Panel noted that the use of bovine brain has not been confirmed by the industries.

As defined in the ChemIDplus database, lecithins are 'A complex mixture of phospholipids, glycolipids and triglycerides with substantial amounts of phosphatidylcholines, phosphatidylethanolamines and phosphatidylinositols, which are sometimes loosely termed as 1,2-diacyl-3-phosphocholines' (ChemIDplus, 2014).

The structural formulae of the main phospholipids in lecithins (E 322) are given in Figure 1. The fatty acid moiety of phospholipids can differ, such as between stearic, palmitic, oleic and linoleic acids (Wendel, 1995; Merck Index, 2006; Tanno, 2012).

Because the fatty acids in lecithins have variable carbon chain lengths, an exact molecular formula and a molecular weight can only be given for individual components. For example, the molecular formula for the phosphatidylcholine containing two linoleate groups is $C_{44}H_{80}O_8NP$ and the molecular weight is 782.1 g/mol.

[11] Commission Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 16.

[12] Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, pp. 1–295.

[13] SciFinder, 2013

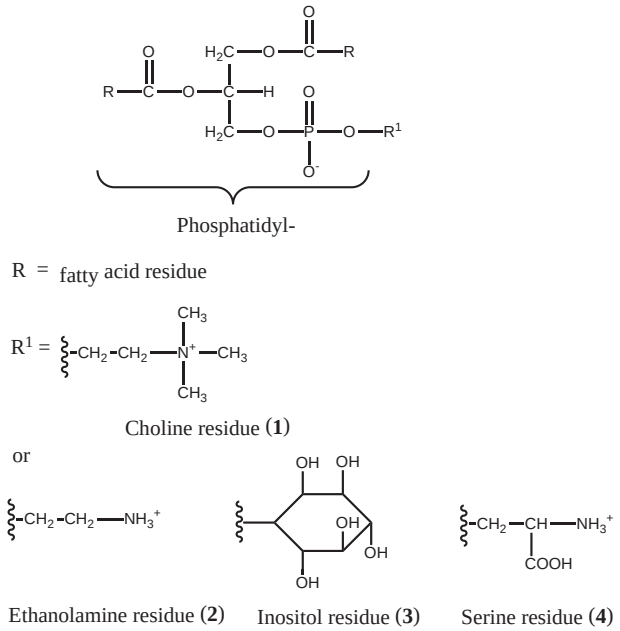

Figure 1: Main structures for phospholipid components in lecithins: phosphatidylcholine (1), phosphatidylethanolamine (2), phosphatidylinositol (3), phosphatidylserine (4). If R^1 = H, the compound is phosphatidic acid

The amount (percentage) of the principal components of lecithins depends on raw material sources (EFEMA, 2013). Food-grade lecithins obtained from soya beans or other sources is a mixture containing about 60% phospholipids and 40% triglycerides, sterols and carbohydrates in various proportions (SCF, 1982).

The phospholipid composition of soya bean lecithin on an oil-free basis is 21% phosphatidylcholine, 22% phosphatidylethanolamine, 19% phosphatidylinositol, 10% phosphatidic acid, 1% phosphatidylserine and 12% glycolipids (Wendel, 1995). Data on phospholipid composition for several batches of soya lecithin (liquid, deoiled, hydrolysed), sunflower lecithin (liquid, deoiled) and rape seed lecithin obtained by ^{31}P nuclear magnetic resonance spectroscopy (^{31}P-NMR) provided by the interested party (Document provided to EFSA n.18) are summarised in Table 2.

Table 2: Summarised data on phospholipid composition of soya lecithin (liquid, de-oiled, hydrolysed), sunflower lecithin (liquid, deoiled) and rape seed lecithin from the European Lecithin Manufacturers Association (ELMA) (Document provided to EFSA n.18)

	Phosphatidyl choline (%)	Phosphatidyl inositol (%)	Phosphatidyl ethanolamine (%)	Phosphatidic acid (%)
Soya lecithin liquid	12.69–16.7	6.47–11.84	6.45–13.57	2.28–5.96
Soya lecithin de-oiled	16.83–22.23	14.66–17.27	10.00–13.67	5.28–8.57
Soya lecithin hydrolysed*	7.66–8.81	6.16–9.15	3.54–5.51	2.09–2.69
Sunflower lecithin liquid	14.34–17.23	12.30–14.92	4.85–6.82	1.32–3.21
Sunflower lecithin de-oiled	24.97–27.57	15.12–21.17	9.91–10.50	2.56–2.80
Rape seed lecithin	16.74–18.18	10.45–12.30	6.46–8.03	2.44–3.59

*: In this product phospholipids are partially hydrolysed. Reported content of liso phosphatidyl choline is 3.85–4.56%, lyso phosphatidyl inositol is 0.88–1.36%, lyso phosphatidyl ethanolamine is 1.67–2.31% and lyso phosphatidic acid is 1.19–1.34%

The content of choline that can theoretically be released from phosphatidylcholine containing two linoleate groups is 13.2% and from lyso phospatidyl choline containing one linoleate group is 20.2%.

Based on the data provided by ELMA (Document provided to EFSA n.18), the calculated content of choline that can theoretically be released from commercial lecithins is given in Table 3.

Table 3: Calculated content of choline that can theoretically be released from commercial lecithins based on the data provided by ELMA (Document provided to EFSA n.18)

	Phosphatidylcholine content (%)	Calculated content of choline, that can theoretically be released from lecithin (%)
Soya lecithin liquid	12.69–16.7	1.67–2.20
Soya lecithin de-oiled	16.83–22.23	2.22–2.93
Soya lecithin hydrolysed	11.51–13.37*	1.51–1.84
Sunflower lecithin liquid	14.34–17.23	1.89–2.27
Sunflower lecithin deoiled	25.57	3.38
Rape seed lecithin	16.74	2.21

*: Total content of phosphatidylcholine and lyso phospatidylcholine.

Wendel (1995) reported that the fatty acid composition of oil-free soya bean lecithins was:

- 18.4% palmitic acid; $CH_3(CH_2)_{14}COOH$,
- 4.0% stearic acid: $CH_3(CH_2)_{16}COOH$,
- 10.7% oleic acid: $CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$),
- 58.0% linoleic acid: $(CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7COOH)$,
- 6.8% linolenic acid: $(CH_3(CH_2CH{=}CH)_3(CH_2)_7CO_2H)$,
- 2.1% others.

Hydrolysed lecithins (lysolecithins) are the products of partial hydrolysis of food-grade lecithins, where the fatty acid in the 2-position of the phospholipids is enzymatically removed. They contain about 51% phospholipids, 18% total free fatty acids, 1% moisture and 24% triglycerides, sterols, commercial pancreatin (enzyme, inactivated) and carbohydrates in various proportions (SCF, 1982).

Refined lecithins with high levels of phospholipids (> 95%), prepared by acetone and alcohol fractionation (see Section 3.1.3), are soft, yellow-brown powders (EFEMA, 2013). The density of commercial crude lecithin is 0.97 g/mL (liquid) and 0.5 g/mL (granule) (Wendel, 1995).

According to JECFA, both lecithin and partially hydrolysed lecithin 'are only partially soluble in water, but readily hydrate to form emulsions; the oil-free phosphatides are soluble in fatty acids, but are practically insoluble in fixed oils' (JECFA, 2007a,b). However, the hydrolysed lecithin (lysolecithin) has an increased solubility in water and greater emulsifying activity for formation of oil-in-water emulsions (Tanno, 2012). The solubilities of soya bean lecithin and some of its individual ingredients are given in Table 4.

Table 4: Solubilities of soya bean lecithins and of various phospholipids (Wendel, 1995; Tanno, 2012)

	Water	Hexane	Alcohol	Acetone
Soya bean lecithins	Insoluble/dispersible	Soluble	Soluble	Insoluble
Phosphatidylcholine	Soluble/dispersible	Soluble	Readily soluble	Sparingly soluble
Phosphatidylethanolamine	Readily soluble/dispersible	Soluble	Soluble	Insoluble
Phosphatidylinositol	Readily soluble/dispersible	Soluble	Insoluble	Insoluble
Lysophospholipids	Soluble	Partially soluble	Soluble	Soluble

The Panel noted that several studies have been conducted with a substance named essential phospholipid (EPL), although, in some studies, the composition of the EPL used was not indicated. The Panel noted that, when given, the composition of the EPL consisted of 75% phosphatidylcholine (fatty acids content as follows: 65% linoleic acid, 5% linolenic acid, 10% oleic acid, 15% palmitic acid and 5% stearic acid). The remaining 25% consisted of 5% phosphatidylethanolamine (kephalins) and 20% accompanying lipids from the soya bean.

No information on the particle size of lecithin powder was provided to the Panel. The FEEDAP Scientific opinion on safety and efficacy of lecithins for all animal species (EFSA FEEDAP Panel, 2016) contains the following information on particle size of lecithin powder: 'Three batches of the de-oiled lecithin powder with different physical characteristics were analysed for particle size distribution (by laser diffraction), showing variable results. The coarser powders had < 0.3% of the particles with a diameter ≤ 200 μm; the finest powder had < 13.9% and < 1.6% of the particles with diameters < 100 μm and 50 μm, respectively'.

3.1.2. Specifications

The specifications for lecithins (E 322) as defined in the Commission Regulation (EU) No 231/2012 and by JECFA (2007a,b) are listed in Table 5.

Table 5: Specifications for lecithins (E 322) according to Commission Regulation (EU) No 231/2012 and JECFA (2007a,b)

	Commission Regulation (EU) No 231/2012	**JECFA (2007a)**	**JECFA (2007b)**
	Lecithins (E 322)	**Lecithin (INS 322i)**	**Lecithin, Partially Hydrolysed (INS 322ii)**
Definition	Lecithins are mixtures or fractions of phosphatides obtained by physical procedures from animal or vegetable foodstuffs; they also include hydrolysed products obtained through the use of harmless and appropriate enzymes. The final product must not show any signs of residual enzyme activity. The lecithins may be slightly bleached in aqueous medium by means of hydrogen peroxide. This oxidation must not chemically modify the lecithin phosphatides	Usually prepared from oil-bearing seeds used for food, especially soybeans; may also be prepared from animal sources; a complex mixture of acetone-insoluble phosphatides which consists chiefly of phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol, combined with various amounts of other substances such as triglycerides, fatty acids, and carbohydrates; refined grades may contain any of these components in varying proportions and combinations depending on the type of fractionation used; its oil-free forms, the preponderance of triglycerides and fatty acids is removed and the product contains 90% or more of phosphatides representing all or certain fractions of the total phosphatide complex	Prepared by partial hydrolysis of lecithin by the use of a suitable lipase. When the desired degree of hydrolysis is attained, the product is heated in order to inactivate the residual enzyme
Assay	Lecithins: not less than 60.0% of substances insoluble in acetone Hydrolysed lecithins: not less than 56.0% of substances insoluble in acetone	Not less than 60% of acetone-insoluble matter (phosphatides)	Not less than 56% of acetone-insoluble matter (phosphatides)
Description	Lecithins: brown liquid or viscous semiliquid or powder Hydrolysed lecithins: light brown to brown viscous liquid or paste	Consistency of both natural grades and refined grades may vary from plastic to fluid, depending upon free fatty acid and oil content, and upon the presence or absence of other diluents; from light yellow to brown, depending on the source, on crop variations, and on whether it is bleached or unbleached; odourless or has a characteristic, slight nut-like odour. Edible diluents, such as cocoa butter and vegetable oils, often replace soybean oil to improve functional and flavour characteristics	Consistency may vary from plastic to fluid, depending upon free fatty acid and oil content, and upon the presence or absence of other diluents. Its colour varies from light yellow to brown, depending on the source, on crop variations, and on whether it is bleached or unbleached; odourless or has a characteristic, slight nutlike odour. Edible diluents, such as cocoa butter and vegetable oils, often replace soybean oil to improve functional and flavour characteristics
Identification			
Tests for choline, for phosphorus and fatty acids	Passes test	Test for phosphorus: Ignite 1 g of the sample with 2 g of anhydrous sodium carbonate. Cool and dissolve the residue in 5 mL of water and 5 mL of nitric acid. Add 5 mL of ammonium molybdate TS and heat to boiling. A yellow precipitate is obtained Test for choline: To 0.5 g of the sample, add 5 mL of diluted hydrochloric acid (1 + 1), heat in a water bath for 2 h, and filter. Use this solution as the test	Test for phosphorus: Ignite 1 g of the sample with 2 g of anhydrous sodium carbonate. Cool and dissolve the residue in 5 mL of water and 5 mL of nitric acid. Add 5 mL of ammonium molybdate TS and heat to boiling. A yellow precipitate is obtained Test for choline: To 0.5 g of the sample, add 5 mL of diluted hydrochloric acid (1 + 1), heat in a water bath for 2 h, and filter. Use this solution as the test

	Commission Regulation (EU) No 231/2012	JECFA (2007a)	JECFA (2007b)
	Lecithins (E 322)	**Lecithin (INS 322i)**	**Lecithin, Partially Hydrolysed (INS 322ii)**
		solution. Perform *Paper Chromatography* with 10 µL of the test solution, using choline chloride solution (1 + 200) as the control solution and *n*-butanol–water–acetic acid mixture (4:2:1) as the developing solvent. A red–orange spot corresponding to the spot obtained from the control solution is observed. For the filter paper, use a No. 2 filter paper for chromatography. Stop the development when the developing solvent rises about 25 cm, air-dry, spray with Dragendorff TS to develop a colour, and observe in daylight Test for fatty acids: Reflux 1 g of the sample for 1 h with 25 mL of 0.5 N ethanolic potassium hydroxide. When cooled to 0°, a precipitate of potassium soap is obtained	solution. Perform *Paper Chromatography* with 10 µL of the test solution, using choline chloride solution (1 + 200) as the control solution and *n*-butanol–water–acetic acid mixture (4:2:1) as the developing solvent. A red–orange spot corresponding to the spot obtained from the control solution is observed. For the filter paper, use a No. 2 filter paper for chromatography. Stop the development when the developing solvent rises about 25 cm, air-dry, spray with Dragendorff TS to develop a colour, and observe in daylight Test for fatty acids: Reflux 1 g of the sample for 1 h with 25 mL of 0.5 N ethanolic potassium hydroxide. When cooled to 0°, a precipitate of potassium soap is obtained
Test for hydrolysed lecithin	To a 800-mL beaker, add 500 mL of water (30–35°C). Then slowly add 50 mL of the sample with constant stirring. Hydrolysed lecithin will form a homogeneous emulsion. Non-hydrolysed lecithin will form a distinct mass of about 50 g	To a 800-mL beaker, add 500 mL of water (30–35°C). Then, slowly add 50 mL of the sample with constant stirring. Hydrolysed lecithin will form a homogeneous emulsion. Non-hydrolysed lecithin will form a distinct mass of about 50 g	To a 800-mL beaker, add 500 mL of water (30–35°C). Then, slowly add 50 mL of the sample with constant stirring. Hydrolysed lecithin will form a homogeneous emulsion. Non-hydrolysed lecithin will form a distinct mass of about 50 g
Solubility	–	Only partially soluble in water; readily hydrates to form emulsions; oil-free phosphatides are soluble in fatty acids, but are practically insoluble in fixed oils	Only partially soluble in water, but readily hydrates to form emulsions; the oil-free phosphatides are soluble in fatty acids, but are practically insoluble in fixed oils
Purity			
Loss on drying	Not more than 2.0% (105°C, 1 h)	Not more than 2% (105°C, 1 h)	Not more than 2% (105°C, 1 h)
Toluene-insoluble Matter	Not more than 0.3%	Not more than 0.3%	Not more than 0.3%
Acid value	Lecithins: not more than 35 mg of potassium hydroxide per gram Hydrolysed lecithins: not more than 45 mg of potassium hydroxide per gram	Not more than 36	Not more than 45
Peroxide value	Equal to or less than 10	Not more than 10	Not more than 10
Arsenic	Not more than 3 mg/kg	–	–
Lead	Not more than 2 mg/kg	Not more than 2 mg/kg	Not more than 2 mg/kg
Mercury	Not more than 1 mg/kg	–	–

According to the information from the interested party (Document provided to EFSA n.18), phospholipids can be modified by enzymes in a wide variety of ways. Phospholipases A and B split off fatty acids, whereas phospholipases C and D attack at the glycerophosphate bond. Specifications for five commercial lipases with a different level of details, developed by recombinant DNA techniques, were submitted (Document provided to EFSA n.18). Concerning the residual enzymatic activity, according to Association of Manufacturers and Formulators of Enzyme Products (AMFEP) it is stated that: 'Depending on production process enzyme activity can be excluded, in our case by drying at 70°C for 24 h and further with a low moisture content of 0.4–0.5%'. As an indicator of residual enzymatic activity, it is also possible to use acid value. If it is stable, there is no enzyme activity (Document provided to EFSA n.18).

The Panel noted that according to the Commission Regulation (EU) No 231/2012 the final product must not show any signs of residual enzyme activity.

Food Chemicals Codex (2010-2011)also contains specifications for hydroxylated lecithins. The Panel noted that the EU specification for E 322 states that 'The lecithins may be slightly bleached in aqueous medium by means of hydrogen peroxide. This oxidation must not chemically modify the lecithin phosphatides'.

In a study of five batches of non-hydrolysed lecithins from 2007 to 2009, provided by industry, measurements of Enterobacteriaceae (negative/1 g), salmonellae (negative/25 g), heavy metals (lead < 0.1 mg/kg, mercury < 0.005 mg/kg and arsenic < 0.1 mg/kg), residual solvents (hexane < 1 mg/kg, ethanol ≤ 3.8 mg/kg, acetone ≤ 2.5 mg/kg), pesticides (not detectable, limit of detection (LOD): 0.01–10 mg/kg), aflatoxins (< 0.2 mg/kg), polycyclic aromatic hydrocarbons (benzo(a)anthracene ≤ 4.4 μg/kg, chrysene ≤ 7.8 μg/kg, benzo(b)fluoranthene < 0.5 μg/kg, benzo(a)pyrene ≤ 1.4 μg/kg), polychlorinated biphenyls (not detectable, LOD not indicated) and dioxins (sum of dioxins ≤ 0.75 pg TEQ (WHO)/g fat and sum of dioxins and dioxin precursors like PCBs ≤ 1.5 pg TEQ (WHO)/g fat) were performed (Document provided to EFSA n.3).

Data on protein content in lecithins provided by ELMA (Document provided to EFSA n.18), as well as literature data, are rather variable due to number of different extraction systems and specific assays have been utilised. Many of these methods have not been validated and, in addition, interferences from residual lipids may confound the chemical assay results. Results for protein content are in the range 115–27,000 mg/kg for crude soya lecithins, 232–1338 mg/kg for in fluid soya lecithin, 65–480 mg/kg for in deoiled soya lecithin and 49 mg/kg for in egg lecithins (Document provided to EFSA n.18; Porras et al., 1985; Müller et al., 1998; Gu et al., 2001; Paschke et al., 2001; Martin-Hernandez et al., 2005).

The Panel noted that there is no specification for the presence of residual proteins from the source material used in the manufacturing of the food additive.

According to EFSA NDA Panel (2014), the lowest MED reported in soya-allergic patients undergoing DBPCFC was 0.2 mg of soya protein, although the majority of patients only reacted to higher doses. MEDs of ingested egg proteins reported to trigger objective reactions in clinical studies range from few micrograms to milligrams. The Panel also noted some case reports of hypersensitivity reactions associated with egg and soya lecithins (see Section 3.5.7). The Panel agree with the opinion from EFSA NDA Panel (2014) that this hypersensitivity is due to the residual proteins in lecithins (E 322) and therefore their content should be reduced as much as possible.

The Panel noted that, according to the EU specifications for lecithins (E 322), impurities of the toxic elements arsenic, lead and mercury are accepted up concentrations of 3, 2 and 1 mg/kg, respectively. Contamination at those levels could have a significant impact on the exposure to these metals, for which the intake is already close to the health-based guidance values established by the EFSA (EFSA CONTAM Panel, 2009a,b, 2010, 2012). The Panel noted that limit for cadmium should be included in the specifications.

According to data provided by industry, concentrations of toxic elements: lead, mercury and arsenic were below the LOD of 0.1, 0.005 and 0.1 mg/kg, respectively (Document provided to EFSA n.3), and between 1 and 2.5 order of magnitude below the limits set in the EU specifications.

3.1.3. Manufacturing process

The commercial production of lecithins used as food additives is based mainly on soya bean oil; other sources, such as cottonseed, corn, sunflower, rapeseed, egg and bovine brain, are of minor importance (Wendel, 1995; Tanno, 2012).

The first step for the production of lecithins from soya bean is the compression of the seeds to obtain the crude soya bean oil. To this crude oil, water is added to hydrate the phosphatides and the water–oil mixture is then heated at 70°C for 30–60 min. Afterwards, the oil-insoluble lecithin fraction (a wet gum known as lecithin hydrate) is separated by centrifugation. The gum is then transferred to a holding tank to allow addition of bleaching agents, if required. Hydrogen peroxide and benzoyl peroxide are used to bleach the lecithin. Bleaching may be carried out either using a 0.3–1.5% hydrogen peroxide solution instead of water for the degumming process, or by the addition of peroxide to the holding tank. Lecithins are separated from the triglycerides by a molecular membrane degumming process (Tanno, 2012). The Panel noted that according to the Commission Regulation (EU) No 231/2012, only hydrogen peroxide may be used as a bleaching agent in the manufacturing of lecithins (E 322).

Crude lecithin generally has high viscosity and is a brown fluid. The composition of crude lecithin can be changed by fractionation with solvents. Most of the triglycerides and fatty acids can be separated from crude lecithin by acetone fractionation to give oil-free lecithin powders. Lecithins can be enriched by alcohol extraction. Phosphatidylcholine is concentrated by extraction with alcohol. This fraction has increased emulsifying activity for the formation of oil-in-water emulsions. The alcohol-insoluble fraction is rich in the hydrophobic phosphatidylinositol and therefore favours the formation of water-in-oil emulsions. Phosphatidylethanolamine is evenly divided between the alcohol soluble and insoluble fractions. High-grade lecithins are also made by removing the hexane-insoluble material by filtration (Tanno, 2012).

There are many parameters which characterise the physical properties of lecithins such as acetone insoluble matter, acid value, moisture content, hexane-insoluble matter, colour, consistency and clarity (Tanno, 2012).

The partly hydrolysed lecithins are industrially produced by the action of the enzyme phospholipase A2, which selectively hydrolyses the fatty acid in the 2-position of the phospholipid (Tanno, 2012). Any enzymatic activity in the final product is inactivated by heating (TemaNord, 2002).

3.1.4. Methods of analysis in food

Because lecithin compounds (including phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol) are too polar to be subjected to direct gas chromatographic analysis, liquid chromatographic methods are usually used for their analysis (Tanno, 2012).

The procedures described by Helmerich and Koehler (2003) are only appropriate for the analysis of technical mixtures. These authors determined phospholipids in eight commercial lecithins and three flour improvers by thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC) and ^{31}P-NMR. Most components could be quantified by TLC. The highest selectivity was provided by ^{31}P-NMR, whereas HPLC was the method with the lowest selectivity. The best sensitivity was observed for HPLC and TLC with detection limits of 20–170 mg/L.

A method for the determination of the total phosphorous content in lecithins is described in AOAC (1980). After extraction of the sample, the amount of phosphorous is determined as P_2O_5.

3.1.5. Stability of the substance, and reaction and fate in food

Information about the stability of lecithins has been provided by industry (Document provided to EFSA n.3). Packed samples of three batches of fluid lecithins were stored under recommended storage conditions (10–35°C, 60% relative humidity), and tested against EU specifications for assay, description, toluene-insoluble matter, acid value and peroxide value at regular time points up to 36 months. All batches were observed to be stable as the measured values were matching the specifications. Additionally, the same samples were tested for aerobic bacteria (< 10 cfu/g) and *Salmonella* (negative in 25 g).

Long-term storage of lecithins at high temperatures in the presence of air leads to oxidation of the unsaturated fatty acids, resulting in an off-flavour and black colouration (Tanno, 2012).

3.2. Authorised uses and use levels

Maximum levels of lecithins (E 322) have been defined in Annex II to Regulation (EC) No 1333/2008[14] on food additives, as amended. In this document, these levels are named maximum permitted levels (MPLs).

Currently, lecithins (E 322) is an authorised food additive in the EU at *quantum satis* (QS) in most foods apart from fats and oils essentially free from water, infant and follow-on formulae, processed cereal-based foods and baby foods for infants and young children, and other foods for young children. Lecithins (E 322) is included in the Group I of food additives authorised at QS.

Table 6 summarises foods that are permitted to contain lecithins (E 322) and the corresponding MPLs as set by Annex II to Regulation (EC) No 1333/2008.

[14] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, pp. 16–33.

Table 6: MPLs of lecithins (E 322) in foods according to the Annex II to Regulation (EC) No 1333/2008

Food category number	Food category name	E-number/ group	Restrictions/exceptions	MPL (mg/L or mg/kg as appropriate)
01.3	Unflavoured fermented milk products, heat-treated after fermentation	Group I		QS
01.4	Flavoured fermented milk products including heat-treated products	Group I		QS
01.5	Dehydrated milk as defined by Directive 2001/114/EC	E 322		QS
01.6.3	Other creams	Group I		QS
01.7.1	Unripened cheese excluding products falling in category 16	Group I	Except mozzarella	QS
01.7.5	Processed cheese	Group I		QS
01.7.6	Cheese products (excluding products falling in category 16)	Group I		QS
01.8	Dairy analogues, including beverage whiteners	Group I		QS
02.1	Fats and oils essentially free from water (excluding anhydrous milkfat)	E 322	Except virgin oils and olive oils	30,000
02.2.2	Other fat and oil emulsions including spreads as defined by Council Regulation (EC) No 1234/2007 and liquid emulsions	Group I		QS
02.3	Vegetable oil pan spray	Group I		QS
03	Edible ices	Group I		QS
04.2.1	Dried fruit and vegetables	Group I		QS
04.2.2	Fruit and vegetables in vinegar, oil, or brine	Group I		QS
04.2.4.1	Fruit and vegetable preparations excluding compote	Group I		QS
04.2.5.4	Nut butters and nut spreads	Group I		QS
04.2.6	Processed potato products	Group I		QS
05.1	Cocoa and Chocolate products as covered by Directive 2000/36/EC	Group I		QS
05.2	Other confectionery including breath refreshening microsweets	Group I		QS
05.3	Chewing gum	Group I		QS
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4	Group I		QS
06.2.2	Starches	Group I		QS
06.3	Breakfast cereals	Group I		QS
06.4.1	Fresh pasta	E 322		QS

Food category number	Food category name	E-number/ group	Restrictions/exceptions	MPL (mg/L or mg/kg as appropriate)
06.4.2	Dry pasta	E 322	Only gluten-free and/or pasta intended for hypoproteic diets in accordance with Directive 2009/39/EC	QS
06.4.3	Fresh precooked pasta	E 322		QS
06.4.4	Potato gnocchi	Group I	Except fresh refrigerated potato gnocchi	QS
06.4.5	Fillings of stuffed pasta (ravioli and similar)	Group I		QS
06.5	Noodles	Group I		QS
06.6	Batters	Group I		QS
06.7	Precooked or processed cereals	Group I		QS
07.1	Bread and rolls	Group I	Except products in 7.1.1 and 7.1.2	QS
07.1.1	Bread prepared solely with the following ingredients: wheat flour, water, yeast or leaven, salt	E 322		QS
07.1.2	Pain courant francais; *Friss búzakenyér, fehér és félbarna kenyerek*	E 322		QS
07.2	Fine bakery wares	Group I		QS
08.3.1	Non-heat-treated meat products	Group I		QS
08.3.2	Heat-treated meat products	Group I	Except *foie gras, foie gras entier, blocs de foie gras, Libamáj, libamáj egészben, libamáj tömbben*	QS
08.3.3	Casings and coatings and decorations for meat	Group I		QS
09.2	Processed fish and fishery products including molluscs and crustaceans	Group I		QS
09.3	Fish roe	Group I	Only processed fish roe	QS
10.2	Processed eggs and egg products	Group I		QS
11.2	Other sugars and syrups	Group I		QS
12.1.2	Salt substitutes	Group I		QS
12.2.2	Seasonings and condiments	Group I		QS
12.3	Vinegars	Group I		QS
12.4	Mustard	Group I		QS
12.5	Soups and broths	Group I		QS
12.6	Sauces	Group I		QS
12.7	Salads and savoury based sandwich spreads	Group I		QS
12.8	Yeast and yeast products	Group I		QS
12.9	Protein products, excluding products covered in category 1.8	Group I		QS
13.1.1	Infant formulae as defined by Directive 2006/141/EC	E 322	(a)	1,000

Food category number	Food category name	E-number/ group	Restrictions/exceptions	MPL (mg/L or mg/kg as appropriate)
13.1.2	Follow-on formulae as defined by Directive 2006/ 141/EC	E 322	(a)	1,000
13.1.3	Processed cereal-based foods and baby foods for infants and young children as defined by Directive 2006/ 125/EC	E 322	Only biscuits and rusks, cereal-based foods, baby foods	10,000
13.1.4	Other foods for young children	E 322	(a)	10,000
13.1.5.1(b)	Dietary foods for infants for special medical purposes and special formulae for infants	E 322	(a)	1,000
13.1.5.2(c)	Dietary foods for babies and young children for special medical purposes as defined in Directive 1999/21/EC	E 322	(a)	1,000
13.1.5.2(d)	Dietary foods for babies and young children for special medical purposes as defined in Directive 1999/21/EC	E 322	Only biscuits and rusks, cereal-based foods, baby foods	10,000
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)	Group I		QS
13.3	Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)	Group I		QS
13.4	Foods suitable for people intolerant to gluten as defined by Regulation (EC) No 41/2009	Group I	Including dry pasta	QS
14.1.2	Fruit juices as defined by Directive 2001/112/EC and vegetable juices	Group I	Only vegetable juices	QS
14.1.3	Fruit nectars as defined by Directive 2001/112/EC and vegetable nectars and similar products	Group I	Only vegetable nectars	QS
14.1.4	Flavoured drinks	Group I		QS
14.1.5.2	Other	Group I	Excluding unflavoured leaf tea; including flavoured instant coffee	QS
14.2.3	Cider and perry	Group I		QS
14.2.4	Fruit wine and made wine	Group I		QS
14.2.5	Mead	Group I		QS
14.2.6	Spirit drinks as defined in Regulation (EC) No 110/2008	Group I	Except whisky or whiskey	QS
14.2.7.1	Aromatised wines	Group I		QS
14.2.7.2	Aromatised wine-based drinks	Group I		QS

Food category number	Food category name	E-number/ group	Restrictions/exceptions	MPL (mg/L or mg/kg as appropriate)
14.2.7.3	Aromatised wine-product cocktails	Group I		QS
14.2.8	Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15% of alcohol	Group I		QS
15.1	Potato-, cereal-, flour- or starch-based snacks	Group I		QS
15.2	Processed nuts	Group I		QS
16	Desserts excluding products covered in 1, 3 and 4	Group I		QS
17.1(e)	Food supplements supplied in a solid form including capsules and tablets and similar forms, excluding chewable forms	Group I		QS
17.2(e)	Food supplements supplied in a liquid form	Group I		QS
17.3(e)	Food supplements supplied in a syrup-type or chewable form	Group I		QS
18	Processed foods not covered by categories 1 to 17, excluding foods for infants and young children	Group I		QS

MPL, maximum permitted level.
(a): If more than one of the substances E 322, E 471, E 472c and E 473 are added to a foodstuff, the maximum level established for that foodstuff for each of those substances is lowered with that relative part as is present of the other substances together in that foodstuff.
(b): The additives of categories 13.1.1 and 13.1.2 are applicable.
(c): The additive of categories 13.1.2 is applicable.
(d): The additive of category 13.1.3 is applicable.
(e): Food Classification System (FCS) 17 refers to food supplements as defined in Directive 2002/46/EC of the European Parliament and of the Council excluding food supplements for infants and young children.

According to Annex III, parts 1, 2, 3, 4 and part 5, section A of Regulation (EC) No 1333/2008, lecithins (E 322) is also authorised as a carrier in food additives such as colours, fat-soluble antioxidants and glazing agents for fruit at QS, as a food additive other than carriers in food additives in all food additive preparations at QS, and as a food additive including carrier for all food enzymes, all food flavourings and all nutrients, except nutrient intended to be used in foodstuffs for infants and young children at QS.

In addition, according to Annex III, part 5, section B of Regulation (EC) No 1333/2008, lecithins (E 322) can be added in all nutrients intended to be used in foodstuff for infants and young children listed in point 13.1 of Annex II to Regulation (EC) No 1333/2008 (Table 4) for uses in nutrient preparations under the condition that the maximum level in foods mentioned in point 13.1 of Part E of Annex II is not exceeded.

3.3. Exposure data

3.3.1. Reported use levels on lecithins (E 322)

Most food additives in the EU are authorised at a specific MPL. However, a food additive may be used at a lower level than the MPL. Therefore, information on actual use levels is required for performing a more realistic exposure assessment, especially for those food additives for which no MPL is set and which are authorised according to QS.

In the framework of Regulation (EC) No 1333/2008 on food additives and of Commission Regulation (EU) No 257/2010 regarding the re-evaluation of approved food additives, EFSA issued public calls[15,16] for occurrence data (usage level and/or concentration data) on lecithins (E 322). In response to this public call, updated information on the actual use levels of lecithins (E 322) in foods was made available to EFSA by industry. No analytical data on the concentration of lecithins (E 322) in foods were made available by the Member States.

3.3.1.1. Summarised data on reported use levels in foods provided by industry

Industry provided EFSA with data on use levels (n = 563) of lecithins (E 322) in foods for 33 out of the 79 food categories in which lecithins (E 322) is authorised.

Updated information on the actual use levels of lecithins (E 322) in foods was made available to EFSA by FoodDrinkEurope (FDE, Document provided to EFSA n.20), BABBI Confectionary Industry (Document provided to EFSA n.25), Specialised Nutrition Europe (SNE, Document provided to EFSA n.27), CHEPLAPHARM Arzneimittel GmbH (Document provided to EFSA n.21), Stollwerck (Document provided to EFSA n.22), the International Chewing Gum Association (ICGA, Document provided to EFSA n.24), the Association of the European Self-Medication Industry (AESPG, Document provided to EFSA n.19), Rudolf Wild GmbH & Co. KG (Document provided to EFSA n.26), the European Lecithin Manufacturers Association (ELMA, Document provided to EFSA n.23) and Nathura (Document provided to EFSA n.28).

The Panel noted that data from ELMA (Document provided to EFSA n.23) and Rudolf Wild (Document provided to EFSA n.26), food additive producers, are not representing food industries using lecithins in their food products, although producers that recommended usage levels to users of lecithins which might, ultimately, use different levels. The data provided by these producers were therefore used in the current exposure assessment only for the regulatory scenario to estimate QS levels when no usage data were reported by industries for food categories with QS levels.

Appendix A displays all data on the use levels of lecithins (E 322) in foods as reported by industry (food industry and lecithins producers).

3.3.2. Summarised data extracted from the Mintel Global New Products Database

The Mintel GNPD is an online database that monitors products introductions in consumer packaged goods markets worldwide. It contains information of over 2 million food and beverage products of which more than 900,000 are or have been available in the European food market. Mintel started covering European Union's food markets in 1996, currently having 20 out of its 28 member countries and Norway present in the Mintel GNPD.[17]

For the purpose of this Scientific Opinion, the Mintel GNPD[18] was used for checking the labelling of products containing lecithin (E 322) within the EU food products because the Mintel GNPD shows the compulsory ingredient information presented in the labelling of products.

According to Mintel, lecithins (E 322) is labelled on more than 52,300 products published in the Mintel GNPD database between 2011 and 2016.

Appendix B presents the percentage of the food products labelled with lecithins (E 322) between 2011 and 2016, out of the total number of food products per food subcategory according to the Mintel food classification.

3.3.3. Food consumption data used for exposure assessment

3.3.3.1. EFSA Comprehensive European Food Consumption Database

Since 2010, the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) has been populated with national data on food consumption at a detailed level. Competent authorities in the European countries provide EFSA with data on the level of food consumption by the individual consumer from the most recent national dietary survey in their country (cf. Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure

[15] Available online: http://www.efsa.europa.eu/en/dataclosed/call/ans091123
[16] Available online: http://www.efsa.europa.eu/sites/default/files/consultation/140310.pdf
[17] Missing Bulgaria, Cyprus, Estonia, Latvia, Lithuania, Luxembourg, Malta and Slovenia.
[18] Mintel Global New Products Database. Available online: http://www.gnpd.com/sinatra/home/. Accessed on 1 November 2016.

Assessment' (EFSA, 2011a). New consumption surveys recently[19] added in the Comprehensive database were also taken into account in this assessment.[10]

The food consumption data gathered by EFSA were collected by different methodologies and thus direct country-to-country comparisons should be interpreted with caution. Depending on the food category and the level of detail used for exposure calculations, uncertainties could be introduced owing to possible subjects' under-reporting and/or misreporting of the consumption amounts. Nevertheless, the EFSA Comprehensive Database represents the best available source of food consumption data across Europe at present.

Food consumption data from the following population groups: infants, toddlers, children, adolescents, adults and the elderly were used for the exposure assessment. For the present assessment, food consumption data were available from 33 different dietary surveys carried out in 19 European countries (Table 7).

Consumption records were codified according to the FoodEx classification system (EFSA, 2011b).

Table 7: Population groups considered for the exposure estimates of lecithins (E 322)

Population	Age range	Countries with food consumption surveys covering more than 1 day
Infants	From 12 weeks on up to and including 11 months of age	Bulgaria, Denmark, Finland, Germany, Italy, UK
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Denmark, Finland, Germany, Italy, Netherlands, Spain, UK
Children[(a)]	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Spain, Sweden, UK
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Italy, Latvia, Spain, Sweden, UK
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Romania, Spain, Sweden, UK
The elderly[(a)]	From 65 years of age and older	Austria, Belgium, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Romania, Sweden, UK

(a): The terms 'children' and 'the elderly' correspond, respectively, to 'other children' and the merge of 'elderly' and 'very elderly' in the Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011a).

Nomenclature from the FoodEx classification system has been linked to the Food Classification System (FCS) as presented in Annex II of Regulation (EC) No 1333/2008, part D, to perform exposure estimates. In practice, FoodEx food codes were matched to the FCS food categories.

3.3.3.2. Food categories selected for the exposure assessment of lecithins (E 322)

The food categories in which the use of lecithins (E 322) is authorised were selected from the nomenclature of the EFSA Comprehensive Database (FoodEx classification system), at the most detailed level possible (up to FoodEx Level 4) (EFSA, 2011b).

Some food categories or their restrictions/exceptions are not referenced in the EFSA Comprehensive Database and could therefore not be taken into account in the present estimate. This may have resulted in an underestimation of the exposure. This was the case for six categories (Appendix C). The food categories which were not taken into account are described below (in ascending order of the FCS codes):

- 02.3 Vegetable oil pan spray;
- 06.6 Batters;
- 06.7 Pre-cooked or processed cereals;
- 08.3.3 Casings and coatings and decorations for meat;
- 12.1.2 Salt substitutes;
- 14.1.3 Fruit nectars as defined by Directive 2001/112/EC and vegetable nectars and similar products, only vegetable nectars.

[19] Available online: http://www.efsa.europa.eu/en/press/news/150428.htm

For the following food categories, the differences between subgroups could not be taken into account, and therefore the whole category was considered in the exposure assessment:

- 08.3 Processed meat
  - 08.3.1 Non-heat treated processed meat;
  - 08.3.2 Heat-treated processed meat.
- 17.1/17.2/17.3 Food supplements, in solid, liquid, syrup-type or chewable form. According to Regulation (EC) No 1333/2008, the food supplement category (FC 17) excludes 'food supplements for infants and young children'. However, in the EFSA Comprehensive database, food supplements are consumed by infants and young children with no information provided on the food supplement type. In the exposure assessment, it was therefore assumed that the food supplements consumed in these population groups were the same as those consumed in the older population groups for which concentration data were supplied, resulting in an overestimation of the exposure to lecithins (E 322) in these two population groups.

For the refined scenario, six additional food categories were not taken into account in the exposure assessment because no concentration data were provided to EFSA (Appendix C). For the remaining food categories, the refinements considering the restrictions/exceptions as set in Annex II to Regulation No 1333/2008 were applied.

Overall, for the maximum level exposure scenario, 35 food categories were included, whereas, for the refined scenarios, 29 food categories were included in the present exposure assessment (Appendix C).

3.4. Exposure estimate

3.4.1. Exposure to lecithins (E 322) from its use as a food additive

The Panel estimated chronic exposure for the following population groups: infants, toddlers, children, adolescents, adults and the elderly. Dietary exposure to lecithins (E 322) was calculated by multiplying lecithins (E 322) concentrations for each food category (Appendix C) by their respective consumption amount per kg body weight for each individual in the Comprehensive Database. The exposure per food category was subsequently added to derive an individual total exposure per day. These exposure estimates were averaged over the number of survey days, resulting in an individual average exposure per day for the survey period. Dietary surveys with only 1 day per subject were excluded because they are considered as not adequate to assess repeated exposure.

This was carried out for all individuals per survey and per population group, resulting in distributions of individual exposure per survey and population group (Table 7). On the basis of these distributions, the mean and 95th percentile of exposure were calculated per survey and per population group. The 95th percentile of exposure was only calculated for those population groups where the sample size was sufficiently large to allow this calculation (EFSA, 2011a). Therefore, in the present assessment, The 95th percentile of exposure for infants from Italy and for toddlers from Belgium, Italy and Spain were not included.

Two exposure scenarios were defined and carried out by the ANS Panel regarding the concentration data of lecithins (E 322) used: (1) maximum levels of data provided to EFSA (defined as *the maximum level exposure assessment scenario*) and (2) the reported use levels (defined as the *refined exposure assessment scenario*). These two scenarios are discussed in detail below.

Because lecithins (E 322) is also authorised in food categories 13.1.5.1 and 13.1.5.2, a refined estimated exposure assessment scenario taking into account these two food categories was performed to estimate the exposure of infants and toddlers who may eat and drink these foods for special medical purposes (FSMP).

Considering that these specific foods are not reported in the EFSA Comprehensive data set, but that foods for infants and young children in good health are, the Panel assumed that the consumption patterns of infants and toddlers who need to eat the FSMP are the same as the ones of infants and toddlers from the general population. Thus, the consumption of FSMP under the food category 13.1.5 was assumed to be the same amount as the formulae and food products of food categories 13.1.1, 13.1.2, 13.1.3 and 13.1.4., e.g. the consumption of 'specific' infant formulae was assumed to be the same amount than the infant formulae of the FC 13.1.1.

Concerning the uses of lecithins (E 322) as carriers, there might be food categories where lecithins (E 322) is used according to annex III and not to annex II. These food categories can only be

addressed by analytical data or limits set in the Regulation (EC) No 1333/2008 that were not available to the Panel. Therefore, a possible additional exposure from the use of lecithins (E 322) as a food additive in Annex III to Regulation (EC) No 1333/2008 was not considered in any of the exposure assessment scenario.

3.4.1.1. Maximum level exposure assessment scenario

The regulatory maximum level exposure assessment scenario is based on the MPLs as set in Annex II to Regulation (EC) No 1333/2008 and listed in Table 6. Because lecithins (E 322) is authorised according to QS in almost all food categories, a 'maximum level exposure assessment' scenario was estimated based on the maximum reported use levels provided by industry, as described in the EFSA Conceptual framework (EFSA ANS Panel, 2014). The maximum levels used in this exposure scenario are listed in Appendix C.

The Panel considers the exposure estimates derived following this scenario as the most conservative because it is assumed that the population group will be exposed to lecithins (E 322) present in food at the MPL use levels over a longer period of time.

3.4.1.2. Refined exposure assessment scenario

In this opinion, the refined exposure assessment scenario is based on use levels reported by industry. This exposure scenario can consider only food categories for which the above data were available to the Panel.

Appendix C summarises the concentration levels of lecithins (E 322) used in the refined exposure assessment scenario. Based on the available data set, the Panel calculated two refined exposure estimates based on different model populations:

- The brand-loyal consumer scenario: It was assumed that a consumer is exposed long-term to lecithins (E 322) present at the maximum reported use level for one food category. This exposure estimate is calculated as follows:
  - Combining food consumption with the maximum of the reported use levels for the main contributing food category at the individual level.
  - Using the mean of the typical reported use levels for the remaining food categories.
- The non-brand-loyal consumer scenario: It was assumed that a consumer is exposed long-term to lecithins (E 322) present at the mean reported use in food. This exposure estimate is calculated using the mean of the typical reported use levels for all food categories.

In addition to these, as mentioned before, for the scenario taking into account the FSMP, considering that it is very specific diet, it is assumed that consumers are brand-loyal and only the results of the brand-loyal scenario are presented.

3.4.1.3. Dietary exposure to lecithins (E 322)

Table 8 summarises the estimated exposure to lecithins (E 322) from their use as food additives in six population groups (Table 7) according to the different exposure scenarios. Detailed results per population group and survey are presented in Appendix D.

Table 8: Summary of dietary exposure to lecithins (E 322) from their use as food additives in the maximum level exposure assessment scenario and in the refined exposure scenarios, in six population groups (minimum–maximum across the dietary surveys in mg/kg bw per day)

	Infants (12 weeks–11 months)	Toddlers (12–35 months)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (≥ 65 years)
Regulatory maximum level exposure assessment scenario						
Mean	50–178	69–365	71–314	32–177	70–118	72–116
95th percentile	109–368	130–520	119–576	59–324	134–237	132–199
Refined estimated exposure assessment scenario						
Brand-loyal scenario						
Mean	18–56	16–78	16–82	7–45	9–34	11–30
95th percentile	49–163	39–175	31–187	15–108	20–84	21–74

	Infants (12 weeks–11 months)	Toddlers (12–35 months)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (≥ 65 years)
Non-brand-loyal scenario						
Mean	15–21	11–22	7–21	4–12	3–9	3–8
95th percentile	39–62	23–41	14–39	8–27	6–19	6–16

Considering the general population, from the *regulatory maximum level exposure assessment scenario*, mean exposure to lecithins (E 322) from its use as a food additive ranged from 32 mg/kg bw per day in adolescents to 365 mg/kg bw per day in toddlers. The 95th percentile of exposure to lecithins (E 322) ranged from 59 mg/kg bw per day in adolescents to 576 mg/kg bw per day in children. From the *refined estimated exposure scenario* considering concentration levels not exceeding the MPLs for food categories listed under Annex II to Regulation No 1333/2008, in the *brand-loyal scenario*, mean exposure to lecithins (E 322) from its use as a food additive ranged from 7 mg/kg bw per day in adolescents to 82 mg/kg bw per day in children. The 95th percentile exposure to lecithins (E 322) ranged from 15 mg/kg bw per day in the adolescents to 187 mg/kg bw per day in children. In the *non-brand-loyal scenario*, mean exposure to lecithins (E 322) from its use as a food additive ranged from 3 mg/kg bw per day in adults/elderly to 22 mg/kg bw per day in toddlers. The 95th percentile of exposure to lecithins (E 322) ranged from 6 mg/kg bw per day in the adults/elderly to 62 mg/kg bw per day in infants.

From the refined estimated exposure scenario taking into account the foods for special medical purposes, in the *brand-loyal scenario*, mean exposure to lecithins (E 322) from its use as a food additive ranged from 24 mg/kg bw per day in toddlers to 85 mg/kg bw per day in infants. The 95th percentile of exposure to lecithins (E 322) ranged from 66 mg/kg bw per day to 232 mg/kg bw per day in toddlers (not presented in Table 8).

3.4.1.4. Main food categories contributing to exposure for the general population (i.e. not taking into account FCS 13.1.5)

Main food categories contributing to exposure to lecithins (E 322) using the maximum level exposure assessment scenario and the refined exposure assessment scenario (Tables 9–11)

Table 9: Main food categories contributing to exposure to lecithins (E 322) using maximum usage levels (> 5% to the total mean exposure) and number of surveys in which each food is contributing

FCS category number	FCS food category	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys)[a]					
01.5	Dehydrated milk as defined by Directive 2001/114/EC	6.5 (1)	–	28.7 (1)	–	–	–
01.6	Cream and cream powder	8.8 (1)	8.7 (1)	9.5 (1)	6.1 (1)	5.8 (1)	–
01.7.1	Unripened cheese excluding products falling in category 16	21.4 (1)	7.5–12.6 (2)	–	–	7.2 (1)	–
01.8	Dairy analogues, including beverage whiteners	10.3 (1)	7.6 (1)	–	–	–	–
02.1	Fats and oils essentially free from water (excluding anhydrous milkfat)	–	–	6.3–10.8 (3)	7–11.3 (2)	7.2–20.6 (3)	12.5–17.4 (2)
02.2.2	Other fat and oil emulsions mainly of type water-in-oil	5.5–23.5 (2)	9.5–19.4 (2)	13.5 (1)	14.3 (1)	–	5.3 (1)
03	Edible ices	–	–	8.6 (1)	–	–	–

FCS category number	FCS food category	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys)[a]					
05.1	Cocoa and chocolate products as covered by Directive 2000/36/EC	5.6 (1)	6.3–10.6 (2)	5.2–14.6 (9)	5.1–17.8 (7)	5.3 (1)	–
05.2	Other confectionery including breath refreshening microsweets	–	–	–	7 (1)	–	–
06.3	Breakfast cereals	6 (1)	10.3 (1)	–	–	–	6.2 (1)
07.1	Bread and rolls	24.8–66.5 (4)	34.4–74.4 (9)	26.1–71.5 (17)	37.1–70.3 (16)	36.7–72.8 (17)	34.6–75.6 (14)
07.2	Fine bakery wares	9.6–24.7 (3)	5.7–34.2 (10)	14.6–38.8 (16)	13.7–35.8 (15)	5.3–28.6 (16)	6.5–36.4 (13)
08.3	Processed meat	7.9 (1)	5–11.1 (5)	5.6–12.6 (6)	5.5–11.8 (8)	5.2–7.6 (8)	5.4–6.5 (4)
12.5	Soups and broths	29.7 (1)	13.8 (1)	11.4–19.3 (2)	5.6–15.4 (3)	5.1–16.3 (7)	6.6–18.6 (7)
12.6	Sauces	–	–	5.2 (1)	5.2–5.8 (3)	5.5–5.6 (3)	5.4 (1)
13.1.1	Foods for infants and young children	12.4–56.6 (6)	5.8–15 (4)	–	–	–	–
13.3	Dietary foods for weight control diets	–	–	–	–	9.8 (1)	–
16	Desserts excluding products covered in categories 1, 3 and 4	–	5.2–7.6 (3)	5.2–6.2 (3)	–	–	–

FCS: Food Classification System.
(a): The total number of surveys may be greater than the total number of countries as listed in Table 7 because some countries submitted more than one survey for a specific population.

Table 10: Main food categories contributing to exposure lecithins (E 322) using the brand-loyal refined exposure scenario (> 5% to the total mean exposure) and number of surveys in which each food category is contributing

Food Classification System (FCS) category number	FCS food category	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys)[a]					
01.8	Dairy analogues, including beverage whiteners	–	5.4 (1)	–	–	–	–
02.2.2	Other fat and oil emulsions mainly of type water-in-oil	–	–	17 (1)	17.1 (1)	–	–
03	Edible ices	–	5.8 (1)	30.4 (1)	11 (1)	–	–
05.1	Cocoa and chocolate products as covered by Directive 2000/36/EC	–	6.1–12.1 (3)	5.7–21.9 (9)	5.3–25.4 (8)	8.4 (1)	–
05.2	Other confectionery including breath refreshening microsweets	–	–	8.2 (1)	16.5 (1)	–	–
05.3	Chewing gum	–	–	–	10.6 (1)	–	–
07.1	Bread and rolls	6.2–36.3 (4)	7.5–74.8 (9)	6.5–70.9 (15)	6–67.3 (16)	7–71.9 (17)	6–75.4 (14)
07.2	Fine bakery wares	20.2–37.4 (3)	8.2–80.7 (10)	7.5–90.2 (17)	7.4–83.5 (16)	9.8–74.3 (17)	13.2–78.1 (14)
12.5	Soups and broths	15.9 (1)	–	6.9 (1)	6.3 (1)	6.1–8 (2)	6.6–11.6 (2)
12.6	Sauces	–	5.1 (1)	5.3–10.7 (2)	8.8–10.4 (3)	5.2–10.5 (6)	5.6–6.3 (2)
13.1	Foods for infants and young children	50.6–93.9 (6)	8.3–65.8 (3)	–	–	–	–

Food Classification System (FCS) category number	FCS food category	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys)[a]					
13.3	Dietary foods for weight control diets intended to replace total daily food intake or an individual	–	–	–	7.2 (1)	6.5–28.8 (5)	16 (1)
14.1.5.2	Coffee, tea, herbal and fruit infusions, chicory; tea, herbal and fruit infusions and chicory extracts; tea, plant, fruit and cereal preparations for infusions, as well as mixes and instant mixes of these products	–	–	–	–	–	5.2–6.6 (2)
16	Desserts excluding products covered in categories 1, 3 and 4	5.3–10.8 (2)	5–20.9 (6)	9–16.1 (3)	5.8 (1)	5.5–7.1 (3)	10.2 (1)

(a): The total number of surveys may be greater than the total number of countries as listed in Table 7 because some countries submitted more than one survey for a specific population.

Table 11: Main food categories contributing to exposure to lecithins (E 322) using the non-brand-loyal refined exposure scenario (> 5% to the total mean exposure) and number of surveys in which each food category is contributing

Food Classification System (FCS) category number	FCS food category	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys)[a]					
01.8	Dairy analogues, including beverage whiteners	–	5.9 (1)	5.7 (1)	–	–	–
02.2.2	Fat and oil emulsions mainly of type water-in-oil	–	5.4–8.1 (3)	5.3–21.8 (6)	5–19.1 (4)	6.1–10.2 (5)	7.9–13.2 (6)
03	Edible ices	–	–	6–13.7 (2)	5.2–6.1 (2)	–	–
05.1	Cocoa and chocolate products as covered by Directive 2000/36/EC	5.6 (1)	7.8–27.6 (7)	5.2–35.2 (18)	7.6–35.7 (17)	5.1–23 (16)	5.5–15.8 (7)
05.2	Other confectionery including breath refreshening microsweets	–	–	5.3 (1)	6.6 (1)	–	–
05.3	Chewing gum	–	–	5.6 (1)	11.2 (1)	–	–
07.1	Bread and rolls	6.1–16 (3)	11.5–44.1 (9)	9.1–36.7 (17)	13.2–34.4 (16)	12–51.5 (17)	13.8–50.9 (14)
07.2	Fine bakery wares	7.4–19.4 (3)	10.5–58.5 (9)	8.1–70.8 (17)	6.9–56.6 (16)	10.7–51.9 (17)	13.4–53.1 (14)
12.5	Soups and broths	–	7.6 (1)	5.6–9.1 (2)	7.7 (1)	5.9–9.7 (2)	5.3–10.2 (4)
12.6	Sauces	–	6.1–6.3 (3)	5.2–8.3 (7)	5.9–11.3 (9)	5.2–12 (10)	5–8.4 (8)
13.1	Foods for infants and young children	66.5–95 (6)	5.3–79.8 (7)	–	–	–	–
13.3	Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)	–	–	–	7.4 (1)	6.8–28.2 (5)	16.3 (1)

Food Classification System (FCS) category number	FCS food category	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys)[a]					
14.1.5.2	Coffee, tea, herbal and fruit infusions, chicory; tea, herbal and fruit infusions and chicory extracts; tea, plant, fruit and cereal preparations for infusions, as well as mixes and instant mixes of these products	9 (1)	5.3–10.3 (2)	6.7–8.4 (2)	5.9–8.9 (3)	5.2–13.3 (6)	6.5–22.5 (7)
16	Desserts excluding products covered in categories 1, 3 and 4	–	5.5–7.6 (2)	5.4–5.9 (2)	–	–	–

(a): The total number of surveys may be greater than the total number of countries as listed in Table 7 because some countries submitted more than one survey for a specific population.

3.4.1.5. Uncertainty analysis

Uncertainties in the exposure assessment of lecithins (E 322) have been discussed above. In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2007), the following sources of uncertainties have been considered and summarised in Table 12.

Table 12: Qualitative evaluation of influence of uncertainties on the dietary exposure estimate

Sources of uncertainties	Direction[a]
Consumption data: different methodologies/representativeness/under-reporting/misreporting/no portion size standard	+/–
Use of data from food consumption survey of a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile)	+
Correspondence of reported use levels and analytical data to the food items in the EFSA Comprehensive Food Consumption Database: uncertainties to which types of food the levels refer to	+/–
Food categories selected for the exposure assessment: exclusion of food categories due to missing FoodEx linkage (n = 6 out of 79 food categories)	–
Food categories included in the exposure assessment: data not available for certain food categories which were excluded from the exposure estimates (n = 48 only for the refined scenarios out of 79 food categories)	–
Concentration data: • levels considered applicable for all items within the entire food category	 +
Maximum level exposure assessment scenario: • food categories which may contain lecithins (E 322) due to carry-over not considered • food categories authorised at MPL according to Annex II to Regulation (EC) No 1333/2008	 – +
Refined exposure assessment scenarios: • food categories which may contain lecithins (E 322) due to carry-over not considered • exposure calculations based on the maximum or mean levels (reported use from industries)	 – +/–
Uncertainty in possible national differences in use levels of food categories	+/–

(a): +, uncertainty with potential to cause overestimation of exposure; –, uncertainty with potential to cause underestimation of exposure.

Overall, the Panel considered that the uncertainties identified would, in general, result in an overestimation of the exposure to lecithins (E 322) as a food additive in European countries for the maximum level exposure scenario and for the refined scenario, if it is considered that the food additive may not be used in food categories for which no usage data have been provided, and considering that usage of lecithins (E 322) according to Annex III to Regulation No 1333/2008 was not taken into account.

Lecithins (E 322) is authorised as a Group I food additive in 79 food categories (Table 2). EFSA was provided with reported use levels for only 33 food categories out of the 79 in which it is authorised. The Panel calculated that, out of the foods authorised to contain lecithins (E 322) according to Annex II to Regulation (EC) No 1333/2008, 33–95% of the amount of food consumed (by weight) per population group was reported to potentially contain lecithins as a food additive. Based on this, the Panel noted that the information from the Mintel GNPD supported the observation that, due to its Group I authorisation, lecithins may not be used in all food categories in which it is authorised. Furthermore, the Panel noted that information from the Mintel's GNPD (Appendix B) indicated that approximately 65% of the food products in which lecithins (E 322) was labelled, were included in the current exposure estimates.

3.4.2. Exposure via the regular diet

According to JECFA (WHO, 1974), the average diet provides a daily intake of several grams of lecithin (approximately 1–5 g corresponding to 14–71 mg/kg bw for a 70-kg adult population).

In the human diet, according to Zeisel (1981), most choline is consumed in the form of lecithin which is highly present in common consumed foods such as liver (850 mg per 100 g), eggs (394 mg per 100 g), soya beans (1,480 mg per 100 g), peanuts (1,113 mg per 100 g) and wheat germ (2,820 mg per 100 g).

In a recent opinion, the EFSA NDA Panel (NDA, April 2016) provided dietary intakes estimates of total choline from the regular diet of the European population group. The choline content of exhaustive food products was calculated as the sum of free choline and choline derived from the choline products from glycerolphosphatocholine, phosphocholine, phosphatidylcholine and sphingomyelin. Phospholipid lecithin (phosphatidylcholine) is known as being the ultimate source of most dietary choline (Zeisel, 1981).

Nutrient intake calculations were performed only on subjects with at least two reporting days. Choline intake from dietary supplements was not assessed. Total choline intake mean estimates ranged from 75 to 127 mg/day in infants < 1 years old (corresponding to 9–16 mg/kg bw per day using the EFSA default body weight), 151 to 210 mg/day in children aged from 1 to < 3 years (corresponding to 13–18 mg/kg bw per day), 177–304 mg/day in children aged from 3 to < 10 years (corresponding to 8–13 mg/kg bw per day) and 244–373 mg/day in children aged from 10 to < 18 years (corresponding to 4–6 mg/kg bw per day). Total choline intake mean estimates ranged from 269 to 468 mg/day in adults aged from 18 to ≥ 75 years (corresponding to 4–7 mg/kg bw per day).

Overall, the Panel considered that dietary intakes of total choline from regular diet could be estimated in average ranging from 4 to 18 mg/kg bw per day across all population age groups.

Moreover, the Panel noted that mean dietary intakes of lecithins from the regular diet are in the range of the mean estimated exposure from the use of the food additive itself (Table 8, non-brand loyal consumer scenario).

3.4.3. Exposure via other sources

Exposure to lecithins due to the following uses was not considered in this opinion.

Lecithins as an ingredient in food supplements and other foods

Lecithin is an ingredient of preparations promoted as tonics and dietary supplements in a wide range of disorders (Radimer et al., 2000; Martindale, 2014). Lecithins are purported to increase brain function, promote energy or prevent arteriosclerosis or cardiovascular disease (Radimer et al., 2000).

Pharmaceutical uses

Lecithins are used in pharmaceutical products as an active ingredient, as well as an excipient (Documentation provided to EFSA n.17).

The average single dosage of lecithins as an active ingredient for adolescents and adults in mono or combination products can be approximately up to 2,000 mg, whereas the average daily dosage might be up to 6,000 mg/day.[20] According to the draft monograph of the HMPC of the EMA, a traditional medicinal usage of soya bean lecithin can result in daily dosages in the range of 1,500–8,100 mg (divided into two or three intakes) (HMPC, 2016b; draft).

According to the EMA, lecithins are used as active ingredients which do not fulfil the criteria of traditional use in daily dosages up to 9,000 mg daily in several Member States of the EU (HMPC, 2016a; draft).

In many national and European authorised products, lecithins are used as an excipient in medicinal products for oral use for adolescents and adults starting from trace amounts up to approximately 30 mg as daily dosage/person.

3.5. Biological and toxicological data

Lecithins are natural constituents of all cells in the human body. Synthesis of phosphatides and the pathway of catabolism of lecithins in humans are well known. Hydrolysed lecithins are produced in the gut as a result of normal digestion of food.

The Panel noted that one of its metabolites, choline, is a precursor of the neurotransmitter acetylcholine. Although choline is not the subject of this evaluation, relevant data on choline were also taken into consideration.

Furthermore, for the toxicological evaluation, the Panel used available data on lecithins as a mixture of different phosphatides and, when available, purified phospholipids, such as phosphatidyl choline and phosphatidyl inositol.

3.5.1. Absorption, distribution, metabolism and excretion

3.5.1.1. Lecithins

In vivo studies

Several *in vivo* studies using radiolabelled lecithins were available in animals and humans.

Animal studies

Rats and monkeys were orally administered with radiolabelled soya phosphatidylcholine (1,2-diacylglycero-3-phosphorylcholine labelled with ^{3}H in the fatty acid moiety or with ^{14}C in the choline moiety) (Documents provided to EFSA n.4 and 5). Rats (four of each sex) and rhesus monkeys (three of each sex) received 250 mg ^{3}H- or ^{14}C-phosphatidylcholine/kg bw as a single dose or as a daily dose for five consecutive days. In these animals, tritium exchange with body water occurred extensively *in vivo* and a part of the ^{3}H radioactivity detected represented $^{3}H_2O$. The tissue distribution was investigated in rats; liver contained the higher amounts of radioactivity, although significant radioactivity was detectable after 6 h in striated muscle, depot fat and the kidneys. After repeated dosing over 5 days, there was a comparable organ distribution with additional small amounts of radioactivity in the lungs, testes, intestines, skin, thymus and thyroid gland. For both rats and monkeys receiving a single oral dose, the faecal excretion of ^{14}C radioactivity within 5 days corresponded to 3–7.4% of the dose, whereas, in rats, 30–47% of a single oral dose was exhaled as $^{14}CO_2$. The urinary excretion of ^{14}C radioactivity within 5 days amounted to 2.9–5.3% and 17–21% of the dose in rats and monkeys, respectively.

Le Kim and Betzing (1976) investigated the fate of polyunsaturated phosphatidylcholine in rats given 1,2-di-[9,10,12,13-$^{3}H_4$]linoleoyl-*sn*-glycero-3-phospho-[N-$^{14}CH_3$]-choline, 1-[1-^{14}C]linoleoyl-2-[9,10,12,13-$^{3}H_4$]-linoleoyl- or 1-[9,10,12,13-$^{3}H_4$]linoleoyl-2-[1-^{14}C]linoleoyl-*sn*-glycero-3-phosphocholine. Wistar rats (four males and four females) were given a single oral dose of 70 mg/kg bw of each radiolabelled substance and were kept in metabolic cages. The absorption rate of radioactivity from the gastrointestinal tract was rapid and 85% of the doses was absorbed within the first 8 h. One half of the orally administered polyunsaturated phosphatidylcholine was hydrolysed to 1-acyl-lysophosphatidylcholine and reacylated to phosphatidylcholine upon entering the mucosa cell. The other half was completely hydrolysed to free fatty acids and glycerophosphocholine. There was a relatively slow rate of degradation of the fatty acid in the 1-position, in contrast to the fatty acid esterified to the 2-position of phosphatidylcholine. In anaesthetised rats (six males), lymph samples

[20] Available online: http://www.kade.de/fileadmin/assets/beipackzettel/buer-lecithin-plus-vitamine-fluessigkeit-dr-kade.pdf

were collected every 1 h up to 24 h. Some 17–25% of the administered radioactivity appeared in the lymph chylomicrons within 6.5 h. This radioactivity was mainly located in phosphatidylcholine and neutral lipids fractions. From these data, it was considered that 'phosphatidylcholine is hydrolyzed to 1-acyl-lysophosphatidylcholine by pancreatic phospholipase A. This acyl-lyso compound is absorbed in the mucosal cells and is reacylated to form phosphatidylcholine by the lysolecithin acyltransferase. Part of the 1-acyl-lysophosphatidylcholine is further hydrolyzed in the intestinal tract by lysophospholipase to form glycerophosphocholine. The fatty acids are also absorbed and enter the Kennedy pathway to form triglycerides before appearing in the lymph chylomicrons'. Regarding tissue distribution, when ^{14}C radiolabelling was located on choline, the liver contained 30% of the applied ^{14}C-radioactivity and almost 10% of the applied ^{3}H-radioactivity. Minor amounts of radioactivity were distributed in all other organs analysed: lung, spleen, kidney, heart and brain. Blood contained 8% and 4% of ^{14}C-radioactivity and ^{3}H-radioactivity doses, respectively, and elimination half-lives for ^{14}C-radioactivity and ^{3}H-radioactivity were 20 and 30 h, respectively. Six hours after dosing, the respiratory excretions of $^{14}CO_2$ were 1.8%, 7.7% or 25% of the dose when ^{14}C-radioactivity was located in choline, in the 1- or 2-position of the fatty acid, respectively. The Panel noted that urinary and faecal excretions of radioactivity were not determined in this study.

Wistar rats (six males and six females) were given a single oral dose of 70 mg/kg bw radiolabelled phosphatidylcholine (Fox et al., 1979). Dilinoleoylphosphatidylcholine was labelled with ^{14}C in either the 1-position or the 2-position in the acyl moiety, or in the choline moiety. The same phosphatidylcholine was also labelled with ^{3}H in the acyl moiety and with ^{14}C in the choline moiety. Up to 84% of phosphatidylcholine was absorbed from the intestine within 19 h. The rates of absorption were equal for both the fatty acids and choline moieties. A considerable amount of radioactivity was found in the intestinal wall (40% of the dose after 3 h). The highest amounts of ^{3}H and ^{14}C radioactivities were found in the liver (38% of the dose after 6 h). Within 5 days after dosing, most of the radioactivity administered remained in the carcass (^{3}H: 58.8%; ^{14}C: 51.3%) or was expired (^{3}H: 6.6%; ^{14}C: 32%). Only minor amounts were excreted via faeces (^{3}H: 8.2%; ^{14}C: 3.2%) or urine (^{3}H: 15.6%; ^{14}C: 6.4%). In another experiment in dogs, using ^{3}H-^{14}C dilinoleoylphosphatidylcholine, it was shown that intestinal absorption of this compound was similar to that in rats and was not influenced by the vehicle in which phosphatidylcholine was administered (Fox et al., 1979).

Human studies

In humans (one female and four male fasted subjects), the metabolic fate of orally administered lecithins (1 g containing 150 μCi ^{3}H-polyenephosphatidylcholine and 50 μCi di[1'-^{14}C]linoleoyl-3-*sn*-glycerophosphocholine) was studied by Zierenberg and Grundy (1982). More than 90% of both isotopes were absorbed from the intestine. In blood, 70–85% of the ^{3}H-radioactivity was linked to phosphatidylcholine and 70% of the ^{14}C-radioactivity was in non-polar lipids (triglycerides and cholesteryl ester). According to the authors, it can be assumed that most of the phosphatidylcholine was hydrolysed to lysolecithin before absorption. After a lag time of about 2 h, radiolabelled lipids were measured in the blood. An examination of lipoproteins showed that the specific radioactivities of phosphatidylcholine in high-density lipoprotein (HDL) were 2–6 times higher than in apolipoproteina B-containing lipoproteins, and 2–20 times higher than that of red blood cells or total blood. This would indicate that absorbed phosphatidylcholine was incorporated preferentially into the HDL fraction of plasma. Within 7 days, only 2% and 4.5% of ^{3}H and ^{14}C, respectively, was excreted via faeces, whereas 6% and 1.2% of ^{3}H and ^{14}C, respectively, were excreted via urine. The Panel noted that, in this study, the radiolabelling of the only acyl moieties of lecithins did not permit an assessment of the fate of the free hydrolysed choline.

3.5.1.2. Metabolism of lecithins into choline

Among lecithins, phosphatidylcholine is hydrolysed to release choline in the cytidine-5-diphosphate-choline pathway in all cells of the body. Choline can also be synthesised *de novo* by the human body. It is a precursor of the neurotransmitter acetylcholine and it plays an important role in the metabolism and transport of lipids and cholesterol by lipoproteins, and is needed for the assembly and secretion of very low-density lipoproteins by the liver (EFSA NDA Panel, 2016).

The EFSA NDA Panel considered a total choline concentration of 145 mg/L for human milk (EFSA NDA Panel, 2016). According to older literature, human milk is reported to contain 160–210 mg (1.5–2 mmol)/L of total choline, delivered as choline, phosphocholine, glycerophosphocholine, phosphatidylcholine and sphingomyelin (Zeisel et al., 1986; Holmes-McNary et al., 1996).

In humans, dietary lecithins, namely phosphatidylcholines, are known to be hydrolysed by phospholipases to liberate choline. According to data from ELMA (Document provided to EFSA n.18), 1–3.38% of choline could theoretically be released from the food additive lecithins (E 322) (see Table 3). Following intestinal hydrolysis of phosphatidylcholine, choline is rapidly absorbed by a carrier-mediated saturable transport system and appears in plasma predominantly as free choline. Lecithins having escaped hydrolysis enter the lymph incorporated into chylomicrons. This metabolism was reviewed by Zeisel (1981) who reported the dietary sources of choline, as well as its biochemistry, physiology and pharmacology, and it was more recently described by EFSA in the scientific opinion on dietary reference values for choline (EFSA, 2016).

In humans, the relationship between dietary lecithin intake and plasma choline levels has been investigated in several studies.

For instance, Hirsch et al. (1978) determined choline serum levels in nine patients receiving either 3 g of choline chloride or a meal supplemented with an equivalent dose in the form of 100 g of lecithin granules, containing 10–20% lecithin and 80–90% mixed neutral lipids. After the consumption of a single meal containing 3 g of choline chloride, serum choline rose by 86%, attaining peak values after 30 min. When the same subjects ate the meal containing an equivalent amount of choline in the form of lecithin, serum choline levels rose by 33% after 30 min, and continued to rise for at least 12 h, to 265% over control values.

In six male subjects, Zeisel et al. (1980) examined plasma choline changes after ingestion of diets composed of common foodstuffs, with choline contents bracketing the average daily intake in the American diet, and the ingestion of diets supplemented with exogenous purified lecithin. A diet with low choline content did not increase plasma choline concentrations. A diet with high choline content doubled plasma choline levels. A lecithin supplemented (25 g of egg or soya lecithin; 80% phosphatidylcholine) low-choline diet increased plasma choline levels by four-fold at the peak value (6 h post-dosing).

Free choline is also found in maternal milk and its concentration changes during the progress of lactation and is influenced by maternal diet as reported in EFSA (2016).

Fischer et al. (2010) investigated, in pregnant women, the response of maternal plasma and breast milk choline concentrations to a phosphatidylcholine supplement (containing 750 mg choline/day per person, $n = 48$, from the 18 gestational weeks to 90 days post partum), compared to placebo ($n = 46$). The supplement was consumed in addition to a mean dietary choline intake of about 350 mg/day. Breast milk and maternal plasma concentrations were measured at 45 days post partum. There was a significant linear correlation between total choline intake (from foods and supplements; range about 150 to > 750 mg/day) and breast milk concentrations of phosphatidylcholine, phosphocholine, free choline and betaine when all subjects were taken into account. Mean breast milk concentrations of phosphocholine (722 vs 553 μmol/L) and free choline (106 vs 83 μmol/L) were significantly higher in the supplemented group than in the placebo group, whereas phosphatidylcholine was not significantly different. According to the authors, the study physician reported that unusual or unexpected events did not occur more frequently in women receiving the supplement compared to those receiving a placebo or to a normal obstetric population, and as well as in their nursed infants.

High doses of choline have been associated with a fishy body odour. This results from the excretion of excessive amounts of trimethylamine, a choline metabolite, as the result of bacterial action in the digestive system. Lecithin, as a group of choline-containing phospholipids, however, does not present a risk of fishy body odour. This is because the intestinal bacteria in general cannot cleave the esters, and hence do not form major amounts of trimethylamine from choline (Zeisel et al., 1983 cited in IOM 1998).

Conclusions

Overall, studies using radiolabelled phosphatidylcholine in animals and humans clearly indicated that, following oral administration, phosphatidylcholine is absorbed unchanged or as lysophosphatidylcholine or choline after intestinal hydrolysis. In intestinal mucosa cells, lysophosphatidylcholine would be reacylated into phosphatidylcholine or hydrolysed to glycerophosphocholine and free fatty acids. The fatty acids would be further utilised for the reassembly of triacylglycerides and phosphatidylcholine found in the chylomicrons. In humans, the absorbed phosphatidylcholine would be incorporated preferentially into the HDL fraction of plasma. Peak levels of phosphatidylcholine in blood are reached within 6 h. Besides the intestinal wall, the major target organ for distribution and metabolism of lecithins is the liver. Only minor amounts of radioactivity were excreted via urine and faeces demonstrating that the administered lecithins would undergo metabolism as for endogenous phospholipids. From the current database, the Panel noted that only minor levels of choline labelling radioactivity were detected in the brain.

In humans, dietary lecithins are known to be hydrolysed by phospholipases to liberate choline, which is rapidly absorbed by a carrier-mediated saturable transport system and appears in plasma predominantly as free choline. Consequently, an increased plasma-free choline concentration has been described as a consequence of increased dietary intake of lecithins. Moreover, a significant increase in breast milk concentrations of free choline was observed in lactating women receiving a phosphatidylcholine supplement in comparison with the placebo group.

3.5.2. Acute toxicity

Unpublished studies on acute oral toxicity of lecithins were presented by Cosmetic Ingredient Review (CIR) (2001), although the information on these data was limited.

In several studies, LD_{50} of more than 16,000 mg/kg bw in mice, more than 5,000 mg/kg bw in rats and 4,750 mg/kg bw in rabbits were reported (FDRL, 1973a,b; Leberco-Celsis Testing, 1997; FDRL, 1973c, as cited in CIR, 2001). The Panel noted that, in these studies, the test substance is not always characterised.

There were no deaths or clinical signs observed in male and female rats to which purified phosphatidylinositol from soya lecithin (Asahi Kasei PI) was orally administrated once in single doses up to 2,000 mg/kg bw (Honda et al., 2009).

3.5.3. Short-term and subchronic toxicity

3.5.3.1. Short-term studies

Rats

The SCF (1982) described a subacute toxicity study performed by Unilever (1978) as follows: 'a 3 week feeding study in rats comparing lecithin, hydrolysed lecithin and a control purified diet containing 10% ground nut oil showed no essential difference between lecithin and hydrolysed lecithin with respect to effects on body weight, food intake and growth. Level of 20% or more in the diet produced adverse effects on hematopoiesis and enlargement of the kidneys'.

Dogs

The effect of different batches of EPL (see Section 3.1.1) (without additional information on the composition) was tested by peroral administration to 18 pure-bred Beagle dogs (three animals per group) over a 6-week period (Document provided to EFSA n.11). Six more dogs received the solvent only and served as controls. The dosages used were 50, 250 and 2,500 mg EPL/kg bw per day in 5 mL of 1% aqueous carboxy ethyl cellulose gel by stomach tube. At all three dosages, the only effect observed was on lipid metabolism. After 6 weeks of treatment, the free cholesterol level was significantly lowered in animals receiving 2,500 mg EPL/kg bw per day. Total cholesterol and total lipid levels in serum were slightly lowered, although the values determined still lay within the normal range. Esterified and non-esterified fatty acids and neutral fats in serum were not affected. Behaviour, external appearances, feed and drinking water consumption, faeces, body weight development, haematological and electrocardiographic investigations, urine composition, examinations of the eyes, hearing and dentition, macroscopic inspection and visual comparison of the internal organs in section showed no evidence of adverse effects, even at the highest EPL dosage (2,500 mg/kg bw per day). Apart from the aforementioned influence on fat metabolism, no certain deviations could be seen in the clinical-biochemical parameters. The histopathological investigations also revealed no indication of injury. None of the animals died. According to the authors, the lowest toxic dose may be expected to be $>$ 2,500 mg EPL/kg bw per day. The alterations of lipid metabolism may be due to the pharmacodynamic properties of the preparation (Document provided to EFSA n.11).

3.5.3.2. Subchronic toxicity studies

Rats

A 90-day study has been performed in rats (Weanling SPF rats of the Carworth Farm E strain) with a mixture of ammonium compounds of phosphatidic acids derived from rapeseed oil and a proportion of triglycerides from the partially hardened oil (Gaunt et al., 1967). The soya bean lecithin (no additional information on the composition available) was used for comparison in this study.

Groups of 15 male and 15 female rats were fed diets containing 0.0% (control) or 6.0% soya bean lecithins, equivalent[21] to 4,860 mg/kg bw per day for males and 5,460 mg/kg bw per day for females, respectively. Body weight and food consumption were recorded weekly. Haematological investigations were made during week 6 with blood collected from the tail veins of 10 animals of each sex from the control, 6% test item and 6% lecithin groups, and terminally on all animals using blood collected from the dorsal aorta.

There was slight anaemia in females receiving 6% lecithin for 6 weeks but this effect was absent terminally. The osmotic fragility of the erythrocytes of rats on the 6% lecithin diet was comparable with that of the controls. There was no deviation from normality in respect of the terminal serum chemistry or renal function tests conducted at 6 or 13 weeks. No significant differences of relative organ weight were noted. At necroscopy, no gross changes were seen. The authors concluded that a minimum no-effect level was 6% of soya bean lecithins, equivalent to 4,860 mg/kg bw per day for male and 5,460 mg/kg bw per day for female rats, respectively.

The effect of EPL was tested in Wistar rats (male and female) over a period of 12 weeks using oral administration (Document provided to EFSA n.12). The test material was described as a product with active principle choline phosphoric acid diglyceride ester of natural origin with predominantly unsaturated fatty acids, particularly linolic acid (approximately 70%), linolenic and oleic acid. Four groups of 20 animals (10 males and 10 females) were administrated 0, 150, 750 and 3,750 mg EPL/kg bw per day. Distilled water was used as a solvent, and the solution was administered in a constant volume of 20.0 mL/kg bw per day by gavage. The control animals received the same volume of distilled water. No effect on behaviour, external appearance, body weight and intake of food and drinking water could be observed during the 12-week duration of the study. No changes were observed in the faeces. No modification of haematological and biochemical parameters, or urinanalysis, was noted. Histopathological examination did not detect changes induced by the test item. The authors concluded that the no-effect daily dose is > 3,750 mg/kg bw per day. The Panel considered that, in this study, the no-observed-adverse effect (NOAEL) was 3,750 mg/kg bw per day, which is the highest dose tested.

In a 13-week study in male and female rats, purified phosphatidylinositol from soya lecithin (Asahi Kasei PI) was administered orally at daily doses of 0, 100, 300 and 1,000 mg/kg bw. Neither death nor any substance-related change with regard to body weight, food consumption, ophthalmoscopy, haematology, blood biochemistry, necropsy, organ weights or histopathology were observed in any of the treatment groups. Based on these results, the authors considered the NOAEL to be 1,000 mg phosphatidylinositol/kg bw per day for male and female rats, the highest dose tested (Honda et al., 2009).

Dogs

The effect of EPL by oral administration of 250, 500 and 1,000 mg/kg bw per day (three male and three female animals per group) in a capsule for 5 days/week for 1 year was investigated in beagle dogs (Document provided to EFSA n.15). A group of six dogs was taken as a control. During the whole treatment period, no visible signs of intolerance were detected. There was a slight but not dose-related increase in body weight in the treatment groups. Besides a slight increase (twice) in the amount of total lipids and a significant increase in triglyceride levels in females, no treatment-related differences in haematological, clinical-chemical, electrocardiographical and clinical data and urinanalysis could be detected. During sacrifice at the end of the study, no gross pathological changes were observed. The histopathological investigations of the tissues showed no significant substance-related differences. It was concluded that, under these experimental conditions, the no-effect dose was higher than 1,000 mg/kg bw per day.

3.5.4. Genotoxicity

No genotoxicity studies using lecithin preparations meeting the EU specifications for the food additive E 322 were available to the Panel. However, a number of *in vitro* and *in vivo* studies were available with a multivitamin preparation containing lecithins.

3.5.4.1. *In vitro*

Lecithin (no additional information on the composition available) was tested in an Ames test with *Salmonella* Typhimurium tester strains TA1535, TA1537 and TA1538 performed both in the absence

[21] EFSA guidance on selected default values. EFSA Journal 2012;10(3):2579, 32 pp.

and presence of S9 metabolic activation prepared from liver, lung and testis of rat, mouse and monkey (*Macaca mulatta*). A concentration of 0.02% was used in the plate test and concentrations of 0.01%, 0.02% and 0.04% were employed in the suspension test. The survival rate at the highest concentrations employed was 50% both in the plate and in suspension tests. No mutagenicity was observed. The Panel noted that the study is limited mainly for the incomplete set of the *S. typhimurium* tester strains employed (Litton Bionetics Inc., 1975).

In an unpublished report (Document provided to EFSA n.10), a multivitamin preparation (ESSENTIALE 303™) containing lecithin (50 mg/mL) was assessed. Lecithin was described as polyunsaturated phosphatidylcholine containing 60% unsaturated fatty acids (linoleic acid 80%, linolenic acid 5% and oleic acid 15%). This preparation was assessed for its mutagenicity in the reverse mutation assay using *S. typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100 and in the forward mutation assay in *Schizosaccharomyces pombe* (strain P1), both in the absence and presence of rat liver S9 metabolism at concentrations of 200, 100, 50 and 25 μL/plate. No mutagenic activity was observed in any of the strains employed.

Another Ames test with *S. typhimurium* TA1535, TA1537, TA1538, TA98 and TA100 tester strains was performed both in the absence and presence of rat liver S9 metabolic activation. The test compound was added at 0.1, 1.0, 10.0 and 500.0 μg/plate as mixed micelles containing 169.3 mg/mL lecithins artificially decomposed by exposure to temperature of 80°C for 250 h (resulting in the decomposition of about 25% lecithins into fatty acids and lysolecithins). No indication of genotoxic activity was observed (Teelmann et al., 1984).

The preparation ESSENTIALE 303™ was also tested for induction of gene conversion in yeasts, both in the absence and presence of metabolic activation in two independent studies. In the first study, *Saccharomyces cerevisiae* (strain D4) was treated with lecithin at concentrations of 1.875%, 3.750% and 7.5% in dimethyl sulfoxide. The survival rate at the highest concentrations employed was 50% and no indication of genotoxic activity was observed (Litton Bionetics Inc., 1975). In the second study, the lecithin preparation (250 mg/5 mL polyunsaturated phosphatidylcholine containing 150 mg/5 mL unsaturated fatty acids) was employed for treatment at 200, 100, 50 and 25 μL for 2 h in *S. cerevisiae* (strain D4 and D7). The survival rate exceeded the value of 80% compared to the untreated control in both strains and at all concentrations assayed. No indication of genotoxicity was observed (Document provided to EFSA n.10).

In a study by Honda et al. (2009) purified phosphatidylinositol from soya lecithin (Asahi Kasei PI) was tested in an Ames test with *S. typhimurium* tester strains TA1535, TA1537, TA1538, TA100 and *Escherichia coli* WP2 uvrA, performed both in the absence and presence of S9 metabolic activation prepared from liver of rats pretreated with phenobarbital and 5,6-benzoflavone. A concentration in the range 315–5,000 μg/plate was employed in two experiments using the preincubation method and no increases in the number of revertant colonies were observed. The Panel noted that the study was performed according to the relevant OECD Guideline no. 471 adopted on 21 July 1997.

In an unscheduled DNA synthesis (UDS) assay in human embryonic epithelium (EUE) cells, the preparation ESSENTIALE 303™ was employed for treatment at 1×10^{-8}%, 1×10^{-6}%, 1×10^{-4}% and 1×10^{-2}%, for 1 h both in the absence and presence of S9 metabolic activation. At the end of the treatment, cultures were washed and ^{3}H-thymidine at 5 μCi/mL was added for 4 h to detect DNA repair events by the autoradiographic method. The results obtained did not indicate any induction of UDS (Document provided to EFSA n.10).

A purified phosphatidylinositol from soya lecithin (Asahi Kasei PI) was also tested for the induction of chromosomal aberration in a Chinese hamster lung fibroblast cell line, both in the absence and presence of S9 metabolic activation prepared from liver of rats pretreated with phenobarbital and 5,6-benzoflavone (Honda et al., 2009). The concentrations used, selected from preliminary dose-range finding experiments, were 1,250, 2,500 and 5,000 μg/mL. The highest dose-level selected, which is 2.5-fold higher than the recommended dose of 2,000 μg/mL in the current OECD Guideline no. 473, did not induce any cytotoxicity or reduction in cell growth. Cells were treated for 6 h both in the presence and absence of S9 metabolic activation with sampling at 24 h from the beginning of treatment in the short-term treatment time and for 24 and 48 h in the long-term treatment time. The results obtained indicated that the incidence of both structural and numerical (polyploidy) chromosomal aberration was similar to the untreated control. The Panel noted that the study was performed essentially in agreement with the current OECD Guideline no. 473.

3.5.4.2. *In vivo*

Groups of five male Swiss CD-1 mice were injected once, intraperitoneally, with 0.1, 1.0 and 2.0 mL/kg bw of the preparation ESSENTIALE 303™, and then kept in metabolic cages, and urine was collected for the following 24 h and filter sterilised. Volumes of 0.2 mL of urine, in the absence and presence of glucuronidase at 1,000 U/mL, were added to cultures of *S. cerevisiae* (strain D7) for induction of gene conversion. The survival rate exceeded the value of 80% compared to the untreated control at all concentrations assayed and no mutagenic activity was detected (Document provided to EFSA n.10).

In a host-mediated assay, groups of five male Swiss CD-1 mice were injected once, subcutaneously, with 0.1, 1.0 and 2.0 mL/kg bw of the preparation ESSENTIALE 303™ for 3 h. Immediately after treatment, 1 mL of a suspension containing about 2×10^9 cells of *S. cerevisiae* (strain D7) was injected into the peritoneum of each mouse. Three hours after the injection of the yeast, the mice were sacrificed and the yeast cells were aseptically washed out of the peritoneum of each animal and suspended in phosphate buffer at pH 7.1. Yeast cells were then plated under standard conditions to detect gene conversion. The survival rate exceeded the value of 85% compared to the untreated control at all concentrations assayed and no mutagenic activity was detected (Document provided to EFSA n.10).

In conclusion, no genotoxicity was observed in different *in vitro* assays with lecithins, which include the bacterial reverse mutation assay (Ames test), test for induction of gene conversion in *S. cerevisiae* (strains D4 and D7), an UDS assay in the human EUE cells *in vitro*, as well as in *in vivo* host-mediated and urinary assays. The Panel noted that investigations of structural and numerical aberrations that are two out of the three endpoints required for the assessment on the genotoxicity (EFSA Scientific Committee Guidance document, 2011) were only available for the purified phosphatidylinositol. However, the Panel considered that read-across from phosphatidylinositol to the other phospholipid components of lecithins was justified. Moreover, the substances known to induce structural chromosomal aberrations frequently also induce UDS and the Panel noted that the available UDS assay was negative. Overall, based on the data available, the Panel concluded that there is no concern with respect to the genotoxicity of lecithins.

3.5.5. Chronic toxicity and carcinogenicity

Mice

A study performed by Szepsenwol (1969) focussing on the development of brain nerve cell tumours in TM strain mice was regarded by the Panel as invalid due to several deficiencies (no complete histopathology performed, unknown mouse strain, no exact specification of the tumour type).

Rats

The effect of orally administered EPL (see Section 3.1.1) was tested in 25 female and male Wistar rats (25 of each sex per group) during 24 weeks (Document provided to EFSA n.13). The dosages were 0, 150, 750 and 3,750 mg EPL/kg bw per day. EPL was diluted in distilled water and the solution was administered in a constant volume of 20 mL/kg bw by gavage. The control animals received the same volume of the solvent. No influence on behaviour, external appearance, body weight, and food and water intake was observed during the test. Faeces did not show changes. There were no substance-related mortalities. The EPL administration did not affect the haematological, clinical-chemical and urinary parameters, nor the relative organ weights. Haemoglobin in the faeces was not detected during the 24-week test. No influence on hearing, growth of teeth and the visual system was observed. Macroscopic changes detected during necropsy were incidental findings and not substance-related. It was concluded that the NOAEL of this study is 3,750 mg EPL/kg bw per day.

The effect of EPL (see Section 3.1.1) was tested in Wistar rats (male and female) over a period of 48 weeks, using oral administration (Document provided to EFSA n.7). Four groups of 25 (male and female) were treated with 0, 150, 750 and 3,750 mg EPL/kg bw per day. Distilled water was used as a solvent, and the solution was administered in a constant volume of 20.0 mL/kg bw per day to the rats by gavage. The control animals received the same volume of distilled water. No influence on the behaviour, external appearance, body weight and the intake of food and drinking water could be observed during the 48-week duration of the test. No changes were observed in the faeces. In total, seven rats died during the study (three control animals, two animals from group I and one animal each from groups II and III), whereby the death of all animals is independent of the administration of

the preparation. An influence resulting from the administration of the substance on hearing, the growth of teeth and the visual apparatus was not detectable. The haematological, clinical-chemical and urinary parameters, as well as the relative weight or the organs, were not influence by the administration of EPL over the 48-week period. Haemoglobin was not detectable in the faeces after the 48-week duration of the test. The macroscopically detected findings of necropsy of all animals at the end of the test can be considered to be chance findings and normal for rat populations, and thus independent of the test. For histological examination, paraffin sections of the following organs, stained with haemalum-eosin, were available: cerebrum, cerebellum, nervus ischiadicus, hypophysis, thyroid gland (2×), thymus gland, lung, heart, liver, oesophagus, stomach, duodenum, jejunum, pancreas, spleen, mesenteric lymph nodes, kidneys (2×), adrenal gland, skeletal muscle, testes, prostate gland, seminal vesicle and ovaries (2×), and uterus (2×). A Prussian-blue reaction of the lung and of the spleen was available for the detection of iron. Frozen sections stained with Sudan III were made for the detection of fat in the heart, liver and kidneys. Five male and female animals were then examined histopathologically in control and the lowest and middle doses, and 10 males and five females at the highest dose. With respect to the fatty changes, there was a tendency towards diffuse fatty changes in the heart in the case of the male higher dosage group (nine of 10 compared to three of five in controls), whereas there was no evidence of dosage-dependent fatty changes in the liver. The fatty changes in the liver were characterised by peripheral fatty deposits in the liver cells. According to the authors, this effect could be a chance finding, and they concluded that under the given circumstances of the test, the 'no-effect' dosage of EPL in Wistar rats may be expected, in the case of a 48-week per oral administration to be above 3,750 mg/kg bw per day.

The Panel noted that similar histopathological changes were observed in the heart in both control and treated animals. The Panel considered that these histopathological changes were likely to be a background finding in rats of this strain and age. Furthermore, the Panel noted that this study has some shortcomings.

In a 2-year study, groups of 48 male (100–130 g) and 48 female (90–120 g) weanling Wistar rats were fed diets containing either 0% (control), 2% or 6% a mixture of ammonium compounds of phosphatidic acids derived from rapeseed oil, and a proportion of triglycerides from the partially hardened oil or 4% soya lecithin (no additional information on the composition available), equal to 1,470 and 2,280 mg soya lecithin/kg bw per day in male and female rats, respectively, for 2 years (Brantom et al., 1973). Body weights and food consumption were recorded at intervals up to week 95. Necropsies were carried out on all rats. The animals were examined for macroscopic abnormalities and the brain, pituitary, thyroid, heart, liver, spleen, stomach, small intestine, caecum, kidneys, adrenal glands and gonads were weighed. Samples of these organs and samples of salivary gland, trachea, lung, aortic arch, skeletal muscle, lymph nodes, colon, rectum, pancreas, spinal cord, bone and uterus and any other tissue that appeared abnormal were preserved in 10% buffered formalin. All tissues from control animals and those fed 4% soya lecithin were prepared for microscopic examination. No abnormalities were seen in the behaviour of the rats. The body weights of females fed 4% soya lecithin were significantly higher than those of controls from week 62 onwards. The food intakes of all treated male groups were slightly higher than those of controls, whereas females from treated and control groups consumed similar amounts of food daily. There were no statistically significant differences between treated and control animals with respect to the results of serum analyses or the tests of renal concentrating ability, and haematological investigations did not reveal any significant differences between treated and control animals. At necropsy, it was noted, mainly in males, that small nodules were present on the surface of the thyroids of four controls and seven or eight animals in each treated group. Histopathological examination of these tissues revealed enlarged hyperplastic parathyroids. This lesion was also found in rats in which no nodules were seen at necropsy. Regarding the incidence of tumours, the commonest was chromophobe adenoma of the pituitary and fibroadenoma of the mammary tissue. Benign tumours affecting the liver, pancreas, pituitary, thyroid, adrenals, testes, skin, brain salivary gland, ovary, uterus, prostate and connective tissue were also found. Malignant tumours were found in all groups affecting the pancreas, thymus, salivary gland, mammary tissue, uterus, skin and connective tissue. However, the incidence of tumours was not influenced by feeding with soya lecithin.

The authors concluded that, although tumours were observed in this study, in no case could these be taken as an indication of a carcinogenic effect of the test item. On the basis of the present study, soya lecithin can be considered as not carcinogenic when fed to rats for 2 years at dietary levels of up to 4%. Similarly, no toxic effects that could be attributed to the ingestion of the soya lecithin were found in this study and a no-untoward effect level of 4% in the diet, equal to 1,470 and 2,280 mg

soya lecithin/kg bw per day in males and females, respectively, was identified by the authors. The Panel agreed with this conclusion.

3.5.6. Reproductive and developmental toxicity

3.5.6.1. Reproductive toxicity studies

No reproductive toxicity studies with lecithins were available.

3.5.6.2. Developmental studies

Several prenatal developmental toxicity studies with lecithins were conducted in CD1 mice, Wistar rats and Dutch belted rabbits (FDA, 1974). Animals were administered different doses of lecithin suspended in anhydrous corn oil by gavage; the control groups were vehicle treated.[22] Body weights were recorded at regular intervals during gestation and all animals were observed daily for appearance and behaviour. All dams were subjected to caesarean section, and the numbers of implantation sites, resorption sites, live and dead fetuses, and body weight of live fetuses were recorded. All fetuses were examined grossly for external abnormalities, one-third underwent detailed visceral examinations and two-thirds were stained and examined for skeletal defects.

Mice

In a mice study, groups of 21–23 pregnant albino CD-1 mice were dosed via gavage with 0, 16, 74.3, 345 or 1,600 mg/kg bw per day lecithin in corn oil (dose volume 10 mL/kg bw) from gestational day (GD) 6 to 15 (FDA, 1974). Body weights were recorded on GD 0, 6, 11 and 15, and at necropsy on GD 17. For both dams and fetuses, no adverse effects were noted at doses of up to 1,600 mg/kg bw per day.

Rats

In a rat study (FDA, 1974), groups of 22–24 pregnant albino Wistar rats were dosed via gavage with 0, 16, 74.3, 345 or 1,600 mg/kg bw per day lecithin in corn oil from GD 6 to 15. The dose volume of the vehicle was 1, 1, 1, 2 or 6.4 mL/kg bw, respectively). Body weights were recorded on days 0, 6, 11 and 15, and at necropsy on day 20. Dams and fetuses were examined as described in the above study with mice. No adverse effects for both dams and fetuses were noted at doses of up to 1,600 mg/kg bw per day.

The effect of a preparation containing choline phosphoric acid diglyceride ester of natural origin with mainly unsaturated fatty acids, particularly linolic acid (approximately 70%), linolenic and oleic acid, was tested on rats. Groups of 25 pregnant rats received, throughout pregnancy and lactation, oral doses of 0, 150, 750 and 3,750 mg/kg bw per day, from GD 16 to the third week of lactation (Document provided to EFSA n.9). Distilled water was used as a solvent, and the solution was administered in a constant volume of 20 mL/kg bw per day to the rats by gavage. No influence of the preparation on behaviour, appearance, body weight, food and water intake or the faeces of the dams was recorded. There were no mortalities. No abnormalities were seen regarding the duration of gestation. The number of dead pups was somewhat higher in the 750 mg/kg bw per day group compared to the control. However, the effect was not dose-dependent and the changes in the 750 mg/kg bw per day group were within the historical background range expected for this strain. No morphological abnormalities could be detected in the offspring. Regarding lactation and viability index, as well as rearing rate, no substance-specific influences were seen. It was stated that this preparation in oral doses up to 3,750 mg/kg bw per day exerts no influence on peri- and postnatal development of rats.

EPL (see Section 3.1.1) was administered to pregnant Wistar rats (n = 24 per group) at doses of 0, 100, 500 and 1,000 mg/kg bw by gavage (dosing volume 10 mL/kg bw) from GD 6 to 15 (Document provided to EFSA n.14). The substance was stirred with distilled water and allowed to swell. Administration of the substance had no effect on behaviour, external appearance, weight development, water consumption and faeces of the dams. A reduction on food intake after the treatment phase in the animals of the mid- and high dose group was considered by the authors of little or no significance because no effect on weight development could be found. There were no deaths. Macroscopic examination at necropsy showed no pathological findings in the dams. Corpora lutea, implantations,

[22] Taking into account the statement from the teratology study in rats (FDRL, 1973a,b,c) that 'the controls were sham treated with the vehicle at a level equivalent to the group receiving the highest test dose', the Panel assumed that control group was treated with the vehicle, corn oil.

resorptions, litter size, fetal and placental weights, and pre- and post-implantation losses showed no marked differences between treated animals and controls. No treatment-related effects were observed on external, visceral or skeletal examination of the fetuses. The Panel agreed with the authors and considered the NOAEL for maternal and developmental effects to be 1,000 mg/kg bw per day (the highest dose tested).

EPL (see Section 3.1.1) US 10% was given intravenously to pregnant Wistar rats (n = 24 per group) in doses of 0, 1.0, 3.16 and 10 mL/kg bw per day (dosing volume 10 mL/kg bw in 0.9% NaCl solution) from GD 6 to 15 (Document provided to EFSA n.8). After the treatment phase (GD 15), an increase in food intake in the animals of the mid- and high dose group was observed. There were no deaths. Macroscopic examination at necropsy showed no pathological findings in the dams. Corpora lutea, implantations, resorptions, litter size, fetal and placental weights, pre- and post-implantation losses showed no marked differences between treated animals and controls. No treatment-related effects were observed on external, visceral or skeletal examination of the fetuses. The Panel agreed with the authors and considered the NOAEL for maternal and developmental effects to be 10 mL/kg bw per day approx. 1,000 mg/kg bw per day (the highest dose tested).

Rabbits

In a rabbit study (FDA, 1974), groups of 10–14 pregnant Dutch-belted rabbits were dosed via gavage with 0, 4.75, 22.1, 100.3 or 475 mg/kg bw per day lecithin in corn oil on GD 6–18. The dose volume of the vehicle was 1, 1, 1, 1 or 2 mL/kg bw. Body weights were determined on days 0, 6, 12 and 18, and at necropsy on GD 29. In addition, live fetuses of each litter were placed in an incubator for 24 h for evaluation of neonatal survival. For both dams and fetuses, no adverse effects were noted at doses of up to 475 mg/kg bw per day.

The effect of PPC-R (containing 95.2% of phosphatidylcholine, 1.3% lysolecithins and 0.13% kephalin) was tested after administration by gavage from GD 1 to 6 in 12 pregnant rabbits per group (Document provided to EFSA n.6). PPC-R was taken up in 0.8% aqueous hydroxypropyl-methylcellulose gel and administered at doses of 0, 250, 500 or 1,000 mg PPC-R/kg bw per day by gavage (volume: 5 mL/kg bw per day). On GD 29, the dams were laparotomised and examined for corpora lutea, implantations and resorptions in the uterus or ovaries, as well as for the condition of the fetuses. The pre-implantation loss was not increased and the development of embryos and fetuses showed no substance-related influence after administration of PPC-R compared to the control group. The authors concluded that PPC-R administration by gavage up to 1,000 mg PPC-R/kg bw per day (treatment from GD 1 to 6) did not influence the implantation in rabbits and the further development of the fetuses. The Panel agreed with this conclusion.

Overall, with respect to reproductive toxicity, no reproductive studies with lecithins are known. In the prenatal developmental studies in mice, rat and rabbits with lecithins, no developmental effects were induced up to the highest dose tested (1,600 mg/kg bw per day, mice and rat and 475 mg/kg bw per day in rabbits). The Panel noted the lack of details in the report of these studies and a lack of description of the statistical methods. In a peri- and post-natal study in rats with a preparation containing choline phosphoric acid diglyceride ester of natural origin with mainly unsaturated fatty acids, particularly linolic acid (approximately 70%), linolenic and oleic acid, no treatment-related effects were observed up to the highest dose tested, 3,750 mg/kg bw per day. PPC-R (containing 95.2% of phosphatidylcholine, 1.3% lysolecithins and 0.13% kephalin) as administered by gavage up to 1,000 mg PPC-R/kg bw per day (treatment from GD 1 to 6) did not influence the implantation in rabbits and the of the fetuses.

3.5.6.3. Neurodevelopmental toxicity studies

Mice

Effects of phospholipids on behavioural maturation were studied in mice by Gozzo et al. (1982). The pregnant females (10 per group) were fed the test diet from GD 14 and continued throughout lactation. At weaning, the pups were fed the control diet until they were sacrificed on post-natal day (PND 60). The control diet contained 10% of lipids (9% made up from margarine and 1% corn oil). The 10% of lipids in the control diet were replaced by commercial soya lecithin in the test diet. Pups were subjected to a series of test of reflex responses, locomotor activity and avoidance leaning between PND 1 and 21. On PND 60, an avoidance learning session of five consecutive days was performed. Body weights of the lactating dams and the pups were not affected (data not shown only for day birth). Fore limb grasping and vibrissae placing were achieved earlier in the pups of the soya

bean lecithin group compared to the control group. On PND 2, 4 and 8, locomotor activity was decreased. The number of avoidances in the learning sessions (from PND 60 onwards) of the soya lecithin group was increased. The number of mice, litters and pups used for each measurement was not clear to the Panel, nor was the selection of these animals for the measurements.

Several studies on the effects of soya lecithin on neurochemical and behavioural effects were reported by the same group (Bell and Lundberg, 1985; Bell and Slotkin, 1985; Bell et al., 1986).

Rats

Against the background that choline availability as a precursor of acetylcholine may possibly influence neurotransmitter systems, Bell and Lundberg (1985) studied the effects of 2% and 5% soya lecithin in the diet of pregnant rats (equivalent to 1,250 or 2,500 mg soya lecithin/kg bw per day). The diets were fed from 2 weeks before mating until weaning of their litters. The control animals were fed AIN 76 diet. After weaning, half of the litters were placed on control litters, whereas the others remained on their respective diets. The authors stated that based on an average consumption of 10 g, the control animals received 8.9 mg and the soya lecithin groups received 14.0 or 22 mg choline/day. Neurobehavioural toxicity in rats was assessed using a developmental test battery from PND 3 to 20. Furthermore, a number of post-weaning tests were performed. Choline acetyltransferase was measured in whole brain of PND 1 pups and in the forebrain on PND 21, 42 and 67. In the 5% group, reflex righting and swimming development were delayed. In this group, the brain to bodyweight and acetylcholine levels were increased. Animals exposed also after weaning to 2% and 5% soya lecithin were shown to be hypoactive and to have neurochemical abnormalities. For several tests, there was no clear dose relationship detected between the 2% and the 5% concentration groups. The results for the measurement of choline acetyltransferase of F1 pups/animals were presented for dams fed lecithin pre- and/or post-natally. From these results, no clear indication can be given which period the F1 pups/animals were more sensitive to changes in this parameter.

Bell and Slotkin (1985) fed control (AIN) or diets containing 5% soya lecithin to pregnant rats (equivalent to 2,500 mg soya lecithin/kg bw per day). The diet was fed from GD 7 until termination of the study. The control diet contained 0.2% choline bitartrate. The authors stated that, based on an average consumption of 10 g, the control received 9 mg choline/day and the soya lecithin group 22 mg choline/day. Latencies for righting responses (measured on PND 1–4) and negative geotaxis (measured on PND 5–8) were shorter in the soya lecithin group. Behavioural differences were still present in adulthood as response to analgesia was reduced in the soya lecithin group at that time. Biochemical markers in the cerebellum and the cerebral cortex were different in the soya lecithin treated groups compared to the control. However, the Panel noted that the number of pregnant animals and the number of litters and the sex of the pups in the control and treated groups used in the assessment for neurotoxicity were not described in sufficient detail. In addition, the length of gestation and the pup weight at birth and during the tests were not presented.

Bell et al. (1986) studied the effects of replacing 5% corn oil with 5% commercial lecithin in the diet of pregnant Sprague–Dawley rats (equivalent to 2,500 mg soya lecithin/kg bw per day). The diet was fed from GD 7 until the end of lactation and pups were also fed the same diet until adulthood. The authors stated that, based on an average consumption of 10 g, the control received 9 mg choline/day and the soya lecithin group 22 mg choline/day. The description and selection of animals, pups/litter and pups for each measurement is not clear to the Panel. Catecholamine, noradrenaline and dopamine levels were measured in several brain regions. The authors concluded that transmitter uptake capabilities in the brain were affected by developmental exposure to soya bean lecithin.

Overall, the Panel noted the following flaws for the study by Gozzo et al. (1982) in mice and the studies of Bell and co-workers in rats (Bell and Lundberg, 1985; Bell and Slotkin, 1985; Bell et al., 1986) with soya lecithin. The number of pregnant animals, the number of litters and the sex of the pups in the control and treated groups as used in the assessment for neurotoxicity was not described in sufficient detail. In addition, the length of gestation and the pup weight at birth and during the tests was not presented in all publications. In neurodevelopmental toxicity studies, the selection of pups, the sex used in the tests, the pup weight and the corresponding developmental windows of the animals are very important. Therefore, the Panel concluded that the relevance of the studies is limited, although, at concentrations of 5% soya lecithin and higher in the diet during the gestation, lactation and post-weaning period, there were indications for alterations in the development of the brain.

The report by the Ministry of Agriculture and Fisheries and Food of the UK (1992) reported the following on these studies rats (Bell and Lundberg, 1985; Bell and Slotkin, 1985; Bell et al., 1986): 'These studies are of limited quality and the results were not considered relevant to the general use of

lecithins as additives in food'. In 1996, the SCF (1997) also addressed the possible behavioural effects described in the studies of Bell and co-workers (Bell and Lundberg, 1985; Bell and Slotkin, 1985; Bell et al., 1986) and proposed that the maximum level of lecithins in infant formulae should be restricted to that of human milk (1 g/L). The Panel agreed with this conclusion.

3.5.7. Hypersensitivity, allergenicity and food intolerance

3.5.7.1. Humans

Adults

There are several case reports and studies available that describe a possible allergenic potential of lecithins (E 322).

In an occupational study, inhaled soya bean lecithin was reported to cause immunological (20 males) and respiratory changes (19 males) (Zuskin et al., 1990, 1991). All workers reacted to intradermal skin tests with soya bean dust and almost all reacted to soya bean antigen prepared after separation of oil (94.7%). Increased levels of soya-specific immunoglobulin (Ig) E were noted in only three of 19 individuals. There was a higher incidence of chronic respiratory symptoms compared to controls not exposed to soya bean dust (significantly different for dyspnoea: 47.4% vs 9.7% in controls).

Lavaud et al. (1994) reported two cases of soya bean-lecithin-induced asthma in bakers. Both individuals tested positive in skin tests and also the radioallergosorbent test gave a positive result for soya bean.

Awazuhara et al. (1998) investigated the antigenicity of soya lecithin and soya oil proteins with regard to soya bean allergy. The proteins present in soya lecithin and soya oil were determined according to an established method and analysed by SDS-PAGE. The IgE- and IgG4-binding abilities of the soya lecithin proteins were investigated by immunoblotting with sera from 30 soya bean-sensitive patients, including seven with a positive challenge test. The results of SDS-PAGE demonstrated the presence of only three proteins, with molecular weights of about 58–67 kDa in soya oil, and suggested that soya lecithin also contains these proteins. The soya lecithin also contained many proteins besides these. The proteins with molecular weights of 58–67 kDa rarely bound to serum IgE. Only one of the patients who presented a positive challenge test had IgE antibodies bound to soya lecithin proteins. Neither the IgE, nor the IgG4 present in the patients' sera reacted to any soya oil protein. The authors concluded that the proteins present in soya lecithin and soya oil have little antigenicity with regard to soya bean allergy.

Gu et al. (2001) isolated soya lecithin proteins following solvent extraction of lipid components and then separated them by SDS-PAGE. The level of protein in six lecithin samples obtained from commercial suppliers ranged from 100 to 1,400 ppm. Immunoblotting with sera from soya-sensitive individuals showed IgE binding to bands corresponding to 7, 12, 20, 39 and 57 kDa. The authors concluded that soya lecithin contains a number of IgE-binding proteins and therefore might represent a source of hidden allergens. According to the authors, these allergens may be a more significant concern for soya-allergic individuals consuming lecithin products as a health supplement.

Müller et al. (1998) investigated six commercial soya lecithins for residual allergenicity and compared with extracts from raw and heat-treated soya bean. The protein content was determined by enzyme-linked immunosorbent assay and allergens were analysed with specific IgE from patients' sera using the enzyme allergosorbent test (EAST). The EAST studies revealed that three of six sera from patients with allergy to soya beans contained IgE to four soya lecithins with the content of residual proteins higher than 20 mg/kg. EAST inhibition showed that the allergens from soya lecithin were immunologically more closely related to allergens from heat-treated soya beans than to those from raw soya beans.

Martin-Hernandez et al. (2005) performed quantification and characterisation of residual proteins in lecithins. The SDS-PAGE protein pattern of the standard soya lecithin was very similar to that of soya flour. The seed maturation protein P34 from the 7S globulin fraction of soya proteins, reported as the most allergenic protein in soya bean, has also been identified in soya lecithins.

According to the EFSA NDA Panel (2014), the prevalence of clinically confirmed soya allergy in unselected populations in Europe appears to be low, although available studies are scarce. Higher rates of anaphylactic reactions to soya protein have been reported among peanut-allergic patients. Serological and clinical cross-reactions have been described between soya and other legumes, with the pollen allergen Bet 1 v, and with bovine casein. Thermal processing, high hydrostatic pressure

treatments and fermentation have been shown to reduce the IgE-binding capacity of soya proteins, depending on the conditions and duration of the processes. The lowest MED reported in soya-allergic patients undergoing DBPCFC was 0.2 mg of soya protein, although the majority of patients only reacted to higher doses.

The possibility of residual allergenicity in food products manufactured using egg lecithin has been reported in a DBPCFC. Both egg white- and egg yolk-derived proteins have been described to trigger clinical allergic reactions. Heat denaturation and other food-processing treatments do not reliably reduce the allergenicity of eggs. The MEDs of ingested egg proteins reported to trigger objective reactions in clinical studies range from few micrograms to milligrams (EFSA NDA Panel, 2014).

Infants and children

According to the Annex II of the Regulation (EU) No 1169/2011[23], soya beans and products thereof and eggs and products thereof are listed as substances or products causing allergies or intolerances, and information on their presence in food should be given to the consumers.

Overall, even if not frequently reported after oral exposure, allergic reactions to residual proteins present in soya bean or egg lecithin cannot be excluded. Therefore, it should be specified that the amount of these residual proteins in the food additive lecithins (E 322) must be kept as low as possible.

The Panel considered it advisable to reduce as much as possible the presence of proteinaceous compounds by introducing appropriate purification steps in the manufacturing process.

3.5.8. Other studies

3.5.8.1. Animal studies

The effect of supplementing the diet with natural/dietary emulsifiers was examined by Lecomte et al. (2016). Four groups of C57BL6 mice (21–23 g and 6 weeks old) received either a low-fat diet (n = 10), a high-fat diet (n = 12), a high-fat diet containing soya bean lecithin (n = 12) or a high-fat diet containing a polar lipid emulsifier from milk (n = 12) for 8 weeks. The three high-fat diet formulations contained the same amount of lipids, proteins and carbohydrates, differing only by the lack or the presence of 1.2% by weight of polar lipids (equivalent to 600 mg/kg bw per day) from soya bean or milk. Compared with the high-fat diet group, the group maintained on a high-fat diet containing soya bean lecithin diet had increased white adipose tissue mass ($p < 0.05$), with larger adipocytes ($p < 0.05$) and increased epididymal adipose expression of tumour necrosis factor α, monochemoattractant protein-1, lipopolysaccharide-binding protein and leptin ($p < 0.05$). These changes were not observed in the group treated with a high-fat diet containing a polar lipid emulsifier from milk. Liver weight did not differ among groups. However, the group fed a high-fat diet containing soybean lecithin had a higher hepatic lipid content compared to the groups fed either a high-fat diet or a high-fat diet containing a polar lipid emulsifier from milk ($p < 0.01$ and $p < 0.05$, respectively). The group fed a high-fat diet containing soya bean lecithin also had a greater proportion of hepatic triglycerides compared to the groups fed either a high-fat diet or a high-fat diet containing a polar lipid emulsifier from milk ($p < 0.001$ and $p < 0.01$, respectively) and a lower proportion of hepatic phospholipids compared to the high-fat group ($p < 0.05$). No differences were observed among groups regarding plasma lipid concentrations. The Panel noted that, when feeding a high-fat diet to mice, addition of soya bean lecithin compared to addition of polar lipid emulsifier lead to an increase in white adipose tissue mass and greater portion of hepatic triglycerides.

3.5.8.2. Human data: information from pharmaceutical uses

Contraindications, warnings and undesirable effects for lecithin as an excipient are not known in dosages used. In the literature, it is always emphasised that the sensitisation of atopic patients is possible due to residual proteins in lecithin, resulting in hypersensitivity (Palm et al., 1999; HMPC, 2006). At higher amounts, such as a daily dosage of 1.5–2.7 g of lecithin (containing 73–79% phosphatidyl-choline), occasional gastrointestinal effects (such as stomach pain, loose stool and diarrhoea) were described (Blumenthal et al., 1998).

[23] Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004.

From the European Economic Area, there are only few cases of a wide range of adverse effects reported without proven cause–effect relationship.[24]

According to the recently published draft monograph of the HMPC of EMA, the traditional medicinal usage of soya bean lecithin (deoiled, enriched phospholipids from soya bean) by oral administration at the dosage of 750–2,700 mg (two or three times daily) corresponding to 1,500–8,100 mg/day could be verified for 'the relief of temporary fatigue and sensation of weakness' in adolescents, adults and elderly. The undesirable effects reported were: 'Allergic reactions including severe anaphylaxis and angioedema have been reported. The frequency is not known. Skin reactions like pruritus, dermatitis, exanthema and urticaria have been reported. The frequency is not known. Gastrointestinal disorders like stomach discomfort and diarrhoea have been reported' (HMPC, 2016b; draft).

Adults

Dechent et al. (1999) studied the effects of oral administration of choline (short-term study) or lecithin (long-term study) on the metabolite concentrations in the human brain. In the short-term study, three women and three men (age 28.0 ± 3.5 years, mean weight 71 ± 10 kg) ingested a single dose of 50 mg/kg bw of free choline as choline bitartrate. These dose levels were chosen because Stoll et al. (1995) and Cohen et al. (1995) observed a doubling of the plasma choline levels at this dose. In the long-term study, three women and three men (aged 27.7 ± 3.8 years; weighing 72.7 ± 10 kg) received 2 × 16 g of lecithin (containing 95% phosphatidylcholine) per day. The choline levels in the brain in both studies were not increased.

Infants

A 3-year-old boy with retarded bodyweight growth due to chronic diarrhoea showed abdominal pain and post-prandial emesis (Renaud et al., 1996). Testing with native soya lecithin caused a diarrhoeal bout, whereas placebo had no effect. During provocation, there was a sharp rise in the urinary lactulose/mannitol ratio compared to a fasting test (4.25% vs 1.34%), which is indicative for an alteration of intestinal permeability. In a test with placebo, there was no significant change in urinary lactulose/mannitol ratio (1.82% vs 1.59%).

Healthy full-term infants were fed from birth exclusively human milk (n = 16), standard term formula (n = 15) or the same formula supplemented with egg yolk lecithin providing docosahexaenoic a(DHA) 0.15% and arachidonic acids (AA) 0.30% (n = 18) (Bondía-Martínez et al., 1998). Fatty acid composition of plasma and erythrocytes were determined at birth, as well as at day 7, 1 month and 3 months. At 1 and 3 months, the infants of the non-supplemented formula group showed a decreased in DHA and AA in the serum. No differences were observed between the group fed breast milk and the group fed supplemented formula during the study period.

4 Discussion

Lecithins are mixtures or fractions of phosphatides obtained by physical procedures from animal or vegetable foodstuffs. They also include the corresponding hydrolysed products obtained through the use of harmless and appropriate enzymes, although the final product must not show any signs of residual enzyme activity. The lecithins may be slightly bleached in aqueous medium by means of hydrogen peroxide, although the oxidation must not chemically modify the lecithin phosphatides (Commission Regulation (EU) No 231/2012).

Lecithins (E 322) are authorised as food additives in the EU and have been previously evaluated by JECFA in 1973 (JECFA, 1974a,b) and by the SCF in 1982 (SCF, 1982). The Panel noted that, although Commission Regulation (EU) No 231/2012 includes both types of lecithins (non-hydrolysed and hydrolysed) under the same food additive (E 322), JECFA differentiated between them and treated them as different food additives (INS 322i and INS 322ii) with separate specifications.

The Panel noted that the protein content in crude, fluid and deoiled soya lecithins are in the range of 115–27,000 mg/kg, 232–1,338 mg/kg and 65–480 mg/kg, respectively, and in egg lecithins 49 mg/kg ((Document provided to EFSA n.18); Porras et al., 1985; Müller et al., 1998; Gu et al., 2001; Paschke et al., 2001; Martin-Hernandez et al., 2005). According to EFSA NDA Panel (2014), the lowest MED reported in soya-allergic patients undergoing DBPCFC was 0.2 mg of soya protein, and from a few micrograms to a few milligrams of egg proteins. The Panel agreed with the opinion from NDA Panel (2014) that the hypersensitivity to soya and egg lecithins is due to the residual proteins in

[24] From the table provided from EMA (Eudravigilance).

lecithins (E 322) and therefore considered it necessary to develop the limit for the presence of residual protein in the EU specifications.

The Panel noted that, based on the data provided by the industry, it is feasible to lower the specification limits for toxic elements: lead, mercury and arsenic. The Panel also noted that the limit for cadmium should be included in the EU specifications.

The Panel noted that the composition of the preparations used in the various studies was different. However, because all of the constituents were qualitatively similar, the Panel considered the studies relevant for the risk assessment of lecithins.

Lecithins are natural constituents of all cells in the human body and also are natural components of the diet. Hydrolysed lecithins are produced in the gut as a result of normal digestion (SCF, 1982). Among lecithins, phosphatidylcholine is hydrolysed in choline in the cytidine-5-diphosphate-choline pathway in all cells of the body. The content of choline that can theoretically be released from phosphatidylcholine containing two linoleate groups is 13.2%. Choline is a precursor of the neurotransmitter acetylcholine and plays an important role in the metabolism and transport of lipids (EFSA NDA Panel, 2016).

For choline, the EFSA NDA Panel (2016) prepared a scientific opinion on DRVs in 2016. In this opinion, the NDA Panel considered dietary choline including choline compounds (e.g. glycerophosphocholine, phosphocholine, phosphatidylcholine, sphingomyelin). The NDA Panel concluded that ARs and PRIs for choline could not be derived for adults, infants (aged 7–11 months) and children, and therefore defined AIs for total choline (free and bound). For infants during the first 6 months of life, the amount of total choline provided in human milk was considered adequate. With regard to an excessive intake of choline, the NDA Panel referenced on the setting of ULs for choline by the US IOM (1998) and noted that no UL was established by IOM for infants. According to IOM, the only source of intake of choline for infants should be from food or formula to prevent high levels of intake.

Studies using radiolabelled phosphatidylcholine in animals and humans clearly indicated that, following oral administration, phosphatidylcholine is absorbed intact or as lysophosphatidylcholine or choline after intestinal hydrolysis. In intestinal mucosa cells, lysophosphatidylcholine would be reacylated into phosphatidylcholine or hydrolysed to glycerophosphocholine and free fatty acids. The fatty acids would be further utilised for the reassembly of triacylglycerides and phosphatidylcholine found in the chylomicrons. In humans, the absorbed phosphatidylcholine would be incorporated preferentially into the HDL fraction of plasma. In humans, dietary lecithins are known to be hydrolysed by phospholipases to liberate choline which is rapidly absorbed by a carrier-mediated saturable transport system and appears in plasma predominantly as free choline. Consequently, an increased plasma-free choline concentration has been described as a consequence of increased dietary intake of lecithins. Moreover, a significant increase in breast milk concentrations of free choline was observed in pregnant women receiving a phosphatidylcholine supplementation compared to the placebo group.

The acute toxicity of lecithins (E 322) in mice, rats and rabbits is low. The Panel noted that in these studies the test substance is not always characterised.

Subchronic toxicity studies in rats and dogs did not report any adverse effect, even at the highest doses tested (3,750 mg EPL (see Section 3.1.1)/kg bw per day, 1,000 mg soya phosphatidylinositol or EPL/kg bw per day in rats and dogs, respectively, and 5,460 mg lecithins/kg bw per day in rats).

The Panel considered the available genotoxicity data on lecithins (E 322) to be sufficient to conclude that there is no concern with respect to genotoxicity.

Chronic toxicity studies in rats did not report any adverse effects, even at the highest dose tested (3,750 mg EPL/kg bw per day). No carcinogenic effects were reported in rats, even at the highest dose tested (1,470 and 2,280 mg soya lecithin/kg bw per day in males and females, respectively) for 2 years.

The Panel considered that no adverse effects were observed in the developmental toxicity studies performed in mice, rat and rabbits up to the highest dose tested. However, the Panel noted that no reproductive toxicity studies were available.

Against the background that choline availability as a precursor of acetylcholine may possibly influence neurotransmitter systems, several neurodevelopmental toxicity studies were conducted with lecithin. The Panel noted that the neurodevelopmental toxicity studies of Gozzo et al. (1982) in mice and the studies of Bell and co-workers in rats (Bell and Lundberg, 1985; Bell and Slotkin, 1985; Bell et al., 1986) had several limitations, such as the number of pregnant animals, the number of litters, and the sex of the pups in the control and treated groups not being described in sufficient detail. In addition, the length of gestation and pup weight at birth, as well as during the tests, were not presented in all publications. Therefore, the Panel concluded that the relevance of the studies is limited

but, at concentrations of 5% soya lecithin and higher in the diet during the gestation, lactation and the post-weaning period, there were indications for alterations in the development of the brain.

The UK Ministry of Agriculture Fisheries and Food (1992) reported the following on these rat studies (Bell and Lundberg, 1985; Bell and Slotkin, 1985; Bell et al., 1986): 'These studies are of limited quality and the results were not considered relevant to the general use of lecithins as additives in food'. In 1996, the SCF (SCF, 1997), also addressed the possible behavioural effects described in the studies of Bell and co-workers (Bell and Lundberg, 1985; Bell and Slotkin, 1985; Bell et al., 1986) and proposed that the maximum level of lecithins in infant formulae should be restricted to that of human milk (1 g/L). The Panel agreed with this conclusion. Furthermore, the Panel considered it prudent that lecithins (E 322) use in infant formulae should not lead to choline intakes higher than the amount of total choline provided in human milk considered adequate by the NDA Panel (EFSA 2016).

The Panel noted that, in Annex II of Regulation (EC) No 1333/2008, the use levels of lecithins (E 322) in food for infants under the age of 12 weeks are included in categories 13.1.1, 13.1.5.1 and 13.1.5.2. The Panel considered that these uses would require a specific risk assessment in line with the recommendations given by JECFA (1978) and the SCF (1998) and endorsed by the Panel (EFSA ANS Panel, 2012). Therefore, the current re-evaluation of lecithins (E 322) as a food additive is not considered to be applicable for infants under the age of 12 weeks.

The present re-evaluation includes the use of lecithins (E 322) in foods for infants from 12 weeks of age and for young children.

Concerning uses of lecithins in food for infants and young children the Panel concurs with the SCF (SCF, 1998, 2003) '... the SCF considered it prudent that the number and amounts of additives used in foods for infants and young children should be kept at the minimum necessary. The SCF confirmed its long standing view that additives should not be permitted in foods specially prepared for infants. Rarely, exceptional technological circumstances may justify the use of an additive.'

The Panel acknowledged that consumption with respect to the concerned food categories would be short and also noted that it is prudent to keep the number of additives used in foods for infants and young children to the minimum necessary and that there should be strong evidence of need, as well as safety, before additives can be regarded as acceptable for use in infant formulae and foods for infants and young children.

The Panel noted that, if lecithins are added in combination with mono- and diglycerides of fatty acids (E 471), citric acid esters of mono- and diglycerides of fatty acids (E 472c) and sucrose esters of fatty acids (E 473) to food of the categories 13.1.1, 13.1.2, 13.1.4 or 13.1.5, the maximum level established for lecithins should not be exceeded by the total concentration of these substances.

To assess the dietary exposure to lecithins (E 322) from its use as a food additive, the exposure was calculated based on (1) maximum levels of data provided to EFSA (defined as *the maximum level exposure assessment scenario*) and (2) the reported use levels (defined as the *refined exposure assessment scenario*). Dietary exposure through this latter scenario was assessed using reported use levels data considering levels not exceeding the MPLs for food categories for which direct addition of lecithins is authorised (Annex II to Regulation No 1333/2008).

Based on the available data set, the Panel calculated two refined exposure estimates based on different assumptions: a *brand-loyal consumer scenario* and a *non-brand-loyal scenario* (see Section 3.4.1).

The main contributing food category to the total mean exposure estimates in the maximum scenario was bread and rolls for all age groups. The Panel noted that the estimated long-term exposures based on this scenario are very likely conservative because this scenario assumes that all foods and beverages listed under the Annex II to Regulation No 1333/2008 contain lecithins (E 322) as a food additive at the maximum reported use levels.

From the *refined estimated exposure scenario* considering only food categories for which direct addition of lecithins (E 322) to food is authorised, in the *brand-loyal scenario*, mean exposure to lecithins (E 322) ranged from 7 mg/kg bw per day in adolescents to 82 mg/kg bw per day in children. The 95th percentile exposure to lecithins (E 322) ranged from 15 mg/kg bw per day in adolescents to 187 mg/kg bw per day in children. In the *non-brand-loyal scenario*, mean exposure to lecithins (E 322) ranged from 3 mg/kg bw per day in adults/elderly to 22 mg/kg bw per day in toddlers. The 95th percentile exposure to lecithins (E 322) ranged from 6 mg/kg bw per day in adults/elderly to 62 mg/kg bw per day in infants. The main contributing food categories in the non-*brand-loyal scenario* were foods for infants and young children for infants and toddlers, fine bakery wares, bread and rolls for children, adolescents, adults and the elderly. The main contributing food categories in the *brand-loyal*

scenario were foods for infants and young children for infants, fine bakery wares, and bread and rolls for the other age groups.

The Panel considered that the refined exposure assessment approach resulted in more realistic long-term exposure estimates compared to the *maximum level exposure assessment scenario*. This approach is based on the extensive range of analytical data available and assumes that people, in the long term, are exposed to foods and beverages that contain the food additive at a mean concentration level for all products (*non-brand-loyal scenario*) or that one product contains the food additive at the maximum concentration level (*brand-loyal scenario*) and the remaining products contain the additive at a mean concentration level. For lecithins (E 322), reported use levels were available. However, not all available data could be included in the assessment as a result of specific restrictions/exceptions regarding products not referenced in the FoodEx classification. This may have resulted in an underestimation of exposure to lecithins (E 322).

The Panel considered that dietary intakes of lecithins from the regular diet could be estimated in average ranging from 4 to 71 mg/kg bw per day across all population age groups.

Moreover, the Panel noted that mean dietary intakes to lecithins from the regular diet are in the range of the mean estimated exposure from the use of the food additive itself for the non-brand loyal consumer scenario.

Lecithins (E 322) is used as emulsifying and stabilising agents of water-oil/fat mixtures in a wide range of foods and it is therefore not expected that brand-loyalty will result in higher exposure in general population, except in specific populations consuming foods for special medical purposes and in infants and young children consuming infant formulae and/or follow-on formulae. The Panel therefore selected the brand-loyal refined scenario as the most relevant exposure scenario for this additive in these specific situations when justified.

Overall, the Panel considered, that in view of the limited information on health effects of excessive intake of lecithins or choline, respectively, especially by infants, children, pregnant and lactating women, estimated total choline intake including the use of lecithins (E 322) as a food additive should not lead to a significant exceedance of AIs for choline for infants or ULs defined by IOM (1998). Maximum levels of lecithins (E 322) in all types of infant formulae should be restricted to that of human milk (1 g/L).

The Panel considered that lecithins added during food processing may increase the average daily per capita consumption of phosphatidylcholine by 1.5 mg/kg of body weight for adults (this corresponds to 0.225 mg/kg of body weight of choline moiety).

5. Conclusions

I. General population

a) Above 1 year of age

Following the conceptual framework for the risk assessment of certain food additives re-evaluated under Commission Regulation (EU) No 257/2010 (EFSA, 2014), and given that:

- adequate exposure data were available and the highest relevant exposure estimate calculated in the refined exposure assessment scenario based on the reported data from food industry was for toddlers (12–35 months) up to 175 mg lecithins/kg bw per day at the 95th percentile (brand-loyal scenario),
- exposure via natural occurrence as reported by JECFA provided a daily mean intake of several grams of lecithin (approximately 1–5 g corresponding to 14–71 mg/kg bw for a 70-kg adult population),
- lecithins are natural constituents of all cells in the human body and also are natural components of the diet,
- toxicity database for lecithins was overall sufficient but not adequate regarding the endpoint of neurobehavioural developmental effects,
- there was no concern with respect to genotoxicity,
- no adverse effects were reported in chronic and carcinogenicity study in rats at the highest dose tested of 3,750 mg lecithins/kg bw per day,

the Panel concluded that there was no need for a numerical ADI for lecithins (E 322) and that there was no safety concern for the general population from more than 1 year of age at the refined exposure assessment for the reported uses of lecithins (E 322) as a food additive.

Moreover, taking into consideration that:

- hydrolysed lecithins and choline are produced in the gut as a result of normal digestion of lecithins. Choline is rapidly absorbed and appears in plasma predominantly as free choline,
- choline is a precursor of the neurotransmitter acetylcholine,
- the content of choline, that can theoretically be released from phosphatidylcholine containing two linoleate groups, is up to 13.2%, and the measured content of choline from commercial lecithins (E 322) up to 3.4%,
- 13.2% release would result in exposure up to 23 mg choline/kg bw per day at the 95th percentile intake of lecithins in toddlers (brand loyal scenario),
- total choline intake considering regular diet (estimated in average ranging from 4 to 18 mg/kg bw per day) across all population age groups and choline intake resulting from lecithins (E 322) used as a food additive are below the UL for choline defined by the IOM (1998),

the Panel concluded that there is no safety concern for the exposure to the choline from lecithins (E 322) as a food additive at use and use levels reported by industry.

b) Infants (from 12 weeks up to 11 months of age)

Taking further into consideration that:

- adequate exposure estimates calculated in the refined exposure assessment scenario based on the reported data from food industry for infants (12 weeks to 11 months) was up to 163 mg/kg bw per day at the 95th percentile (brand-loyal scenario),
- 13.2% release would result in exposure up to 22 mg choline/kg bw per day at the 95th percentile dietary exposure of lecithins (E 322) in infants (brand loyal scenario),
- total choline intake considering regular diet in the same population group (estimated in average ranging from 9 to 16 mg/kg bw per day), and choline intake resulting from lecithins used as a food additive were in the same order as the adequate intake levels (AI) (EFSA NDA, 2016),

the Panel concluded that there was no safety concern at the refined exposure assessment for the reported uses of lecithins (E 322) as a food additive and for the choline from lecithins (E 322) as a food additive at use and use levels reported by industry.

II. Infants and young children consuming foods for special medical purposes

Taking further into consideration that:

- with respect to the exposure estimates calculated based on the reported data from food industry for infants (12 weeks to 11 months) and young children, the highest exposure was 232 mg lecithins/kg bw per day for toddlers (12–35 months) at the 95th percentile (brand-loyal scenario),
- 13.2% release would result in exposure up to 31 mg choline/kg bw per day at the 95th percentile dietary exposure of lecithins (E 322) in toddlers (brand loyal scenario),
- total choline intake considering regular diet in the same population group (estimated on average as ranging from 13–18 mg/kg bw per day), and choline intake resulting from lecithins used as a food additive, are in the same order as the AI (EFSA NDA, 2016),

the Panel concluded that there was no safety concern with respect to the refined exposure assessment for the reported uses of lecithins (E 322) as a food additive and for exposure to choline resulting from these uses of lecithins (E 322).

6. Recommendations

The Panel recommended that the maximum limits for the impurities of toxic elements (lead, mercury and arsenic) in the EU specification for lecithins (E 322) should be revised in order to ensure that lecithins (E 322) as a food additive will not be a significant source of exposure to those toxic elements in food. The Panel recommended that the limit for cadmium should be included in the specifications.

The Panel noted some case reports of hypersensitivity reactions associated with soya and egg lecithins (see Section 3.5.7). The Panel agree with the opinion from EFSA NDA Panel (2014) that this

hypersensitivity is due to the residual proteins in lecithins (E 322) and therefore their content should be reduced as much as possible.

Regarding the results of the inadequate neurobehavioural studies, to clarify the relevance of the data, a study with lecithins (E 322) in compliance with the current OECD TG 426 would be warranted.

In case the food additive lecithins (E 322) is used in infant formulae and follow-on formulae supplemented with choline or choline salts (see Section 1.2), the Panel recommended that the intake of choline from all sources including the use of the food additive lecithins (E 322) via infant formulae (category 13.1.1), follow-on formulae (category 13.1.2) or other food should be in the order of the AIs defined by the EFSA NDA Panel (2016).

The Panel noted discrepancies between the data reported from industry and the Mintel database, where lecithins (E 322) is labelled in more products than in food categories for which data were reported from industry. Therefore, the Panel recommended collection of data of usage and use levels of lecithins (E 322) in order to perform a more realistic exposure assessment. Moreover, there are several authorised uses that are not supported by data submitted by industry nor by the Mintel database.

Documentation provided to EFSA

1) Pre-evaluation documents on Lecithins (E 322). Frauenhofer ITEM. July 2012.
2) Mars Chocolate, 2010. Reply to EFSA: Call for data on emulsifiers, stabilisers and gelling agents. Information on "Present usage". Submitted on 19 May 2010.
3) ELMA (European Lecithin Manufacturers Association), 2010. Reply to EFSA: Call for data on emulsifiers, stabilisers and gelling agents. Information on "Reaction and fate in food; present usage and exposure". Submitted on 19 October 2010.
4) Sanofi-Aventis Deutschland GmbH, 2013. Chasseaud LF, Down WH, Sacharin RM and Franklin ER, 1976. The metabolic fate 3H: 14C-essential phospholipids (EPL) in the rat. Nattermann Internal Report NTN 4/75379 (unpublished).
5) Sanofi-Aventis Deutschland GmbH, 2013. Chasseaud LF, Down WH and Sacharin RM, 1976. The metabolic fate ^{3}H: ^{14}C-essential phospholipids (EPL) in the rhesus monkey. Nattermann Internal Report NTN 5/75497(unpublished).
6) Sanofi-Aventis Deutschland GmbH, 2013. Neumann V, Leuschner F, Leuschner A, Mitterer KE, Klie R and Hubsher F, 1983. Influence of PPC-R, batch 82/002 on the development of implantations and on the embryonal and foetal development in pregnant rabbits by using oral administration. Treatment from 1st to 6th day of pregnancy. Nattermann Internal Report No 0050/84 (unpublished).
7) Sanofi-Aventis Deutschland GmbH, 2013. Friehe H, Fontaine R, Messow C, Schulz LC and Wenzel E, 1976. 48 Week Test for Toxicity of EPL in Wistar Rats in the case of per oral administration. Nattermann Internal Report No. 840124(unpublished).
8) Sanofi-Aventis Deutschland GmbH, 2013. Friehe H and Fontaine R, 1978. Teratogenicity and embryotoxicity of EPL US 10% following intravenous administration to Wistar-rats. Nattermann Internal Report No. 840160 (unpublished).
9) Sanofi-Aventis Deutschland GmbH, 2013. Friehe H and Fontaine R, 1978. Peri- and postnatal toxicity of EPL following oral administration to Wistar-rats. Nattermann Internal Report No 840170 (unpublished).
10) Sanofi-Aventis Deutschland GmbH, 2013. Gaggi R and Biagi, GL, 1983. An investigation to assess potential mutagenic activity on the part of polyunsaturated phosphatidylcholine (EPL, Nattermann) which is the active principle of "Essentiale 303" (presented as capsules and vials), "Lipostabil" (presented as capsules and vials), "Essaven" (presented as capsules and gel). Nattermann Internal Report No 842153 (unpublished).
11) Sanofi-Aventis Deutschland GmbH, 2014. Leuschner F, Leuschner A, Schwerdtfeger W and Dontenwill W, 1973. 6-weeks toxicity of EPL, batch nos. 72/006 and 72/009 (herein briefly designated 'EPL') administered by gastric tube to beagle dogs. Lab. Pharmacol. Toxicol. Hamburg. Internal Report No. 840129 (unpublished).
12) Sanofi-Aventis Deutschland GmbH, 2014. Friehe H, Fontaine R, Messow C, Schulz LC and Wenzel S, 1977. 12-wöchige Toxizitätsprüfung von EPL an Wistar-Ratten bei peroraler Applikation. Nattermann Internal Report No 840131(unpublished).
13) Sanofi-Aventis Deutschland GmbH, 2014. Friehe H, Fontaine R, Messow C, Schulz LC and Wenzel S, 1976. 24 week toxicity of EPL in Wistar rats upon peroral administration. Nattermann Internal Report No 840134 (unpublished).

14) Sanofi-Aventis Deutschland GmbH, 2014. Friehe H and Fontaine R, 1978. Test for teratogenicity and embryotoxicity of EPL following oral administration to Wistar rats. Nattermann Internal Report No 840157 (unpublished).
15) Sanofi-Aventis Deutschland GmbH, 2014. Wetzig H and Fontaine R, 1980. Toxicity of EPL spezial in Beagle dogs by oral administration for 360 days. Nattermann Internal Report No 840117 (unpublished).
16) Sanofi-Aventis Deutschland GmbH, 2015. Reply to EFSA: Call for data on lecithins (E 322) permitted as a food additive in the EU. Report on general information available on lecithins. Submitted on 13 July 2015.
17) EMA (European Medicines Agency): communication to EFSA request for information on a certain group of substances used as food additives, May 2015.
18) ELMA (European Lecithin Manufacturers Association), 2016. Reply to EFSA: Call for data on lecithins (E 322) permitted as a food additive in the EU. Information on the identity of the substance. Submitted on 4th January 2016.
19) AESGP (Association of the European Self-Medication Industry), 2013. Data on usage levels of lecithins (E 322) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2014). Submitted to EFSA on 9 September 2013.
20) FDE (Food Drink Europe), 2013. Data on usage levels of lecithins (E 322) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2014). Submitted to EFSA on 29 November 2013.
21) CHEPLAPHARM Arzneimittel GmbH, 2014. Data on usage levels of lecithins (E 322) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2014). Submitted to EFSA on 21 August 2014.
22) Stollwerck GMBH, 2014. Data on usage levels of lecithins (E 322) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2014). Submitted to EFSA on 28 August 2014.
23) ELMA (European Lecithin Manufacturers Association), 2014. Data on usage levels of lecithins (E 322) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2014). Submitted to EFSA on 30 September 2014.
24) ICGA (International Chewing Gum Association), 2014. Data on usage levels of lecithins (E 322) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2014). Submitted to EFSA on 30 September 2014.
25) BABBI Confectionery Industry, 2014. Data on usage levels of lecithins (E 322) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2014). Submitted to EFSA on 12 August 2014.
26) Rudolf Wild GmbH & Co. KG, 2014. Data on usage levels of lecithins (E 322) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2014). Submitted to EFSA on 29 September 2014.
27) SNE (Specialised Nutrition Europe), 2014. Data on usage levels of lecithins (E 322) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2014). Submitted to EFSA on 30 September 2014.
28) Nathura, 2014. Data on usage levels of lecithins (E 322) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2014). Submitted to EFSA on 19 September 2014.

References

AOAC (Association of Official Agricultural Chemists), 1980. AOAC Official Method 13.045. In: Horwitz W (ed.). *Official Methods of Analysis of AOAC International*. 13th Edition. 3 pp.

Awazuhara H, Kawai H, Baba M, Matsui T and Komiyama A, 1998. Antigenicity of the proteins in soy lecithin and soy oil in soybean allergy. Clinical and Experimental Allergy, 28, 1559–1564.
Bell JM and Lundberg PK, 1985. Lecithin preparation on development of sensorimotor behavior and brain biochemistry in the rat. Developmental Phychobiology, 18, 59–66.
Bell JM and Slotkin TA, 1985. Perinatal dietary supplementation with a commercial soy lecithin preparation: effects on behavior and brain biochemistry in the developing rat. Developmental Psychobiology, 18, 383–394.
Bell JM, Withmore WL, Cowdery T and Slotkin TA, 1986. Perinatal dietary supplementation with a soy lecithin preparation: effects on development of central catecholaminergic neurotransmitter systems. Brain Research Bulletin, 17, 189–195.
Blumenthal M, Busse WR, Goldeberg A, Gruenwald J, Hall T, Riggins CW and Rister RS (eds.), 1998. *The Complete German Commission E Monographs*. The American Botanical Council, Austin, Texas.
Bondía-Martínez E, López-Sabater MC, Castellote-Bargalló AI, Rodríguez-Palmero M, González-Corbella MJ, Rivero-Urgell M, Campoy-Folgoso C and Bayés-García R, 1998. Fatty acid composition of plasma and erythrocytes in term infants fed human milk and formulae with and without docosahexaenoic and arachidonic acids from egg yolk lecithin. Early Human Development, 53 (Suppl. S), 109–119.
Brantom PG, Gaunt IF, Hardy J, Grasso P and Gangolli SD, 1973. Long-term feeding and reproduction studies on emulsifier YN in rats. Food and Cosmetics Toxicology, 11, 755–769.
ChemIDplus, 2014. Lecithin. Available online: http://chem.sis.nlm.nih.gov/chemidplus/rn/8002-43-5
CIR (Cosmetic Ingredient Review), 2001. Final report on the safety assessment of lecithin and hydrogenated lecithin. International Journal of Toxicology, 20 (Suppl 1), 21–45.
Cohen BM, Renshaw PF, Stoll AL, Wurtman RJ, Yurgelun-Todd D and Babb SM, 1995. Decrease brain choline uptake in older adults. An in vivo proton magnetic resonance spectroscopy study. JAMA, 274, 902–907.
Dechent P, Pouwels PJW and Frahan J, 1999. Neither short-term nor long-term administration of oral choline alters metabolic concentrations in human brain. Society of Biological Psychiatry, 46, 406–411.
EFEMA (European Food Emulsifier Manufacturers' Association), 2013. *Index of food emulsifiers, June 2013*, 6th Edition. Available online: http://www.emulsifiers.org/files/Index_of_food_emulsifiers_June_2013_Edition.pdf
EFSA (European Food Safety Authority), 2007. Opinion of the Scientific Committee related to uncertainties in dietary exposure assessment. EFSA Journal 2007;5(1):438, 54 pp. doi:10.2903/j.efsa.2007.438
EFSA (European Food Safety Authority), 2011a. Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment. EFSA Journal, 2011;9(3):2097, 34 pp. doi:10.2903/j.efsa.2011.2097
EFSA (European Food Safety Authority), 2011b. Evaluation of the FoodEx, the food classification system applied to the development of the EFSA Comprehensive European Food Consumption Database. EFSA Journal 2011; 9 (3):1970, 27 pp. doi:10.2903/j.efsa.2011.1970
EFSA ANS Panel (European Food Safety Authority Panel on Food Additives and Nutrient Sources added to Food), 2012. Guidance for submission for food additive evaluations. EFSA Journal 2012;10(7):2760, 60 pp. doi: 10.2903/j.efsa.2012.2760. Available online: http://www.efsa.europa.eu/efsajournal
EFSA ANS Panel (EFSA ANS Panel on Food Additives and Nutrient Sources added to Food), 2014. Statement on a conceptual framework for the risk assessment of certain food additives re-evaluated under Commission Regulation (EU) No 257/2010. EFSA Journal 2014;12(6):3697, 11 pp. doi:10.2903/j.efsa.2014.3697
EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2009a. Scientific Opinion on cadmium in food. EFSA Journal 2009;7(10):980, 139 pp. doi:10.2903/j.efsa.2009.980
EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2009b. Scientific Opinion on arsenic in food. EFSA Journal 2009;7(10):1351, 199 pp. doi:10.2903/j.efsa.2009.1351
EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2010. Scientific Opinion on lead in food. EFSA Journal 2010;8(4):1570, 151 pp. doi:10.2903/j.efsa.2010.1570
EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2012. Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food. EFSA Journal 2012;10(12):2985, 241 pp. doi:10.2903/j.efsa.2012.2985
EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2016. Scientific opinion on the safety and efficacy of lecithins for all animal species. EFSA Journal 2016;14(8):4561, 13 pp. doi:10.2903/j.efsa.2016.4561
EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2014. Scientific Opinion on the evaluation of allergenic foods and food ingredients for labelling purposes. EFSA Journal 2014;12(11):3894, 286 pp. doi:10.2903/j.efsa.2014.3894
EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2016. Scientific Opinion on Dietary Reference Values for choline. EFSA Journal 2016;14(8):4484, 70 pp. doi:10.2903/j.efsa.2016.4484
EFSA Scientific Committee, 2009. Guidance of the Scientific Committee on Transparency in the Scientific Aspects of Risk Assessments carried out by EFSA. Part 2: General Principles. EFSA Journal 2009;7(7):1051, 22 pp. doi:10.2903/j.efsa.2009.1051
EFSA Scientific Committee, 2012. Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA Journal 2012;10(3):2579, 32 pp. doi:10.2903/j.efsa.2012.2579. Available online: www.efsa.europa.eu

FDA (Food and Drug Administration), 1974. Teratological test results in three species of animals (mice, rats and rabbits), PB234-874. Food and Drug Administration, Washington, DC. 42 pp.
FDRL (Food and Drug Research Laboratories, Inc.), 1973a. Approximate LD50 of FDA 71-88 (Lecithin) in rats. Report prepared under DHEW contract no. FDA 71-260; laboratory no. 1763. Dated October 16. Submitted by FDA in response to a FOI request dated 11/27/95 (2 pp.), cited in CIR, 2001.
FDRL (Food and Drug Research Laboratories, Inc.), 1973b. Approximate LD50 of FDA 71-88 (Lecithin) in mice. Report prepared under DHEW contract no. FDA 71-260; laboratory no. 1762. Dated October 16. Submitted by FDA in response to a Freedom of Information (FOI) request dated 11/27/95 (2 pp.), cited in CIR, 2001.
FDRL (Food and Drug Research Laboratories, Inc.), 1973c. Approximate LD50 of FDA 71-88 (Lecithin) in rabbits. Report prepared under DHEW contract no. FDA 71-260; laboratory no. 1764. Dated November 29. Submitted by FDA in response to a FOI request dated 11/27/95 (2 pp.), cited in CIR, 2001.
Fisher LM, da Costa K-A, Kwock L, Galanko J and Zeisel SH, 2010. Dietary choline requirements of women: effects of estrogen and genetic variation. The American Journal of Clinical Nutrition, 92, 1113–1119.
Food Chemicals Codex, 2010-2011. *Hydroxylated lecithins*. 7th Edition. United States Pharmacopeial Convention, Rockville, USA. pp. 501–503.
Fox JM, Betzing H and Lekim D, 1979. Pharmacokinetics of orally ingested phosphatidylcholine. In: Barbeau A, Growdon JH, Wurtman RJ (eds.). *Choline and Lecithin in Brain Disorders. Nutrition and the Brain*, Vol. 5. Raven Press, New York. pp. 95–108.
Gaunt IF, Grasso P and Gangolli SD, 1967. Short-term toxicity study of emulsifier YN in rats. Food and Cosmetics Toxicology, 5, 623–629.
Gozzo S and Oliverio A, 1982. Effects of dietary phospholipids and odd-chain fatty acids on the behavioral maturation of mice. Food and Chemical Toxicology, 20, 153–157.
Gu X, Beardslee T, Zeece M, Sarath G and Markwell J, 2001. Identification of IgE-binding proteins in soy lecithin. International Archives of Allergy and Immunology, 126, 218–225.
Helmerich G and Koehler P, 2003. Comparison of methods for the quantitative determination of phospholipids in lecithins and flour improvers. Journal of Agricultural and Food Chemistry, 51, 6645–6651.
Hirsch MJ, Growdon JH and Wurtman R, 1978. Relations between dietary choline or lecithins intake, serum choline levels, and various metabolic indexes. Metabolism, 27, 953–960.
HMPC (Committee on Herbal Medicinal Products), 2006. Public statement on the allergenic potency of herbal medicinal products containing soya or peanut protein (EMA/HMPC/138139/2005). Available online: http://www.ema.europa.eu/ema/pages/includes/document/open_document.jsp?webContentId=WC500089954
HMPC (Committee on Herbal Medicinal Products), 2016a. Assessment report on Glycine max (L.) Merr., lecithin. Draft. (EMA/HMPC/220598/2016). Available online: http://www.ema.europa.eu/docs/en_GB/document_library/Herbal_-_HMPC_assessment_report/2016/07/WC500211086.pdf
HMPC (Committee on Herbal Medicinal Products), 2016b. European Union herbal monograph on Glycine max (L.) Merr., lecithin. Draft. (EMA/HMPC/220599/2016). Available online: http://www.ema.europa.eu/docs/en_GB/document_library/Herbal_-_Herbal_monograph/2016/07/WC500211088.pdf
Holmes-McNary MQ, Cheng WL, Mar MH, Fussell S and Zeisel SH, 1996. Choline and choline esters in human and rat milk and in infant formulas. American Journal of Clinical Nutrition, 64, 572–576.
Honda K, Enoshima T, Oshikata T, Kamiya K, Hamamura M, Yamaguchi N, Nakamura K, Oguma Y, Fujiwara S, Takabe M, Sono A, Kawasaki T, Nasu M, Otsubo K and Wakigawa K, 2009. Toxicity studies of Asahi Kasei PI, purified phosphatidylinositol from soy lecithin. The Journal of Toxicological Sciences, 34, 265–280.
IOM (Institute of Medicine), 1998. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline. Food and Nutrition Board. National Academy Press, Washington, DC.
IPCS, 2004. IPCS Risk Assessment Terminology (Part 1: IPCS/OECD Key Generic terms used in Chemical Hazard/Risk Assessment. Available online: http://www.inchem.org/documents/harmproj/harmproj/harmproj1.pdf
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1972. WHO Technical Report Series, Evaluation of certain food additives and the contaminants mercury, lead, and cadmium. Sixteenth report of the Joint FAO/WHO Expert Committee on Food Additives. No. 505. World Health Organization, Geneva.
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1974a. 299. Lecithin. WHO Food Additives Series 5. 2 pp.
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1974b. 297. Hydroxylated Lecithin. WHO Food Additives Series 5. 2 pp.
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1978. Evaluation of certain food additives. Twentyfirst report of the Joint FAO/WHO Expert Committee on Food Additives. No. 617. World Health Organization, Geneva.
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2000. 976. Lead. WHO Food Additives Series 44, 39 pp.
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2007a. Lecithin. Published in FAO JECFA Monographs 4. Joint FAO/WHO Expert Committee on Food Additives. 4 pp.
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2007b. Lecithin, Partially Hydrolyzed. Published in FAO JECFA Monographs 4. Joint FAO/WHO Expert Committee on Food Additives. 4 pp.

Lavaud F, Perdu D, Prevost A, Vallerand H, Cossart C and Passemard F, 1994. Baker's asthma related to soybean lecithin exposure. Allergy, 49, 159–162.
Le Kim D and Betzing H, 1976. Intestinal absorption of polyunsaturated phosphatidylcholine in the rat. Hoppe-Seylers Zeitschrift fur Physiologische Chemie, 357, 1321–1331.
Leberco-Celsis Testing, 1997. Acute oral toxicity of hydrogenated lecithin (Basis LP-20H). Assay no. 973555 dated Apr 21. Unpublished data submitted by the Cosmetic, Toiletry, and Fragrance Association (CTFA) (4 pp.), cited in CIR, 2001.
Lecomte M, Couëdelo L, Meugnier E, Plaisancié P, Létisse M, Benoit B, Gabert L, Penhoat A, Durand A, Pineau G, Joffre F, Geloen A, Vaysse C, Laugerette F and Michalski MC, 2016. Dietary emulsifiers from milk and soybean differently impact adiposity and inflammation in association with modulation of colonic goblet cells in high-fat fed mice. Molecular Nutrition & Food Research, 60, 609–620.
Litton Bionetics Inc., 1975. Mutagenic evaluation of compound FDA 71-88, Lecithin. LBI Project #2468. Prepared for the Food and Drug Administration under contract no. 223-74-2104. Report dated 15 April. National Technical Information Service (NTIS) #: PB-245 478.
Martindale, 2014. The Complete Drug Reference, 38th Edition. Pharmaceutical Press, London, UK.
Martin-Hernandez C, Bénet S and Marvin-Guyl LF, 2005. Characterization and quantification of proteins in lecithins. Journal of Agricultural and Food Chemistry, 53, 8607–8613.
Merck Index, 2006. Lecithins. In: The Merck Index, an Encyclopedia of Chemicals, Drugs, and Biologicals. Available online: http://themerckindex.cambridgesoft.com/themerckindex/Forms/Search/ContentArea/C
Müller U, Weber W, Hoffmann A, Franke S, Lange R and Vieths S, 1998. Commercial soybean lecithins: a source of hidden allergens? Zeitschrift für Lebensmittel-Untersuchung und -Forschung A, 207, 341–351.
Palm M, Moneret-Vautrin DA, Kanny G, Denery-Papini S and Frémont S, 1999. Food allergy to egg and soy lecithins. Allergy, 54, 1116.
Paschke A, Zunker K, Wigtzki M and Steinhart H, 2001. Determination of the IgE-binding activity of soy lecithin and refined soybean oils. Journal of Chromatography B, 756, 249–254.
Porras O, Carlsson SP, Fällström SP and Hanson LA, 1985. Detection of soy protein in soy lecithin, margarine and occasionally, soy oil. International Archives of Allergy and Applied Immunology, 78, 30–32.
Radimer KL, Subar AF and Thompson FE, 2000. Nonvitamin, nonmineral dietary supplements: issues and findings from NHANES III. Journal of the American Dietetic Association, 100, 447–454.
Renaud C, Cardiet C and Dupont C, 1996. Allergy to soy lecithin in a child. Journal of Pediatric Gastroenterology Nutrition, 22, 328–329.
SCF (Scientific Committee for Food), 1982. Reports of the Scientific Committee for Food (13th series). Opinion expressed 1981. Food Science and Techniques.
SCF (Scientific Committee for Food), 1983. First Report of the Scientific Committee for Food on the essential requirements of infant formulae and follow-up milks based on cows' milk proteins (14th series). Opinion expressed 27 April 1983.
SCF (Scientific Committee for Food), 1996. Opinion on additives in nutrient preparations for use in infant formulae, follow-on formulae and weaning foods. 7 June 1996. Available online: http://ec.europa.eu/food/fs/sc/scf/reports/scf_reports_40.pdf
SCF (Scientific Committee for Food), 1997. Reports from the Scientific Committee for Food (40th series). Opinion expressed 7 June 1996. Food Science and Techniques.
SCF (Scientific Committee for Food), 1998. Opinion of the Scientific Committee of Food on the applicability of the ADI (Acceptable Daily Intake) for food additives to infants. 17 September 1998. Available online: http://ec.europa.eu/food/fs/sc/scf/out13_en.htmLmL
SCF (Scientific Committee on Food), 2001 Guidance on submissions for food additive evaluations by the Scientific Committee on Food. Opinion expressed on 11 July 2001.
SCF (Scientific Committee for Food), 2003. Report of the Scientific Committee on Food on the revision of essential requirements of infant formulae and follow-on formulae. Adopted on 4 April 2003. SCF/CS/NUT/IF/65 Final. 18 May 2003.
Stoll AL, Renshaw PF, De Micheli E, Wurtman R, Pillay SS and Cohen B, 1995. Choline ingestion increases the resonance of choline-containing compounds in human brain: an in vivo proton magnetic resonance study. Society of Biological Psychiatry, 37, 170–174.
Szepsenwol J, 1969. Brain nerve cell tumors in mice on diets supplemented with various lipids. Pathologia et Microbiologia, 34, 1–9.
Tanno H, 2012. Lecithin. In: *Ullmann's Encyclopedia of Industrial Chemistry*, Vol. 12 Online version. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. 6 pp, doi:10.1002/14356007.a15_293
Teelmann K, Schläppi B, Schüpbach M and Kistler A, 1984. Preclinical safety evaluation of intravenously administered mixed micelles. Arzneimittel-Forschung, 34, 1517–1523.
TemaNord, 2002. E 322 Lecithins. In: Food Additives in Europe 2000 – Status of safety assessments of food additives presently permitted in the EU. 318–320.
UK Ministry of Agriculture Fisheries and Food, 1992. Food Advisory Committee Report on the Review of the Use of Additives in Foods Specially Prepared for Infants and Young Children. (FdAC/REP/12). HMSO, London.

Unilever, 1978. Unpublished study, Biological safety evaluation of hydrolysed lecithin (BOLEC M) submitted to the EEC Commission, cited in SCF, 1982.
Wendel A, 1995. Lecithin. In: *Kirk-Othmer Encyclopedia of Chemical Technology*, Vol. 15. 4th Edition. Wiley-Interscience, New York. pp. 192–210.
WHO (World Health Organization), 1974. Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers and thickening agents. WHO Food Additives Series No. 5. Available online: http://www.inchem.org/documents/jecfa/jecmono/v05je42.htm
Zeizel SH, Growdon JH, Wurtman RJ, Magil SG and Logue M, 1980. Normal plasma choline responses to ingested lecithins. Neurology, 30, 1226–1229.
Zeizel SH, 1981. Dietary choline, biochemistry, physiology, and pharmacology. Annual Review of Nutrition, 1, 95–121.
Zeisel SH, Wishnok JS and Blusztajn JK, 1983. Formation of methylamines from ingested choline and lecithin. Journal of Pharmacology and Experimental Therapeutics, 225, 320–324.
Zeisel SH, Char D and Sheard NF, 1986. Choline, phosphatidylcholine and sphingomyelin in human and bovine milk and infant formulas. Journal of Nutrition, 116, 50–58.
Zierenberg O and Grundy SM, 1982. Intestinal absorption of polyenephosphatidylcholine in man. Journal of Lipid Research, 23, 1136–1142.
Zuskin E, Kanceljak B, Godnic-Cvar J, Tonkovic-Lojovic M and Turcic N, 1990. Immunological and respiratory changes in soybean workers. Arhiv Za Higijenu Rada i Toksikologiju, 41, 7–18.
Zuskin E, Kanceljak B, Schachter EN, Witek TJ, Marom Z, Goswami S and Maayani S, 1991. Immunological and respiratory changes in soy bean workers. International Archives of Occupational and Environmental Health, 63, 15–20.

Glossary [and/or] Abbreviations

AA	arachidonic acid
ADI	acceptable daily intake
AESGP	Association of the European Self-Medication Industry
AI	adequate intake
AMFEP	Association of Manufacurers and Formulators of Enzyme Products
ANS	Panel on Food Additives and Nutrient Sources added to Food
AOAC	Association of Analytical Communities
AR	average requirement
CAS	Chemical Abstracts Service
cfu	colony-forming unit
CIR	Cosmetic Ingredient Review
DBPCFC	double-blind placebo-controlled food challenge
DHA	docosahexaenoic acid
DRV	dietary reference value
EAST	enzyme allergosorbent test
EFEMA	European Food Emulsifiers Manufacturers Association
EFSA FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
EFSA NDA	EFSA Panel on Dietetic Products, Nutrition and Allergies
EINECS	European Inventory of Existing Commercial Chemical Substances
ELMA	European Lecithin Manufacturers Association
EMA	European Medicines Agency
EPL	essential phospholipid
EUE	human embryonic epithelium cells
FAO/WHO	Food and Agriculture Organization/World Health Organisation
FCS	Food Classification System
FDA	Food and Drug Administration
FDE	Food Drink Europe
FDRL	Food and Drug Research Laboratories
FSMP	foods for special medical purposes
GD	gestational day
GNPD	Global New Products Database
GRAS	'Generally Recognised As Safe'
HDL	high-density lipoprotein
HMPC	Committee on Herbal Medicinal Products
HPLC	high-performance liquid chromatography
ICGA	International Chewing Gum Association

Ig	immunoglobulin
INS	International Numbering System for Food Additives
IOM	Institute of Medicine
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LD_{50}	lethal dose, 50%, i.e. dose that causes death among 50% of treated animals
LOD	limit of detection
MED	minimum eliciting dose
MPL	maximum permitted level
NMR	nuclear magnetic resonance (spectroscopy)
NOAEL	no-observed-adverse effect
PND	post-natal day
PRI	population reference intake
QS	*quantum satis*
SCF	Scientific Committee on Food
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SNE	Specialised Nutrition Europe
TLC	thin-layer chromatography
UDS	unscheduled DNA synthesis
UL	upper intake level

Appendix A – Summary of reported use levels of lecithins (E 322) (mg/kg or mg/L as appropriate) provided by industry

Food category number[(a)]	Food category name	MPL	Restrictions/exceptions	Information provided by	N	Typical mean	Maximum
01.5	Dehydrated milk as defined by Directive 2001/114/EC	QS		European Lecithin Manufacturers Association	3	1,000	12,000
01.6.3	Cream and cream powder	QS		European Lecithin Manufacturers Association	1	1,000	12,000
01.7.1	Unripened cheese excluding products falling in category 16	QS	Except mozzarella	European Lecithin Manufacturers Association	1	1,000	12,000
01.8	Dairy analogues, including beverage whiteners	QS		FDE Food and Drink Europe	10	1,222	2,750
01.8	Dairy analogues, including beverage whiteners	QS		European Lecithin Manufacturers Association	2	2,000	10,000
02.2.2	Other fat and oil emulsions including spreads as defined by Council Regulation (EC) No 1234/2007 and liquid emulsions	QS		FDE Food and Drink Europe	6	2,296	7,500
02.2.2	Other fat and oil emulsions including spreads as defined by Council Regulation (EC) No 1234/2007 and liquid emulsions	QS		European Lecithin Manufacturers Association	4	2,450	15,000
03	Edible ices	QS		BABBI Confectionary Industry	1	3,000	3,000
03	Edible ices	QS		FDE Food and Drink Europe	26	902	6,497
03	Edible ices	QS		European Lecithin Manufacturers Association	1	1,000	5,000
04.2.5.4	Nut butters and nut spreads	QS		European Lecithin Manufacturers Association	1	5,000	5,000
04.2.5.4	Nut butters and nut spreads	QS		FDE Food and Drink Europe	1	1,000	10,000
05.1	Cocoa and Chocolate products as covered by Directive 2000/36/EC	QS		Rudolf Wild GmbH & Co. KG	1	600	600
05.1	Cocoa and Chocolate products as covered by Directive 2000/36/EC	QS		Stollwerck	1	6,000	6,500
05.1	Cocoa and chocolate products as covered by Directive 2000/36/EC	QS		FDE Food and Drink Europe	146	6,978	12,283
05.1	Cocoa and chocolate products as covered by Directive 2000/36/EC	QS		European Lecithin Manufacturers Association	11	2,486	30,000

Food category number[(a)]	Food category name	MPL	Restrictions/exceptions	Information provided by	N	Typical mean	Maximum
05.1	Cocoa and chocolate products as covered by Directive 2000/36/EC	QS		BABBI Confectionary Industry	1	2,000	2,000
05.2	Other confectionery including breath freshening microsweets	QS		FDE Food and Drink Europe	3	862	7,000
05.2	Other confectionery including breath freshening microsweets	QS		European Lecithin Manufacturers Association	3	2,250	8,000
05.3	Chewing gum	QS		European Lecithin Manufacturers Association	2	13,000	50,000
05.3	Chewing gum	QS		INTERNATIONAL CHEWING GUM ASSOCIATION	1	13,600	50,000
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4	QS		FDE Food and Drink Europe	62	3,811	25,943
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4	QS		European Lecithin Manufacturers Association	2	1,000	12,000
06.3	Breakfast cereals	QS		European Lecithin Manufacturers Association	1	3,000	3,000
07.1	Bread and rolls	QS	Except products in 7.1.1 and 7.1.2	FDE Food and Drink Europe	3	887	5,000
07.1	Bread and rolls	QS	Except products in 7.1.1 and 7.1.2	European Lecithin Manufacturers Association	3	3,000	30,000
07.2	Fine bakery wares	QS		Rudolf Wild GmbH & Co. KG	1	10	10
07.2	Fine bakery wares	QS		FDE Food and Drink Europe	37	3,342	20,000
07.2	Fine bakery wares	QS		BABBI Confectionary Industry	1	3,000	3,000
07.2	Fine bakery wares	QS		European Lecithin Manufacturers Association	13	2,000	25,000
08.3.2	Heat-treated meat products	QS	Except foie gras, foie gras entier, blocs de foie gras, Libamáj, libamáj egészben, libamáj tömbben	FDE Food and Drink Europe	1	165	318

Food category number[a]	Food category name	MPL	Restrictions/exceptions	Information provided by	N	Typical mean	Maximum
08.3.2	Heat-treated meat products	QS	Except foie gras, foie gras entier, blocs de foie gras, Libamáj, libamáj egészben, libamáj tömbben	European Lecithin Manufacturers Association	3	1,000	10,000
12.2.2	Seasonings and condiments	QS		European Lecithin Manufacturers Association	3	1,000	12,000
12.5	Soups and broths	QS		FDE Food and Drink Europe	5	336	2,839
12.5	Soups and broths	QS		European Lecithin Manufacturers Association	1	2,000	10,000
12.6	Sauces	QS		FDE Food and Drink Europe	13	1,459	10,688
12.6	Sauces	QS		European Lecithin Manufacturers Association	1	2,000	10,000
13.1.1	Infant formulae as defined by Commission Directive 2006/141/EC	1,000	[b]	FDE Food and Drink Europe	3	383	600
13.1.1	Infant formulae as defined by Commission Directive 2006/141/EC	1,000	[b]	SNE Specialised Nutrition Europe	7	455	1,000
13.1.1	Infant formulae as defined by Commission Directive 2006/141/EC	1,000	[b]	European Lecithin Manufacturers Association	1	1,000	1,000
13.1.2	Follow-on formulae as defined by Directive 2006/141/EC	1,000	[b]	FDE Food and Drink Europe	1	550	600
13.1.2	Follow-on formulae as defined by Directive 2006/141/EC	1,000	[b]	SNE Specialised Nutrition Europe	5	399	950
13.1.3	Processed cereal-based foods and baby foods for infants and young children as defined by Commission Directive 2006/125/EC	1,0000	Only biscuits and rusks, cereal-based foods, baby foods	SNE Specialised Nutrition Europe	3	1,412	2,600
13.1.4	Other foods for young children	1,0000	[b]	European Lecithin Manufacturers Association	2	1,000	10,000
13.1.4	Other foods for young children	1,0000	[b]	SNE Specialised Nutrition Europe	2	285	500
13.1.5.1	Dietary foods for infants for special medical purposes and special formulae for infants	1,000	[b]	SNE Specialised Nutrition Europe	9	513	1,000

Food category number[a]	Food category name	MPL	Restrictions/exceptions	Information provided by	N	Typical mean	Maximum
13.1.5.2	Dietary foods for babies and young children for special medical purposes as defined in Directive 1999/21/EC	1,000	[b]	SNE Specialised Nutrition Europe	6	572	930
13.1.5.2	Dietary foods for babies and young children for special medical purposes as defined in Directive 1999/21/EC	1,0000	Only biscuits and rusks, cereal-based foods, baby foods[b]	SNE Specialised Nutrition Europe	40	2,057	8,000
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)	QS		European Lecithin Manufacturers Association	1	1,000	30,000
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)	QS		FDE Food and Drink Europe	1	25	484
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)	QS		SNE Specialised Nutrition Europe	74	1,753	4,215
13.3	Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)	QS		SNE Specialised Nutrition Europe	5	34,173	100,000
13.3	Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)	QS		FDE Food and Drink Europe	2	5,619	10,397
13.3	Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)	QS		European Lecithin Manufacturers Association	1	1,000	30,000
14.1.4	Flavoured drinks	QS		FDE Food and Drink Europe	4	25	25
14.1.5.2	Other	QS	Excluding unflavoured leaf tea; including flavoured instant coffee	FDE Food and Drink Europe	1	369	437
14.2.8	Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15% of alcohol	QS		FDE Food and Drink Europe	1	20	20

Food category number[(a)]	Food category name	MPL	Restrictions/exceptions	Information provided by	N	Typical mean	Maximum
15.2	Processed nuts	QS		FDE Food and Drink Europe	1	62	62
16	Desserts excluding products covered in categories 1, 3 and 4	QS		FDE Food and Drink Europe	7	536	8,280
17.1	Food supplements supplied in a solid form including capsules and tablets and similar forms, excluding chewable forms	QS		CHEPLAPHARM Arzneimittel GmbH	1	0.02	0.02
17.1	Food supplements supplied in a solid form including capsules and tablets and similar forms, excluding chewable forms	QS		Nathura	4	715	1,350
17.1	Food supplements supplied in a solid form including capsules and tablets and similar forms, excluding chewable forms	QS		FDE Food and Drink Europe	2	5,226	15,000
17.1	Food supplements supplied in a solid form including capsules and tablets and similar forms, excluding chewable forms	QS		European Lecithin Manufacturers Association	2	12,750	20,000
17.3	Food supplements supplied in a syrup-type or chewable form	QS		AESGP – Association of the European Self-Medication Industry	1	14,706	18,387

MPL: maximum permitted levels; QS: *quantum satis*.
(a): FCS, Food Categorisation System (food nomenclature) presented in the Annex II to Regulation (EC) No 1333/2008.
(b): If more than one of the substances E 322, E 471, E 472c and E 473 is added to a foodstuff, the maximum level established for that foodstuff for each of those substances is lowered with that relative part as is present of the other substances together in that foodstuff.

Appendix B – Number and percentage of food products labelled with lecithins (E 322) out of the total number of food products present in Mintel GNPD per food subcategory between 2011 and 2016

Mintel sub-category[a]	Total number of products	Products labelled with lecithins (E 322)	
		Number	%
Individually wrapped chocolate pieces	2,380	2,044	85.9
Chocolate countlines	2,112	1,804	85.4
Seasonal chocolate	5,171	4,359	84.3
Non-individually wrapped chocolate pieces	4,798	3,832	79.9
Chocolate spreads	991	791	79.8
Other chocolate confectionery	266	212	79.7
Baby formula (6–12 months)	251	181	72.1
Gum	1,332	949	71.2
Growing up milk (1–4 years)	227	159	70
Baby formula (0–6 months)	235	162	68.9
Chocolate tablets	7,566	5,184	68.5
Growing up milk (4+ years)	3	2	66.7
Sweet biscuits/cookies	15,850	8,848	55.8
Snack/cereal/energy bars	4,345	2,205	50.7
Other frozen desserts	1,471	716	48.7
Margarine & other blends	903	429	47.5
Caramel & cream spreads	245	105	42.9
Cakes, pastries & sweet goods	11,977	5,047	42.1
Toffees, caramels & nougat	1,757	740	42.1
Dairy-based frozen products	7,236	2,991	41.3
Malt & other hot beverages	930	331	35.6
Meal replacements & other drinks	1,010	303	30
Soy-based frozen products	73	21	28.8
Mixed assortments	276	78	28.3
Beverage mixes	798	219	27.4
Cold cereals	5,621	1,539	27.4
Popcorn	991	223	22.5
Baby biscuits & rusks	274	55	20.1
Chilled desserts	5,726	1,152	20.1
Nut spreads	651	130	20
Other sugar confectionery	963	183	19
Baking ingredients & mixes	8,180	1,409	17.2
Rice snacks	363	52	14.3
Baby cereals	629	89	14.1
Dessert toppings	575	81	14.1
Wheat & other grain-based snacks	1,748	237	13.6
Rice/nut/grain & seed based drinks	963	130	13.5
Other snacks	118	15	12.7
Soy yogurt	364	44	12.1
Savoury biscuits/crackers	4,298	515	12
Snack mixes	1308	142	10.9
Lollipops	342	37	10.8
Hot cereals	1,022	96	9.4
Standard & power mints	808	67	8.3

Mintel sub-category[a]	Total number of products	Products labelled with lecithins (E 322)	
		Number	%
Boiled sweets	870	71	8.2
Shelf-stable desserts	2,972	241	8.1
Cream	1,471	116	7.9
Soft cheese desserts	1,397	103	7.4
Spoonable yogurt	9,079	590	6.5
Bread & bread products	9,063	555	6.1
Water-based frozen desserts	1,097	66	6
Pizzas	3,995	227	5.7
Sandwiches/wraps	2,457	136	5.5
Flavoured milk	1,316	70	5.3
Pastry dishes	1,755	91	5.2
Pastilles, gums, jellies & chews	3,411	174	5.1
Hors d'oeuvres/canapes	3,704	183	4.9
Liquorice	690	34	4.9
Stocks	1,276	60	4.7
Potato snacks	4,500	201	4.5
Marshmallows	436	18	4.1
Instant noodles	1,014	40	3.9
Medicated confectionery	931	29	3.1
Corn-based snacks	1,981	57	2.9
Meal kits	1,851	49	2.6
Baby fruit products, desserts & yogurts	1,423	35	2.5
Baby juices & drinks	339	8	2.4
Oils	3,880	87	2.2
Baby snacks	262	5	1.9
Cassava & other root-based snacks	269	5	1.9
Dry soup	1,516	29	1.9
Processed cheese	1,913	37	1.9
Fresh cheese & cream cheese	2,519	45	1.8
Other sauces & seasonings	862	15	1.7
Prepared meals	10,058	172	1.7
RTD (iced) coffee	810	11	1.4
Shortening & lard	72	1	1.4
Coffee	6,932	88	1.3
Butter	1,294	15	1.2
Liqueur	1,476	18	1.2
Sports drinks	728	9	1.2
Bean-based snacks	183	2	1.1
Instant pasta	567	6	1.1
Fish products	11,023	111	1
Meat substitutes	1,949	20	1
Tea	8,103	79	1
Rice	2,986	26	0.9
Wet soup	3,817	33	0.9
Cooking sauces	4,528	34	0.8
Fruit snacks	2,912	24	0.8
Instant rice	124	1	0.8
Liquid dairy other	119	1	0.8

Mintel sub-category[a]	Total number of products	Products labelled with lecithins (E 322)	
		Number	%
Meat pastes & pates	2,785	23	0.8
Poultry products	5,535	44	0.8
Soy based drinks	619	5	0.8
Stuffing, polenta & other side dishes	2,024	17	0.8
Pasta	9,091	63	0.7
Potato products	2,943	22	0.7
Sandwich fillers/spreads	910	6	0.7
Sweetened condensed milk	134	1	0.7
White milk	2,004	12	0.6
Creamers	189	1	0.5
Dark rum	219	1	0.5
Dips	1,306	7	0.5
Nuts	4,091	19	0.5
Pasta sauces	3,483	16	0.5
Savoury vegetable pastes/spreads	1,469	7	0.5
Seasonings	8,604	44	0.5
Artificial sweeteners	273	1	0.4
Eggs & egg products	1,303	5	0.4
Noodles	492	2	0.4
Fortified & other wines	386	1	0.3
Meat products	14,094	36	0.3
Table sauces	5,470	18	0.3
Whisky	688	2	0.3
Baby savoury meals & dishes	1,546	3	0.2
Confiture & fruit spreads	4,375	9	0.2
Dressings & vinegar	3,125	6	0.2
Drinking yogurt & liquid cultured milk	2,967	7	0.2
Mayonnaise	816	2	0.2
Soft cheese & semi-soft cheese	5,070	9	0.2
Vegetable snacks	517	1	0.2
Vegetables	9,418	20	0.2
Vodka	496	1	0.2
Energy drinks	1,539	2	0.1
Flavoured alcoholic beverages	1,816	1	0.1
Fruit	2,488	3	0.1
Fruit/flavoured still drinks	2,637	2	0.1
Hard cheese & semi-hard cheese	5,973	5	0.1
Honey	1,541	1	0.1
Nectars	3,633	5	0.1
Salads	2,378	2	0.1
Sucrose	983	1	0.1
Carbonated soft drinks	5,024	1	0
Juice	7,067	1	0
Pickled condiments	5,050	1	0
Wine	3,589	1	0
Total sample	**373,237**	**52,373**	**14.0**[b]

(a): According to the Mintel food categorisation.
(b): In total, around 14% of the foods available on the Mintel GNPD are labelled with lecithins (E 322) between 2011 and 2016.

Appendix C – Concentration levels of food additive lecithins (E 322) used in the refined exposure scenarios (mg/kg or mL/kg as appropriate)

FCS category number	FCS food category	Restrictions/exception	MPL	Concentration levels used in the refined exposure assessment scenario (only reported use levels)		Comments
				Mean	Maximum	
01.3	Unflavoured fermented milk products, heat-treated after fermentation		QS	–	–	Not taken into account (no concentration data)
01.4	Flavoured fermented milk products including heat treated products		QS	–	–	Not taken into account (no concentration data)
01.5	Dehydrated milk as defined by Directive 2001/114/EC		QS[(a)]	–	–	Not taken into account in the refined scenarios (no concentration data)
01.6.3	Cream and cream powder		QS[(a)]	–	–	Not taken into account in the refined scenarios (no concentration data)
01.7.1	Unripened cheese excluding products falling in category 16	Except mozzarella	QS[(a)]	–	–	Not taken into account in the refined scenarios (no concentration data)
01.7.5	Processed cheese		QS	–	–	Not taken into account (no concentration data)
01.7.6	Cheese products (excluding products falling in category 16)		QS	–	–	Not taken into account (no concentration data)
01.8	Dairy analogues, including beverage whiteners		QS	1,222	2,750	
02.1	Fats and oils essentially free from water (excluding anhydrous milkfat)	Except virgin oils and olive oils	30,000	–	–	Not taken into account in the refined scenarios (no concentration data)
02.2.2	Other fat and oil emulsions including spreads as defined by Council Regulation (EC) No 1234/2007 and liquid emulsions		QS	2,296	7,500	

FCS category number	FCS food category	Restrictions/exception	MPL	Concentration levels used in the refined exposure assessment scenario (only reported use levels)		Comments
				Mean	Maximum	
02.3	Vegetable oil pan spray		QS	–	–	Not taken into account (no consumption and no concentration data)
03	Edible ices		QS	981	6,497	
04.2.1	Dried fruit and vegetables		QS	–	–	Not taken into account (no concentration data)
04.2.2	Fruit and vegetables in vinegar, oil, or brine		QS	–	–	Not taken into account (no concentration data)
04.2.4.1	Fruit and vegetable preparations excluding compote		QS	–	–	Not taken into account (no concentration data)
04.2.5.4	Nut butters and nut spreads		QS	1,000	10,000	
04.2.6	Processed potato products		QS	–	–	Not taken into account (no concentration data)
05.1	Cocoa and chocolate products as covered by Directive 2000/36/EC		QS	6,894	12,283	
05.2	Other confectionery including breath freshening microsweets		QS	862	7,000	
05.3	Chewing gum		QS	13,600	50,000	
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4		QS	3,811	25,943	
06.2.2	Starches		QS	–	–	Not taken into account (no concentration data)
06.3	Breakfast cereals		QS[(b)]	–	–	Not taken into account in the refined scenarios (no concentration data)
06.4.1	Fresh pasta		QS	–	–	Not taken into account (no concentration data)

FCS category number	FCS food category	Restrictions/exception	MPL	Concentration levels used in the refined exposure assessment scenario (only reported use levels)		Comments
				Mean	Maximum	
06.4.2	Dry pasta	Only gluten-free and/or pasta intended for hypoproteic diets in accordance with Directive 2009/39/EC	QS	–	–	Not taken into account (no concentration data)
06.4.3	Fresh pre-cooked pasta		QS	–	–	Not taken into account (no concentration data)
06.4.4	Potato Gnocchi	Except fresh refrigerated potato gnocchi	QS	–	–	Not taken into account (no concentration data)
06.4.5	Fillings of stuffed pasta (ravioli and similar)		QS	–	–	Not taken into account (no concentration data)
06.5	Noodles		QS	–	–	Not taken into account (no concentration data)
06.6	Batters		QS	–	–	Not taken into account (no consumption and no concentration data)
06.7	Precooked or processed cereals		QS	–	–	Not taken into account (no consumption and no concentration data)
07.1	Bread and rolls	Except products in 7.1.1 and 7.1.2	QS	887	5,000	
07.1.1	Bread prepared solely with the following ingredients: wheat flour, water, yeast or leaven, salt		QS	–	–	Not taken into account (no concentration data)
07.1.2	Pain courant francais; Friss búzakenyér, fehér és félbarna kenyerek		QS	–	–	Not taken into account (no concentration data)
07.2	Fine bakery wares		QS	3,247	20,000	
08.3	Processed meat	Except foie gras, foie gras entier, blocs de foie gras, Libamáj, libamáj egészben, libamáj tömbben	QS	165	318	

FCS category number	FCS food category	Restrictions/exception	MPL	Concentration levels used in the refined exposure assessment scenario (only reported use levels)		Comments
				Mean	Maximum	
09.2	Processed fish and fishery products including molluscs and crustaceans		QS	–	–	Not taken into account (no concentration data)
09.3	Fish roe	Only processed fish roe	QS	–	–	Not taken into account (no concentration data)
10.2	Processed eggs and egg products		QS	–	–	Not taken into account (no concentration data)
11.2	Other sugars and syrups		QS	–	–	Not taken into account (no concentration data)
12.1.2	Salt substitutes		QS	–	–	Not taken into account (no consumption and no concentration data)
12.2.2	Seasonings and condiments		QS[(a)]	–	–	Not taken into account in the refined scenarios (no concentration data)
12.3	Vinegars		QS	–	–	Not taken into account (no concentration data)
12.4	Mustard		QS	–	–	Not taken into account (no concentration data)
12.5	Soups and broths		QS	336	2,839	
12.6	Sauces		QS	1,459	10,688	
12.7	Salads and savoury based sandwich spreads		QS	–	–	Not taken into account (no concentration data)
12.8	Yeast and yeast products		QS	–	–	Not taken into account (no concentration data)
12.9	Protein products, excluding products covered in category 1.8		QS	–	–	Not taken into account (no concentration data)
13.1.1	Infant formulae as defined by Commission Directive 2006/141/EC		1,000	433	1,000	

FCS category number	FCS food category	Restrictions/exception	MPL	Concentration levels used in the refined exposure assessment scenario (only reported use levels)		Comments
				Mean	Maximum	
13.1.2	Follow-on formulae as defined by Directive 2006/141/EC		1,000	424	950	
13.1.3	Processed cereal-based foods and baby foods for infants and young children as defined by Commission Directive 2006/125/EC	Only biscuits and rusks, cereal-based foods, baby foods	10,000	1,412	2,600	
13.1.4	Other foods for young children		10,000	285	500	
13.1.5.1	Dietary foods for infants for special medical purposes and special formulae for infants		1,000	513	1,000	
13.1.5.2	Dietary foods for babies and young children for special medical purposes as defined in Directive 1999/21/EC		1,000	572	930	
13.1.5.2	Dietary foods for babies and young children for special medical purposes as defined in Directive 1999/21/EC	Only biscuits and rusks, cereal-based foods, baby foods	10,000	2,057	8,000	
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)		QS	1,730	4,215	
13.3	Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)		QS	26,014	100,000	
13.4	Foods suitable for people intolerant to gluten as defined by Regulation (EC) No 41/2009	Including dry pasta	QS	–	–	Not taken into account (no concentration data)

FCS category number	FCS food category	Restrictions/exception	MPL	Concentration levels used in the refined exposure assessment scenario (only reported use levels)		Comments
				Mean	Maximum	
14.1.2	Fruit juices as defined by Directive 2001/112/EC and vegetable juices	Only vegetable juices	QS	–	–	Not taken into account (no concentration data)
14.1.3	Fruit nectars as defined by Directive 2001/112/EC and vegetable nectars and similar products	Only vegetable nectars	QS	–	–	Not taken into account (no consumption and no concentration data)
14.1.4	Flavoured drinks		QS	25	25	
14.1.5.2	Other non-alcoholic beverages	Excluding unflavoured leaf tea; including flavoured instant coffee	QS	369	437	
14.2.3	Cider and perry		QS	–	–	Not taken into account (no concentration data)
14.2.4	Fruit wine and made wine		QS	–	–	Not taken into account (no concentration data)
14.2.5	Mead		QS	–	–	Not taken into account (no concentration data)
14.2.6	Spirit drinks as defined in Regulation (EC) No 110/2008	Except whisky or whiskey	QS	–	–	Not taken into account (no concentration data)
14.2.7.1	Aromatised wines		QS	–	–	Not taken into account (no concentration data)
14.2.7.2	Aromatised wine-based drinks		QS	–	–	Not taken into account (no concentration data)
14.2.7.3	Aromatised wine-product cocktails		QS	–	–	Not taken into account (no concentration data)
14.2.8	Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15% of alcohol		QS	20	20	
15.1	Potato-, cereal-, flour- or starch-based snacks		QS	–	–	Not taken into account (no concentration data)

FCS category number	FCS food category	Restrictions/exception	MPL	Concentration levels used in the refined exposure assessment scenario (only reported use levels)		Comments
				Mean	Maximum	
15.2	Processed nuts		QS	62	62	
16	Desserts excluding products covered in categories 1, 3 and 4		QS	536	8,280	
17.1/17.2/17.3	Food supplements		QS	4,002	18,387	
18	Processed foods not covered by categories 1 to 17, excluding foods for infants and young children		QS	–	–	Not taken into account (no concentration data)

FCS: Food Classification System; MPL: maximum permitted level; QS: *quantum satis*.
(a): A level of 12,000 mg/kg was used in the maximum scenario.
(b): A level of 3,000 mg/kg was used in the maximum scenario.

Appendix D – Summary of total estimated exposure of lecithins (E 322) from their use as a food additives for the maximum level exposure scenario and the refined exposure assessment scenarios per population group and survey: mean and 95th percentile (mg/kg bw per day)

Population group	Number of subjects	MPL scenario		Brand-loyal scenario		Non-brand-loyal scenario	
		Mean	P95	Mean	P95	Mean	P95
Infants							
Bulgaria (NUTRICHILD)	659	140	337	56	163	19	62
Germany (VELS)	159	178	368	44	108	21	49
Denmark (IAT 2006_07)	826	140	306	32	67	17	39
Finland (DIPP_2001_2009)	500	50	109	18	49	15	42
United Kingdom (DNSIYC_2011)	1,369	124	273	36	88	20	48
Italy (INRAN_SCAI_2005_06)	12	67	–	36	–	16	–
Toddlers							
Belgium (Regional_Flanders)	36	365	–	78	–	22	–
Bulgaria (NUTRICHILD)	428	295	520	76	175	18	36
Germany (VELS)	348	257	422	63	136	21	41
Denmark (IAT 2006_07)	917	253	392	39	65	13	23
Spain (enKid)	17	187	–	45	–	17	–
Finland (DIPP_2001_2009)	500	69	130	16	39	11	29
United Kingdom (NDNS-RollingProgrammeYears1-3)	185	228	401	60	137	16	32
United Kingdom (DNSIYC_2011)	1,314	208	395	50	122	15	35
Italy (INRAN_SCAI_2005_06)	36	158	–	51	–	12	–
Netherlands (VCP_kids)	322	320	517	73	162	21	38
Children							
Austria (ASNS_Children)	128	257	442	64	151	17	38
Belgium (Regional_Flanders)	625	291	482	71	141	20	34
Bulgaria (NUTRICHILD)	433	314	576	82	187	19	39
Czech Republic (SISP04)	389	231	396	60	135	17	34
Germany (EsKiMo)	835	186	317	37	80	13	27
Germany (VELS)	293	250	379	64	135	21	38
Denmark (DANSDA 2005-08)	298	215	338	34	55	12	23
Spain (enKid)	156	203	353	53	131	15	32
Spain (NUT_INK05)	399	211	331	46	99	14	25
Finland (DIPP_2001_2009)	750	71	119	16	31	7	14
France (INCA2)	482	213	362	73	145	19	35
United Kingdom (NDNS-RollingProgrammeYears1-3)	651	206	350	58	130	15	29
Greece (Regional_Crete)	838	261	476	69	165	14	31
Italy (INRAN_SCAI_2005_06)	193	182	373	54	117	13	27
Latvia (EFSA_TEST)	187	216	475	56	124	15	33
Netherlands (VCP_kids)	957	283	453	66	148	19	35
Netherlands (VCPBasis_AVL2007_2010)	447	250	408	61	140	18	33
Sweden (NFA)	1,473	205	346	59	133	16	32
Adolescents							
Austria (ASNS_Children)	237	147	276	35	86	9	19
Belgium (Diet_National_2004)	576	124	234	31	70	9	18
Cyprus (Childhealth)	303	88	165	24	57	6	14

Population group	Number of subjects	MPL scenario		Brand-loyal scenario		Non-brand-loyal scenario	
		Mean	P95	Mean	P95	Mean	P95
Czech Republic (SISP04)	298	177	324	45	108	11	27
Germany (National_Nutrition_Survey_II)	1,011	118	235	29	77	8	20
Germany (EsKiMo)	393	145	256	28	62	10	20
Denmark (DANSDA 2005-08)	377	113	212	18	35	7	15
Spain (AESAN_FIAB)	86	94	189	25	57	6	14
Spain (enKid)	209	148	300	35	85	10	22
Spain (NUT_INK05)	651	138	248	31	67	9	17
Finland (NWSSP07_08)	306	32	59	7	15	4	8
France (INCA2)	973	122	234	39	90	10	21
United Kingdom (NDNS-RollingProgrammeYears1-3)	666	115	215	37	86	9	18
Italy (INRAN_SCAI_2005_06)	247	115	234	32	80	8	18
Latvia (EFSA_TEST)	453	169	316	41	96	11	25
Netherlands (VCPBasis_AVL2007_2010)	1,142	163	290	40	87	12	23
Sweden (NFA)	1,018	137	239	40	93	11	21
Adults							
Austria (ASNS_Adults)	308	118	237	34	84	9	19
Belgium (Diet_National_2004)	1,292	107	204	25	59	7	14
Czech Republic (SISP04)	1,666	111	211	25	66	6	14
Germany (National_Nutrition_Survey_II)	10,419	106	202	26	65	8	17
Denmark (DANSDA 2005-08)	1,739	81	139	13	23	4	9
Spain (AESAN)	410	72	143	18	52	5	11
Spain (AESAN_FIAB)	981	70	142	18	47	4	11
Finland (FINDIET2012)	1,295	87	164	21	50	6	13
France (INCA2)	2,276	92	169	24	55	6	13
United Kingdom (NDNS-RollingProgrammeYears1-3)	1,266	75	136	22	53	6	12
Hungary (National_Repr_Surv)	1,074	101	179	14	28	4	8
Ireland (NANS_2012)	1,274	88	156	19	42	5	11
Italy (INRAN_SCAI_2005_06)	2,313	73	145	17	43	4	9
Latvia (EFSA_TEST)	1,271	117	235	25	65	7	15
Netherlands (VCPBasis_AVL2007_2010)	2,057	112	199	26	56	8	16
Romania (Dieta_Pilot_Adults)	1,254	71	134	9	20	3	6
Sweden (Riksmaten 2010)	1,430	87	172	29	78	8	17
The elderly							
Austria (ASNS_Adults)	92	116	198	30	74	8	16
Belgium (Diet_National_2004)	1,215	110	199	22	48	7	14
Germany (National_Nutrition_Survey_II)	2,496	108	197	26	64	7	16
Denmark (DANSDA 2005-08)	286	77	132	12	21	4	7
Finland (FINDIET2012)	413	81	160	19	48	5	11
France (INCA2)	348	93	174	22	43	6	10
United Kingdom (NDNS-RollingProgrammeYears1-3)	305	73	133	20	50	5	10
Hungary (National_Repr_Surv)	286	95	163	14	29	3	8
Ireland (NANS_2012)	226	86	160	21	45	5	11
Italy (INRAN_SCAI_2005_06)	518	72	143	15	32	3	8
Netherlands (VCPBasis_AVL2007_2010)	173	106	182	23	45	7	16
Netherlands (VCP-Elderly)	739	103	169	21	39	7	12

Population group	Number of subjects	MPL scenario		Brand-loyal scenario		Non-brand-loyal scenario	
		Mean	P95	Mean	P95	Mean	P95
Romania (Dieta_Pilot_Adults)	128	81	170	11	25	3	6
Sweden (Riksmaten 2010)	367	81	152	24	54	6	13

MPL: maximum permitted level; P95: 95th percentile.
–: P95 of exposure was only calculated for those population groups where the sample size was sufficiently large to allow this calculation (EFSA, 2011a).

Safety Assessment of Lecithin and Other Phosphoglycerides as Used in Cosmetics

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ABSTRACT: The phosphoglycerides considered in this safety assessment function mainly as skin and hair conditioning agents, emulsifying agents and surfactants in cosmetic products, and are used up to a maximum reported concentration of 50%. Although phospholipids exert physiologic effects, these are not reproduced by application of phospholipids to the skin. Given the possibility that lecithin may be derived from animal sources, it should be noted that the Food and Drug Administration does not permit the use of ingredients made from bovine specified risk materials in cosmetic products. The Panel concluded that the 17 phosphoglycerides are safe in the present practices of use and concentration in cosmetics, as described in this safety assessment. This conclusion supersedes the 15% concentration limit on lecithin and hydrogenated lecithin for leave-on products specified in the 2001 Cosmetic Ingredient Review final report on the safety assessment of these two ingredients in cosmetic products.

INTRODUCTION

The safety of lecithin and other phosphoglycerides, listed below, in cosmetics is reviewed in this safety assessment. These 17 ingredients function mainly as skin and hair conditioning agents, emulsifying agents and surfactants in cosmetic products.

- Lecithin
- Hydrogenated Lecithin
- Lysolecithin
- Hydrogenated Lysolecithin
- Phospholipids
- Hydrolyzed Phospholipids
- Phosphatidic Acid
- Lysophosphatidic Acid
- Phosphatidylglycerol
- Lysophosphatidylglycerol
- Phosphatidylserine
- Ammonium Phosphatidyl Rapeseedate
- Phosphatidylcholine
- Hydrogenated Phosphatidylcholine
- Hydrogenated Lysophosphatidylcholine
- Lysophosphatidylethanolamine
- Phosphatidylinositol

The Cosmetic Ingredient Review (CIR) Expert Panel has evaluated the safety of lecithin and hydrogenated lecithin in cosmetics and issued a final report (published in 2001) with the following conclusion: Lecithin and hydrogenated lecithin are safe as used in rinse-off products, safe for use in leave-on products at concentrations of $\leq$15%, and the data are insufficient to determine the safety of use in cosmetic products where lecithin and hydrogenated lecithin are likely to be inhaled; lecithin and hydrogenated lecithin should not be used in cosmetic products in which *N*-nitroso compounds may be formed.[1] The qualification relating to the formation of *N*-nitroso compounds was based on concern over *N*-nitrosation of a potential bacterial metabolite. Please note that the conclusion reached in this safety assessment supersedes the conclusion on lecithin and hydrogenated lecithin in the 2001 CIR final report on the safety assessment of these 2 ingredients. The 15% concentration limit and the qualification relating to the formation of *N*-nitroso compounds are no longer applicable, and the available data addressing cosmetics that may be inhaled are sufficient.

The Expert Panel considered that phospholipids are the ubiquitous components of cell membranes and play a role in physiological processes. Because phospholipids have a physiologic role only when they are found in certain configurations in cell membranes, placing these compounds on the skin will not result in the same physiologic effect that results from these compounds as incorporated into cell membranes.

CHEMISTRY

Definition and Structure

The ingredients in this report are glycerides of fatty acids, linked to phosphoric acid or to a phosphoric ester. Lecithin, for example, is a complex mixture of phosphatides, consisting chiefly of phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositol, with varying amounts of triglycerides, fatty acids, and carbohydrates isolated from animal or vegetable sources.[1] In naturally occurring lecithins, the phosphoric acid is attached to the glycerol at the α-position. However, the phosphoric acid can also be attached in the *β*-position of glycerin, as a by-product of synthesis.[2] A structural scheme that is representative of the systematic nature of the phosphoglycerides family is presented in Figure 1.

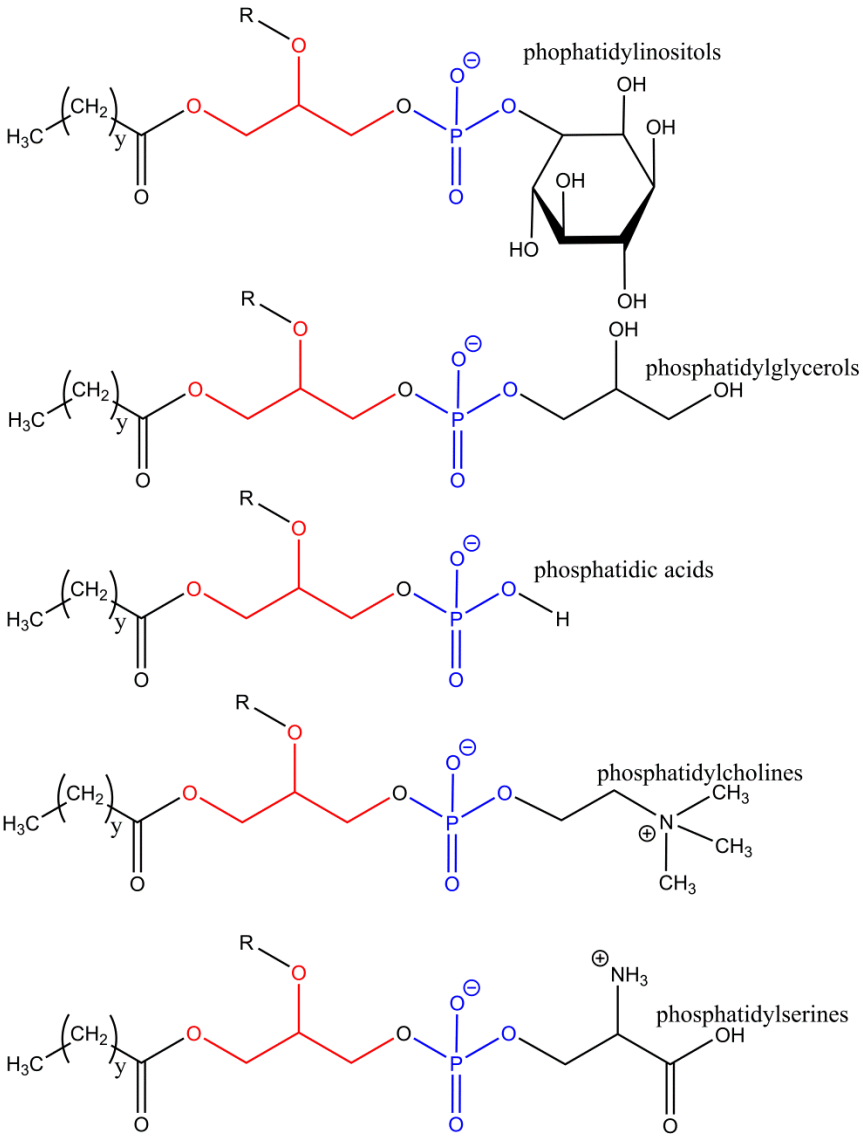


Figure 1. Phosphoglycerides

Phosphatidylserine is one example of a phosphoglyceride substituted with a fatty acid ester at the secondary alcohol residue of glycerin (i.e., is not a lysophosphoglyceride) and an amino acid (i.e., serine) attached through the phosphate group, as depicted in Figure 2.

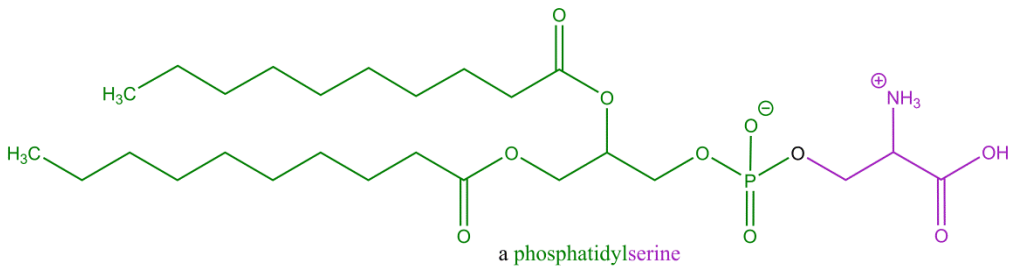


Figure 2. One example of a phosphoglyceride, phosphatidylserine (depicted is just one example of the possible fatty acyl chain lengths).

Hydrogenated lecithin (CAS No. 92128-87-5) is the end product of the controlled hydrogenation of lecithin.[1]

Despite the ionic, or even zwitterionic, natures of these ingredients, they are mostly insoluble in water.[3] These ingredients typically are waxy, hygroscopic substances that swell in contact with water to form, dependent on their molecular composition and structure, liposomes, micelles, or mixed micelles.

The main sources of naturally-occurring phosphoglycerides, such as lecithin, as used in personal care products, are maize, egg yolk, and soybean.[4] Phospholipids constitute 0.3% to 0.6% of soybean seed, or 1.5% to 3.0% of crude soybean oil. The composition of phospholipids in soybeans has been reported as follows:[5]

- Phosphatidylcholine (12% to 46%)
- Phosphatidylethanolamine (8% to 34%)
- Phosphatidylinositol (1.7% to 21%)
- Phosphatidic Acid (0.2% to 14%)
- Phosphatidylserine (0.2% to 6.3%)
- Lysophosphatidylcholine (1.5% to 8.5%)
- Lysophosphatidylinositol (0.4% to 1.8%)
- Lysophosphatidylserine (1%)
- Lysophosphatidic acid (1%)

The definitions, structures and functions of the phosphoglycerides reviewed in this safety assessment are included in Table 1.[6] There is some overlap among the ingredients in this report. While phosphatidylserine and phosphatidylcholine are listed as separate ingredients, they are likely components of the ingredient named phospholipids.

The ingredients in this report form a systematic, logical grouping that interrelates on numerous levels. All of these ingredients: 1) are glycerides of fatty acids, linked to phosphoric acid or to a phosphoric ester; 2) conform to Figure1; 3) are ionic and mostly insoluble in water; 4) are typically waxy, hygroscopic substances that swell, when in contact with water, to form liposomes; and 5) either come from sources such as maize, egg yolk, and soybean, or are synthesized via transphosphatidylation of phosphatidylcholine (which itself is sourced from soy).

Chemical and Physical Properties

Specifications for lecithin and related ingredients are presented in Table 2.[7,8,9,10,11,12,13,14,15,16] Included are chemical and physical properties data and microbiological specifications.

Method of Manufacture

Lecithin and Lecithin (enzyme-modified)

Commercial lecithin is isolated as a gum following hydration of solvent-extracted soy, safflower, or corn oils.[17] Lecithin is bleached, if desired, by hydrogen peroxide and benzoyl peroxide, and dried by heating. During the manufacture of lecithin derived from soy, most, if not all, of the soy protein is removed. If present, soy allergens would be found in the protein fraction.[18] According to another source, soy lecithin is usually produced from the hexane extract of soybean.[19]

In addition to the commercial lecithin mentioned above, it should be noted that another form of lecithin, enzyme-modified lecithin, is prepared by treating lecithin with either phospholipase A2 or pancreatin.[20]

Phosphoglycerides

Synthetic phosphoglycerides can be produced via phospholipase D-catalyzed transphosphatidylation of phosphatidylcholine (which is abundant in soy lecithin) with the desired phosphate substituent (e.g., myo-inositol for the synthesis of phosphatidylinositol).[21]

Phosphatidylglycerol

Phosphatidylcholine (minimum purity of 90%) in the presence of enzyme and glycerin yields phosphatidylglycerol (minimum purity of 85%) and choline.[22]

Phosphatidylserine

Soy-derived phosphatidylserine (phosphatidylserine complex derived from soy lecithin) consists of serine-substituted soy lecithin phospholipids and other phospholipids occurring naturally in lecithin.[23] Production of such soy lecithin phosphatidylserine complex involves the enzymatic transphosphatidylation of phosphatidylcholine and phosphatidylethanolamine from soy lecithin (via cabbage-derived phospholipase in the presence of exogenous serine) to phosphatidylserine. The production of the phosphatidylserine-enriched complex proceeds without the use of solvents during the manufacturing process. Thus, the final soy lecithin phosphatidylserine complex is solvent-free.

In addition to the preceding methods for manufacturing phospholipids, it should be noted that various other methods have been described in detail.[24]

Composition

Lecithin and Lecithin (enzyme-modified)

Composition data on various phosphoglyceride tradename materials are included in Table 2.

Commercial lecithin is a naturally-occurring mixture of the phosphatides of choline, ethanolamine, and inositol, with smaller amounts of other lipids.[17] Practically all of the lecithin in commerce is derived from soybeans. Phosphoglycerides are the major constituents of lecithin, and commercial lecithin may contain up to 35% triglycerides.[25]

Impurities

As an approved direct food additive for human food consumption, lecithin (enzyme-modified) meets the following specifications:[20]

- Acetone-insoluble matter (phosphatides), not less than 50%
- Acid value, not more than 40
- Lead, not more than 1.0 part per million, as determined by atomic absorption spectroscopy
- Heavy metals (as Pb), not more than 20 ppm
- Hexane-insoluble matter, not more than 0.3%
- Peroxide value, not more than 20
- Water, not more than 4%
- Lysolecithin, 50 to 80 mole% of total phosphatides

The Food Chemicals Codex[26] stipulates that food grade lecithin must not contain more than 0.3% hexane-insoluble matter. Because the protein fraction of lecithin would reside in this insoluble material, this specification limits the amount of protein in food grade lecithin to 0.3% or 300 mg/100 grams of lecithin.

The United States Pharmacopeia (USP) stipulates that sunflower lecithin in food contain not more than 1% hexane-insoluble matter.[27] It is also stipulated in the USP that soy lecithin contain not more than 20 ppm heavy metals and not more than 10 ppm lead.

Potential impurities are included in the specifications for various phosphoglyceride tradename materials that are summarized in Table 2.[7,8,9,10,11,12,13,14,15,16]

Nitrosamine Formation

Lecithin

Dimethylnitrosamine (DMNA) was reportedly formed in a model system in which 22.8 mmol sodium nitrite in 15 ml of water was added to a buffered solution, pH 5.6, containing 4.56 mmol of lecithin and stirred at 78˚C for 4 h.[28] The amount of DMNA formed (mg DMNA/kg of compound), confirmed by mass spectrometry, with various lecithins was as follows: soy lecithin (edible), 2.05 ppm; soy lecithin (commercial), 0.70 ppm; vegetable lecithin, 1.02 ppm; egg lecithin, 5.40 ppm; bovine lecithin (purified), 1.66 ppm; bovine lecithin (60%), 30.76 ppm; and synthetic lecithin, 319.7 ppm.

Lecithin is metabolized to choline by bacterial phospholipases and the released choline is dealkylated to dimethylamine, which is N-nitrosatable in the presence of nitrate.[29]

Of concern in cosmetics is the conversion (nitrosation) of nitrogen-bearing ingredients into *N*-nitroso chemicals that may be carcinogenic. Of the approximately 209 nitrosamines tested, 85% have been shown to produce cancer in laboratory animals.[30] Nitrosation can occur under physiologic conditions.[31] Depending on the nitrosating agent and the substrate, nitrosation can occur under acidic, neutral, or alkaline conditions. Atmospheric NO_2 may also participate in nitrosation in aqueous solution.[32]

USE

Cosmetic

The ingredients reviewed in this safety assessment function mainly as skin and hair conditioning agents, emulsifying agents, and surfactants in cosmetic products.[6] According to information supplied to the Food and Drug Administration (FDA) by industry as part of the Voluntary Cosmetic Registration Program (VCRP), and the results from a survey of ingredient use concentrations conducted by the Personal Care Products Council (Council), the following phosphoglycerides are being used in cosmetic products at maximum concentrations ranging, for different product categories, from 0.00000008% (lecithin in skin cleansing products and face products) to 50% (lecithin in foot products [leave-on]):[33,34] lecithin, hydrogenated lecithin, lysolecithin, lysophosphatidic acid, phosphatidylcholine, and phospholipids. The reported highest maximum use concentrations for other ingredients evaluated in this safety assessment are as follows, all relating to use in leave-on products: hydrogenated lecithin (5%, face and neck products [not spray]), lysolecithin (0.2%, in face and neck products [not spray]), phosphatidylcholine (0.8%, in body and hand products [not spray]), and phospholipids (0.75%, in face and neck products [not spray]). Use frequency and concentration of use data are presented in Table 3.

According to the CIR final safety assessment on lecithin and hydrogenated lecithin published in 2001, data received from FDA in 1984 indicated that the maximum reported use concentration of lecithin was in the 25% to 50% concentration range; use concentration data on hydrogenated lecithin were not included.[1] Concentration of use data provided by the Personal Care Products Council (formerly the Cosmetic, Toiletry, and Fragrance Association [CTFA]) in 1996 indicated that 65% lecithin was used at concentrations of 0.1% to 3%; use concentration data on hydrogenated lecithin were not provided.[1]

Cosmetic products containing phosphoglycerides may be applied to the skin and hair or, incidentally, may come in contact with the eyes and mucous membranes. Products containing these ingredients may be applied as frequently as several times per day and may come in contact with the skin or hair for variable periods following application. Daily or occasional use may extend over many years.

Lecithin is used in perfumes at maximum concentrations up to 0.0021%, and in hairspray formulations at maximum concentrations up to 0.000014% (aerosol) and up to 0.00015% (pump spray); hydrogenated lecithin is used in pump hair spray formulations at maximum concentrations up to 0.8%. Lecithin is also used in spray deodorants at maximum concentrations up to 0.0029% (aerosol) and up to 0.03% (pump spray). Phospholipids are used in aerosol hair spray at maximum concentrations up to 0.8%, and lysolecithin, phosphatidylcholine, and hydrogenated lecithin are used in body and hand sprays at maximum concentrations up to 0.1%, 0.8%, and 0.65%, respectively. Hydrogenated lecithin is used in moisturizing sprays and face and neck sprays at maximum concentrations of 0.65% and 0.5%, respectively. Ingredient use in face powders is also reported for lecithin (up to 1%) and hydrogenated lecithin (up to 0.56%). Because phosphoglycerides are used in products that are sprayed or in powder form, they could possibly be inhaled. In practice, 95% to 99% of the

droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters >10 µm, with propellant sprays yielding a greater fraction of droplets/particles below 10 µm, compared with pump sprays.[35,36,37,38] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.[35,36] There is some evidence indicating that deodorant spray products can release substantially larger fractions of particulates having aerodynamic equivalent diameters in the range considered to be respirable.[36] However, the information is not sufficient to determine whether significantly greater lung exposures result from the use of deodorant sprays, compared to other cosmetic sprays.

Because of concerns about potential transmission of bovine spongiform encephalopathy (BSE), cosmetic products are not permitted to contain ingredients made from bovine specified risk materials, which includes the central nervous system.[39]

Noncosmetic

Commercial lecithin, defined as a naturally-occurring mixture of the phosphatides of choline, ethanolamine, and inositol, is a direct food substance affirmed as generally recognized as safe (GRAS).[17] Additionally, enzyme-modified lecithin is listed among the substances added directly to human food affirmed as GRAS.[20] Food uses of lecithin include: emulsifier, stabilizer, dispersing aid, and release agent for baked goods.[18] Lecithin is also used in topical medicaments.

The Food Allergen Labeling and Consumer Protection act (FALCPA) of 2004 altered the way in which lecithin derived from soy must be declared on food labels. Whether intended to have a technical or functional effect in the finished food or to be used as an incidental additive (such as a release agent), lecithin that is derived from soy must be declared as an ingredient, using its common or usual name, and with the food source ("soy," "soya", or "soybeans") declared as required by section 403(w) of the Act.[18]

Lecithin (source not stated) is listed as an inactive ingredient in inhaled (aerosol, metered; maximum potency = 0.0002%) drug products that have been approved by FDA, and the same is true for soybean lecithin (aerosol, metered; maximum potency = 0.1%). Additionally, the approval of hydrogenated soy lecithin as an inactive ingredient in inhaled (aerosol, metered; maximum potency = 0.28%) drug products by FDA is pending.[40]

Phosphatidylcholine is the most abundant phospholipid in mammalian cellular membranes, bile, and lipoproteins.[41] The injection of a phosphatidylcholine and deoxycholic acid preparation is widely used as an alternative to liposuction for the reduction of subcutaneous fat.[42]

TOXICOKINETICS

Non-Human

Lecithin and Lysolecithin

The distribution of intravenously (i.v.) injected 1-^{14}C palmitoyl ^{32}P-lysolecithin was studied using male albino rats (number not stated).[43] The animals were injected i.v. with 1 ml of rat serum containing endogenously-labeled ^{32}P-lysolecithin or with 1 ml of rat serum containing endogenously-labeled ^{32}P phospholipids. The amount of lysolecithin incorporated into 1 ml of serum ranged between 550 and 850 µg. In some of the experiments, lysolecithin labeled with both ^{32}P and ^{14}C (in fatty acid moiety) was used. At 20 and 60 minutes post-injection, 45% and 77% of injected radioactive material was removed from the blood, respectively. There was an uptake of labeled phospholipids by heart and skeletal muscle. The percentage of ^{32}P lysolecithin in these organs was much higher than in the injected material. The authors noted that the high percentage of labeled lysolecithin in the skeletal and heart muscle indicated that lysolecithin might leave the vascular compartment more rapidly than lecithin and be metabolized by the tissues. The per cent composition of labeled phospholipids found in the liver resembled that of the injected serum, with lecithin as the major labeled component. However, the percentage of lysolecithin was lower than that in the starting material. The authors noted that, based on these results, it seems more likely that the liver takes up phospholipids indiscriminately and that lysolecithin is rapidly converted to lecithin.

In subsequent experiments in which rats (number not stated) were injected with serum containing ^{32}P -lysolecithin, the injected labeled lysolecithin disappeared from the bloodstream very rapidly ($t_{1/2} \approx 2$ minutes). Considerable amounts of lysolecithin were recovered in the liver and skeletal muscle at a time when the serum radioactivity decreased to negligible

levels. The conversion of lysolecithin to lecithin was observed in all the tissues examined, and this reaction was most rapid in the liver. *In vivo*-injected lysolecithin taken up by the liver was converted to lecithin by an acylation reaction.[43]

Lysohosphatidic Acid

Lysophosphatidic acid was degraded to glycerophosphate and orthophosphate by phosphatidases and phosphatases, respectively, in an enzyme preparation, i.e., cytoplasmic particulate fraction of guinea pig brain or liver.[44]

Phosphatidylserine

Following i.v. administration to rats and mice, phosphatidylserine was eliminated from plasma in a biphasic manner and largely distributed to several major organs, including the liver spleen, and brain.[45,46,47,48,49] Conversely, orally administered phosphatidylserine was extensively hydrolyzed by phospholipase A_2 to lysophosphatidylserine in the gastrointestinal tract prior to absorption, as is the case for all other dietary phospholipids.[45,50,51] In rats, approximately 60% of an orally administered dose of phosphatidylserine (20 mg/kg body weight) was recovered in the feces, of which 50% was identified as lysophosphatidylserine. Approximately 10% of the orally administered dose was detected in the urine.[48]

Studies in which animals were injected i.v. with phosphatidylserine also indicate that phospholipids undergo hydrolytic cleavage to the monoacyl derivative lysophosphatidylserine in the plasma, as well as decarboxylation of the serine moiety to phosphatidylethanolamine in circulating blood cells.[46,47,49] Lysophosphatidylserine and phosphatidylethanolamine were also detected in the liver and brain after i.v. administration. However, in all organs, the majority of radioactivity (~90%) was consistently accounted for as unmetabolized phosphatidylserine.[48,49] Conversely, at 60 minutes post oral dosing of phosphatidylserine (20 mg/kg) in rats, the majority of the circulating plasma radioactivity consisted of phosphatidylserine; the radioactivity at 24 h was attributed primarily to phosphatidylserine degradation products.[48] Furthermore, less than 20% of the administered dose recovered in the mesenteric lymph of rats after oral administration of phosphatidylserine (560 mg/kg body weight of [^{3}H]-glycerol-labeled, brain-derived phosphatidylserine) was liposoluble, with phospholipids comprising 11% of the liposoluble fraction.[51] The majority of the radioactivity was recovered as triglycerides and, to a smaller extent, diacylglycerol, indicating complete degradation of orally administered phosphatidylserine.

In the mitochondria of mammalian cells, phosphatidylserine may undergo decarboxylation to phosphatidylethanolamine, which is followed by potential re-formation of phosphatidylserine through exchange of the ethanolamine moiety with serine in the endoplasmic reticulum or mitochondria-associated membrane.[52] Thus, in the intestinal absorptive cells, lysophosphatidylserine may be reacylated to yield phosphatidylserine and ultimately converted to phosphatidylethanolamine.[51] Re-esterified phospholipids are subsequently incorporated into intestinal lipoproteins (i.e., chylomicrons) or directly transported as lysophospholipids via the portal system to the liver.[53,54] As the chylomicrons circulate in the blood, their components, including phospholipids, are degraded via lipoprotein lipase hydrolytic activity.[54] Ultimately, the phosphatidylserine degradation products (i.e., free fatty acids, serine, glycerol, and phosphorus-containing substances) enter common physiological pathways of amino acid and lipid metabolism. In turn, intact phospholipids are excreted in the bile, and, thus, may be subject to enterohepatic circulation.

Pharmacokinetic studies indicate exogenous phosphatidylserine crosses the blood-brain barrier, where it appears to have an affinity for the hypothalamus.[55] Oral administration results in peak levels in 1 to 4 h.

Human

In 8 human subjects, the oral consumption of 500 mg phosphatidylserine (as soy lecithin phosphatidylserine capsules) resulted in peak plasma phosphatidylserine levels of 3.95% of the total phospholipid plasma concentration, compared to background phosphatidylserine levels of 1.8% to 2.2% of total plasma phospholipids.[23]

Skin Penetration Enhancement

The effects of phosphatidylcholine and hydrogenated phosphatidylcholine on the permeation of indomethacin through hairless rat skin were studied using liquid paraffin and a gel prepared with liquid paraffin and hydrogenated soybean phospholipid.[56] Indomethacin (1%) was mixed with liquid paraffin and phosphatidylcholine or hydrogenated phosphatidylcholine, and heated at 95°C for 30 minutes. The mixture was then cooled to room temperature and allowed to stand for 1 day. Skin permeation was measured using a modified Franz-type diffusion cell apparatus. Permeation rates for indomethacin from the liquid paraffin suspension with phosphatidylcholine or hydrogenated phosphatidylcholine were determined. For liquid paraffin without phosphatidylcholine or hydrogenated phosphatidylcholine, permeation of

indomethacin was observed only after 10 h. However, within 10 minutes, indomethacin permeated at rates of ~ 10 μg/cm^2 and 5 μg/cm^2 from liquid paraffin with phosphatidylcholine and hydrogenated phosphatidylcholine, respectively.

The effect of hydrophilic groups of phospholipids on the percutaneous penetration of indomethacin *in vitro* was examined in a Franz-type diffusion chamber, using dorsal skin from guinea pigs.[57] The following phospholipids were evaluated for enhancement of indomethacin skin penetration: phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, phosphatidylglycerol, phosphatidic acid and sphingomyelin. Phospholipid-induced enhancement of IM percutaneous penetration was in the following order: phosphatidylglycerol > phosphatidylethanolamine > phosphatidylcholine > phosphatidylserine > phosphatidic acid > phosphatidylinositol > control > sphingomyelin.

TOXICOLOGY

Single Dose (Acute) Toxicity

Oral

Phosphatidylserine

The oral administration of a purified phospholipid preparation obtained from bovine cerebral cortex (in phosphate buffer suspension) to Sprague-Dawley rats (CD strain; number not stated) indicated that the LD_{50} is > 5 g/kg body weight.[58]

Intravenous

Phosphatidylserine

Following the i.v. dosing of Sprague-Dawley rats (CD strain; number not stated) with phosphatidylserine derived specifically from bovine cerebral cortex (in phosphate buffer suspension), an LD_{50} of 236 mg/kg body weight was reported.[58]

Subcutaneous

Phosphatidylserine

When Sprague-Dawley rats (CD strain; number not stated) were dosed subcutaneously (s.c.) with phosphatidylserine derived specifically from bovine cerebral cortex (in phosphate buffer suspension), the LD_{50} was reported to be > 5 g/kg body weight.[58]

Repeated Dose Toxicity

Inhalation

Phosphatidylcholine

The effects of chronic exposure to liposome aerosols on lung histology and alveolar macrophage function were studied.[59] Liposomes were made from hydrogenated soy phosphatidylcholine (50 mg/mL). Groups of 30 mice (strain not stated) were placed in a nose-only exposure module and exposed to liposome (20 ml total volume, 50 mg lipid/mL phosphate-buffered saline) or saline aerosols 1 h per day, 5 days per week, for 4 weeks. Five mice of both the experimental and control groups were removed weekly and their lungs examined. The animals were killed and bronchoalveolar lavage (BAL) was performed through a tracheostomy. *In vivo* uptake of liposomes by alveolar macrophages was documented by fluorescence microscopy and flow cytometry of BAL. A consistent amount of 1 to 3 μg of lipid inhaled per dosing per mouse was estimated from fluorescence measurements. No histologic changes of the lungs or untoward effects on general health or survival of animals were noted. Alveolar macrophage phagocytic function was not affected. Transmission electron microscopy and morphometry showed no treatment-related alterations.

Oral

Non-Human

Lecithin

A group of 48 male and 48 female SPF Wistar rats was fed 4% (soya) lecithin for 2 years, while a control group was fed commercial diet only.[60] Feed consumption and body weights were determined prior to dosing, at intervals up to week 95, at week 102, and at study termination. The mean lecithin intake was 1470 and 2280 mg/kg/day for males and females, respectively. No stastistically significant differences were observed in mortality, feed consumption, or body weight between the treated and control groups, but it was noted that feed consumption and body weight were sometimes greater in the treated group when compared to controls. Hematology values of animals of the treated group were similar to those of control animals, as were organ weights and gross and microscopic alterations. Increased parathyroid gland hyperplasia, particularly in the males, was attributed to an increased phosphate intake. The incidence of tumor formation was similar in the treated and control groups. A slightly increased incidence of myocardial fibrosis was associated with parathyroid gland hyperplasia.

Lecithin, Phosphatidylserine, Phosphatidylcholine, Phosphatidylethanolamine, Phosphatidylinositol, and Phosphatidic Acid

A 90-day feeding study was performed to evaluate the safety of dietary soy lecithin transphosphatidylated phosphatidylserine [soybean-derived phosphatidylserine (SB-PS)], with or without fish oil-derived long-chain polyunsaturated fatty acids (LC-PUFA) mixed or conjugated to the glyceride backbone.[61] One-hundred-two male Wistar rats (wild type, pathogen free) were randomly assigned to 6 groups. The 5 groups consumed 100 mg chow containing each of the following components, respectively, incorporated in 1 ml of milk-based supplement matrix:

- medium-chain triglycerides (MCT group)
- fish oil diluted with MCT to yield 30% (w/w) of omega-3 long-chain polyunsaturated fatty acids [LC-PUFA] (omega-3 group)
- soybean 78% powdered SB-PS (final concentration of 20% SB-PS (w/w)) emulsified with 13% phosphatidylcholine (PC), 2% phosphatidylethanolamine, 1% phosphatidylinositol, 4% phosphatidic acid and further diluted with MCT (SB-PS group)
- fish oil mixed with soybean 78% powdered SB-PS and diluted with MCT to yield a final concentration of 20% SB-PS (w/w) and 30% (w/w) of omega-3 LC-PUFA (omega-PS group)
- 20% phosphatidylserine (w/w) consisting largely of molecular species of palmitic acid (16:0) and docosahexanoic acid [DHA] (22:6) or eicosapentanoic acid (20:5), resulting in 30% (w/w) of omega-3 LC-PUFA (PS-DHA group).

The control group consumed normal chow. Blood samples were drawn and hematological parameters evaluated. Signs of toxicity were not observed during the feeding period. At the end of the study, gross examination of organs was performed. The following mortalities were reported: 1 rat (control group), 2 rats (MCT group), 1 rat (omega-3 group), and 1 rat (PS-DHA group). Pathological examinations did not reveal a specific cause of death; however it was concluded that the deaths were not treatment-related. Hematological parameters were normal in all treatment groups. At gross pathological examination, there were mild signs of liver enlargement in 5 of 102 rats, and these were considered unrelated to treatment. Possible early signs of lung metastasis (pale color nodes and different tissue consistency) were observed in 4 of 102 rats, but these findings were considered typical and abundant in rats of this age (15 months old). It was noted that none of these pathological findings occurred in PS-fed rats. It was concluded that no adverse effects were associated with diets fed in this study.

Phosphatidylserine

The repeated dose toxicity of phosphatidylserine derived specifically from bovine cerebral cortex (in phosphate buffer suspension) was studied using 3 groups of Sprague-Dawley rats of the CD strain (20 males, 20 females/group).[58] The 3 groups received doses of 10, 100, and 1,000 mg/kg/day, respectively, by gavage for 26 weeks. The control group was dosed with phosphate buffer only. Body weight gain and food consumption in all dose groups were comparable to the control group. No significant hematological changes were observed. At week 13, slightly elevated alkaline phosphatase levels in male and female rats, and slightly lowered serum albumin levels in males was observed in the 1,000 mg/kg/day dose group. Elevated potassium and lower sodium values were reported for males at week 13. Terminal studies indicated no major problems, and there were no significant morphological changes. It was concluded that phosphatidylserine derived specifically from bovine cerebral cortex did not cause significant toxicity in this study.

Another repeated dose toxicity study involved groups of 40 beagle dogs (20 males, 20 females).[58] Three groups received phosphatidylserine derived specifically from bovine cerebral cortex (in corn oil) orally at doses of 10, 100, and 1,000 mg/kg/day (dose volume = 5 ml/kg), respectively, for 1 year. The control group was dosed with corn oil. None of the

animals died. At the highest dose administered, blood glucose and cholesterol levels were significantly lowered. There were no significant macroscopic findings, and organ weights were within normal range. Histopathological examination of tissues did not indicate treatment-related changes. It was concluded that phosphatidylserine derived specifically from bovine cerebral cortex did not cause significant toxicity in this study.

Human

Phosphatidylserine

Human subjects (120) received soy lecithin-derived phosphatidylserine (300 or 600 mg orally) daily in a 12-week study.[62] There were no clinically-significant variations in blood chemistry or hematology. Additionally, there were no differences in the occurrence of side effects between test and placebo groups.

Intravenous

Phosphatidylserine

Phosphatidylserine derived specifically from bovine cerebral cortex (in phosphate buffer suspension) was administered i.v. to groups of Sprague-Dawley rats of the CD strain (10 males, 10 females/group).[58] The 3 groups received doses of 5, 20, and 80 mg/kg/day (dose volume = 2.5 ml/kg), respectively, for 4 weeks. The control group was dosed with vehicle only. Except for females of the 5 mg/kg/day group, reddening and swelling of the paws and around the muscle region were observed in all dose groups. The following hematological changes were observed in male and female rats of the 80 mg/kg/day dose group: significant lowering of the erythrocyte count, hemoglobin concentration, and packed cell volume, and increased neutrophil and lymphocyte counts. An increase in spleen weight in males and females of the 80 mg/kg dose group and males of the 20 mg/kg/day dose group was reported. Kidney weights of males dosed with 80 or 20 mg/kg/day were also increased when compared to controls. Adrenal weights of males and females of the 80 mg/kg dose group were marginally increased. Results at microscopic examination revealed an injection site thrombosis in some rats in all dose groups, with an increase in severity in rats dosed with 20 mg/kg/day or 80 mg/kg/day. Whether or not this finding was reported for the control group was not stated. An increase in the incidence of extramedullary hematopoiesis was observed in the 80 mg/kg/day dose group. It was concluded that phosphatidylserine derived specifically from bovine cerebral cortex did not cause significant toxicity in this study.

The i.v. toxicity of phosphatidylserine derived specifically from bovine cerebral cortex (in phosphate buffer suspension) was evaluated using groups of 24 (12 males, 12 females) Beagle dogs.[58] Three groups were injected i.v. with phosphatidylserine derived specifically from bovine cerebral cortex at doses of 5, 15, and 40 mg/kg/day, respectively, for 4 weeks. A fourth group was dosed with vehicle only. Generalized tremors of body muscles were observed in animals of the 15 or 40 mg/kg/day group. A significant increase in total white cell count and a reduction in total serum protein values were reported for dogs dosed with 40 mg/kg/day. At gross examination, hemorrhage was observed around the injection sites. There were no significant group differences in organ weights. Microscopic examination of the liver revealed centrilobular and periportal sinusoidal aggregations of polymorphonuclear leukocytes in one animal of the 15/mg/kg/day dose group and in 4 animals of the 40 mg/kg/day dose group. It was concluded that phosphatidylserine derived specifically from bovine cerebral cortex did not cause significant toxicity in this study.

Intramuscular

Phosphatidylserine

The intramuscular toxicity of phosphatidylserine derived specifically from bovine cerebral cortex (in phosphate buffer suspension) was evaluated using groups of 32 (16 males, 16 females) Beagle dogs.[58] Three groups were injected intramuscularly with phosphatidylserine derived specifically from bovine cerebral cortex at doses of 5, 10, and 15 mg/kg/day, respectively, for 6 weeks. A fourth group served as the vehicle control. None of the animals died. Subcutaneous hardening and/or swelling of injection sites was observed in the 10 and 15 mg/kg/day dose groups. Hematological analyses indicated elevation of the erythrocyte sedimentation rate and an increase in total white blood cell count in the 15 mg/kg dose group. At gross examination, subcutaneous hemorrhage and adhesion between the skin and muscles (at injection site) was reported for all groups, including the control group. This finding was considered dose-related. Organ weights were within normal ranges. Muscle degeneration, and subcutaneous and intramuscular acute inflammatory cell infiltration and necrosis were also observed at injection sites. It was concluded that phosphatidylserine derived specifically from bovine cerebral cortex did not cause significant toxicity, i.e., there were no significant signs of systemic toxicity.

Cytotoxicity

Lysolecithin

Lysolecithin has been described as a powerful hemolytic and cytolytic phosphoglyceride.[63,64] Furthermore, the toxic effect of many snake venoms is attributable to their content of phosphatidase A, an enzyme capable of converting plasma phosphatides into lysophosphatides, one of which is lysolecithin.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Phosphatidylserine

In a teratogenicity study, phosphatidylserine derived from bovine cerebral cortex was administered by gavage to pregnant Sprague-Dawley rats (CD strain; number not stated) at doses of 0, 10, 100, and 200 mg/kg/day on days 6 through 18 of gestation.[58] The animals were killed on day 20 of gestation, and litters were examined for skeletal and visceral abnormalities. At terminal necropsy, there were no treatment-related gross changes. Additionally, the following litter values were not affected by treatment with phosphatidylserine: litter size, post-implantation loss, litter and mean fetal weights, and embryonic and fetal development.

Phosphatidylserine derived from bovine cerebral cortex was also administered by gavage to pregnant New Zealand White rabbits (number not stated) at doses of 0, 50, 150, and 450 mg/kg/day on days 6 through 18 of gestation.[58] On gestation day 29, the animals were killed and litters subjected to gross examination. Fetuses were examined externally and internally for evidence of visceral and skeletal malformations. There was no evidence of systemic effect, and neither pregnancy nor mortality was affected by treatment. At the highest dose, mean fetal weights were slightly lower when compared to control values, but the difference was not statistically significant. Additionally, there were no treatment-related effects on embryonic and fetal development.

Lysophosphatidic Acid

Lysophosphatidic acid and sphingosine 1-phosphate are both lysophospholipids.[65] Because lysophosphatidic acid promotes prostaglandin synthesis, mediators in the lysophosphatidic acid pathway may also play a significant role in implantation and parturition. Sphingosine 1-phosphate signaling is thought to be essential in vascular formation within the uteroplacental unit and in fetomaternal immunologic interactions. Derangements in either one of these lysophospholipid signaling pathways could result in pregnancy complications that may include implantation failure, preeclampsia, and preterm labor.

Immature germinal vesicle stage oocytes from 5- to 6-week-old female BDF-1 mice were incubated for 17–18 h in *in vitro* maturation (IVM) medium containing 0, 1, 10 or 30 µM lysophosphatidic acid and then either fertilized *in vitro* with epididymal sperm or assessed for spindle morphology or mitochondrial membrane potential.[66] Chromosomal aneuploidy in the resultant blastocysts and the production of normal pups were not assessed. The fertilized embryos were grown in *vitro* to assess blastocyst-formation rates, differential cell counts and apoptosis. The supplementation of IVM with 30 µM lysophosphatidic acid enhanced the maturation and developmental competence of mouse oocytes. Rates of maturation, fertilization and blastocyst formation and hatching were significantly higher in the 30 µM lysophosphatidic acid-supplemented group (94.3%, 96.3%, 79.1 and 51.3%, respectively) than in the unsupplemented control (0 mM) group (80.5%, 87.5%, 61.3% and 37.8%, respectively), and more comparable to that of the *in vivo* matured oocytes (100%, 96.5%, 95.3% and 92.9%, respectively). Lysophosphatidic acid did not adversely affect mitochondrial activity, spindle integrity, or blastocyst cell number. The results of this study imply that the supplementation of IVM medium with 30 µM lysophosphatidic acid may enhance the developmental competence of mouse oocytes without affecting apoptosis, spindle normalcy or mitochondrial integrity.

GENOTOXICITY

In Vitro

Hydrogenated Lecithin

The genotoxicity of Lpsm-Flln or Lpsm (313–5000 μg/plate) was examined, with and without metabolic activation, using *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2uvrA (pKM101).[67] Neither liposome was genotoxic in this assay, with or without metabolic activation. The following 5 positive controls were genotoxic: sodium azide, *N*-ethyl-*N*'-nitro-*N*-nitrosoguanidine, 2-nitrofluorene, 9-aminoacridine, and 2-aminoanthracene.

Phosphatidylserine

Human lymphocyte cultures were incubated with phosphatidylserine derived from bovine cerebral cortex concentrations up to 165.6 μg/ml, with and without metabolic activation.[58] Cyclophosphamide served as the positive control. Phosphatidylserine derived specifically from bovine cerebral cortex was not genotoxic with or without metabolic activation.

In the mouse lymphoma assay, phosphatidylserine derived from bovine cerebral cortex was not genotoxic to mouse lymphoma L5178Y cells with or without metabolic activation.[58] Test concentrations were not stated.

Phosphatidylserine derived from bovine cerebral cortex was evaluated in a DNA repair assay involving human epithelioid cells (HELA S_3 cells), with and without metabolic activation.[58] Test concentrations were not stated. Increases in the number of silver grains found in autoradiographic film over cell nuclei served as indicators of repair synthesis. There was no evidence of DNA repair synthesis with or without metabolic activation.

In Vivo

In the micronucleus test, phosphatidylserine derived from bovine cerebral cortex was administered orally (by gavage) to mice at doses up to 300 mg/kg body weight.[58] Two equal doses were administered, separated by a 24-h interval. Mitomycin C served as the positive control. Bone marrow smears were examined for the presence of micronuclei in 1,000 polychromatic erythrocytes per mouse, and for the ratio of normochromatic to polychromatic erythrocytes. Phosphatidylserine derived from bovine cerebral cortex was neither cytotoxic nor genotoxic to bone marrow cells.

Modulation of Gene Expression

Phospholipids

The capacity of a formulation of grape seed extract and soy phospholipids (formulation identified as SBD.5HC) to trigger a regenerative response in the dermis and epidermis through a selective action on the hypodermis was investigated using human skin (from breast reduction surgeries).[68] SBD.5HC was prepared by combining grape seed extract (95% proanthocyanidins-grade) and soy lecithin (95% - 98% phospholipids-grade) at a ratio of 1:3 w/w. After 5 days of culture under control conditions, full-thickness human skin biopsies showed marked degradation, characterized by pyknotic nuclei in fibroblasts and basal keratinocytes, as well as intercellular gaps in spiny and granular layers of the epidermis. The inclusion of SBD.5HC (100 μg/ml) in the medium bathing the hypodermal layer of the biopsies resulted in an improved overall morphology. Treated skin samples had mostly normal, elongated fibroblasts, a decreased number of dying basal keratinocytes at the dermal-epidermal junction, less gaping spaces in the stratum spinosum, and better preserved granulosum and stratum corneum. Thus, study results suggested that the application of SBD.5HC to the hypodermal layer of skin triggered modulation of gene expression in the upper layers of skin, and resulted in morphological changes in the dermis and epidermis.

CARCINOGENICITY

Lecithin

TM strain mice were fed 5 to 10 mg lecithin mixed with sugar (for palatability), and a second group was fed lecithin (5 to 10 mg) and cholesterol (4 to 5 mg).[69] The mice were bred and their offspring dosed following the same procedures; dosing continued until all mice became moribund or had died. A control group was given laboratory feed *ad libitum*. The total number of mice fed lecithin, lecithin and cholesterol, or control feed was 166, 212, and 360, respectively. Animals were killed and brain necropsies performed. It was noted that the brains of moribund animals or animals found dead were removed and necropsied, but necropsy results were not reported. Brain nerve cell tumors (2-5 mm) were found in 18 of 73 examined animals fed lecithin and in 27 of 88 examined animals fed lecithin and cholesterol, whereas, no brain nerve cell tumors were found in 188 control animals.

Groups of female dd mice were dosed s.c. as follows: Fifty mice were given 0.1 ml of a 0.25% mixture of 4-nitroquinoline1-oxide (in 10% aqueous lecithin) in a single injection until the total dose was 2.5 mg.[70] The injections were repeated weekly, each time in a different site on the back. Thirty mice were dosed (10 times) with a lecithin-water mixture at the same total dose as in the previous group. Twenty mice were not dosed and served as controls. The mice were killed after 221 to 296 days. Animals dosed with 4-nitroquinoline 1-oxide/lecithin that survived more than 221 days after dose initiation (36/50) had pulmonary neoplasms; skin neoplasia at the injection site (1 animal) and leukemia (1 animal) were also observed in this group. No surviving mice dosed with lecithin-water or untreated control mice had pulmonary or any other type of neoplasia. However 3/28 animals of the lecithin-water group and 3/18 control animals had lung adenomas; these were considered spontaneous.

In the same study, groups of female Buffalo rats were dosed s.c. as follows: Twenty-five rats were given 0.2 ml of a 0.25% mixture of 4-nitroquinoline 1-oxide (in 10% aqueous lecithin) in a single injection until the dose reached 10 mg; the injections were repeated weekly. Fifteen rats were dosed (20 times) with a lecithin-water mixture, having received the same total dose. The rats were killed after 264 to 329 days. Nineteen of the 25 animals dosed with 4-nitroquinoline 1-oxide/lecithin that survived more than 264 days after dose initiation had pulmonary neoplasms, with 11 s.c. sarcomas and 2 endometrial sarcomas also reported. No neoplasms were found in any of the 13/15 surviving rats dosed with lecithin-water.[70]

IRRITATION AND SENSITIZATION

Ocular Irritation

Lecithin

Lecithin 65% (solution of 65% lecithin) and products containing 2.25% or 3.0% Lecithin 65% were non- to minimally irritating to unrinsed rabbit eyes. A soap containing 0.83% lecithin powder (tested at 25%) was moderately irritating, and lecithin-containing liposomes were practically nonirritating in a Draize test.[1]

Skin Irritation and Skin Sensitization

Non-Human

Lecithin and Hydrogenated Lecithin

In single-insult occlusive patch tests (rabbits), lecithin 65% (solution of 65% lecithin) was minimally irritating, products containing 3% lecithin 65% were practically non- to mildly irritating, and a product containing 2.25% lecithin 65% was non-irritating to the skin of rabbits. In a guinea pig immersion study, 0.5% of a soap containing 0.83% lecithin powder was practically non-irritating. Hydrogenated lecithin was not a primary dermal irritant in rabbits.[1]

Human

Lecithin and Hydrogenated Lecithin

In clinical irritation studies, cosmetic formulations containing 0.3% or 3% lecithin 65% (solution of 65% lecithin), a soap containing 0.83% lecithin powder (tested at 0.5%), and lecithin liposomes were generally non-irritating. Barely perceptible erythema was the most severe reaction observed. Hydrogenated lecithin also was not an irritant, and hydrogenated lecithin (15% in petrolatum) was not a sensitizer. Additionally, a tanning oil containing 3% lecithin 65%, a mascara containing 0.1% lecithin 65%, and a foundation containing 0.3% lecithin 65% were non-sensitizing.[1]

Lysolecithin

The intracutaneous injection of 0.04 µM to 0.25 µM lysolecithin, derived from beef serum, human serum, or beef brain lecithin, caused typical wheal and erythema reactions in the 3 subjects tested.[71] Lysolecithin (0.125 and 0.17 µM) produced wheal and erythema reactions that were roughly equivalent to that produced by the injection of histamine (0.5 µg). These reactions consisted of a pale, elevated central swelling (occasionally with small pseudopods), surrounded by a bright red zone of erythema. The lower concentrations of lysolecithin (0.085 and 0.043 µM) caused minor reactions that were smaller than those obtained with 0.3 µg histamine, but slightly greater than those caused by 0.1 µg histamine (slight threshold reaction). A faint, but definite, reaction was observed at concentrations as low as 0.013 µM in another experiment.

Allergenicity

Lecithin

The antigenicity of soy lecithin was studied using 30 soybean-sensitive patients and 22 controls.[19] One control group (11 subjects) consisted of nonatopic individuals, and the other control group (11 subjects) consisted of allergic patients with negative IgE to soybean (radioallergosorbent test [RAST] score = 0). The IgE- and IgG4-binding activities of the soy lecithin proteins were evaluated by immunoblotting with sera obtained from the patients, 7 of whom had a positive challenge test. In 100 grams of sample, the soy lecithin contained 2.8 mg of proteins. The proteins present in soy lecithin were analyzed by sodium dodecyl sulfate polyacrylamide gel elelectrophoresis. For the soy lecithin, the detection rate of only one protein (molecular weight: 31 kDa) by the serum IgE of patients was statistically significantly different when compared to serum from the 2 control groups combined (detection rates: 40% [patient sera] and 4.5% [control sera]). Proteins in the molecular weight range of 58 to 67 kDa were rarely bound to serum IgE. Only one of the patients with a positive challenge test had IgE antibodies to soy lecithin proteins. The presence of IgG4-binding proteins in soy lecithin was described as rare. It was concluded that the proteins present in soy lecithin have little antigenicity with respect to soybean allergy.

Photocytotoxicity

Non-Human

Hydrogenated Lecithin

The photocytotoxicity of Lpsm-Flln, 0.2% aqueous, was studied using Balb/3T3 fibroblastic cells.[67] Bacterial assay results for this liposome are included in the Genotoxicity section. Lpsm-Flln had the following composition: hydrogenated lecithin, glycine soja (soybean) sterols, and fullerene C60 (C60) in the weight ratio of 89.7:10:0.3 (i.e., contains 89.7% hydrogenated lecithin). Results were compared with that of a 0.2% liposome solution (Lpsm) that did not contain C60, described as follows: hydrogenated lecithin and glycine soja (soybean) sterols in the weight ratio of 90:10 (i.e., contains 90% hydrogenated lecithin). The fibroblasts (in Lpsm-Flln or Lpsm at doses of 0.49 to 1,000 µg/mL) were exposed to sham-irradiation or UVA light (5 J/cm^2, 320–400 nm, λ_{max} = 360 nm) for 50 minutes. Cell viability of Balb/3T3 fibroblastic cells in Lpsm-Flln was 96.3 - 158.5% for the UVA group and 94.5 - 149.6% for the sham group, and did not decrease dose-dependently. Also, cell viability in Lpsm was similar to that in Lpsm-Flln. These results show that Lpsm-Flln (89.7% hydrogenated lecithin) or Lpsm (90% hydrogenated lecithin) at a concentration of 0.2% was not photocytotoxic to Balb/3T3 fibroblasts.

Phototoxicity/Photosensitization

Lecithin and Hydrogenated Lecithin

A foundation containing 0.3% lecithin 65% (solution of 65% lecithin) was not a photosensitizer in human subjects. The subjects were exposed for 1 minute to aUV light source (360 nm peak output), at a distance of 12 inches, after removal of the 1st, 4th, 7th, and 10th induction patches and challenge patches. Photosensitization reactions were determined 48 h after exposure. Lecithin and hydrogenated lecithin (both at 15% in petrolatum) were not phototoxic or photosensitizing in human subjects. On days 1, 4, and 7, of induction, patches were removed and test sites were irradiated with a dose of 3 MED of UVA. A 4th induction patch was also applied, followed by irradiation of the test site. Challenge patch sites were exposed to a dose of 9.5 MED and 0.5 MED of simulated solar light (UVA and UVB).[1]

Case Report

Lecithin

A 3-year-old boy with a history of asthma and peanut allergy was treated for asthma that developed after an upper respiratory tract infection.[72] He developed respiratory distress and generalized urticaria within an hour after receiving the second of 2 inhalations of an ipratropium bromide inhaler. All signs regressed within 48 h of withdrawal of the drug. Soy lecithin, an excipient in the metered dose inhaler, was strongly suspected of causing the adverse events.

OTHER STUDIES

Phospholipid Signaling

The organization of biological systems involves communication between cells and, in multicellular organisms, these interactions are mediated through cell-cell contacts.[73] In order for a cell to react, a signal must be detected and converted across the physical boundary that is defined by the cell membrane. Membrane lipids have important roles in signaling reactions. These ligands can act as signaling molecules in various ways, and the binding of a ligand to a cell surface receptor is an initiating event of cellular signaling. Transmembrane signaling frequently includes the activation of enzymes that act on metabolizing lipids in the vicinity of the respective receptor, leading to the generation of membrane-bound and diffusible metabolites. Receptor stimulation is often accompanied by the activation of downstream factors that are regulated by products of lipid metabolism. Some of the phospholipids involved in signaling include phosphatidylinositol, phosphatidylcholine, lysophosphatidic acid, and phosphatidylserine. Signaling effects relating to these phospholipids are summarized below.

Minor products of inositol phospholipid metabolism, such as phosphatidylinositol-3,4,5-triphosphate, serve as key intermediates in cell signaling.[74] Furthermore, phosphoinositides, a family of lipid molecules derived from the phosphorylation of phosphatidylinositol, control important cellular processes, including cell proliferation, apoptosis, metabolism, and migration.[75] Phosphoinositides make up only a small fraction of cellular phospholipids, yet they control almost all aspects of a cell's life and death.[76] The specific interaction of phosphoinositides with proteins is critical for a plethora of cellular processes, including cytoskeleton remodeling, mitogenic signaling, ion channel regulation, and membrane trafficking.[77] Phosphoinositide homeostasis is tightly regulated by a large number of inositol kinases and phosphatases that have been implicated in regulating membrane trafficking, and the dysregulation of these enzymes has been linked to a number of human diseases, ranging from cancer and diabetes to neurological disorders and asthma.

Cancer cells display sensitivity to ablation of fatty acid synthesis, possibly as a result of the diminished capacity to synthesize complex lipids involved in signaling or growth pathways.[78] Evidence has accrued that phosphatidylcholine, the major phospholipid component of eukaryotic membranes, as well as choline metabolites derived from its synthesis and catabolism, contribute to both proliferative growth and programmed cell death. Coordinated changes in substrate availability, gene expression, and enzyme activity lead to altered phosphatidylcholine synthesis in cancer.

Lysophosphatidic acid is capable of stimulating a plethora of different cellular responses through the activation of its family of cognate G protein-coupled receptors.[79] It mediates a wide range of biological effects in many tissue types, including vasculogenesis, angiogenesis, and vascular maturation, and has also been implicated in the regulation of pathophysiologic vascular responses. For example, lysophosphatidic acid was found to signal through $G\alpha_q$ to promote the growth and migration of vascular smooth muscle cells, which is essential for the development of intimal hyperplasia after vascular injury.

Phophatidylserine-specific binding is important in the function of A-, B- and C-Raf kinases, which are important regulators of many signal transduction pathways. Raf kinases are generally downstream from the Ras GTPases, and transmit information to activate mitogen-activated protein kinase signaling.[80] The activation of protein kinase B, Raf-1, and protein kinase C signaling, which supports neuronal survival and differentiation, requires the interaction of these proteins with phosphatidylserine.[81] Phosphatidylserine, exposed extracellularly, is instrumental in triggering blood clotting and also serves as a signal for the clearance of apoptotic cells.[80]

Skin Composition

Lecithin, Lysolecithin, Phosphatidylethanolamine, and Phosphatidylserine

Lecithin, phosphatidylethanolamine, and phosphatidylserine comprise the major phospholipid components of skin from young adult female albino rabbits.[82] Polyglycerolphosphatides, lysolecithin, and sphingomyelin are also present.

In a study in which the total lipid concentration, distribution of all major lipid species, and the fatty acid composition in human stratum corneum were assessed, the following lipids were found: phospholipids (phosphatidylethanolamine), cholesterol sulfate, neutral lipids (free sterols, free fatty acids, triglycerides, sterol and wax esters, squalene, and n-alkanes), and sphingolipids.[83] The neutral lipids contributed the greatest proportion to the stratum corneum lipids. Values for the

phospholipid composition (lipid weight %) at the following 4 skin sites were: abdomen (4.9 ± 1.6), leg (5.2 ± 1.1), face (3.3 ± 0.3) and plantar (3.2 ± 0.89).

SUMMARY

The safety of the following 17 ingredients in cosmetics is reviewed in this safety assessment: lecithin, hydrogenated lecithin, lysolecithin, hydrogenated lysolecithin, phospholipids, hydrolyzed phospholipids, phosphatidic acid, lysophosphatidic acid, phosphatidylglycerol, lysophosphatidylglycerol, phosphatidylserine, ammonium phosphatidyl rapeseedate, phosphatidylcholine, hydrogenated phosphatidylcholine, hydrogenated lysophosphatidylcholine, lysophosphatidylethanolamine, and phosphatidylinositol. These ingredients function mainly as skin and hair conditioning agents, emulsifying agents and surfactants in cosmetic products. Frequency of use data from FDA and the results of an industry survey indicate that the following ingredients are being used in cosmetic products: lecithin, hydrogenated lecithin, lysolecithin, lysophosphatidic acid, phosphatidylcholine, and phospholipids. Of these ingredients, the highest maximum concentration of use is 50% lecithin in a leave-on foot product.

The fate of i.v.-injected 1-^{14}C palmitoyl ^{32}P-lysolecithin was studied using male albino rats. A high percentage of labeled lysolecithin was detected in skeletal and heart muscle, and it is likely that that lysolecithin is rapidly converted to lecithin in the liver. Following i.v. administration to rats and mice, phosphatidylserine was eliminated from plasma in a biphasic manner and largely distributed to several major organs, including the liver spleen, and brain tissue. In rats, approximately 60% of an orally administered dose of phosphatidylserine (20 mg/kg body weight) was recovered in the feces, of which 50% was identified as lysophosphatidylserine. Approximately 10% of this orally administered dose was detected in the urine. In humans, the oral consumption of soy lecithin phosphatidylserine capsules (total of 500 mg phosphatidylserine) resulted in peak plasma phosphatidylserine levels of 3.95% of the total phospholipid plasma concentration, when compared to background phosphatidylserine levels of 1.8% to 2.2% of total plasma phospholipids.

The effect of the following phospholipids on the percutaneous penetration of indomethacin was evaluated *in vitro* using dorsal skin from guinea pigs: phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, phosphatidylglycerol, phosphatidic acid and sphingomyelin. Phospholipid-induced enhancement of IM percutaneous penetration was in the following order: phosphatidylglycerol > phosphatidylethanolamine > phosphatidylcholine > phosphatidylserine > phosphatidic acid > phosphatidylinositol > control > sphingomyelin.

In a study in which a purified phospholipid preparation obtained from bovine brain (phosphatidylserine derived specifically from bovine cerebral cortex, in phosphate buffer suspension) was administered orally to Sprague-Dawley rats, the LD_{50} was > 5 g/kg body weight.

In a repeated dose inhalation toxicity study involving mice exposed to phosphatidylcholine liposomes, no histologic changes of the lungs or untoward effects on general health or survival of animals were noted. In a two-year feeding study on 4% lecithin involving rats, no significant differences were observed for mortality, feed consumption, or body weight between the treated and control groups. Additionally, there were no differences in gross or microscopic findings when the groups were compared.

In a 12-week study in which human subjects received soy lecithin-derived phosphatidylserine daily, there were no clinically-significant variations in blood chemistry or hematology. Additionally, there were no differences in the occurrence of side effects between test and placebo groups.

Lecithin 65% (solution of 65% lecithin) and products containing 2.25% or 3.0% Lecithin 65% were non- to minimally irritating to unrinsed rabbit eyes. In single-insult occlusive patch tests (rabbits), lecithin 65% was minimally irritating, products containing 3% lecithin 65% were practically non- to mildly irritating, and a product containing 2.25% lecithin 65% was non-irritating to the skin of rabbits.

The photocytotoxicity of liposome-fullerene (Lpsm-Flln, 0.2% aqueous) was studied using Balb/3T3 fibroblastic cells; results were negative. A foundation containing 0.3% lecithin 65% (solution of 65% lecithin) was not a photosensitizer. Lecithin and hydrogenated lecithin (both at 15% in petrolatum) were not phototoxic or photosensitizing.

In oral teratogenicity studies on phosphatidylserine derived specifically from bovine cerebral cortex involving rats and rabbits, there were no treatment-related effects on embryonic and fetal development. Lysophosphatidic acid (30 μM) enhanced the maturation and developmental competence of BDF-1 mouse oocytes *in vitro*.

Hydrogenated lecithin was not genotoxic to *Salmonella typhimurium* or *Escherichia coli* bacterial strains with or without metabolic activation. The results for phosphatidylserine in mammalian cell assays (i.e., mouse lymphoma, DNA repair [HELA cells], micronucleus assays) were also negative.

TM strain mice were fed 5 to 10 mg lecithin mixed with sugar, and a second group was fed lecithin and 4 to 5 mg cholesterol. Brain nerve cell tumors (2-5 mm) were found in 18 of 73 examined animals fed lecithin and in 27 of 88 examined animals fed lecithin and cholesterol; brain nerve cell tumors were not found in 188 control animals. In another study, groups of female dd mice were dosed s.c. with a 0.25% mixture of 4-nitroquinoline1-oxide (in 10% aqueous lecithin). No surviving mice dosed with lecithin-water or untreated control mice had pulmonary or any other type of neoplasia. However, 3/28 animals of the lecithin-water group and 3/18 control animals had adenomas, which were considered spontaneous.

Membrane lipids, i.e., phospholipids, have important roles in signaling reactions. However, these effects are not relevant to the use of phospholipids as cosmetic ingredients.

DISCUSSION

The Panel acknowledged their previous conclusion, published in 2001, that lecithin and hydrogenated lecithin are safe as used in rinse-off products and safe for use in leave-on products at concentrations of ≤15%, and the data are insufficient to determine the safety of use in cosmetic products where lecithin and hydrogenated lecithin are likely to be inhaled; lecithin and hydrogenated lecithin should not be used in cosmetic products in which *N*-nitroso compounds may be formed. This 15% concentration limit was the highest concentration evaluated in tests for skin irritation, sensitization, phototoxicity, and photosensitization potential in human subjects, all of which were negative. The Panel also noted that the highest maximum use concentration of lecithin reported in 2014 was 50% in leave-on cosmetic products. The Panel agreed that there is little sensitization potential at this concentration, based on extensive clinical experience indicating no problems associated with the application of lecithin to the skin. Thus, the Panel determined that the concentrations of lecithin, hydrogenated lecithin, and other phosphoglycerides reviewed in this safety assessment need not be limited to 15% in cosmetic products. This decision, based, in part, on clinical experience with lecithin, is applicable across the phosphoglycerides reviewed in this safety assessment because lecithin is a complex mixture consisting primarily of phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositol, with varying amounts of triglycerides, fatty acids, and carbohydrates from vegetable or animal sources.

The Panel discussed the potential for incidental inhalation exposures to phosphoglycerides in products that are sprayed or in powder form and agreed that, based on the results of the repeated dose inhalation toxicity study, likely airborne particle size distributions and concentrations in the breathing zone and ingredient use, incidental inhalation would not lead to local respiratory effects or systemic effects. The Panel also considered the safe use of lecithin as an inactive ingredient in FDA-approved aerosolized drug products. Thus, it was agreed that the previous conclusion should be amended, acknowledging that the the data are no longer insufficient to determine the safety of use in cosmetic products where lecithin and hydrogenated lecithin are likely to be inhaled.

Additionally, in the previous safety assessment, concerns about the formation of *N*-nitroso compounds in cosmetic products containing lecithin and hydrogenated lecithin were based on experimental conditions that do not represent plausible use conditions. For example, lecithin has been reported to be metabolized to choline by bacterial phospholipases in a model system, and the released choline can be dealkylated to dimethylamine, which is N-nitrosatable in the presence of nitrate. The Panel has determined that these experimental conditions do not reflect ingredient use in cosmetic products. Thus, it was agreed that the previous conclusion should be amended, removing the restriction that lecithin and hydrogenated lecithin should not be used in cosmetic products in which *N*-nitroso compounds may be formed.

The Panel expressed concern about animal tissue as a potential source of phosphoglycerides, particularly bovine brain as a source of phosphatidylserine and lysolecithin. However, the Panel determined that these phosphoglycerides are safe as used, noting that ingredients derived from bovine central nervous system tissues are not permitted for use in cosmetic products. Concern about pesticide residues and heavy metals that may be present in botanical ingredients was also expressed.

The Panel stressed that the cosmetics industry should continue to use current good manufacturing practices to limit impurities.

Phosphoglycerides are known to enhance the dermal penetration of drugs. The Panel noted that formulators should be aware of the potential for enhancing the dermal penetration of other ingredients in cosmetic formulations that contain the ingredients that are being evaluated in this safety assessment, especially in products intended for use on infants.

Acknowledging the involvement of cell-membrane lipids in cellular signaling cascades, the Panel noted that these signaling effects are not relevant to the use of phosphoglycerides as cosmetic ingredients. The Panel also acknowledged that derangements in phosphoglyceride metabolism can be associated with prostate, breast, or ovarian cancer, but noted that these changes are artifacts of cancer and are not relevant for assessing the safety of cosmetic ingredients. Furthermore, systemic toxicity is not a concern because, among other reasons, phospholipids are the ubiquitous components of cell membranes and are generally recognized as safe for human consumption.

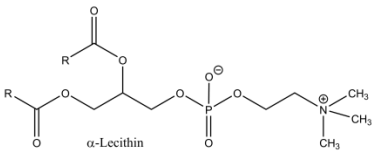
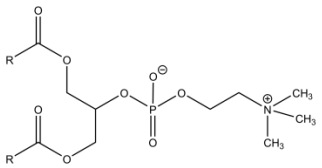
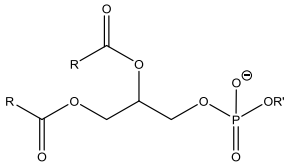
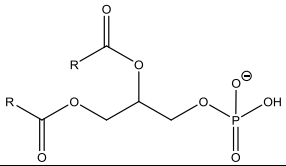
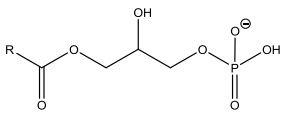
CONCLUSION

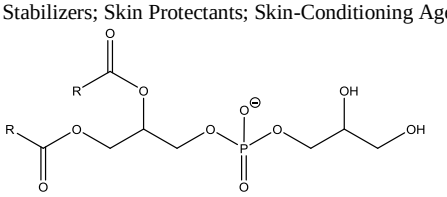
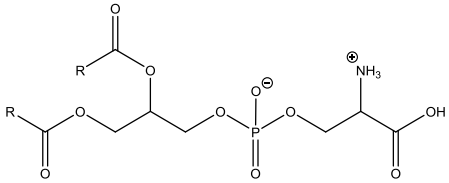
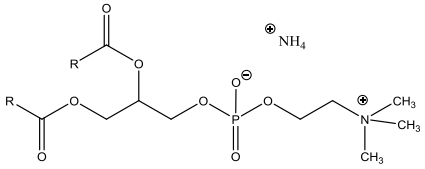
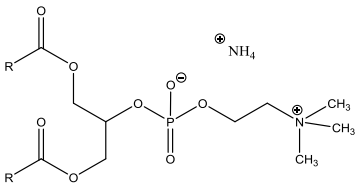
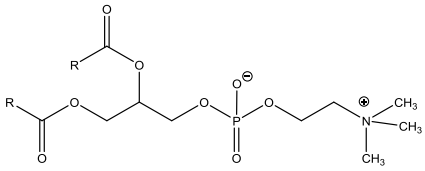
The CIR Expert Panel concluded that the following 17 ingredients are safe in the present practices of use and concentration in cosmetics, as described in this safety assessment. This conclusion supersedes the conclusion that is stated in the 2001 published CIR final report on the safety assessment of lecithin and hydrogenated lecithin.

Lecithin
Hydrogenated Lecithin
Lysolecithin
Hydrogenated Lysolecithin*
Phospholipids
Hydrolyzed Phospholipids*
Phosphatidic Acid*
Lysophosphatidic Acid
Phosphatidylglycerol*
Lysophosphatidylglycerol*
Phosphatidylserine*
Ammonium Phosphatidyl Rapeseedate*
Phosphatidylcholine
Hydrogenated Phosphatidylcholine*
Hydrogenated Lysophosphatidylcholine*
Lysophosphatidylethanolamine*
Phosphatidylinositol*

*Not reported to be in current use. Were ingredients in this group not in current use to be used in the future, the expectation is that they would be used in product categories and at concentrations comparable to others in this group.

Table 1. Names, CAS Registry Numbers, Structures, and Definitions of the Phosphoglyceride Ingredients (INCI Dictionary; Staff)[6]

Ingredient & CAS No.	Definitions, Structures, and Functions
Lecithin 8002-43-5 8030-76-0 93685-90-6	Lecithin is a complex mixture of phosphatides, consisting chiefly of phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositol, with varying amounts of triglycerides, fatty acids, and carbohydrates isolated from animal or vegetable sources **Functions:** Skin-Conditioning Agents - Miscellaneous; Surfactants - Emulsifying Agents  α-Lecithin  β-Lecithin wherein RC(O)- represents the residue of a naturally occurring fatty acid.
Hydrogenated Lecithin 92128-87-5 [308068-11-3]	Hydrogenated Lecithin is the end-product of the controlled hydrogenation of Lecithin. **Functions:** Dispersing Agents - Nonsurfactant; Skin-Conditioning Agents - Miscellaneous; Surfactants - Emulsifying Agents
Lysolecithin (Hydrolyzed Lecithin) 9008-30-4 [85711-58-6]	Lysolecithin is the product obtained from acid, enzyme or other method of hydrolysis of lecithin. **Functions:** Surfactants - Emulsifying Agents
Hydrogenated Lysolecithin	Hydrogenated Lysolecithin is the product obtained by the controlled hydrogenation of Lysolecithin. **Functions:** Surfactants - Emulsifying Agents
Phospholipids 123465-35-0	Phospholipids are complex lipids in which one of the primary hydroxyl groups of glycerin is esterified with phosphoric acid which carries an additional ester grouping. The two remaining hydroxyl groups are esterified with long chain, saturated or unsaturated fatty acids. **Functions:** Skin-Conditioning Agents - Miscellaneous  wherein RC(O)- represents the residue of a naturally occurring fatty acid and R'(either a hydrogen, choline, serine, ethanolamine, or inositol) is "an additional ester grouping."
Hydrolyzed Phospholipids	Hydrolyzed Phospholipids is the hydrolysate of Phospholipids derived by acid, enzyme or other method of hydrolysis. **Functions:** Skin-Conditioning Agents - Miscellaneous
Phosphatidic Acid [308069-40-1]	Phosphatidic Acid is the phospholipid in which one of the primary hydroxyl groups of glycerin is esterified with phosphoric acid; and the two remaining hydroxyl groups are esterified with long chain, saturated or unsaturated fatty acids. **Functions:** Skin-Conditioning Agents - Emollient; Surfactants - Emulsifying Agents 
Lysophosphatidic Acid	Lysophosphatidic Acid is the organic compound that conforms to the formula below **Functions:** Hair Conditioning Agents; Humectants; Skin Protectants; Skin-Conditioning Agents – Miscellaneous  where RCO- represents a long chain saturated or unsaturated fatty acid.

Ingredient & CAS No.	Definitions, Structures, and Functions
Phosphatidylglycerol 92347-24-5	Phosphatidylglycerol is the phospholipid that conforms to the following formula. **Functions:** Emulsion Stabilizers; Skin Protectants; Skin-Conditioning Agents - Miscellaneous; Surfactants - Emulsifying Agents  wherein RC(O)- represents the residue of a naturally occurring fatty acid.
Lysophosphatidylglycerol	Lysophosphatidylglycerol is the hydrolysate of phosphatidylglycerol obtained by the reaction of phospholipase A2. **Functions:** Skin-Conditioning Agents - Humectant; Surfactants - Emulsifying Agents
Phosphatidylserine [1446756-47-3]	Phosphatidylserine is the phospholipid that conforms to the following formula. **Functions:** Emulsion Stabilizers; Hair Conditioning Agents; Skin Protectants; Skin-Conditioning Agents - Miscellaneous  wherein RC(O)- represents the residue of a naturally occurring fatty acid.
Ammonium Phosphatidyl Rapeseedate 100085-59-4	Ammonium Phosphatidyl Rapeseedate is the product formed by the reaction of ammonium phosphatide and hydrogenated rapeseed oil. **Functions:** Emulsion Stabilizers; Skin-Conditioning Agents - Emollient; Surfactants - Emulsifying Agents   wherein RC(O)- represents the residue of a naturally occurring fatty acid common to hydrogenated rapeseed oil.
Phosphatidylcholine [97281-48-6]	Phosphatidylcholine is a purified grade of Lecithin containing no less than 95% of the phospholipid that conforms to the following formula. **Functions:** Skin-Conditioning Agents - Miscellaneous; Surfactants - Emulsifying Agents  wherein RC(O)- represents the residue of a naturally occurring fatty acid.
Hydrogenated Phosphatidylcholine 97281-48-6 [97281-45-3] [92129-12-9]	Hydrogenated Phosphatidylcholine is the end-product of the controlled hydrogenation of Phosphatidylcholine. **Functions:** Skin-Conditioning Agents - Miscellaneous; Surfactants - Emulsifying Agents
Hydrogenated Lysophosphatidylcholine [1332834-64-6]	Hydrogenated Lysophosphatidylcholine is the end-product of the controlled hydrogenation of lysophosphatidylcholine. **Functions:** Emulsion Stabilizers; Skin-Conditioning Agents - Miscellaneous; Surfactants - Emulsifying Agents
Lysophosphatidylethanolamine	Lysophosphatidylethanolamine is the hydrolysate of phosphatidylethanolamine obtained by acid, enzyme or other method of hydrolysis. **Functions:** Humectants; Skin Bleaching Agents; Skin Protectants; Skin-Conditioning Agents - Miscellaneous

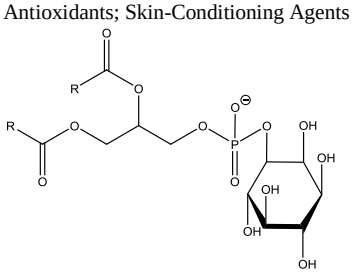
Ingredient & CAS No.	Definitions, Structures, and Functions
Phosphatidylinositol 383907-36-6 [Na salt]	Phosphatidylinositol is the phospholipid that conforms to the following formula **Functions:** Antioxidants; Skin-Conditioning Agents - Miscellaneous; Surfactants - Emulsifying Agents  wherein RC(O)- represents naturally occurring fatty acids.

Table 2. Specifications for Lecithin and Related Ingredients

Chemical	Organoleptic Characteristics	Physico-chemical Characteristics	Microbiological Characteristics
Lecithin trade name material defined as liquid soybean lecithin	Viscous, brown liquid with characteristic odor	Acid insolubles (60-100%); Hexane Insolubles (≤ 0.1%); Acid Value (0-32% KOH/g); Peroxide Value (0-5 mEq/Kg); Gardner color value (<14, when undiluted); Moisture (0-0.8%); Viscosity at 25°C (0-10 Pa·s)	Total Plate Count (< 1,000/g); Yeasts (<30/g); Molds (<30/g); *S. aureus* (absent); *P. aeruginosa* (absent); *C. albicans* (absent).[7]
Lecithin trade name material defined as soybean phospholipids powder	Yellow-brown powder with characteristic odor	Acetone Insolubles (97-100%); Toluene Insolubles (0-0.1%); Hexane Insolubles (≤ 0.3%); Phosphatidylethanolamine (17-22%); Phosphatidic Acid (2-9%); Phosphatidylinositol (12-18%); Phosphatidylcholine (22-27%); Acid Value (0-35 KOH/g); Peroxide Value (0-5 mEq/Kg); Moisture (0-1.5%); pH (6-7 at 1%)	Total Plate Count (<500/g); Molds and Yeasts (<100/g); Coliforms (absent); *Salmomellae* (absent).[8]
Hydrogenated Lecithin trade name material defined as hydrogenated deoiled soybean lecithin	Beige-gray powder with characteristic odor	Phosphatidylcholine (18-26%); Phosphatidylethanolamine (15-22%); Phosphatidylinositol (10-16%); Phosphorus (2.9-3.1%);Residual Protein (undetectable, based on toluene insolubles value); Iodine Value (0-12, using WIJS method); moisture (0-1.5%); pH (6-7.5 at 1%)	Total Plate Count (<1,000/g); Molds and Yeasts (<100/g); *Enterobacteriaceae* (absent); *Salmonellae* (absent).[9]
Lecithin trade name material defined as purified soybean phospholipids	Brown wax with characteristic odor	Toluene Insolubles (< 0.3%); Phosphatidylcholine (45-100%); Phosphatidylethanolamine (10-100%); Phosphatidic Acid (0-3%); Phosphatidylinositol (0-3%); Peroxide Value (0.5 mEq/Kg); Iodine Color Value (0-45 at 10% in toluene); Moisture (0-1%)	Total Plate Count (<1,000/g); Molds and Yeasts (<100/g); *Enterobacteriaceae* (absent); *Salmonellae* (absent).[10]
Phosphatidylcholine trade name material defined as purified soybean phosphatidylcholine	Light yellow flakes with characteristic odor	Toluene Insolubles (< 0.3%); Phosphatidylcholine (92-100%); Lyso-phosphatidylcholine (0-3%); Phosphorus (3.6-3.9%); Peroxide Value (0-5 mEq/Kg); Iodine Value (>95 mg/g); Moisture (0-0.8%)	Total Plate Count (<1,000/g); Molds and Yeasts (<100/g); *Enterobacteriaceae* (absent); *Salmonellae* (absent).[11]
Hydrogenated Lecithin trade name material defined as purified hydrogenated soybean phosphatidylcholine	White powder with characteristic odor	Phosphatidylcholine (94-100%); Lyso-phosphatidylcholine (0-1%); Phosphorus (3.7-4%); Protein (undetectable, using Bradford method); Peroxide Value (0-5 mEq/Kg); Iodine Value (0-3 mg/g); Moisture (0-0.5%)	Total Plate Count (<1,000/g); Molds (<50/g); Yeasts (<50/g); *Enterobacteriaceae* (absent); *Salmonellae* (absent).[12]
Lysolecithin trade name material defined as hydrolyzed soybean lecithin	Viscous brown liquid with characteristic odor	Acetone Insolubles (56-100%); Acid Value (0-40 mg KOH/g); Peroxide Value (0-5 mEq/Kg); Moisture (0-1%); Viscosity at 25°C (0-10 Pa.s); Iodine Color Value (0-65 at 10% in toluene)	Total Plate Count (<1,000/g); Molds and Yeasts (<60/g); *Enterobacteriaceae* (absent); *Salmonellae* (absent).[13]

Table 2. Specifications for Lecithin and Related Ingredients

Chemical	Organoleptic Characteristics	Physico-chemical Characteristics	Microbiological Characteristics
Lecithin trade name material defined as egg lecithin powder	Yellow-brown paste with characteristic odor	Phosphatidylcholine (59-100%); Phosphatidylethanolamine (6-100%); Moisture (0-2.5%); Acid Value (0-25 mg KOH/g); Iodine Value (65-100 mg/g); Peroxide Value (0-5 mEq/Kg); Iodine Color Value (<60 at 10% in toluene); Triglycerides (0-15%); Cholesterol (0-8%)	Total Plate Count (<1,000/g); Molds and Yeasts (<50/g); *Enterobacteriaceae* (absent); *Salmonellae* (absent).[14]
Lecithin trade name material defined as egg oil	Yellow-brown semisolid with characteristic odor	Phospholipids (>30%); Cholesterol (0-5%); Acid Value (0-25 mg KOH/g); Peroxide Value (0-3 mEq/Kg); Iodine Value (>70 g/100 g); Iodine Color Value (<55 at 10% in toluene); Moisture (0-2%)	Total Plate Count (<500/g); Molds and Yeasts (absent); *S. aureus (absent); Enterobacteriaceae* (absent); *Salmonella* (absent).[15]
Phospholipids trade name material defined as lecithin rich in phosphatidylserine	Yellowish powder with characteristic odor	Phosphatidylserine (20-100%); Phosphatidylcholine (10-100%); Phosphatidylethanolamine (0-12%); Phosphatidylinositol (0-13%); Peroxide Value (maximum of 5 mEq/Kg); and moisture (0-2%)	Total Plate Count (< 1,000/g); Molds and Yeasts (<100/g).[16]

Table 3. Current Frequency and Concentration of Use According to Duration and Type of Exposure.[33,34]

	Lecithin		**Hydrogenated Lecithin**		**Lysolecithin**	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Totals/Conc. Range	2045	0.00000008-50	684	0.000001-5	75	0.0001-0.2
Duration of Use						
Leave-On	866	0.00000008-50	605	0.000001-5	62	0.0001-0.2
Rinse off	328	0.0000008-11.5	78	0.00055-5	13	NR
Diluted for (bath) Use	2	0.35	NR	0.2	NR	NR
Exposure Type						
Eye Area	381	0.0005-2.5	79	0.000005-1.5	1	0.0001
Incidental Ingestion	123	0.01-3.4	21	0.001-0.14	3	NR
Incidental Inhalation- Sprays	NR	0.00003-1*	366	0.00003-0.8*	50	0.1
Incidental Inhalation- Powders	1	0.0025-1**	365	0.005-0.56**	46	0.00011-0.2**
Dermal Contact	859	0.00000008-50	640	0.000001-5	72	0.0001-0.2
Deodorant (underarm)	NR	0.000075-0.03	NR	NR	NR	NR
Hair - Non-Coloring	277	0.0000028-2	17	0.00003-1.3	NR	NR
Hair-Coloring	27	0.035	NR	NR	NR	NR
Nail	11	0.002-0.5	NR	0.001	NR	NR
Mucous Membrane	150	0.01-3.4	22	0.001-0.95	3	NR
Baby Products	1	0.015-0.055	2	NR	NR	NR
	Phosphatidylcholine		**Phospholipids**		**Lysophosphatidic Acid**	
	# of Uses	Conc. (%)				
Totals/Conc. Range	34	0.000008-0.8	543	0.000013-0.75	2	NR
Duration of Use						
Leave-On	33	0.26-0.8	275	0.000013-0.75	2	NR
Rinse off	1	0.000008	66	0.0005-0.17	NR	NR
Diluted for (bath) Use	NR	NR	4	0.09	NR	NR
Exposure Type						
Eye Area	11	NR	51	0.0012-0.2	NR	NR
Incidental Ingestion	NR	NR	82	0.6	NR	NR
Incidental Inhalation- Sprays	17	0.8	81	0.001	2***	NR
Incidental Inhalation- Powders	17	NR	82	0.0015-0.75**	2***	NR
Dermal Contact	33	0.000008-0.8	233	0.000013-0.75	NR	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	1	NR	25	0.0005-0.01	NR	NR
Hair-Coloring	NR	NR	5	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	107	0.015-0.6	NR	NR
Baby Products	1	NR	NR	NR	NR	NR

Lecithin is used in perfumes at max. concentrations up to 0.0021%.
Lecithin is used in hairsprays at max. concentrations up to 0.000014% (aerosol) and up to 0.00015% (pump spray).
Lecithin is used in spray deodorants at max. concentrations up to 0.0029% (aerosol) and up to 0.03% (pump spray).
Lecithin is used in face powders at max. concentrations up to 1%.
Hydrogenated lecithin is used in pump hair sprays at max. concentrations up to 0.8%.
Hydrogenated lecithin is used in moisturizing products (sprays) at a max. concentration of 0.65%.
Hydrogenated lecithin is used in face and neck products (sprays) at a max. concentration of 0.5%.
Hydrogenated lecithin is used in body and hand products (sprays) at max. concentrations up to 0.65%.
Hydrogenated lecithin is used in face powders at max. concentrations up to 0.56%.
Lysolecithin is used in body and hand products (sprays) at a max. concentration of 0.1%.
Phosphatidylcholine is used in body and hand products (spray) at a max. concentration of 0.8%.
Phospholipids are used in aerosol hair sprays at max. concentrations up to 0.001%.

NR = Not Reported; Totals = Rinse-off + Leave-on + Diluted for (Bath) Use Product Uses.
*It is possible that these products may be sprays, but it is not specified whether the reported uses are sprays.
**It is possible that these products may be powders, but it is not specified whether the reported uses are powders.
***Not specified whether a powder or spray, so this information is captured for both categories of incidental inhalation.
Note: Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure type uses may not equal the sum total uses.

References

1. Andersen, F. A. Final report on the safety assessment of lecithin and hydrogenated lecithin. *International Journal of Toxicology*. 2001;20(1):21-45.

2. Gennaro, A. R. Remington's Pharmaceutical Sciences. 18th *ed*. Easton: Mack Publishing, 1990.

3. Schlossman, M. L. The chemistry and manufacture of cosmetics. 3rd *ed*. Carol Stream: Allured Publishing Corp., 2002.

4. Barel, A. Paye M. and Maibach H. I. Handbook of cosmetic science and technology. 3rd *ed*. Boca Raton: CRC Press, 2009.

5. Liu, D. and Ma F. Soybean phospholipids, recent trends for enhancing the diversity and quality of soybean products. Chapter 22. Soybean phospholipids. Prof. D. Krezhova (Ed.). ISBN: 978-953-307-533-4. http://www.intechopen.com/books//recent-trends-for-enhancing-the-diversity-and-quality-of-soybean-products/soybean-phospholipids.

6. Nikitakis, J. and Breslawec H. P. International Cosmetic Ingredient Dictionary and Handbook. 14 *ed*. Washington, DC: Personal Care Products Council, 2014.

7. Lucas Meyer Cosmetics S.A.S. Emulmetik™ 100 (lecithin) specification criteria. Unpublished data submitted by the Personal Care Products Council on 10-9-2014. 2013. pp.1-2.

8. Lucas Meyer Cosmetics S.A.S. Emulmetik™ 300 (lecithin) specification criteria. Unpublised data submitted by the Personal Care Products Council on 10-9-2014. 2014. pp.1-2.

9. Lucas Meyer Cosmetics S.A.S. Emulmitek™ 320 (hydrogenated lecithin) specification criteria. Unpublished data submitted by the Personal Care Products Council on 10-9-2014. 2012. pp.1

10. Lucas Meyer Cosmetics S.A.S. Emulmitek™ 900 (lecithin) specification criteria. Unpublished data submitted by the Personal Care Products Council on 10-8-2014. 2012. pp.1

11. Lucas Meyer Cosmetics S.A.S. Emulmetik™ 930 (phosphatidylcholine) specification criteria. Unpublished data submitted by the Personal Care Products Council on 10-9-2014. 2012. pp.1

12. Lucas Meyer Cosmetics S.A.S. Emulmitek™ 950 (hydrogenated lecithin) specification criteria. Unpublished data submitted by the Personal Care Products Council on 10-9-2014. 2013. pp.1

13. Lucas Meyer Cosmetics S.A.S. Emulmetik™ 120 (lysolecithin) specification criteria. Unpublished data submitted by the Personal Care Products Council on 10-9-2014. 2012. pp.1

14. Lucas Meyer Cosmetics S.A.S. Emulmetik™ 970 (lecithin) specification criteria. Unpublished data submitted by the Personal Care Products Council on 10-9-2014. 2014. pp.1

15. Lucas Meyer Cosmetics S.A.S. Ovothin™ 120 (lecithin) specification criteria. Unpublished data submitted by the Personal Care Products Council on 10-9-2014. 2013. pp.1-2.

16. Lucas Meyer Cosmetics S.A.S. LECI-PS 20 P IP (phospholipids) specification criteria. Unpublished data submitted by the Personal Care Products Council on 10-9-2014. 2012. pp.1

17. Food and Drug Administration (FDA). Direct food substances affirmed as generally recognized as safe. Lecithin. 21CFR 184.1400.

18. Food and Drug Administration (FDA). Withdrawn: Guidance on the labeling of certain uses of lecithin derived from soy under section 403(w) of the Federal Food, Drug, and Cosmetic Act. http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/LabelingNutrition/ucm059065.htm.

19. Awazuhara, H. Kawai B. Baba M. Matsui T. and Komiyama A. Antigenicity of the proteins in soy lecithin and soy oil in soybean allergy. *Clin.Exp.Allergy*. 1998;28(12):1559-1564.

20. Food and Drug Administration (FDA). Substances added directly to human food affirmed as generally recognized as safe. Enzyme-modified lecithin. 21CFR 184.1063. 2013.

21. Damnjanovic, J. Nakano H. and Iwasaki Y. Simple and efficient profiling of phospholipids in phospholipase D-modified soy lecithin by HPLC with charged aerosol detection. *Journal of the American Oil Chemists' Society*. 2013;90(7):951-957.

22. Yokozeki Oil & Fat Industries Co., Ltd. Method of manufacture and composition: Phosphatidylglycerol. Unpublished data submitted by the Personal Care Products Council on 10-30-2014. 2014. pp.1

23. Lipogen Products (9000) Ltd. Notification of GRAS determination for soy lecithin phosphatidylserine complex. 2005. pp.1-91.

24. Van Hoogevest, P. and Wendel A. The use of natural and synthetic phospholipids as pharmaceutical excipients. *Eur.J.Lipid Sci.Technol.* 2014;116:1088-1107.

25. Food and Drug Administration (FDA). Database of Select Committee on GRAS substances (SCOGS) reviews. Lecithin. http://www.fda.gov. Date Accessed 8-10-2014.

26. The United States Pharmacopeial Convention. Food Chemicals Codex. 8th *ed*. Rockville: The United States Pharmacopeial Convention, 2012.

27. The United States Pharmacopeial Convention. USP 37. NF 32. 37 *ed*. Rockville: United States Pharmacopoeial Convention, 2014.

28. Pensabene, J. W. Fiddler W. Doerr R. C. Lakritz L. and Wasserman A. E. Formation of dimethylnitrosamine from commercial lecithin and its components in a model system. *J.Agric.Food Chem.* 1975;23:979-980.

29. Hill, M. J. Bacterial metabolism and colon cancer. *Nutr.Cancer*. 1979;1:46-50.

30. Shank RC and Magee PN. Toxicity and carcinogenicity of N-nitroso compounds. Chapter: 1. Shank, R. C. In: *Mycotoxins and N-Nitroso Compounds: Environmental Risks*. Boca Raton, FL: CRC Press, Inc.; 1981:185-217.

31. Rostkowska K, Zwierz K, Rozanski A, Moniuszko-Jakoniuk J, and Roszczenko A. Formation and metabolism of N-nitrosamines. *Polish Journal of Environmental Studies*. 1998;7(6):321-325.

32. Challis BC, Shuker DE, Fine DH, Goff EU, and Hoffman GA. Amine nitration and nitrosation by gaseous nitrogen dioxide. *IARC Sci Publ*. 1982;41:11-20.

33. Food and Drug Administration (FDA). Information supplied to FDA by industry as part of the VCRP FDA database. 2015. Washington, D.C.: FDA.

34. Personal Care Products Council. Concentration of use by FDA product category: Lecithin and other phosphoglycerides. Unpublished data submitted by the Personal Care Products Council on 7-30-2014. 2014. pp.1

35. Rothe H, Fautz R, Gerber E, Neumann L, Rettinger K, Schuh W, and Gronewold C. Special aspects of cosmetic spray safety evaluations: Principles on inhalation risk assessment. *Toxicol Lett*. 2011;205(2):97-104.

36. Bremmer HJ, Prud'homme de Lodder LCH, and van Engelen JGM. Cosmetics Fact Sheet: To assess the risks for the consumer; Updated version for ConsExpo 4. 20200. http://www.rivm.nl/bibliotheek/rapporten/320104001.pdf. Date Accessed 8-24-2011. Report No. RIVM 320104001/2006. pp. 1-77.

37. Rothe H. Special aspects of cosmetic spray evaluation. Unpublished information presented to the 26 September CIR Expert Panel. Washington D.C. 2011.

38. Johnsen MA. The Influence of Particle Size. *Spray Technology and Marketing*. 2004;14(11):24-27.

39. Food and Drug Administration (FDA). Use of prohibited cattle materials in cosmetic products. 21 CFR 700.27. 2014.

40. Food and Drug Administration (FDA). Inactive Ingredients Database. Inactive ingredient search for approved drug products. http://www.accessdata.fda.gov/scripts/cder/iig/getiigWEB.cfm.

41. da Silva, R. P. Kelly K. B. Al Rajabi A. and Jacobs R. L. Novel insights on interactions between folate and lipid metabolism. *Biofactors*. 2014;40(3):277-283.

42. Won, T. J. Nam Y. Lee H. S. Chumng S. Lee J. H. Chung Y. H. Park E. S. Hwang K. W. and Jeong J. H. Injection of phosphatidylcholine and deoxycholic acid regulates gene expression of lipolysis-related factors, pro-inflammatory cytokines, and hormones on mouse fat tissue. *Food and Chemical Toxicology*. 2013;60:263-268.

43. Stein, O. and Stein Y. The fate of intravenously injected 1-C^{14} palmitoyl P^{32}-lysolecithin in the rat. *Life Sciences*. 1965;4:203-208.

44. Pieringer, R. A. and Hokin L. E. Biosynthesis of phosphatidic acid from lysophosphatidic acid and palmitoyl coenzyme A. *Th Journal of Biological Chemistry*. 1962;237(3):659-663.

45. Orlando, P. Cerrito F. and Zirilli P. The fate of double-labelled brain phospholipids administered to mice. *Farmaco*. 1975;30(9):451-458.

46. Mazzari, S. Zanotti A. Orlando P. Raciti R. and Toffano G. Pharmacokinetics and pharmacodynamics of phosphatidylserine liposomes in mice. Antolini, R. Gliozzi A. and Gorio A. In: *Transport in Biomembranes: Model Systems and Reconstitution*. New York: Raven Press; 1982:257-263.

47. Toffano, G. Battistella A. Mazzari S. Orlando P. Massari P. and Giordano C. Fate of phosphatidyl-L-(U-14C) serine in mice. Horrocks, L. A. Ansell G. B. and Porcellati G. In: *Phospholipids in the Nervous System. Volume 1*. New York: Raven Press; 1982:173-180.

48. Toffano, G. Battistella A. and Orlando P. Pharmacokinetics of radiolabelled brain phosphatidylserine. *Clin.Trials J.* 1987;24(1):18-24.

49. Palatini, P. Viola G. Bigon E. Menegus A. M. and Bruni A. Pharmacokinetic characterization of phosphatidylserine liposomes in the rat. *Br.J.Pharmacol.* 1991;102:345-350.

50. Bruni, A. Bellinin F. Mietto L. Boarato E. and Viola G. Phospholipid absorption and diffusion through membranes. Hanin, I. and Pepeu G. In: *Phospholipids. Biochemical, Pharmaceutical, and Analytical Considerations.* New York: Plenum Press; 1990:59-68.

51. Bruni, A. Orlando P. Mietto L. and Viola G. Phospholipid metabolism in rat intestinal mucosa after oral administration of lysophospholipids. Bazan, N. G. In: *Neurobiology of Essential Fatty Acids*. New York: Plenum Press; 1992:

52. Kuge, O. and Nishijima M. Biosynthetic regulation and intracellular transport of phosphatidylserine in mammalian cells. *J.Biochem.* 2003;133(4):397-403.

53. Linder, M. C. Nutrition and metabolism of fats. Linder, M. C. In: *Nutritional Biochemistry and Metabolism: With Clinical Applications*. 2nd ed. New York: Elsevier Science Publishing company, Inc.; 2014:51-84.

54. Krummel, D. Lipids [and Polyunsaturated fatty acids]. Philadelphia/Toronto: W.B. Saunders Company; 1996:49-62.

55. Thorne Research, Inc. Phosphatidylserine. *Alternative Medicine Review*. 2008;13(3):245-247.

56. Fujii, M. Shiozawa K. Watanabe Y. and Matsumoto M. Effect of phosphatidylcholine on skin permeation of indomethacin from gel prepared with liquid paraffin and hydrogenated phospholipid. *International Journal of Pharmaceutics*. 2001;222:57-64.

57. Yokomizo, Y. and Sagitani H. Effects of phospholipids on the perctaneous penetration of indomethacin through the dorsal skin of guinea pigs in vitro. *Journal of Controlled Release*. 1996;38:267-274.

58. Heywood, R. Cozens D. D. and Richold M. Toxicology of a phosphatidylserine preparation from bovine brain (BC-PS). *Clin.Trials J.* 2014;24(1):25-32.

59. Myers, M. A. Thomas D. A. Straub L. Soucy D. W. Niven R. W. Kaltenbach M. Hood C. I. Schreier H. and Gonzalez-Rothi R. J. Pulmonary effects of chronic exposure to liposome aerosols in mice. *Experimental Lung Research*. 1993;19:1-19.

60. Brantom, P. G. Gaunt I. F. Jardy J. Grasso P. and Gangolli S. D. Long-term feeding and reproduction studies on emulsifier YN in rats. *Food Cosmet.Toxico.* 1973;11:755-769.

61. Enzymotec, Ltd. Subchronic toxicity evaluation of phosphatidylserine in elderly rat model. www.enzymotec.com. Date Accessed 7-7-2014.

62. Jorissen, B. L. Brouns F. Van Boxtel M. P. and Riedel W. J. Safety of soy-derived phosphatidylserine in elderly people. *Nutr.Neurosci.* 2002;5(5):337-343.

63. Jeannet, M. and Hassig A. The role of lysophosphatides and fatty acids in hemolysis. *Vox.Sang.* 1964;9:113-127.

64. Robinson, N. Review article. Lysolecithin. *J.Pharm.Pharmacol.* 1961;13(1):321-354.

65. Nagamatsu, T. Iwasawa-Kawai Y. Ichikawa M. Kawana K. Yamashita T. Osuga Y. Fujii T. and Schust D. J. Emerging roles for lysophospholipid mediators in pregnancy. *American Journal of Reproductive Immunology*. 2014;72:182-191.

66. Jo, J. W. Jee B. C. Suh C. S. and Kim S. H. Addition of lysophosphatidic acid to mouse oocyte maturation media can enhance fertilization and developmental competence. *Human Reproduction*. 2014;29(2):234-241.

67. Kato, S. Aoshima H. Saitoh Y. and Miwa N. Biological safety of liposome-fullerene consisting of hydrogenated lecithin, glycine soja sterols, and fullerene-C60 upon photocytotoxicity and bacterial reverse mutagenicity. *Toxicology and Industrial Health*. 2009;25:197-203.

68. Bojanowski, K. Hypodermal delivery of cosmetic actives for improved facial skin morphology and functionality. *International Journal of Cosmetic Science*. 2013;35:562-567.

69. Szepsenwol, J. Brain nerve cell tumors in mice on diets supplemented with various lipids. *Pathol.Miocrobiol.* 1969;34:1-9.

70. Mori, K. Kondo M. and Suzuki S. Induction of lung cancer in mice and rats by injections of 4-nitroquinoline 1-oxide in lecithin. *Gann*. 1966;57:559-561.

71. Middleton, E. Jr. and Phillips G. B. Release of histamine activity in human skin by lysolecithin. *Laboratory and Clinical Medicine*. 1964;64(6):889-894.

72. Beliveau, S. Gaudreault P. Goulet L. Primeau M. N. and Marcoux D. Type I hypersensitivity in an asthmatic child allergic to peanuts: was soy lecithin to blame? *J.Cutan.Med.Surg.* 2008;12(1):27-30.

73. Fernandis, A. Z. and Wenk M. R. Membrane lipids as signaling molecules. *Curr.Opin.Lipidol.* 2007;18(2):121-128.

74. Hannun, Y. A. and Obeid L. M. Principles of bioactive lipid signalling: lessons from sphingolipids. *Molecular Cell Biology*. 2008;9:139-150.

75. Wymann, M. P. and Schneiter R. Lipid signalling in disease. *Nature*. 2008;9:162-176.

76. Balla, T. Phosphoinositides: tiny lipids with giant impact on cell regulation. *Physiol.Rev.* 2013;93(3):1019-1137.

77. Billcliff, P. G. and Lowe M. Inositol lipid phosphatases in membrane trafficking and human disease. *Biochem.J.* 2014;461:159-175.

78. Ridgway, N. D. The role of phosphatidylcholine and choline metabolites to cell proliferation and survival. *Crit.Rev.Biochem.Mol.Biol.* 2013;48(1):20-38.

79. Teo, S. T. Yung Y. C. Herr D. R. and Chun J. Lysophosphatidic acid in vascular development and disease. *IUBMB Life*. 2009;61(8):791-799.

80. Kay, J. G. and Grinstein S. Phosphatidylserine-mediated cellular signaling. *Adv.Exp.Med.Biol.* 2014;991:177-193.

81. Kim, H. Y. Huang B. X. and Spector A. A. Phosphatidylserine in the brain: Metabolism and function. *Prog.Lipid Res.* 2014;56:1-18.

82. Schwartz, H. P. Dreisbach L. Stambaugh R. Kleschick A. and Barrionievo M. Chromatography of the phospholipids of rabbit skin. *Archives of Biochemistry and Biophysics*. 1960;87:171-178.

83. Lampe, M. A. Burlingame A. L. Whitney J. Williams M. L. Brown B. E. Roitman E. and Elias P. M. Human stratum corneum lipids: characterization and regional variations. *Journal of Lipid Research*. 1983;24:120-130.

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Safety Assessment of Lecithin and Other Phosphoglycerides as Used in Cosmetics

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Abstract

The phosphoglycerides considered in this safety assessment are reported to function primarily as skin and hair conditioning agents, emulsifying agents, and surfactants in cosmetic products and are used up to a maximum reported concentration of 50%. Although phospholipids exert physiologic effects, these are not reproduced by application of phospholipid ingredients to the skin. Given the possibility that Lecithin may be derived from animal sources, it should be noted that the Food and Drug Administration does not permit the use of ingredients made from bovine specified risk materials in cosmetic products. The Expert Panel for Cosmetic Ingredient Safety concluded that the 17 phosphoglycerides are safe in the present practices of use and concentration in cosmetics, as described in this safety assessment.

Keywords

lecithin, safety, cosmetics, phosphoglycerides

Introduction

The safety of Lecithin and other phosphoglycerides, listed below, in cosmetics is reviewed in this safety assessment. These 17 ingredients are reported to function primarily as skin and hair conditioning agents, emulsifying agents, and surfactants in cosmetic products.

- Lecithin
- Hydrogenated Lecithin
- Lysolecithin
- Hydrogenated Lysolecithin
- Phospholipids
- Hydrolyzed Phospholipids
- Phosphatidic Acid
- Lysophosphatidic Acid
- Phosphatidylglycerol
- Lysophosphatidylglycerol
- Phosphatidylserine
- Ammonium Phosphatidyl Rapeseedate
- Phosphatidylcholine (PC)
- Hydrogenated PC
- Hydrogenated Lysophosphatidylcholine
- Lysophosphatidylethanolamine
- Phosphatidylinositol

The Expert Panel for Cosmetic Ingredient Safety (Panel) had previously evaluated the safety of Lecithin and Hydrogenated Lecithin in cosmetics and issued a final report (published in 2001) with the following conclusion: Lecithin and Hydrogenated Lecithin are safe as used in rinse-off products, safe for use in leave-on products at concentrations of $\leq 15\%$, and the data are insufficient to determine the safety of use in cosmetic products where Lecithin and Hydrogenated Lecithin are likely to be inhaled; Lecithin and Hydrogenated Lecithin should not be used in cosmetic products in which *N*-nitroso compounds may be formed.[1] The qualification relating to the formation of *N*-nitroso compounds was based on concern over *N*-nitrosation of a potential bacterial metabolite. The 15% concentration limit and the qualification relating to the formation of *N*-nitroso compounds are, however, no longer applicable, and the available data addressing cosmetics that may be inhaled are sufficient.

The Panel considered that phospholipids are the ubiquitous components of cell membranes and play a role in physiological processes. Because phospholipids have a physiologic role only when they are found in certain configurations in cell membranes, dermal application of these cosmetic ingredients does

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not result in the same physiologic effect that result from these chemicals as incorporated into cell membranes.

If it is known that the substance discussed is specifically a cosmetic ingredient, the Ingredient Nomenclature Committee (INC) nomenclature (eg, "Phospholipids") will be used; capitalization of the first letter of each word is used in INC names. However, in cases where a term is used for its general, customary meaning (eg, "phospholipids" as in the paragraph above), standard naming will be used (ie, no capitalization); for chemicals discussed that are not cosmetic ingredients in this assessment (eg, phosphatidylethanolamine), INC-type capitalization will not be used.

Chemistry

Definition and Structure

The ingredients in this report are glycerides of fatty acids, linked to phosphoric acid or to a phosphoric ester. Lecithin, for example, is a complex mixture of phosphatides, consisting chiefly of PC, phosphatidylethanolamine, Phosphatidylserine, and Phosphatidylinositol, with varying amounts of triglycerides, fatty acids, and carbohydrates isolated from animal or vegetable sources.[1] In naturally occurring lecithins, the phosphoric acid is attached to the glycerol at the α-position. However, the phosphoric acid moiety can also be attached in the *β*-position of glycerin, as a by-product of synthesis.[2] A structural scheme that is representative of the systematic nature of the phosphoglycerides family is presented in Figure 1.

Phosphatidylserine is one example of a phosphoglyceride substituted with a fatty acid ester at the secondary alcohol residue of glycerin (ie, is not a lysophosphoglyceride) and an amino acid (ie, serine) attached through the phosphate group, as depicted in Figure 2.

Hydrogenated Lecithin (CAS No. 92128-87-5) is the end product of the controlled hydrogenation of Lecithin.[1] Despite the ionic, or even zwitterionic, natures of these ingredients, these ingredients are mostly insoluble in water.[3] These ingredients typically are waxy, hygroscopic substances that swell in contact with water to form, dependent on their molecular composition and structure, liposomes, micelles, or mixed micelles.

The main sources of naturally occurring phosphoglycerides, such as Lecithin, as used in personal care products, are maize, egg yolk, and soybean.[4] Phospholipids constitute 0.3% to 0.6% of soybean seed, or 1.5% to 3.0% of crude soybean oil. The composition of phospholipids in soybeans has been reported as follows[5]:

- PC (12%-46%)
- Phosphatidylethanolamine (8%-34%)
- Phosphatidylinositol (1.7%-21%)
- Phosphatidic Acid (0.2%-14%)
- Phosphatidylserine (0.2%-6.3%)
- Lysophosphatidylcholine (1.5%-8.5%)
- Lysophosphatidylinositol (0.4%-1.8%)
- Lysophosphatidylserine (1%)
- Lysophosphatidic Acid (1%)

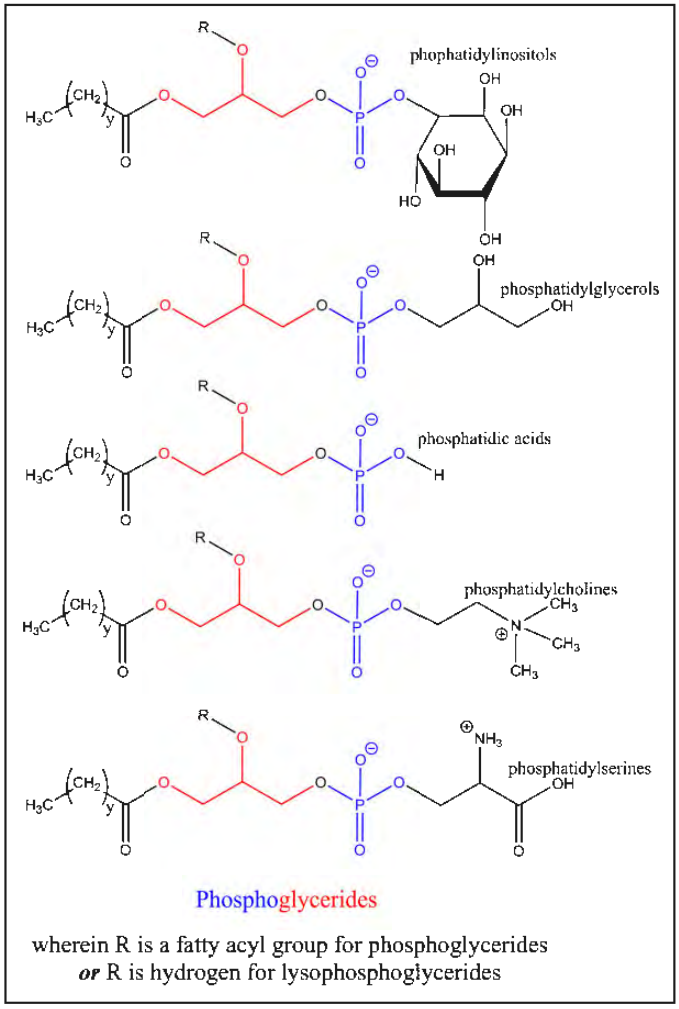


Figure 1. Phosphoglycerides.

The definitions, structures, and functions of the phosphoglycerides reviewed in this safety assessment are included in Table 1.[6] There is some overlap among the ingredients in this report. While Phosphatidylserine and PC are listed as separate ingredients, they are likely components of the ingredient named Phospholipids.

The ingredients in this report form a systematic, logical grouping that interrelates on numerous levels. All of these ingredients: (1) are glycerides of fatty acids, linked to phosphoric acid, or to a phosphoric ester; (2) conform to Figure 1; (3) are ionic and mostly insoluble in water; (4) are typically waxy, hygroscopic substances that swell, when in contact with water, to form liposomes; and (5) either come from sources such as maize, egg yolk, and soybean, or are synthesized via transphosphatidylation of PC (which itself is sourced from soy).

Chemical Properties

Specifications for Lecithin and related ingredients are presented in Table 2.[7-16] Included are chemical properties data and microbiological specifications.

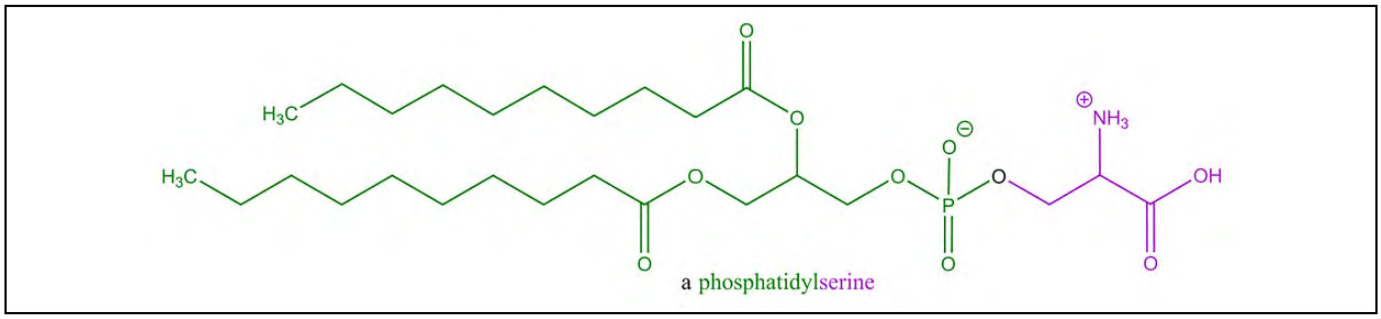


Figure 2. One example of a phosphoglyceride, Phosphatidylserine (depicted is just 1 example of the possible fatty acyl chain lengths).

Method of Manufacture

Lecithin and Lecithin (Enzyme-Modified)

Commercial Lecithin is isolated as a gum following hydration of solvent-extracted soy, safflower, or corn oils.[17] Lecithin is bleached, if desired, by hydrogen peroxide and benzoyl peroxide, and dried by heating. During the manufacture of Lecithin derived from soy, most, if not all, of the soy protein is removed. If present, soy allergens would be found in the protein fraction.[18] According to another source, soy Lecithin is usually produced from the hexane extract of soybean.[19]

In addition to the commercial Lecithin mentioned above, it should be noted that another form of Lecithin, enzyme-modified Lecithin (ie, Lysolecithin), is prepared by treating Lecithin with either phospholipase A2 or pancreatin.[20]

Phosphoglycerides

Synthetic phosphoglycerides can be produced via phospholipase D-catalyzed transphosphatidylation of PC (which is abundant in soy Lecithin) with the desired phosphate substituent (eg, myo-inositol for the synthesis of Phosphatidylinositol).[21]

Phosphatidylglycerol

Phosphatidylcholine (minimum purity of 90%) in the presence of enzyme and glycerin yields Phosphatidylglycerol (minimum purity of 85%) and choline.[22]

Phosphatidylserine

Soy-derived Phosphatidylserine (phosphatidylserine complex derived from soy lecithin) consists of serine-substituted soy lecithin phospholipids and other phospholipids occurring naturally in lecithin.[23] Production of such soy lecithin phosphatidylserine complex involves the enzymatic transphosphatidylation of PC and phosphatidylethanolamine from soy lecithin (via cabbage-derived phospholipase in the presence of exogenous serine) to Phosphatidylserine. The production of the phosphatidylserine-enriched complex proceeds without the use of solvents during the manufacturing process. Thus, the final soy lecithin phosphatidylserine complex is solvent-free.

In addition to the preceding methods for manufacturing phospholipids, it should be noted that various other methods have been described in detail.[24]

Composition

Lecithin and Lecithin (enzyme-modified)

Composition data on various phosphoglyceride tradename materials are included in Table 2. Commercial Lecithin is a naturally occurring mixture of the phosphatides of choline, ethanolamine, and inositol, with smaller amounts of other lipids.[17] Practically all of the Lecithin in commerce is derived from soybeans. Phosphoglycerides are the major constituents of Lecithin, and commercial Lecithin may contain up to 35% triglycerides.[25]

Impurities

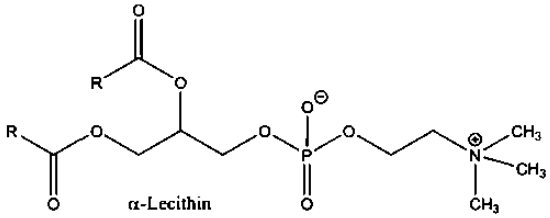
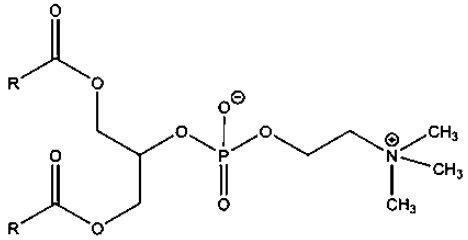
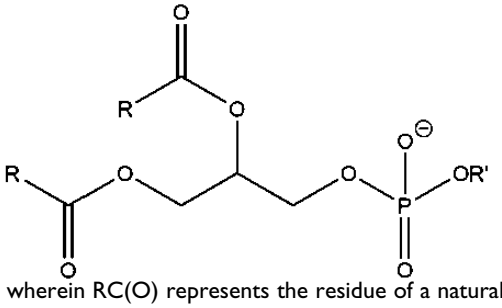
As US Food and Drug Administration (FDA) approved direct food additive for human food consumption, Lecithin (enzyme-modified) meets the following specifications[20]:

- Acetone-insoluble matter (phosphatides), not less than 50%
- Acid value, not more than 40
- Lead, not more than 1.0 part per million, as determined by atomic absorption spectroscopy
- Heavy metals (as Pb), not more than 20 ppm
- Hexane-insoluble matter, not more than 0.3%
- Peroxide value, not more than 20
- Water, not more than 4%
- Lysolecithin, 50 to 80 mole% of total phosphatides

The Food Chemicals Codex[26] stipulates that food grade Lecithin must not contain more than 0.3% hexane-insoluble matter. Because the protein fraction of Lecithin would reside in this insoluble material, this specification limits the amount of protein in food grade Lecithin to 0.3% or 300 mg/100 g of Lecithin.

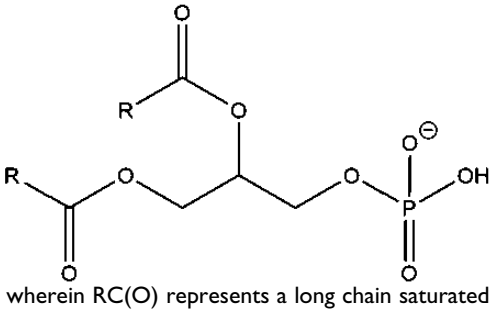
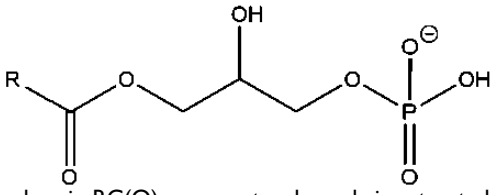
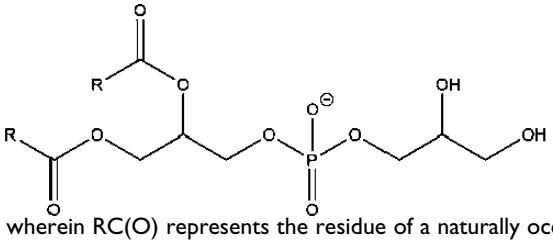
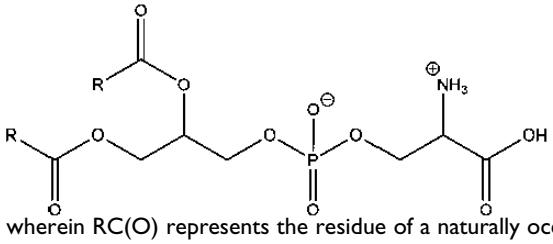
The United States Pharmacopeia (USP) stipulates that sunflower Lecithin in food contain not more than 1% hexane-insoluble matter.[27] It is also stipulated in the USP that soy Lecithin contain not more than 20 ppm heavy metals and not more than 10 ppm lead. Potential impurities are included in the

Table 1. Names, CAS Registry Numbers, Structures, and Definitions of the Phosphoglyceride Ingredients.[6]

Ingredient and CAS No.	Definitions, structures, and reported functions
Lecithin 8002-43-5 8030-76-0 93685-90-6	Lecithin is a complex mixture of phosphatides, consisting chiefly of PC, phosphatidylethanolamine, Phosphatidylserine, and Phosphatidylinositol, with varying amounts of triglycerides, fatty acids, and carbohydrates isolated from animal or vegetable sources **Reported Functions:** Skin-Conditioning Agents—Miscellaneous; Surfactants—Emulsifying Agents  α-Lecithin  β-Lecithin wherein RC(O) represents the residue of a naturally occurring fatty acid.
Hydrogenated Lecithin 92128-87-5 308068-11-3	Hydrogenated Lecithin is the end product of the controlled hydrogenation of Lecithin. **Reported Functions:** Dispersing Agents—Nonsurfactant; Skin-Conditioning Agents—Miscellaneous; Surfactants—Emulsifying Agents
Lysolecithin (Hydrolyzed Lecithin) 9008-30-4 85711-58-6	Lysolecithin is the product obtained from acid, enzyme, or other method of hydrolysis of Lecithin. **Reported Functions:** Surfactants—Emulsifying Agents
Hydrogenated Lysolecithin	Hydrogenated Lysolecithin is the product obtained by the controlled hydrogenation of Lysolecithin. **Reported Functions:** Surfactants—Emulsifying Agents
Phospholipids 123465-35-0	Phospholipids are complex lipids in which one of the primary hydroxyl groups of glycerin is esterified with phosphoric acid which carries an additional ester grouping. The 2 remaining hydroxyl groups are esterified with long chain, saturated, or unsaturated fatty acids. **Reported Functions:** Skin-Conditioning Agents—Miscellaneous  wherein RC(O) represents the residue of a naturally occurring fatty acid and R'(either a hydrogen, choline, serine, ethanolamine, or inositol) is "an additional ester grouping."
Hydrolyzed Phospholipids	Hydrolyzed Phospholipids is the hydrolysate of Phospholipids derived by acid, enzyme, or other method of hydrolysis. **Reported Functions:** Skin-Conditioning Agents—Miscellaneous

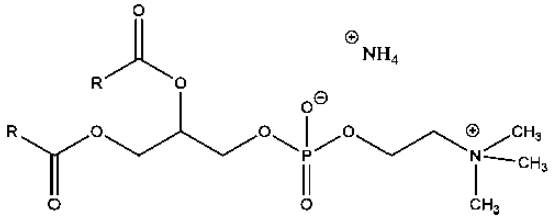
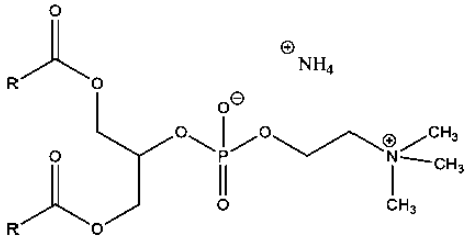
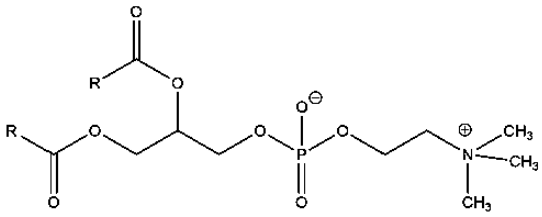
(continued)

Table 1. (continued)

Ingredient and CAS No.	Definitions, structures, and reported functions
Phosphatidic Acid 308069-40-1	Phosphatidic Acid is the phospholipid in which one of the primary hydroxyl groups of glycerin is esterified with phosphoric acid; and the two remaining hydroxyl groups are esterified with long chain, saturated, or unsaturated fatty acids. **Reported Functions:** Skin-Conditioning Agents—Emollient; Surfactants—Emulsifying Agents  wherein RC(O) represents a long chain saturated or unsaturated fatty acid.
Lysophosphatidic Acid	Lysophosphatidic Acid is the organic compound that conforms to the formula below. **Reported Functions:** Hair Conditioning Agents; Humectants; Skin Protectants; Skin-Conditioning Agents—Miscellaneous  wherein RC(O) represents a long chain saturated or unsaturated fatty acid.
Phosphatidylglycerol 92347-24-5	Phosphatidylglycerol is the phospholipid that conforms to the following formula. **Reported Functions:** Emulsion Stabilizers; Skin Protectants; Skin-Conditioning Agents—Miscellaneous; Surfactants—Emulsifying Agents  wherein RC(O) represents the residue of a naturally occurring fatty acid.
Lysophosphatidylglycerol	Lysophosphatidylglycerol is the hydrolysate of Phosphatidylglycerol obtained by the reaction of phospholipase A2. **Reported Functions:** Skin-Conditioning Agents—Humectant; Surfactants—Emulsifying Agents
Phosphatidylserine (1446756-47-3)	Phosphatidylserine is the phospholipid that conforms to the following formula. **Reported Functions:** Emulsion Stabilizers; Hair Conditioning Agents; Skin Protectants; Skin-Conditioning Agents—Miscellaneous  wherein RC(O) represents the residue of a naturally occurring fatty acid.

(continued)

Table 1. (continued)

Ingredient and CAS No.	Definitions, structures, and reported functions
Ammonium Phosphatidyl Rapeseedate 100085-59-4	Ammonium Phosphatidyl Rapeseedate is the product formed by the reaction of ammonium phosphatide and hydrogenated rapeseed oil. **Reported Functions:** Emulsion Stabilizers; Skin-Conditioning Agents—Emollient; Surfactants—Emulsifying Agents   wherein RC(O) represents the residue of a naturally occurring fatty acid common to hydrogenated rapeseed oil.
PC 97281-48-6	PC is a purified grade of Lecithin containing no less than 95% of the phospholipid that conforms to the following formula. **Reported Functions:** Skin-Conditioning Agents—Miscellaneous; Surfactants—Emulsifying Agents  wherein RC(O) represents the residue of a naturally occurring fatty acid.
Hydrogenated PC 97281-48-6 97281-45-3 92129-12-9	Hydrogenated PC is the end product of the controlled hydrogenation of PC. **Reported Functions:** Skin-Conditioning Agents—Miscellaneous; Surfactants—Emulsifying Agents
Hydrogenated Lysophosphatidylcholine 1332834-64-6	Hydrogenated Lysophosphatidylcholine is the end-product of the controlled hydrogenation of lysophosphatidylcholine. **Reported Functions:** Emulsion Stabilizers; Skin-Conditioning Agents—Miscellaneous; Surfactants—Emulsifying Agents
Lysophosphatidylethanolamine	Lysophosphatidylethanolamine is the hydrolysate of phosphatidylethanolamine obtained by acid, enzyme, or other method of hydrolysis. **Reported Functions:** Humectants; Skin Bleaching Agents; Skin Protectants; Skin-Conditioning Agents—Miscellaneous

(continued)

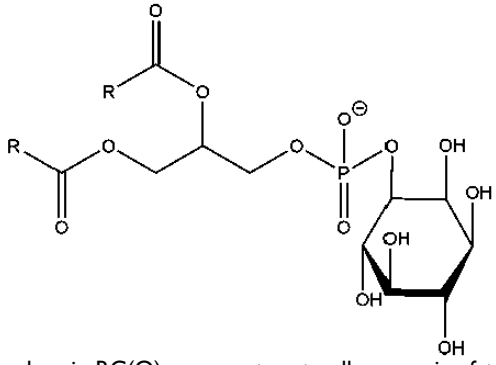
specifications for various phosphoglyceride tradename materials that are summarized in Table 2.[7-16]

Nitrosamine Formation

Lecithin

Dimethyl nitrosamine (DMNA) was reportedly formed in a model system in which 22.8 mmol sodium nitrite in 15 mL of water was added to a buffered solution, pH 5.6, containing 4.56 mmol of Lecithin and stirred at 78 °C for 4 hours.[28] The amount of DMNA formed (mg DMNA/kg of compound), confirmed by mass spectrometry, with various lecithins was as follows: soy Lecithin (edible), 2.05 ppm; soy Lecithin (commercial), 0.70 ppm; vegetable Lecithin, 1.02 ppm; egg Lecithin, 5.40 ppm; bovine Lecithin (purified), 1.66 ppm; bovine Lecithin (60%), 30.76 ppm; and synthetic Lecithin, 319.7 ppm.

Table 1. (continued)

Ingredient and CAS No.	Definitions, structures, and reported functions
Phosphatidylinositol 383907-36-6 (sodium salt)	Phosphatidylinositol is the phospholipid that conforms to the following formula. **Reported Functions:** Antioxidants; Skin-Conditioning Agents—Miscellaneous; Surfactants—Emulsifying Agents  wherein RC(O) represents naturally occurring fatty acids.

Lecithin is metabolized to choline by bacterial phospholipases and the released choline is dealkylated to dimethylamine, which is *N*-nitrosatable in the presence of nitrate.[29] Of concern in cosmetics is the conversion (nitrosation) of nitrogen-bearing ingredients into *N* nitroso chemicals that may be carcinogenic. Of the approximately 209 nitrosamines tested, 85% have been shown to produce cancer in laboratory animals.[30] Nitrosation can occur under physiologic conditions.[31] Depending on the nitrosating agent and the substrate, nitrosation can occur under acidic, neutral, or alkaline conditions. Atmospheric NO_2 may also participate in nitrosation in aqueous solution.[32]

Use

Cosmetic

The ingredients reviewed in this safety assessment function mainly as skin and hair conditioning agents, emulsifying agents, and surfactants in cosmetic products.[6] According to information supplied to the US FDA by industry as part of the Voluntary Cosmetic Registration Program, and the results from a survey of ingredient use concentrations conducted by the Personal Care Products Council (Council), the following phosphoglycerides are used in cosmetic products at maximum concentrations ranging, for different product categories, from 0.00000008% (Lecithin in skin cleansing products and face products) to 50% (Lecithin in foot products [leave-on])[33,34]: Lecithin, Hydrogenated Lecithin, Lysolecithin, Lysophosphatidic Acid, PC, and Phospholipids. The reported highest maximum use concentrations for other ingredients evaluated in this safety assessment are as follows, all relating to use in leave-on products: Hydrogenated Lecithin (5%, face and neck products [not spray]), Lysolecithin (0.2%, in face and neck products [not spray]), PC (0.8%, in body and hand products [not spray]), and Phospholipids (0.75%, in face and neck products [not spray]). Use frequency and concentration of use data are presented in Table 3.

According to the Panel final safety assessment on Lecithin and Hydrogenated Lecithin published in 2001, data received from FDA in 1984 indicated that the maximum reported use concentration of Lecithin was in the 25% to 50% concentration range; use concentration data on Hydrogenated Lecithin were not included.[1] Concentration of use data provided by the Council in 1996 indicated that 65% Lecithin was used at concentrations of 0.1% to 3%; use concentration data on Hydrogenated Lecithin were not provided.[1]

Cosmetic products containing phosphoglycerides may be applied to the skin and hair or, incidentally, may come in contact with the eyes and mucous membranes. Products containing these ingredients may be applied as frequently as several times per day and may come in contact with the skin or hair for variable periods following application. Daily or occasional use may extend over many years.

Lecithin is used in perfumes at maximum concentrations up to 0.0021% and in hairspray formulations at maximum concentrations up to 0.000014% (aerosol) and up to 0.00015% (pump spray); Hydrogenated Lecithin is used in pump hair spray formulations at maximum concentrations up to 0.8%. Lecithin is also used in spray deodorants at maximum concentrations up to 0.0029% (aerosol) and up to 0.03% (pump spray). Phospholipids are used in aerosol hair spray at maximum concentrations up to 0.8%, and Lysolecithin, PC, and Hydrogenated Lecithin are used in body and hand sprays at maximum concentrations up to 0.1%, 0.8%, and 0.65%, respectively. Hydrogenated Lecithin is used in moisturizing sprays and face and neck sprays at maximum concentrations of 0.65% and 0.5%, respectively. Ingredient use in face powders is also reported for Lecithin (up to 1%) and Hydrogenated Lecithin (up to 0.56%). Because phosphoglycerides are used in products that are sprayed or in powder form, these could be incidentally inhaled. In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters > 10 μm, with propellant sprays yielding a greater

Table 2. Specifications for Lecithin and Related Ingredients.

Chemical	Organoleptic characteristics	Physicochemical characteristics	Microbiological characteristics
Lecithin trade name material defined as liquid soybean Lecithin	Viscous, brown liquid with characteristic odor	Acid insolubles (60-100%); Hexane Insolubles (≤ 0.1%); Acid Value (0%-32% KOH/g); Peroxide Value (0-5 mEq/Kg); Gardner color value (<14, when undiluted); Moisture (0-0.8%); Viscosity at 25 °C (0-10 Pa·s)	Total Plate Count (< 1,000/g); Yeasts (<30/g); Molds (<30/g); *Staphylococcus aureus* (absent); *Pseudomonas aeruginosa* (absent); *Candida albicans* (absent).[7]
Lecithin trade name material defined as soybean phospholipids powder	Yellow-brown powder with characteristic odor	Acetone Insolubles (97%-100%); Toluene Insolubles (0%-0.1%); Hexane Insolubles (≤ 0.3%); Phosphatidylethanolamine (17%-22%); Phosphatidic Acid (2%-9%); Phosphatidylinositol (12%-18%); PC (22%-27%); Acid Value (0-35 KOH/g); Peroxide Value (0-5 mEq/Kg); Moisture (0%-1.5%); pH (6-7 at 1%)	Total Plate Count (<500/g); Molds and Yeasts (<100/g); Coliforms (absent); *Salmonellae* (absent).[8]
Hydrogenated Lecithin trade name material defined as hydrogenated deoiled soybean Lecithin	Beige-gray powder with characteristic odor	PC (18-26%); Phosphatidylethanolamine (15%-22%); Phosphatidylinositol (10%-16%); Phosphorus (2.9%-3.1%); Residual Protein (undetectable, based on toluene insolubles value); Iodine Value (0-12, using WIJS method); moisture (0%-1.5%); pH (6-7.5 at 1%)	Total Plate Count (<1,000/g); Molds and Yeasts (<100/g); *Enterobacteriaceae* (absent); *Salmonellae* (absent).[9]
Lecithin trade name material defined as purified soybean phospholipids	Brown wax with characteristic odor	Toluene Insolubles (< 0.3%); PC (45%-100%); Phosphatidylethanolamine (10%-100%); Phosphatidic Acid (0%-3%); Phosphatidylinositol (0%-3%); Peroxide Value (0.5 mEq/Kg); Iodine Color Value (0-45 at 10% in toluene); Moisture (0-1%)	Total Plate Count (<1,000/g); Molds and Yeasts (<100/g); *Enterobacteriaceae* (absent); *Salmonellae* (absent).[10]
PC trade name material defined as purified soybean PC	Light yellow flakes with characteristic odor	Toluene Insolubles (< 0.3%); PC (92%-100%); Lysophosphatidylcholine (0%-3%); Phosphorus (3.6%-3.9%); Peroxide Value (0-5 mEq/Kg); Iodine Value (>95 mg/g); Moisture (0%-0.8%)	Total Plate Count (<1,000/g); Molds and Yeasts (<100/g); *Enterobacteriaceae* (absent); *Salmonellae* (absent).[11]
Hydrogenated Lecithin trade name material defined as purified hydrogenated soybean PC	White powder with characteristic odor	PC (94%-100%); Lysophosphatidylcholine (0%-1%); Phosphorus (3.7%-4%); Protein (undetectable, using Bradford method); Peroxide Value (0-5 mEq/Kg); Iodine Value (0-3 mg/g); Moisture (0%-0.5%)	Total Plate Count (<1,000/g); Molds (<50/g); Yeasts (<50/g); *Enterobacteriaceae* (absent); *Salmonellae* (absent).[12]
Lysolecithin trade name material defined as hydrolyzed soybean Lecithin	Viscous brown liquid with characteristic odor	Acetone Insolubles (56%-100%); Acid Value (0-40 mg KOH/g); Peroxide Value (0-5 mEq/Kg); Moisture (0%-1%); Viscosity at 25 °C (0-10 Pa.s); Iodine Color Value (0-65 at 10% in toluene)	Total Plate Count (<1,000/g); Molds and Yeasts (<60/g); *Enterobacteriaceae* (absent); *Salmonellae* (absent).[13]
Lecithin trade name material defined as egg Lecithin powder	Yellow-brown paste with characteristic odor	PC (59%-100%); Phosphatidylethanolamine (6%-100%); Moisture (0%-2.5%); Acid Value (0-25 mg KOH/g); Iodine Value (65-100 mg/g); Peroxide Value (0-5 mEq/Kg); Iodine Color Value (<60 at 10% in toluene); Triglycerides (0%-15%); Cholesterol (0%-8%)	Total Plate Count (<1,000/g); Molds and Yeasts (<50/g); *Enterobacteriaceae* (absent); *Salmonellae* (absent).[14]
Lecithin trade name material defined as egg oil	Yellow-brown semisolid with characteristic odor	Phospholipids (>30%); Cholesterol (0%-5%); Acid Value (0-25 mg KOH/g); Peroxide Value (0-3 mEq/Kg); Iodine Value (>70 g/100 g); Iodine Color Value (<55 at 10% in toluene); Moisture (0%-2%)	Total Plate Count (<500/g); Molds and Yeasts (absent); *S aureus (absent); Enterobacteriaceae* (absent); *Salmonella* (absent).[15]
Phospholipids trade name material defined as Lecithin rich in Phosphatidylserine	Yellowish powder with characteristic odor	Phosphatidylserine (20%-100%); PC (10%-100%); Phosphatidylethanolamine (0%-12%); Phosphatidylinositol (0%-13%); Peroxide Value (maximum of 5 mEq/Kg); and moisture (0%-2%)	Total Plate Count (< 1,000/g); Molds and Yeasts (<100/g).[16]

fraction of droplets/particles below 10 μm, compared with pump sprays.[35-38] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (ie, they would not enter the lungs) to any appreciable amount.[35,36] There is some evidence indicating that deodorant spray products can release substantially larger fractions of particulates having aerodynamic equivalent diameters in the range considered to be respirable.[36] However, the information is not sufficient to determine whether significantly greater lung exposures result from the use of deodorant sprays, compared to other cosmetic sprays.

Because of concerns about potential transmission of bovine spongiform encephalopathy, cosmetic products are not permitted to contain ingredients made from bovine specified risk materials, which includes the central nervous system.[39]

Non Cosmetic

Commercial Lecithin, defined as a naturally occurring mixture of the phosphatides of choline, ethanolamine, and inositol, is a direct food substance affirmed as generally recognized as safe (GRAS).[17] Additionally, enzyme-modified Lecithin is listed among the substances added directly to human food affirmed as GRAS.[20] Food uses of Lecithin include emulsifier, stabilizer, dispersing aid, and release agent for baked goods.[18] Lecithin is also used in topical medicaments.

The Food Allergen Labeling and Consumer Protection act (FALCPA) of 2004 altered the way in which Lecithin derived from soy must be declared on food labels. Whether intended to have a technical or functional effect in the finished food or to be used as an incidental additive (such as a release agent), Lecithin that is derived from soy must be declared as an ingredient, using its common or usual name, and with the food source ("soy," "soya," or "soybeans") declared as required by section 403(w) of the Act.[18]

Lecithin (source not stated) is listed as an inactive ingredient in inhaled (aerosol, metered; maximum potency = 0.0002%) drug products that have been approved by FDA, and the same is true for soybean Lecithin (aerosol, metered; maximum potency = 0.1%). Additionally, the approval of soy Hydrogenated Lecithin as an inactive ingredient in inhaled (aerosol, metered; maximum potency = 0.28%) drug products by FDA was pending at the time of this review.[40]

Phosphatidylcholine is the most abundant phospholipid in mammalian cellular membranes, bile, and lipoproteins.[41] The injection of a PC and deoxycholic acid preparation is widely used as an alternative to liposuction for the reduction of subcutaneous fat.[42]

Toxicokinetics

Nonhuman

Lecithin and Lysolecithin. The distribution of intravenously (IV) injected 1-[14C] palmitoyl-[32P]-lysolecithin was studied using male albino rats (number not stated).[43] The animals were injected IV with 1 mL of rat serum containing endogenously labeled [32P] Lysolecithin or with 1 mL of rat serum containing endogenously labeled [32P]-phospholipids. The amount of Lysolecithin incorporated into 1 mL of serum ranged between 550 and 850 μg. In some of the experiments, Lysolecithin labeled with both [32P] and [14C] (in fatty acid moiety) was used. At 20 and 60 minutes postinjection, 45% and 77% of injected radioactive material was removed from the blood, respectively. There was an uptake of labeled phospholipids by heart and skeletal muscle. The percentage of [32P]-Lysolecithin in these organs was much higher than in the injected material. The authors noted that the high percentage of labeled Lysolecithin in the skeletal and heart muscle indicated that Lysolecithin might leave the vascular compartment more rapidly than Lecithin and be metabolized by the tissues. The percent composition of labeled phospholipids found in the liver resembled that of the injected serum, with Lecithin as the major labeled component. However, the percentage of Lysolecithin was lower than that in the starting material. The authors noted that, based on these results, it seems more likely that the liver takes up phospholipids indiscriminately and that Lysolecithin is rapidly converted to Lecithin.

In subsequent experiments in which rats (number not stated) were injected with serum containing [32P] Lysolecithin, the labeled Lysolecithin disappeared from the bloodstream very rapidly ($t_{1/2} \sim 2$ minutes). Considerable amounts of Lysolecithin were recovered in the liver and skeletal muscle at a time when the serum radioactivity decreased to negligible levels. The conversion of Lysolecithin to Lecithin was observed in all the tissues examined, and this reaction was most rapid in the liver. Lysolecithin taken up by the liver was converted to Lecithin by an acylation reaction.[43]

Lysophosphatidic Acid. Lysophosphatidic Acid was degraded to glycerophosphate and orthophosphate by phosphatidases and phosphatases, respectively, in an enzyme preparation, that is, cytoplasmic particulate fraction of guinea pig brain or liver.[44]

Phosphatidylserine. Following IV administration to rats and mice, Phosphatidylserine was eliminated from plasma in a biphasic manner and largely distributed to several major organs, including the liver spleen and brain.[45-49] Conversely, orally administered Phosphatidylserine was extensively hydrolyzed by phospholipase A_2 to lysophosphatidylserine in the gastrointestinal tract prior to absorption, as is the case for all other dietary phospholipids.[45,50,51] In rats, approximately 60% of an orally administered dose of Phosphatidylserine (20 mg/kg body weight) was recovered in the feces, of which 50% was identified as lysophosphatidylserine. Approximately 10% of the orally administered dose was detected in the urine.[48]

Studies in which animals were injected IV with radiolabled Phosphatidylserine also indicate that phospholipids undergo hydrolytic cleavage to the monoacyl derivative lysophosphatidylserine in the plasma as well as decarboxylation of the serine moiety to phosphatidylethanolamine in circulating blood cells.[46,47,49] Lysophosphatidylserine and

Table 3. Current Frequency and Concentration of Use According to Duration and Type of Exposure.[33,34]

	Lecithin		Hydrogenated lecithin		Lysolecithin	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Totals/Conc. range	2045	0.00000008-50	684	0.000001-5	75	0.0001-0.2
Duration of use						
Leave on	866	0.00000008-50	605	0.000001-5	62	0.0001-0.2
Rinse off	328	0.0000008-11.5	78	0.00055-5	13	NR
Diluted for (bath) use	2	0.35	NR	0.2	NR	NR
Exposure type						
Eye area	381	0.0005-2.5	79	0.000005-1.5	1	0.0001
Incidental ingestion	123	0.01-3.4	21	0.001-0.14	3	NR
Incidental inhalation—sprays	NR	0.00003-1*	366	0.00003-0.8*	50	0.1
Incidental inhalation—powders	1	0.0025-1**	365	0.005-0.56**	46	0.00011-0.2**
Dermal contact	859	0.00000008-50	640	0.000001-5	72	0.0001-0.2
Deodorant (underarm)	NR	0.000075-0.03	NR	NR	NR	NR
Hair—non-coloring	277	0.0000028-2	17	0.00003-1.3	NR	NR
Hair-coloring	27	0.035	NR	NR	NR	NR
Nail	11	0.002-0.5	NR	0.001	NR	NR
Mucous membrane	150	0.01-3.4	22	0.001-0.95	3	NR
Baby products	1	0.015-0.055	2	NR	NR	NR

	PC		Phospholipids		Lysophosphatidic Acid	
	# of Uses	Conc. (%)				
Totals/conc. range	34	0.000008-0.8	543	0.000013-0.75	2	NR
Duration of use						
Leave on	33	0.26-0.8	275	0.000013-0.75	2	NR
Rinse off	1	0.000008	66	0.0005-0.17	NR	NR
Diluted for (bath) use	NR	NR	4	0.09	NR	NR
Exposure type						
Eye area	11	NR	51	0.0012-0.2	NR	NR
Incidental ingestion	NR	NR	82	0.6	NR	NR
Incidental inhalation—sprays	17	0.8	81	0.001	2***	NR
Incidental inhalation—powders	17	NR	82	0.0015-0.75**	2***	NR
Dermal contact	33	0.000008-0.8	233	0.000013-0.75	NR	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair—non-coloring	1	NR	25	0.0005-0.01	NR	NR
Hair-coloring	NR	NR	5	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous membrane	NR	NR	107	0.015-0.6	NR	NR
Baby products	1	NR	NR	NR	NR	NR

Lecithin is used in perfumes at max. concentrations up to 0.0021%.
Lecithin is used in hairsprays at max. concentrations up to 0.000014% (aerosol) and up to 0.00015% (pump spray).
Lecithin is used in spray deodorants at max. concentrations up to 0.0029% (aerosol) and up to 0.03% (pump spray).
Lecithin is used in face powders at max. concentrations up to 1%.
Hydrogenated Lecithin is used in pump hair sprays at max. concentrations up to 0.8%.
Hydrogenated Lecithin is used in moisturizing products (sprays) at a max. concentration of 0.65%.
Hydrogenated Lecithin is used in face and neck products (sprays) at a max. concentration of 0.5%.
Hydrogenated Lecithin is used in body and hand products (sprays) at max. concentrations up to 0.65%.
Hydrogenated Lecithin is used in face powders at max. concentrations up to 0.56%.
Lysolecithin is used in body and hand products (sprays) at a max. concentration of 0.1%.
Phosphatidylcholine is used in body and hand products (spray) at a max. concentration of 0.8%.
Phospholipids are used in aerosol hair sprays at max. concentrations up to 0.001%.
NR = Not Reported; Totals = Rinse-off + Leave-on + Diluted for (Bath) Use Product Uses.
*It is possible that these products may be sprays, but it is not specified whether the reported uses are sprays.
**It is possible that these products may be powders, but it is not specified whether the reported uses are powders.
***Not specified whether a powder or spray, so this information is captured for both categories of incidental inhalation.
Note: Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure type uses may not equal the sum-total uses.

phosphatidylethanolamine were also detected in the liver and brain after IV administration. However, in all organs, the majority of radioactivity (~90%) was consistently accounted for as unmetabolized Phosphatidylserine.[48,49] Conversely, at 60 minutes post oral dosing of radiolabled Phosphatidylserine (20 mg/kg) in rats, the majority of the circulating plasma radioactivity consisted of Phosphatidylserine; the radioactivity at 24 hours was attributed primarily to Phosphatidylserine degradation products.[48] Furthermore, less than 20% of the administered dose recovered in the mesenteric lymph of rats after oral administration of Phosphatidylserine (560 mg/kg body weight of [3 H]-glycerol-labeled, brain-derived Phosphatidylserine) was liposoluble, with phospholipids comprising 11% of the liposoluble fraction.[51] The majority of the radioactivity was recovered as triglycerides and, to a smaller extent, diacylglycerol, indicating complete degradation of orally administered Phosphatidylserine.

In the mitochondria of mammalian cells, Phosphatidylserine may undergo decarboxylation to phosphatidylethanolamine, which is followed by potential reformation of Phosphatidylserine through exchange of the ethanolamine moiety with serine in the endoplasmic reticulum or mitochondria-associated membrane.[52] Thus, in the intestinal absorptive cells, lysophosphatidylserine may be reacylated to yield Phosphatidylserine and ultimately converted to phosphatidylethanolamine.[51] Re-esterified phospholipids are subsequently incorporated into intestinal lipoproteins (ie, chylomicrons) or directly transported as lysophospholipids via the portal system to the liver.[53,54] As the chylomicrons circulate in the blood, their components, including phospholipids, are degraded via lipoprotein lipase hydrolytic activity.[54] Ultimately, the Phosphatidylserine degradation products (ie, free fatty acids, serine, glycerol, and phosphorus-containing substances) enter common physiological pathways of amino acid and lipid metabolism. In turn, intact phospholipids are excreted in the bile and thus may be subject to enterohepatic circulation.

Pharmacokinetic studies indicate exogenous Phosphatidylserine crosses the blood–brain barrier, where it appears to have an affinity for the hypothalamus.[55] Oral administration results in peak levels in 1 to 4 hours.

Human. In 8 human subjects, the oral consumption of 500 mg Phosphatidylserine (as soy lecithin phosphatidylserine capsules) resulted in peak plasma Phosphatidylserine levels of 3.95% of the total phospholipid plasma concentration, compared to background Phosphatidylserine levels of 1.8% to 2.2% of total plasma phospholipids.[23]

Skin Penetration Enhancement

The effects of PC and Hydrogenated PC on the permeation of indomethacin through hairless rat skin were studied using liquid paraffin and a gel prepared with liquid paraffin and hydrogenated soybean phospholipid.[56] Indomethacin (1%) was mixed with liquid paraffin and PC or Hydrogenated PC and heated at 95 °C for 30 minutes. The mixture was then cooled to room temperature and allowed to stand for 1 day. Skin permeation was measured using a modified Franz-type diffusion cell apparatus. Permeation rates for indomethacin from the liquid paraffin suspension with PC or Hydrogenated PC were determined. For liquid paraffin without PC or Hydrogenated PC, permeation of indomethacin was observed only after 10 hours. However, within 10 minutes, indomethacin permeated at rates of ~ 10 μg/cm^2 and 5 μg/cm^2 from liquid paraffin with PC and Hydrogenated PC, respectively.

The effect of hydrophilic groups of phospholipids on the percutaneous penetration of indomethacin in vitro was examined in a Franz-type diffusion chamber, using dorsal skin from guinea pigs.[57] The following phospholipids were evaluated for enhancement of indomethacin skin penetration: PC, phosphatidylethanolamine, Phosphatidylinositol, Phosphatidylserine, Phosphatidylglycerol, Phosphatidic Acid, and sphingomyelin. Phospholipid-induced enhancement of indomethacin percutaneous penetration was in the following order: Phosphatidylglycerol > phosphatidylethanolamine > PC > Phosphatidylserine > Phosphatidic Acid > Phosphatidylinositol > control > sphingomyelin.

Toxicology

Single-Dose (Acute) Toxicity

Oral

Phosphatidylserine. The oral administration of a purified phospholipid preparation obtained from bovine cerebral cortex (in phosphate buffer suspension) to Sprague-Dawley rats (CD strain; number not stated) indicated that the LD_{50} is > 5 g/kg body weight.[58]

Intravenous

Phosphatidylserine. Following the IV dosing of Sprague-Dawley rats (CD strain; number not stated) with Phosphatidylserine derived specifically from bovine cerebral cortex (in phosphate buffer suspension), an LD_{50} of 236 mg/kg body weight was reported.[58]

Subcutaneous

Phosphatidylserine. When Sprague-Dawley rats (CD strain; number not stated) were dosed subcutaneously (SC) with Phosphatidylserine derived specifically from bovine cerebral cortex (in phosphate buffer suspension), the LD_{50} was reported to be > 5 g/kg body weight.[58]

Repeated Dose Toxicity

Inhalation

Phosphatidylcholine. The effects of chronic exposure to liposome aerosols on lung histology and alveolar macrophage function were studied.[59] Liposomes were made from hydrogenated soy PC (50 mg/mL). Groups of 30 (strain not stated) mice were placed in a nose-only exposure module and exposed to liposome (20-mL total volume, 50-mg lipid/mL phosphate-buffered saline) or saline aerosols 1 hour per day, 5 days per

week, for 4 weeks. Five mice of both the experimental and control groups were removed weekly and their lungs examined. The animals were killed and bronchoalveolar lavage (BAL) was performed through a tracheostomy. In vivo uptake of liposomes by alveolar macrophages was documented by fluorescence microscopy and flow cytometry of BAL. A consistent amount of 1 to 3 μg of lipid inhaled per dosing per mouse was estimated from fluorescence measurements. No histologic changes of the lungs or untoward effects on general health or survival of animals were noted. Alveolar macrophage phagocytic function was not affected. Transmission electron microscopy and morphometry showed no treatment-related alterations.

Oral

Nonhuman

Lecithin. A group of 48 male and 48 female SPF Wistar rats was fed 4% (soya) Lecithin for 2 years, while a control group was fed commercial diet only.[60] Feed consumption and body weights were determined prior to dosing, at intervals up to week 95, at week 102, and at study termination. The mean Lecithin intake was 1,470 and 2,280 mg/kg/d for males and females, respectively. No statistically significant differences were observed in mortality, feed consumption, or body weight between the treated and control groups, but it was noted that feed consumption and body weight were sometimes greater in the treated group when compared to controls. Hematology values of animals of the treated group were similar to those of control animals, as were organ weights and gross and microscopic alterations. Increased parathyroid gland hyperplasia, particularly in the males, was attributed to an increased phosphate intake. The incidence of tumor formation was similar in the treated and control groups. A slightly increased incidence of myocardial fibrosis was associated with parathyroid gland hyperplasia.

Lecithin, Phosphatidylserine, PC, Phosphatidylethanolamine, Phosphatidylinositol, and Phosphatidic Acid. A 90-day feeding study was performed to evaluate the safety of dietary soy lecithin transphosphatidylated phosphatidylserine (soybean-derived Phosphatidylserine [SB-PS]), with or without fish oil-derived long-chain polyunsaturated fatty acids (LC-PUFA) mixed or conjugated to the glyceride backbone.[61] One hundred two male Wistar rats (wild type, pathogen free) were randomly assigned to 6 groups. The 5 groups consumed 100 mg chow containing each of the following components, respectively, incorporated in 1 mL of milk-based supplement matrix:

- medium-chain triglycerides (MCT group)
- fish oil diluted with MCT to yield 30% (wt/wt) of omega-3 LC-PUFA (omega-3 group)
- soybean 78% powdered SB-PS (final concentration of 20% SB-PS [wt/wt]) emulsified with 13% PC, 2% Phosphatidylethanolamine, 1% Phosphatidylinositol, 4% Phosphatidic Acid, and further diluted with MCT (SB-PS group)
- fish oil mixed with soybean 78% powdered SB-PS and diluted with MCT to yield a final concentration of 20% SB-PS (wt/wt) and 30% (wt/wt) of omega-3 LC-PUFA (omega-PS group)
- 20% Phosphatidylserine (wt/wt) consisting largely of molecular species of palmitic acid (16:0) and docosahexaenoic acid (DHA) (22:6) or eicosapentaenoic acid (20:5), resulting in 30% (wt/wt) of omega-3 LC-PUFA (PS-DHA group).

The control group consumed normal chow. Blood samples were drawn, and hematological parameters evaluated. Signs of toxicity were not observed during the feeding period. At the end of the study, gross examination of organs was performed. The following mortalities were reported: 1 rat (control group), 2 rats (MCT group), 1 rat (omega-3 group), and 1 rat (PS-DHA group). Pathological examinations did not reveal a specific cause of death; however, the authors concluded that the deaths were not treatment-related. Hematological parameters were normal in all treatment groups. At gross pathological examination, there were mild signs of liver enlargement in 5 of 102 rats, but these were considered unrelated to treatment. Possible early signs of lung metastasis (pale color nodes and different tissue consistency) were observed in 4 of 102 rats, but these findings were considered typical and abundant in rats of this age (15 months old). It was noted that none of these pathological findings occurred in PS-fed rats. It was concluded that no adverse effects were associated with diets fed in this study.

Phosphatidylserine. The repeated dose toxicity of Phosphatidylserine derived specifically from bovine cerebral cortex (in phosphate buffer suspension) was studied using 3 groups of Sprague-Dawley rats of the CD strain (20 males, 20 females/group).[58] The 3 groups received doses of 10, 100, and 1,000 mg/kg/d, respectively, by gavage for 26 weeks. The control group was dosed with phosphate buffer only. Body weight gain and food consumption in all dose groups were comparable to the control group. No significant hematological changes were observed. At week 13, slightly elevated alkaline phosphatase levels in male and female rats and slightly lowered serum albumin levels in males was observed in the 1,000 mg/kg/d dose group. Elevated potassium and lower sodium values were reported for males at week 13. Terminal studies indicated no major problems, and there were no significant morphological changes. It was concluded that Phosphatidylserine derived specifically from bovine cerebral cortex did not cause significant toxicity in this study.

Another repeated dose toxicity study involved groups of 40 beagle dogs (20 males, 20 females).[58] Three groups received Phosphatidylserine derived specifically from bovine cerebral cortex (in corn oil) orally at doses of 10, 100, and 1,000 mg/kg/d (dose volume = 5 mL/kg), respectively, for 1 year. The control group was dosed with corn oil. None of the animals died. At the highest dose administered, blood glucose and cholesterol levels were significantly lowered. There were no significant macroscopic findings, and organ weights were within

normal range. Histopathological examination of tissues did not indicate treatment-related changes. It was concluded that Phosphatidylserine derived specifically from bovine cerebral cortex did not cause significant toxicity in this study.

Human

Phosphatidylserine. Human subjects (120) received soy lecithin-derived Phosphatidylserine (300 or 600 mg orally) daily in a 12-week study.[62] There were no clinically significant variations in blood chemistry or hematology. Additionally, there were no differences in the occurrence of side effects between test and placebo groups.

Intravenous

Phosphatidylserine. Phosphatidylserine derived specifically from bovine cerebral cortex (in phosphate buffer suspension) was administered IV to groups of Sprague-Dawley rats of the CD strain (10 males, 10 females/group).[58] The 3 groups received doses of 5, 20, and 80 mg/kg/d (dose volume = 2.5 mL/kg), respectively, for 4 weeks. The control group was dosed with vehicle only. Except for females of the 5 mg/kg/d group, reddening and swelling of the paws and around the muscle region were observed in all dose groups. The following hematological changes were observed in male and female rats of the 80 mg/kg/d dose group: significant lowering of the erythrocyte count, hemoglobin concentration, and packed cell volume and increased neutrophil and lymphocyte counts. An increase in spleen weight in males and females of the 80 mg/kg dose group and males of the 20 mg/kg/d dose group was reported. Kidney weights of males dosed with 80 or 20 mg/kg/d were also increased when compared to controls. Adrenal weights of males and females of the 80 mg/kg dose group were marginally increased. Results at microscopic examination revealed an injection site thrombosis in some rats in all dose groups, with an increase in severity in rats dosed with 20 mg/kg/d or 80 mg/kg/d. Whether or not this finding was reported for the control group was not stated. An increase in the incidence of extramedullary hematopoiesis was observed in the 80 mg/kg/d dose group. It was concluded that Phosphatidylserine derived specifically from bovine cerebral cortex did not cause significant toxicity in this study.

The IV toxicity of Phosphatidylserine derived specifically from bovine cerebral cortex (in phosphate buffer suspension) was evaluated using groups of 24 (12 males, 12 females) Beagle dogs.[58] Three groups were injected IV with Phosphatidylserine derived specifically from bovine cerebral cortex at doses of 5, 15, and 40 mg/kg/d, respectively, for 4 weeks. A fourth group was dosed with vehicle only. Generalized tremors of body muscles were observed in animals of the 15 or 40 mg/kg/d group. A significant increase in total white cell count and a reduction in total serum protein values were reported for dogs dosed with 40 mg/kg/d. At gross examination, hemorrhage was observed around the injection sites. There were no significant group differences in organ weights. Microscopic examination of the liver revealed centrilobular and periportal sinusoidal aggregations of polymorphonuclear leukocytes in 1 animal of the 15 mg/kg/d dose group and in 4 animals of the 40 mg/kg/d dose group. It was concluded that Phosphatidylserine derived specifically from bovine cerebral cortex did not cause significant toxicity in this study.

Intramuscular

Phosphatidylserine. The intramuscular toxicity of Phosphatidylserine derived specifically from bovine cerebral cortex (in phosphate buffer suspension) was evaluated using groups of 32 (16 males, 16 females) Beagle dogs.[58] Three groups were injected intramuscularly with Phosphatidylserine derived specifically from bovine cerebral cortex at doses of 5, 10, and 15 mg/kg/d, respectively, for 6 weeks. A fourth group served as the vehicle control. None of the animals died. Subcutaneous hardening and/or swelling of injection sites was observed in the 10 and 15 mg/kg/d dose groups. Hematological analyses indicated elevation of the erythrocyte sedimentation rate and an increase in total white blood cell count in the 15 mg/kg dose group. At gross examination, subcutaneous hemorrhage and adhesion between the skin and muscles (at injection site) was reported for all groups, including the control group. This finding was considered dose-related. Organ weights were within normal ranges. Muscle degeneration and subcutaneous and intramuscular acute inflammatory cell infiltration and necrosis were also observed at injection sites. It was concluded that Phosphatidylserine derived specifically from bovine cerebral cortex did not cause significant toxicity, that is, there were no significant signs of systemic toxicity.

Cytotoxicity

Lysolecithin. Lysolecithin has been described as a powerful hemolytic and cytolytic phosphoglyceride.[63,64] Furthermore, the toxic effect of many snake venoms is attributable to their content of phosphatidase A, an enzyme capable of converting plasma phosphatides into lysophosphatides, one of which is Lysolecithin.

Reproductive and Developmental Toxicity

Phosphatidylserine

In a teratogenicity study, Phosphatidylserine derived from bovine cerebral cortex was administered by gavage to pregnant Sprague-Dawley rats (CD strain; number not stated) at doses of 0, 10, 100, and 200 mg/kg/d on days 6 through 18 of gestation.[58] The animals were killed on day 20 of gestation, and litters were examined for skeletal and visceral abnormalities. At terminal necropsy, there were no treatment-related gross changes. The following litter values were not affected by treatment with Phosphatidylserine: litter size, postimplantation loss, litter and mean fetal weights, and embryonic and fetal development.

Phosphatidylserine derived from bovine cerebral cortex was also administered by gavage to pregnant New Zealand White rabbits (number not stated) at doses of 0, 50, 150, and 450 mg/kg/d on days 6 through 18 of gestation.[58] On gestation

day 29, the animals were killed and litters subjected to gross examination. Fetuses were examined externally and internally for evidence of visceral and skeletal malformations. There was no evidence of systemic effect, and neither pregnancy nor mortality was affected by treatment. At the highest dose, mean fetal weights were slightly lower when compared to control values, but the difference was not statistically significant. There were no treatment-related effects on embryonic and fetal development.

Lysophosphatidic Acid

Lysophosphatidic acid and sphingosine-1-phosphate are both lysophospholipids.[65] Because Lysophosphatidic Acid promotes prostaglandin synthesis, mediators in the lysophosphatidic acid pathway may also play a significant role in implantation and parturition. Sphingosine-1-phosphate signaling is thought to be essential in vascular formation within the uteroplacental unit and in fetomaternal immunologic interactions. Derangements in either one of these lysophospholipid signaling pathways could result in pregnancy complications that may include implantation failure, preeclampsia, and preterm labor.

Immature germinal vesicle stage oocytes from 5- to 6-week-old female BDF-1 mice were incubated for 17 to 18 hours in in vitro maturation (IVM) medium containing 0-, 1-, 10-, or 30-μM Lysophosphatidic Acid and then either fertilized in vitro with epididymal sperm or assessed for spindle morphology or mitochondrial membrane potential.[66] Chromosomal aneuploidy in the resultant blastocysts and the production of normal pups were not assessed. The fertilized embryos were grown in vitro to assess blastocyst formation rates, differential cell counts and apoptosis. The supplementation of IVM with 30-μM Lysophosphatidic Acid enhanced the maturation and developmental competence of mouse oocytes. Rates of maturation, fertilization, and blastocyst formation and hatching were significantly higher in the 30-μM Lysophosphatidic Acid-supplemented group (94.3%, 96.3%, 79.1%, and 51.3%, respectively) than in the unsupplemented control (0 mM) group (80.5%, 87.5%, 61.3%, and 37.8%, respectively), and more comparable to that of the in vivo matured oocytes (100%, 96.5%, 95.3%, and 92.9%, respectively). Lysophosphatidic acid did not adversely affect mitochondrial activity, spindle integrity, or blastocyst cell number. The results of this study imply that the supplementation of IVM medium with 30-μM Lysophosphatidic Acid may enhance the developmental competence of mouse oocytes without affecting apoptosis, spindle normalcy or mitochondrial integrity.

Genotoxicity

In Vitro

Hydrogenated Lecithin. The genotoxicity of liposome-fullerene (Lpsm-Flln) or liposome solution (Lpsm; 313-5,000 μg/plate) was examined, with and without metabolic activation, using *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2uvrA (pKM101).[67] Neither liposome was genotoxic in this assay, with or without metabolic activation. The following 5 positive controls were genotoxic: sodium azide, *N*-ethyl-*N*'-nitro-*N*-nitrosoguanidine, 2-nitrofluorene, 9-aminoacridine, and 2 aminoanthracene.

Phosphatidylserine. Human lymphocyte cultures were incubated with Phosphatidylserine derived from bovine cerebral cortex concentrations up to 165.6 μg/mL, with and without metabolic activation.[58] Cyclophosphamide served as the positive control. Phosphatidylserine derived specifically from bovine cerebral cortex was not genotoxic with or without metabolic activation.

In the mouse lymphoma assay, Phosphatidylserine derived from bovine cerebral cortex was not genotoxic to mouse lymphoma L5178Y cells with or without metabolic activation.[58] Test concentrations were not stated.

Phosphatidylserine derived from bovine cerebral cortex was evaluated in a DNA repair assay involving human epithelioid cells (HeLa S_3 cells), with and without metabolic activation.[58] Test concentrations were not stated. Increases in the number of silver grains found in autoradiographic film over cell nuclei served as indicators of repair synthesis. There was no evidence of DNA repair synthesis, with or without metabolic activation.

In Vivo

In the micronucleus test, Phosphatidylserine derived from bovine cerebral cortex was administered orally (by gavage) to mice at doses up to 300 mg/kg body weight.[58] Two equal doses were administered, separated by a 24-hour interval. Mitomycin C served as the positive control. Bone marrow smears were examined for the presence of micronuclei in 1,000 polychromatic erythrocytes per mouse and for the ratio of normochromatic to polychromatic erythrocytes. Phosphatidylserine derived from bovine cerebral cortex was neither cytotoxic nor genotoxic to bone marrow cells.

Modulation of Gene Expression

Phospholipids. The capacity of a formulation of grape seed extract and soy Phospholipids (formulation identified as SBD.5HC) to trigger a regenerative response in the dermis and epidermis through a selective action on the hypodermis was investigated using human skin (from breast reduction surgeries).[68] SBD.5HC was prepared by combining grape seed extract (95% proanthocyanidins grade) and soy Lecithin (95%-98% phospholipids grade) at a ratio of 1:3 wt/wt. After 5 days of culture under control conditions, full-thickness human skin biopsies showed marked degradation, characterized by pyknotic nuclei in fibroblasts and basal keratinocytes as well as intercellular gaps in spiny and granular layers of the epidermis. The inclusion of SBD.5HC (100 μg/mL) in the medium bathing the hypodermal layer of the biopsies resulted in an improved overall morphology. Treated skin samples had mostly normal, elongated fibroblasts, fewer dying basal

keratinocytes at the dermal–epidermal junction, less gaping spaces in the stratum spinosum, and better-preserved granulosum and stratum corneum. Thus, study results suggested that the application of SBD.5HC to the hypodermal layer of skin triggered modulation of gene expression in the upper layers of skin and resulted in morphological changes in the dermis and epidermis.

Carcinogenicity

Lecithin

TM strain mice were fed 5- to 10-mg Lecithin mixed with sugar (for palatability), and a second group was fed Lecithin (5-10 mg) and cholesterol (4-5 mg).[69] The mice were bred and their offspring dosed following the same procedures; dosing continued until all mice became moribund or had died. A control group was given laboratory feed ad libitum. The total number of mice fed Lecithin, Lecithin and cholesterol, or control feed was 166, 212, and 360, respectively. Animals were killed and brain necropsies performed. It was noted that the brains of moribund animals or animals found dead were removed and necropsied, but necropsy results were not reported. Brain nerve cell tumors (2-5 mm) were found in 18 of 73 examined animals fed Lecithin and in 27 of 88 examined animals fed Lecithin and cholesterol, whereas no brain nerve cell tumors were found in 188 control animals.

Groups of female dd mice were dosed SC as follows: 50 mice were given 0.1 mL of a 0.25% mixture of 4 nitroquinoline-1-oxide (in 10% aqueous Lecithin) in a single injection until the total dose was 2.5 mg.[70] The injections were repeated weekly, each time in a different site on the back. Thirty mice were dosed (10 times) with a Lecithin water mixture at the same total dose as in the previous group. Twenty mice were not dosed and served as controls. The mice were killed after 221 to 296 days. Animals dosed with 4-nitroquinoline-1-oxide/Lecithin that survived more than 221 days after dose initiation (36/50) had pulmonary neoplasms; skin neoplasia at the injection site (1 animal) and leukemia (1 animal) were also observed in this group. No surviving mice dosed with Lecithin water or untreated control mice had pulmonary or any other type of neoplasia. However, 3/28 animals of the Lecithin water group and 3/18 control animals had lung adenomas; these were considered spontaneous.

In the same study, groups of female Buffalo rats were dosed SC as follows: 25 rats were given 0.2 mL of a 0.25% mixture of 4-nitroquinoline 1-oxide (in 10% aqueous Lecithin) in a single injection until the dose reached 10 mg; the injections were repeated weekly. Fifteen rats were dosed (20 times) with a Lecithin water mixture, having received the same total dose. The rats were killed after 264 to 329 days. Nineteen of the 25 animals dosed with 4-nitroquinoline 1-oxide/Lecithin that survived more than 264 days after dose initiation had pulmonary neoplasms, with 11 SC sarcomas and 2 endometrial sarcomas also reported. No neoplasms were found in any of the 13/15 surviving rats dosed with Lecithin water.[70]

Irritation and Sensitization

Ocular Irritation

Lecithin. Lecithin 65% (solution of 65% Lecithin) and products containing 2.25% or 3.0% Lecithin 65% were non- to minimally irritating to unrinsed rabbit eyes. A soap containing 0.83% Lecithin powder (tested at 25%) was moderately irritating, and Lecithin-containing liposomes were practically nonirritating in a Draize test.[1]

Skin Irritation and Skin Sensitization

Nonhuman

Lecithin and Hydrogenated Lecithin. In single-insult occlusive patch tests (rabbits), Lecithin 65% (solution of 65% Lecithin) was minimally irritating, products containing 3% Lecithin 65% were practically non- to mildly irritating, and a product containing 2.25% Lecithin 65% was non-irritating to the skin of rabbits. In a guinea pig immersion study, 0.5% of a soap containing 0.83% Lecithin powder was practically nonirritating. Hydrogenated Lecithin was not a primary dermal irritant in rabbits.[1]

Human

Lecithin and Hydrogenated Lecithin. In clinical irritation studies, cosmetic formulations containing 0.3% or 3% Lecithin 65% (solution of 65% Lecithin), a soap containing 0.83% Lecithin powder (tested at 0.5%), and Lecithin liposomes were generally nonirritating. Barely perceptible erythema was the most severe reaction observed. Hydrogenated Lecithin also was not an irritant, and Hydrogenated Lecithin (15% in petrolatum) was not a sensitizer. Additionally, a tanning oil containing 3% Lecithin 65%, a mascara containing 0.1% Lecithin 65%, and a foundation containing 0.3% Lecithin 65% were non-sensitizing.[1]

Lysolecithin. The intracutaneous injection of 0.04-μM to 0.25-μM Lysolecithin, derived from beef serum, human serum, or beef brain Lecithin, caused typical wheal and erythema reactions in the 3 subjects tested.[71] Lysolecithin (0.125 and 0.17 μM) produced wheal and erythema reactions that were roughly equivalent to that produced by the injection of histamine (0.5 μg). These reactions consisted of a pale, elevated central swelling (occasionally with small pseudopods), surrounded by a bright red zone of erythema. The lower concentrations of Lysolecithin (0.085 and 0.043 μM) caused minor reactions that were smaller than those obtained with 0.3-μg histamine, but slightly greater than those caused by 0.1-μg histamine (slight threshold reaction). A faint, but definite, reaction was observed at concentrations as low as 0.013 μM in another experiment.

Allergenicity

Lecithin. The antigenicity of soy Lecithin was studied using 30 soybean-sensitive patients and 22 controls.[19] One control group (11 subjects) consisted of nonatopic individuals, and the other

control group (11 subjects) consisted of allergic patients with negative IgE to soybean (radioallergosorbent test [RAST] score = 0). The IgE- and IgG4-binding activities of the soy Lecithin proteins were evaluated by immunoblotting with sera obtained from the patients, 7 of whom had a positive challenge test. In 100 grams of sample, the soy Lecithin contained 2.8 mg of proteins. The proteins present in soy Lecithin were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis. For the soy Lecithin, the detection rate of only one protein (molecular weight: 31 kDa) by the serum IgE of patients was statistically significantly different when compared to serum from the 2 control groups combined (detection rates: 40% [patient sera] and 4.5% [control sera]). Proteins in the molecular weight range of 58 to 67 kDa were rarely bound to serum IgE. Only one of the patients with a positive challenge test had IgE antibodies to soy Lecithin proteins. The presence of IgG4-binding proteins in soy Lecithin was described as rare. It was concluded that the proteins present in soy Lecithin have little antigenicity with respect to soybean allergy.

Photocytotoxicity

Nonhuman

Hydrogenated Lecithin. The photocytotoxicity of Lpsm-Flln, 0.2% aqueous, was studied using Balb/3T3 fibroblastic cells.[67] Bacterial assay results for this liposome are included in the Genotoxicity section of this report. Lpsm-Flln had the following composition: Hydrogenated Lecithin, glycine soja (soybean) sterols, and fullerene C60 (C60) in the weight ratio of 89.7:10:0.3 (ie, contains 89.7% Hydrogenated Lecithin). Results were compared with that of a 0.2% Lpsm that did not contain C60, described as follows: Hydrogenated Lecithin and glycine soja (soybean) sterols in the weight ratio of 90:10 (ie, contains 90% Hydrogenated Lecithin). The fibroblasts (in Lpsm-Flln or Lpsm at doses of 0.49 to 1,000 μg/mL) were exposed to sham-irradiation or long-wavelength ultraviolet light (UVA; 5 J/cm^2; 320-400 nm; $\lambda_{max} = 360$ nm) for 50 minutes. Cell viability of Balb/3T3 fibroblastic cells in Lpsm-Flln was 96.3% to 158.5% for the UVA group and 94.5% to 149.6% for the sham group and did not decrease dose-dependently. Also, cell viability in Lpsm was like that in Lpsm-Flln. These results show that Lpsm-Flln (89.7% Hydrogenated Lecithin) or Lpsm (90% Hydrogenated Lecithin) at a concentration of 0.2% was not photocytotoxic to Balb/3T3 fibroblasts.

Phototoxicity/Photosensitization

Lecithin and Hydrogenated Lecithin. A foundation containing 0.3% Lecithin 65% (solution of 65% Lecithin) was not a photosensitizer in human subjects. The subjects were exposed for 1 minute to a UV light source (360 nm peak output), at 12 inches, after removal of the first, fourth, seventh, and 10th induction patches and challenge patches. Photosensitization reactions were determined 48 hours after exposure. Lecithin and Hydrogenated Lecithin (both at 15% in petrolatum) were not phototoxic or photosensitizing in human subjects. On days 1, 4, and 7 of induction, patches were removed, and test sites were irradiated with a dose of 3 MED of UVA. A fourth induction patch was also applied, followed by irradiation of the test site. Challenge patch sites were exposed to a dose of 9.5 MED and 0.5 MED of simulated solar light (UVA and mid-wavelength UV [UVB]).[1]

Case Report

Lecithin. A 3-year-old boy with a history of asthma and peanut allergy was treated for asthma that developed after an upper respiratory tract infection.[72] He developed respiratory distress and generalized urticaria within an hour after receiving the second of 2 inhalations of an ipratropium bromide inhaler. All signs regressed within 48 hours of withdrawal of the drug. Soy Lecithin, an excipient in the metered dose inhaler, was strongly suspected of causing the adverse events.

Other Studies

Phospholipid Signaling

The organization of biological systems involves communication between cells, and in multicellular organisms, these interactions are mediated through cell–cell contacts.[73] In order for a cell to react, a signal must be detected and converted across the physical boundary that is defined by the cell membrane. Membrane lipids have important roles in signaling reactions. These ligands can function as signaling molecules in various ways, and the binding of a ligand to a cell surface receptor is an initiating event of cellular signaling. Transmembrane signaling frequently includes the activation of enzymes that act on metabolizing lipids in the vicinity of the respective receptor, leading to the generation of membrane-bound and diffusible metabolites. Receptor stimulation is often accompanied by the activation of downstream factors that are regulated by products of lipid metabolism. Some of the phospholipids involved in signaling include Phosphatidylinositol, PC, Lysophosphatidic Acid, and Phosphatidylserine. Signaling effects relating to these phospholipids are summarized below.

Minor products of inositol phospholipid metabolism, such as phosphatidylinositol-3,4,5-triphosphate, serve as key intermediates in cell signaling.[74] Furthermore, phosphoinositides, a family of lipid molecules derived from the phosphorylation of Phosphatidylinositol, control important cellular processes, including cell proliferation, apoptosis, metabolism, and migration.[75] Phosphoinositides make up only a small fraction of cellular phospholipids, yet they control almost all aspects of a cell's life and death.[76] The specific interaction of phosphoinositides with proteins is critical for a plethora of cellular processes, including cytoskeleton remodeling, mitogenic signaling, ion channel regulation, and membrane trafficking.[77] Phosphoinositide homeostasis is tightly regulated by a large number of inositol kinases and phosphatases that have been implicated in regulating membrane trafficking, and the dysregulation of these enzymes has been linked to a number of

human diseases, ranging from cancer and diabetes to neurological disorders and asthma.

Cancer cells display sensitivity to ablation of fatty acid synthesis, possibly as a result of the diminished capacity to synthesize complex lipids involved in signaling or growth pathways.[78] Evidence has accrued that PC, the major phospholipid component of eukaryotic membranes, as well as choline metabolites derived from its synthesis and catabolism, contributes to both proliferative growth and programmed cell death. Coordinated changes in substrate availability, gene expression, and enzyme activity lead to altered PC synthesis in cancer.

Lysophosphatidic acid is capable of stimulating a plethora of different cellular responses through the activation of its family of cognate G protein-coupled receptors.[79] It mediates a wide range of biological effects in many tissue types, including vasculogenesis, angiogenesis, and vascular maturation, and has also been implicated in the regulation of pathophysiologic vascular responses. For example, Lysophosphatidic Acid was found to signal through G protein α q subunit (Gαq) to promote the growth and migration of vascular smooth muscle cells, which is essential for the development of intimal hyperplasia after vascular injury.

Phosphatidylserine-specific binding is important in the function of A-, B-, and C-Raf kinases, which are important regulators of many signal transduction pathways. Raf kinases are generally downstream from the Ras GTPases and transmit information to activate mitogen-activated protein kinase signaling.[80] The activation of protein kinase B, Raf-1, and protein kinase C signaling, which supports neuronal survival and differentiation, requires the interaction of these proteins with Phosphatidylserine.[81] Phosphatidylserine, exposed extracellularly, is instrumental in triggering blood clotting and also serves as a signal for the clearance of apoptotic cells.[80]

Skin Composition

Lecithin, Lysolecithin, Phosphatidylethanolamine, and Phosphatidylserine. Lecithin, phosphatidylethanolamine, and Phosphatidylserine comprise the major phospholipid components of skin from young adult female albino rabbits.[82] Polyglycerolphosphatides, Lysolecithin, and sphingomyelin are also present.

In a study in which the total lipid concentration, distribution of all major lipid species, and the fatty acid composition in human stratum corneum were assessed, the following lipids were found: phospholipids (phosphatidylethanolamine), cholesterol sulfate, neutral lipids (free sterols, free fatty acids, triglycerides, sterol and wax esters, squalene, and n-alkanes), and sphingolipids.[83] The neutral lipids contributed the greatest proportion to the stratum corneum lipids. Values for the phospholipid composition (lipid weight %) at the following 4 skin sites were: abdomen (4.9 ± 1.6), leg (5.2 ± 1.1), face (3.3 ± 0.3), and plantar (3.2 ± 0.89).

Summary

The safety of the following 17 ingredients in cosmetics is reviewed in this safety assessment: Lecithin, Hydrogenated Lecithin, Lysolecithin, Hydrogenated Lysolecithin, Phospholipids, Hydrolyzed Phospholipids, Phosphatidic Acid, Lysophosphatidic Acid, Phosphatidylglycerol, Lysophosphatidylglycerol, Phosphatidylserine, Ammonium Phosphatidyl Rapeseedate, PC, Hydrogenated PC, Hydrogenated Lysophosphatidylcholine, Lysophosphatidylethanolamine, and Phosphatidylinositol. These ingredients are reported to function primarily as skin and hair conditioning agents, emulsifying agents, and surfactants in cosmetic products. Frequency of use data from FDA and the results of an industry survey indicate that the following ingredients are being used in cosmetic products: Lecithin, Hydrogenated Lecithin, Lysolecithin, Lysophosphatidic Acid, PC, and Phospholipids. Of these ingredients, the highest maximum concentration of use is 50% Lecithin in a leave-on foot product.

The fate of IV-injected 1-[14C]-palmitoyl-[32P]-Lysolecithin was studied using male albino rats. A high percentage of labeled Lysolecithin was detected in skeletal and heart muscle, and it is likely that that Lysolecithin is rapidly converted to Lecithin in the liver. Following IV administration to rats and mice, Phosphatidylserine was eliminated from plasma in a biphasic manner and largely distributed to several major organs, including the liver spleen and brain tissue. In rats, approximately 60% of an orally administered dose of Phosphatidylserine (20 mg/kg body weight) was recovered in the feces of which 50% was identified as lysophosphatidylserine. Approximately 10% of this orally administered dose was detected in the urine. In humans, the oral consumption of soy lecithin Phosphatidylserine capsules (total of 500 mg Phosphatidylserine) resulted in peak plasma Phosphatidylserine levels of 3.95% of the total phospholipid plasma concentration, when compared to background Phosphatidylserine levels of 1.8% to 2.2% of total plasma phospholipids.

The effect of the following phospholipids on the percutaneous penetration of indomethacin was evaluated in vitro using dorsal skin from guinea pigs: PC, phosphatidylethanolamine, Phosphatidylinositol, Phosphatidylserine, Phosphatidylglycerol, Phosphatidic Acid, and sphingomyelin. Phospholipid-induced enhancement of indomethacin percutaneous penetration was in the following order: Phosphatidylglycerol > phosphatidylethanolamine > PC > Phosphatidylserine > Phosphatidic Acid > Phosphatidylinositol > control > sphingomyelin.

In a study in which a purified phospholipid preparation obtained from bovine brain (Phosphatidylserine derived specifically from bovine cerebral cortex, in phosphate buffer suspension) was administered orally to Sprague-Dawley rats, the LD_{50} was > 5 g/kg body weight.

In a repeated dose inhalation toxicity study involving mice exposed to PC liposomes, no histologic changes of the lungs or untoward effects on general health or survival of animals were noted. In a 2-year feeding study on 4% Lecithin involving rats,

no significant differences were observed for mortality, feed consumption, or body weight between the treated and control groups. Additionally, there were no differences in gross or microscopic findings when the groups were compared.

In a 12-week study in which human subjects received soy lecithin-derived Phosphatidylserine daily, there were no clinically significant variations in blood chemistry or hematology. Additionally, there were no differences in the occurrence of side effects between test and placebo groups.

Lecithin 65% (solution of 65% Lecithin) and products containing 2.25% or 3.0% Lecithin 65% were non- to minimally irritating to unrinsed rabbit eyes. In single-insult occlusive patch tests (rabbits), Lecithin 65% was minimally irritating, products containing 3% Lecithin 65% were practically non- to mildly irritating, and a product containing 2.25% Lecithin 65% was nonirritating to the skin of rabbits.

The photocytotoxicity of Lpsm-Flln (0.2% aqueous) was studied using Balb/3T3 fibroblastic cells; results were negative. A foundation containing 0.3% Lecithin 65% (solution of 65% Lecithin) was not a photosensitizer. Lecithin and Hydrogenated Lecithin (both at 15% in petrolatum) were not phototoxic or photosensitizing.

In oral teratogenicity studies on Phosphatidylserine derived specifically from bovine cerebral cortex involving rats and rabbits, there were no treatment-related effects on embryonic and fetal development. Lysophosphatidic acid (30 μM) enhanced the maturation and developmental competence of BDF-1 mouse oocytes in vitro.

Hydrogenated Lecithin was not genotoxic to *Salmonella typhimurium* or *E coli* bacterial strains with or without metabolic activation. The results for Phosphatidylserine in mammalian cell assays (ie, mouse lymphoma, DNA repair [HeLa cells], micronucleus assays) were also negative.

TM strain mice were fed 5- to 10-mg Lecithin mixed with sugar, and a second group was fed Lecithin and 4- to 5-mg cholesterol. Brain nerve cell tumors (2-5 mm) were found in 18 of 73 examined animals fed Lecithin and in 27 of 88 examined animals fed Lecithin and cholesterol; brain nerve cell tumors were not found in 188 control animals. In another study, groups of female dd mice were dosed SC with a 0.25% mixture of 4-nitroquinoline1-oxide (in 10% aqueous Lecithin). No surviving mice dosed with Lecithin water or untreated control mice had pulmonary or any other type of neoplasia. However, 3/28 animals of the Lecithin water group and 3/18 control animals had adenomas, which were considered spontaneous.

Membrane lipids, that is, phospholipids, have important roles in signaling reactions. However, these effects are not relevant to the use of phospholipids as cosmetic ingredients.

Discussion

The Panel acknowledged their previous conclusion, published in 2001, that Lecithin and Hydrogenated Lecithin are safe as used in rinse-off products and safe for use in leave-on products at concentrations of $\leq$ 15% and that the data are insufficient to determine the safety cosmetic products where Lecithin and Hydrogenated Lecithin are likely to be inhaled; and Lecithin and Hydrogenated Lecithin should not be used in cosmetic products in which *N*-nitroso compounds may be formed. This 15% concentration limit was the highest concentration evaluated in tests for skin irritation, sensitization, phototoxicity, and photosensitization potential in human subjects, all of which were negative. The Panel also noted that the highest maximum use concentration of Lecithin reported in 2014 was 50% in leave-on cosmetic products. The Panel agreed that there is little sensitization potential at this concentration, based on extensive clinical experience indicating no problems associated with the application of Lecithin to the skin. Thus, the Panel determined that the concentrations of Lecithin, Hydrogenated Lecithin, and other phosphoglycerides reviewed in this safety assessment need not be limited to 15% in cosmetic products. This decision, based in part, on clinical experience with Lecithin, is applicable across the phosphoglycerides reviewed in this safety assessment because Lecithin is a complex mixture consisting primarily of PC, phosphatidylethanolamine, Phosphatidylserine, and Phosphatidylinositol, with varying amounts of triglycerides, fatty acids, and carbohydrates from vegetable or animal sources.

The Panel discussed the potential for incidental inhalation exposures to phosphoglycerides in products that are sprayed or in powder form and agreed that, based on the results of the repeated dose inhalation toxicity study, likely airborne particle size distributions and concentrations in the breathing zone and ingredient use, incidental inhalation would not lead to local respiratory effects or systemic effects. The Panel also considered the safe use of Lecithin as an inactive ingredient in FDA-approved aerosolized drug products. Thus, it was agreed that the previous conclusion should be amended, acknowledging that the data are no longer insufficient to determine the safety of cosmetic products where Lecithin and Hydrogenated Lecithin are likely to be inhaled.

Additionally, in the previous safety assessment, concerns about the formation of *N*-nitroso compounds in cosmetic products containing Lecithin and Hydrogenated Lecithin were based on experimental conditions that do not represent plausible cosmetic use conditions. For example, Lecithin has been reported to be metabolized to choline by bacterial phospholipases in a model system, and the released choline can be dealkylated to dimethylamine, which is *N*-nitrosatable in the presence of nitrate. The Panel determined that these experimental conditions do not reflect ingredient use in cosmetic products and thus agreed that the previous conclusion should be amended, removing the restriction that Lecithin and Hydrogenated Lecithin should not be used in cosmetic products in which *N*-nitroso compounds may be formed.

The Panel initially expressed concern about animal tissue as a potential source of phosphoglycerides, particularly bovine brain as a source of Phosphatidylserine and Lysolecithin. However, the Panel determined that these phosphoglycerides are safe as used, noting that ingredients derived from bovine central nervous system tissues are not permitted for use in cosmetic products. Concern about pesticide residues and heavy metals

that may be present in botanical ingredients was also expressed. The Panel stressed that the cosmetics industry should continue to use current good manufacturing practices to limit such impurities.

Phosphoglycerides are known to enhance the dermal penetration of some drugs. The Panel noted that formulators should be aware of the potential for enhancing the dermal penetration of other ingredients in cosmetic formulations that contain the ingredients that are evaluated in this safety assessment, especially in products intended for use on infants.

Acknowledging the involvement of cell-membrane lipids in cellular signaling cascades, the Panel noted that these signaling effects are not relevant to the use of phosphoglycerides as cosmetic ingredients. The Panel also acknowledged that derangements in phosphoglyceride metabolism can be associated with prostate, breast, or ovarian cancer but noted that these changes are artifacts of cancer and are not relevant for assessing the safety of cosmetic ingredients. Furthermore, concern over systemic toxicity is mitigated due to, among other reasons, phospholipids are the ubiquitous components of cell membranes and are GRAS for human consumption.

Conclusion

The Panel concluded that the following 17 ingredients are safe in the present practices of use and concentration in cosmetics, as described in this safety assessment.

Lecithin
Hydrogenated Lecithin
Lysolecithin
Hydrogenated Lysolecithin*
Phospholipids
Hydrolyzed Phospholipids*
Phosphatidic Acid*
Lysophosphatidic Acid
Phosphatidylglycerol*
Lysophosphatidylglycerol*
Phosphatidylserine*
Ammonium Phosphatidyl Rapeseedate*
PC
Hydrogenated PC*
Hydrogenated Lysophosphatidylcholine*
Lysophosphatidylethanolamine*
Phosphatidylinositol*

*Not reported to be in current use. Were ingredients in this group not in current use to be used in the future, the expectation is that those ingredients would be used in product categories and at concentrations comparable to others in this group.

Authors' Note

Unpublished sources cited in this report are available from the Executive Director, Cosmetic Ingredient Review, 1620 L Street, NW, Suite 1200, Washington, DC 20036, USA.

Author Contribution

Johnson, W. contributed to conception and design; acquisition, analysis, and interpretation; drafted manuscript; and critically revised manuscript. Heldreth, B. contributed to conception and design; acquisition, analysis, and interpretation; and critically revised manuscript. Bergfeld, W., Belsito, D., Hill, R., Klaassen, C., Liebler, D., Marks, J., Shank, R., Slaga, T., and Snyder, P. contributed to conception and design, analysis and interpretation, and critically revised manuscript. All authors gave final approval and agree to be accountable for all aspects of work ensuring integrity and accuracy.

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References

1. Andersen FA. Final report on the safety assessment of lecithin and hydrogenated lecithin. *Int J Toxicol*. 2001;20(1):21-45.
2. Gennaro AR. *Remington's Pharmaceutical Sciences*. 18th ed. Mack Publishing; 1990.
3. Schlossman ML. *The Chemistry and Manufacture of Cosmetics*. 3rd ed. Allured Publishing Corp; 2002.
4. Barel A, Paye M, Maibach HI. *Handbook of Cosmetic Science and Technology*. 3rd ed. CRC Press; 2009.
5. Liu D, Ma F. Soybean phospholipids, recent trends for enhancing the diversity and quality of soybean products. In: Krezhova D, ed. *Chapter 22. Soybean Phospholipids*. ISBN: 978-953-307-533-4. http://www.intechopen.com/books//recent-trends-for-enhancing-the-diversity-and-quality-of-soybean-products/soybean-phospholipids
6. Nikitakis J, Breslawec HP. *International Cosmetic Ingredient Dictionary and Handbook*. 14 ed. Personal Care Products Council; 2014.
7. Lucas Meyer Cosmetics SAS. Emulmetik™ 100 (Lecithin) specification criteria. Unpublished data submitted by the Personal Care Products Council on 10-9-2014. 2013:1-2.
8. Lucas Meyer Cosmetics S.A.S. Emulmetik™ 300 (Lecithin) specification criteria. Unpublished data submitted by the Personal Care Products Council on 10-9-2014. 2014:1-2.
9. Lucas Meyer Cosmetics S.A.S. Emulmitek™ 320 (Hydrogenated Lecithin) specification criteria. Unpublished data submitted by the Personal Care Products Council on 10-9-2014. 2012:1.
10. Lucas Meyer Cosmetics S.A.S. Emulmitek™ 900 (Lecithin) specification criteria. Unpublished data submitted by the Personal Care Products Council on 10-8-2014. 2012:1.
11. Lucas Meyer Cosmetics S.A.S. Emulmetik™ 930 (phosphatidylcholine) specification criteria. Unpublished data submitted by the Personal Care Products Council on 10-9-2014. 2012:1.

12. Lucas Meyer Cosmetics S.A.S. Emulmitek™ 950 (Hydrogenated Lecithin) specification criteria. Unpublished data submitted by the Personal Care Products Council on 10-9-2014. 2013:1.
13. Lucas Meyer Cosmetics S.A.S. Emulmetik™ 120 (Lysolecithin) specification criteria. Unpublished data submitted by the Personal Care Products Council on 10-9-2014. 2012:1.
14. Lucas Meyer Cosmetics S.A.S. Emulmetik™ 970 (Lecithin) specification criteria. Unpublished data submitted by the Personal Care Products Council on 10-9-2014. 2014:1.
15. Lucas Meyer Cosmetics S.A.S. Ovothin™ 120 (Lecithin) specification criteria. Unpublished data submitted by the Personal Care Products Council on 10-9-2014. 2013:1-2.
16. Lucas Meyer Cosmetics S.A.S. LECI-PS 20 P IP (phospholipids) specification criteria. Unpublished data submitted by the Personal Care Products Council on 10-9-2014. 2012:1.
17. Food and Drug Administration. Direct food substances affirmed as generally recognized as safe. Lecithin. 21CFR 184.1400.
18. Food and Drug Administration. Withdrawn: guidance on the labeling of certain uses of lecithin derived from soy under section 403(w) of the federal food, drug, and cosmetic act. Updated 2014. http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/LabelingNutrition/ucm059065.htm
19. Awazuhara H, Kawai B, Baba M, Matsui T, Komiyama A. Antigenicity of the proteins in soy Lecithin and soy oil in soybean allergy. *Clin Exp All*. 1998;28(12):1559-1564.
20. Food and Drug Administration. Substances added directly to human food affirmed as generally recognized as safe. Enzyme-modified Lecithin. 21CFR 184.1063. 2013.
21. Damnjanovic J, Nakano H, Iwasaki Y. Simple and efficient profiling of phospholipids in phospholipase D-modified soy Lecithin by HPLC with charged aerosol detection. *J The Ame Oil Chem' Society*. 2013;90(7):951-957.
22. Yokozeki Oil & Fat Industries Co., Ltd. Method of manufacture and composition: Phosphatidylglycerol. Unpublished data submitted by the Personal Care Products Council on 10-30-2014. 2014:1.
23. Lipogen Products (9000) Ltd. *Notification of GRAS Determination for Soy Lecithin Phosphatidylserine Complex*; 2005:1-91.
24. Van Hoogevest P, Wendel A. The use of natural and synthetic phospholipids as pharmaceutical excipients. *Eur J Lipid Sci Technol*. 2014;116(6):1088-1107.
25. Food and Drug Administration. Database of select committee on GRAS substances (SCOGS) reviews. *Lecithin*. Updated 2014. Accessed August 10, 2014. http://www.fda.gov
26. The United States Pharmacopeial Convention. *Food Chemicals Codex*. 8th ed. The United States Pharmacopeial Convention; 2012.
27. The United States Pharmacopeial Convention. *USP 37. NF 32*. 37 ed. United States Pharmacopoeial Convention, 2014.
28. Pensabene JW, Fiddler W, Doerr RC, Lakritz L, Wasserman AE. Formation of di methyl nitrosamine from commercial Lecithin and its components in a model system. *J Agric Food Chem*. 1975;23(1):979-980.
29. Hill MJ. Bacterial metabolism and colon cancer. *Nutr Cancer*. 1979;1(10):46-50.
30. Shank RC, Magee PN. Toxicity and carcinogenicity of N-nitroso compounds. Chapter: 1 In: Shank RC, ed. *Mycotoxins and N-Nitroso Compounds: Environmental Risks*. CRC Press, Inc.; 1981:185-217.
31. Rostkowska K, Zwierz K, Rozanski A, Moniuszko-Jakoniuk J, Roszczenko A. Formation and metabolism of N-nitrosamines. *Polish J Enviro Stud*. 1998;7(6):321-325.
32. Challis BC, Shuker DE, Fine DH, Goff EU, Hoffman GA. Amine nitration and nitrosation by gaseous nitrogen dioxide. *IARC Sci Publ*. 1982;(41):11-20.
33. Food and Drug Administration. *Information Supplied to FDA by Industry as Part of the VCRP FDA Database*. FDA; 2015.
34. Personal Care Products Council. Concentration of use by FDA product category: Lecithin and other phosphor glycerides. Unpublished data submitted by the Personal Care Products Council on 7-30-2014. 2014:1.
35. Rothe H, Fautz R, Gerber E, et al. Special aspects of cosmetic spray safety evaluations: principles on inhalation risk assessment. *Toxicol Lett*. 2011;205(2):97-104.
36. Bremmer HJ, Homme de Lodder LCH, van Engelen JGM. Cosmetics fact sheet: to assess the risks for the consumer; updated version for cons expo 4. 20200. Report No. RIVM 320104001/. 2006:1-77. Accessed August 24, 2011. http://www.rivm.nl/bibliotheek/rapporten/320104001.pdf
37. Rothe H. Special aspects of cosmetic spray evaluation. Unpublished information presented to the 26 September CIR Expert Panel; Washington, DC. 2011.
38. Johnsen MA. The influence of particle size. *Spray Technol Market*. 2004;14(11):24-27.
39. Food and Drug Administration. Use of prohibited cattle materials in cosmetic products. 21 CFR 700.27. 2014.
40. Food and Drug Administration. Inactive ingredients database. Inactive ingredient search for approved drug products. Updated 2014. http://www.accessdata.fda.gov/scripts/cder/iig/getiigWEB.cfm
41. Da Silva RP, Kelly KB, Al Rajabi A, Jacobs RL. Novel insights on interactions between folate and lipid metabolism. *Biofactors*. 2014;40(3):277-283.
42. Won TJ, Nam Y, Lee HS, et al. Injection of phosphatidylcholine and deoxycholic acid regulates gene expression of lipolysis-related factors, pro-inflammatory cytokines, and hormones on mouse fat tissue. *Food Chem Toxicol*. 2013;60(2):263-268.
43. Stein O, Stein Y. The fate of intravenously injected 1-C^{14} palmitoyl P^{32}-Lysolecithin in the rat. *Life Sci*. 1965;4(1):203-208.
44. Pieringer RA, Hokin LE. Biosynthesis of phosphatidic acid from lysophosphatidic acid and palmitoyl coenzyme A. *J Biol Chem*. 1962;237(3):659-663.
45. Orlando P, Cerrito F, Zirilli P. The fate of double-labelled brain phospholipids administered to mice. *Farm*. 1975;30(9):451-458.
46. Mazzari S, Zanotti A, Orlando P, Raciti R, Toffano G. Pharmacokinetics and pharmacodynamics of phosphatidylserine liposomes in mice. In: Antolini R, Gliozzi A, Gorio A, ed. *Transport in Biomembranes: Model Systems and Reconstitution*. Raven Press; 1982:257-263.
47. Toffano G, Battistella A, Mazzari S, Orlando P, Massari P, Giordano C. Fate of phosphatidyl-L-(U-14C) serine in mice. In:

Horrocks LA, Ansell GB, Porcellati G, ed. *Phospholipids in the Nervous System. Volume 1*. Raven Press; 1982:173-180.
48. Toffano G., Battistella A, Orlando P. Pharmacokinetics of radiolabelled brain phosphatidylserine. *Clin Trials J*. 1987;24(1): 18-24.
49. Palatini P, Viola G, Bigon E, Menegus AM, Bruni A. Pharmacokinetic characterization of phosphatidylserine liposomes in the rat. *Br J Pharmacol*. 1991;102(2):345-350.
50. Bruni A, Bellinin F, Mietto L, Boarato E, Viola G. Phospholipid absorption and diffusion through membranes. In: Hanin I, Pepeu G, ed. *Phospholipids. Biochemical, Pharmaceutical, and Analytical Considerations*. Plenum Press; 1990:59-68.
51. Bruni A, Orlando P, Mietto L, Viola G. Phospholipid metabolism in rat intestinal mucosa after oral administration of lysophospholipids. In: Bazan NG, ed. *Neurobiology of Essential Fatty Acids*. Plenum Press; 1992.
52. Kuge O, Nishijima M. Biosynthetic regulation and intracellular transport of phosphatidylserine in mammalian cells. *J Biochem*. 2003;133(4):397-403.
53. Linder MC. Nutrition and metabolism of fats. In: Linder MC, ed. *Nutritional Biochemistry and Metabolism: With Clinical Applications*. 2nd ed. Elsevier Science Publishing company, Inc; 2014:51-84.
54. Krummel D. *Lipids [and Polyunsaturated Fatty Acids]*. W.B. Saunders Company; 1996:49-62.
55. Thorne Research, Inc. Phosphatidylserine. *Alter Med Rev*. 2008; 13(3):245-247.
56. Fujii M, Shiozawa K, Watanabe Y, Matsumoto M. Effect of phosphatidylcholine on skin permeation of indomethacin from gel prepared with liquid paraffin and hydrogenated phospholipid. *Int J Pharm*. 2001;222(1):57-64.
57. Yokomizo Y, Sagitani H. Effects of phospholipids on the perctaneous penetration of indomethacin through the dorsal skin of guinea pigs in vitro. *J Control Release*. 1996;38(2-3):267-274.
58. Heywood R, Cozens DD, Richold M. Toxicology of a phosphatidylserine preparation from bovine brain (BC-PS). *Clin Trials J*. 2014;24(1):25-32.
59. Myers MA, Thomas DA, Straub L, et al. Pulmonary effects of chronic exposure to liposome aerosols in mice. *Exp Lung Res*. 1993;19(3):1-19.
60. Brantom PG, Gaunt IF, Jardy J, Grasso P, Gangolli SD. Long-term feeding and reproduction studies on emulsifier YN in rats. *Food Cosmet Toxico*. 1973;11(5):755-769.
61. Enzymotec, Ltd. Sub chronic toxicity evaluation of phosphatidylserine in elderly rat model. Updated 2010. Accessed July 7, 2014. www.enzymotec.com
62. Jorissen BL, Brouns F, Van Boxtel MP, Riedel WJ. Safety of soy-derived phosphatidylserine in elderly people. *Nutr Neurosci*. 2002;5(5):337-343.
63. Jeannet M, Hassig A. The role of lysophosphatides and fatty acids in hemolysis. *Vox Sang*. 1964;9(3):113-127.
64. Robinson N.Review article. Lysolecithin. *J Pharm Pharm*. 1961; 13(1):321-354.
65. Nagamatsu T, Iwasawa-Kawai Y, Ichikawa M, et al. Emerging roles for lysophospholipid mediators in pregnancy. *Ame J Rep Immunol*. 2014;72(3):182-191.
66. Jo JW, Jee BC, Suh CS, Kim SH. Addition of lysophosphatidic acid to mouse oocyte maturation media can enhance fertilization and developmental competence. *Human Reproduction*. 2014; 29(2):234-241.
67. Kato S., Aoshima H, Saitoh Y, Miwa N. Biological safety of liposome-fullerene consisting of hydrogenated lecithin, glycine soja sterols, and fullerene-C60 upon photocytotoxicity and bacterial reverse mutagenicity. *Toxicol Indus Health*. 2009;25(3): 197-203.
68. Bojanowski K. Hypodermal delivery of cosmetic actives for improved facial skin morphology and functionality. *Int J Cos Sci*. 2013;35(6):562-567.
69. Szepsenwol J. Brain nerve cell tumors in mice on diets supplemented with various lipids. *Pathol Miocrobiol*. 1969;34(1):1-9.
70. Mori K, Kondo M, Suzuki S. Induction of lung cancer in mice and rats by injections of 4-nitroquinoline 1-oxide in Lecithin. *Gann*. 1966;57(5):559-561.
71. Middleton E Jr, Phillips GB. Release of histamine activity in human skin by Lysolecithin. *Lab Clin Med*. 1964;64(6):889-894.
72. Beliveau S, Gaudreault P, Goulet L, Primeau MN, Marcoux D. Type I hypersensitivity in an asthmatic child allergic to peanuts: was soy Lecithin to blame? *J Cutan Med Surg*. 2008;12(1):27-30.
73. Fernandis AZ, Wenk MR. Membrane lipids as signaling molecules. *Curr Opin Lipidol*. 2007;18(2):121-128.
74. Hannun YA, Obeid LM. Principles of bioactive lipid signalling: lessons from sphingolipids. *Mole Cell Biol*. 2008;9(2):139-150.
75. Wymann MP, Schneiter R. Lipid signalling in disease. *Nature*. 2008;9(2):162-176.
76. Balla T. Phosphoinositides: tiny lipids with giant impact on cell regulation. *Physiol Rev*. 2013;93(3):1019-1137.
77. Billcliff PG, Lowe M. Inositol lipid phosphatases in membrane trafficking and human disease. *Biochem J*. 2014;461(2):159-175.
78. Ridgway ND. The role of phosphatidylcholine and choline metabolites to cell proliferation and survival. *Crit Rev Biochem Mol Biol*. 2013;48(1):20-38.
79. Teo ST, Yung YC, Herr DR, Chun J. Lysophosphatidic acid in vascular development and disease. *IUBMB Life*. 2009;61(8): 791-799.
80. Kay JG, Grinstein S. Phosphatidylserine-mediated cellular signaling. *Adv Exp Med Biol*. 2014;991:177-193.
81. Kim HY, Huang BX, Spector AA. Phosphatidylserine in the brain: metabolism and function. *Pro Lipid Res*. 2014;56:1-18.
82. Schwartz HP, Dreisbach L, Stambaugh R, Kleschick A, Barrionievo M. Chromatography of the phospholipids of rabbit skin. *Arch Biochem Biophys*. 1960;87:171-178.
83. Lampe MA, Burlingame AL, Whitney J, et al. Human stratum corneum lipids: characterization and regional variations. *J Lipid Res*. 1983;24(2):120-130.

SAFETY DATA SHEET

according to Regulation UK SI 2019/758 and UK SI 2020/1577 as amended

Creation Date 26-Jan-2011 **Revision Date** 24-Jan-2024 **Revision Number** 4

SECTION 1: IDENTIFICATION OF THE SUBSTANCE/MIXTURE AND OF THE COMPANY/UNDERTAKING

1.1. Product identifier

Product Description:	**Lecithin, 90%, soybean**
Cat No. :	**J61675**
Synonyms	Phosphatidylcholine
CAS No	8002-43-5
EC No	232-307-2
Molecular Formula	C42 H80 N O8 P
REACH registration number	-

1.2. Relevant identified uses of the substance or mixture and uses advised against

Recommended Use	Laboratory chemicals.
Uses advised against	No Information available

1.3. Details of the supplier of the safety data sheet

Company

Avocado Research Chemicals Ltd.
(Part of Thermo Fisher Scientific)
Shore Road, Heysham
Lancashire, LA3 2XY,
United Kingdom
Office Tel: +44 (0) 1524 850506
Office Fax: +44 (0) 1524 850608

E-mail address begel.sdsdesk@thermofisher.com

1.4. Emergency telephone number

For information **US** call: 001-800-227-6701 / **Europe** call: +32 14 57 52 11
Emergency Number **US:**001-201-796-7100 / **Europe:** +32 14 57 52 99
CHEMTREC Tel. No. **US:**001-800-424-9300 / **Europe:**001-703-527-3887

SECTION 2: HAZARDS IDENTIFICATION

2.1. Classification of the substance or mixture

CLP Classification - According to GB-CLP Regulations UK SI 2019/720 and UK SI 2020/1567

Physical hazards

Based on available data, the classification criteria are not met

Health hazards

Based on available data, the classification criteria are not met

Environmental hazards
Based on available data, the classification criteria are not met

Full text of Hazard Statements: see section 16

2.2. Label elements
None required

2.3. Other hazards

This product does not contain any known or suspected endocrine disruptors

SECTION 3: COMPOSITION/INFORMATION ON INGREDIENTS

3.1. Substances

Component	CAS No	EC No	Weight %	CLP Classification - According to GB-CLP Regulations UK SI 2019/720 and UK SI 2020/1567
Soybean lecithin	8002-43-5	EEC No. 232-307-2	>95	-

REACH registration number	-

Full text of Hazard Statements: see section 16

SECTION 4: FIRST AID MEASURES

4.1. Description of first aid measures

Eye Contact Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes. Get medical attention.

Skin Contact Wash off immediately with plenty of water for at least 15 minutes. Get medical attention immediately if symptoms occur.

Ingestion Do NOT induce vomiting. Clean mouth with water and drink afterwards plenty of water. Get medical attention if symptoms occur.

Inhalation Remove to fresh air. If not breathing, give artificial respiration. Get medical attention immediately if symptoms occur.

Self-Protection of the First Aider No special precautions required.

4.2. Most important symptoms and effects, both acute and delayed

None reasonably foreseeable.

4.3. Indication of any immediate medical attention and special treatment needed

Notes to Physician Treat symptomatically.

SECTION 5: FIREFIGHTING MEASURES

5.1. Extinguishing media

Suitable Extinguishing Media
Water spray, carbon dioxide (CO2), dry chemical, alcohol-resistant foam.

Extinguishing media which must not be used for safety reasons
No information available.

5.2. Special hazards arising from the substance or mixture

Thermal decomposition can lead to release of irritating gases and vapors.

Hazardous Combustion Products
Nitrogen oxides (NOx), Carbon monoxide (CO), Carbon dioxide (CO_2), Oxides of phosphorus.

5.3. Advice for firefighters

As in any fire, wear self-contained breathing apparatus pressure-demand, MSHA/NIOSH (approved or equivalent) and full protective gear.

SECTION 6: ACCIDENTAL RELEASE MEASURES

6.1. Personal precautions, protective equipment and emergency procedures

Use personal protective equipment as required. Ensure adequate ventilation. Avoid dust formation.

6.2. Environmental precautions

Should not be released into the environment. See Section 12 for additional Ecological Information.

6.3. Methods and material for containment and cleaning up

Sweep up and shovel into suitable containers for disposal. Avoid dust formation.

6.4. Reference to other sections

Refer to protective measures listed in Sections 8 and 13.

SECTION 7: HANDLING AND STORAGE

7.1. Precautions for safe handling

Wear personal protective equipment/face protection. Ensure adequate ventilation. Avoid contact with skin, eyes or clothing. Avoid ingestion and inhalation. Avoid dust formation. Wash hands before breaks and immediately after handling the product.

Hygiene Measures
Handle in accordance with good industrial hygiene and safety practice. Keep away from food, drink and animal feeding stuffs. Do not eat, drink or smoke when using this product. Remove and wash contaminated clothing and gloves, including the inside, before re-use. Wash hands before breaks and after work.

7.2. Conditions for safe storage, including any incompatibilities

Keep containers tightly closed in a dry, cool and well-ventilated place.

Technical Rules for Hazardous Substances (TRGS) 510 Storage Class (LGK) (Germany) Class 11

7.3. Specific end use(s)

Use in laboratories

SECTION 8: EXPOSURE CONTROLS/PERSONAL PROTECTION

8.1. Control parameters

Exposure limits
This product, as supplied, does not contain any hazardous materials with occupational exposure limits established by the region specific regulatory bodies

Biological limit values
This product, as supplied, does not contain any hazardous materials with biological limits established by the region specific regulatory bodies

Derived No Effect Level (DNEL) / Derived Minimum Effect Level (DMEL)
No information available

Predicted No Effect Concentration (PNEC)
No information available.

8.2. Exposure controls

Engineering Measures
None under normal use conditions.

Personal protective equipment

Eye Protection Wear safety glasses with side shields (or goggles) (European standard - EN 166)

Hand Protection Protective gloves

Glove material	Breakthrough time	Glove thickness	EU standard	Glove comments
Natural rubber Nitrile rubber Neoprene PVC	See manufacturers recommendations	-	EN 374	(minimum requirement)

Skin and body protection Wear appropriate protective gloves and clothing to prevent skin exposure.

Inspect gloves before use.
Please observe the instructions regarding permeability and breakthrough time which are provided by the supplier of the gloves. (Refer to manufacturer/supplier for information)
Ensure gloves are suitable for the task: Chemical compatability, Dexterity, Operational conditions, User susceptibility, e.g. sensitisation effects, also take into consideration the specific local conditions under which the product is used, such as the danger of cuts, abrasion.
Remove gloves with care avoiding skin contamination.

Respiratory Protection	No protective equipment is needed under normal use conditions.
Large scale/emergency use	Use a NIOSH/MSHA or European Standard EN 136 approved respirator if exposure limits are exceeded or if irritation or other symptoms are experienced **Recommended Filter type:** Particle filter
Small scale/Laboratory use	Maintain adequate ventilation
Environmental exposure controls	No information available.

SECTION 9: PHYSICAL AND CHEMICAL PROPERTIES

9.1. Information on basic physical and chemical properties

Physical State	Solid	
Appearance	Beige	
Odor	Odorless	
Odor Threshold	No data available	
Melting Point/Range	No data available	
Softening Point	No data available	
Boiling Point/Range	No information available	
Flammability (liquid)	Not applicable	Solid
Flammability (solid,gas)	No information available	
Explosion Limits	No data available	
Flash Point	No information available	**Method -** No information available
Autoignition Temperature	No data available	
Decomposition Temperature	No data available	
pH	6.8	1% aq.sol
Viscosity	Not applicable	Solid
Water Solubility	practically insoluble	
Solubility in other solvents	No information available	
Partition Coefficient (n-octanol/water)		
Vapor Pressure	No data available	
Density / Specific Gravity	No data available	
Bulk Density	No data available	
Vapor Density	Not applicable	Solid
Particle characteristics	No data available	

9.2. Other information

Molecular Formula	C42 H80 N O8 P
Molecular Weight	758.06
Evaporation Rate	Not applicable - Solid

SECTION 10: STABILITY AND REACTIVITY

10.1. Reactivity

None known, based on information available

10.2. Chemical stability

Stable under normal conditions. Hygroscopic.

10.3. Possibility of hazardous reactions

Hazardous Polymerization	Hazardous polymerization does not occur.
Hazardous Reactions	None under normal processing.

10.4. Conditions to avoid

Incompatible products. Excess heat. Avoid dust formation. Exposure to moist air or water.

10.5. Incompatible materials

Strong oxidizing agents.

10.6. Hazardous decomposition products

Nitrogen oxides (NOx). Carbon monoxide (CO). Carbon dioxide (CO_2). Oxides of phosphorus.

SECTION 11: TOXICOLOGICAL INFORMATION

11.1. Information on hazard classes as defined in Regulation (EC) No 1272/2008

Product Information No acute toxicity information is available for this product

(a) acute toxicity;

Oral Based on available data, the classification criteria are not met
Dermal No data available
Inhalation No data available

Component	LD50 Oral	LD50 Dermal	LC50 Inhalation
Soybean lecithin	>8 g/kg (Rat)	-	-

(b) skin corrosion/irritation; No data available

(c) serious eye damage/irritation; No data available

(d) respiratory or skin sensitization;

Respiratory No data available
Skin No data available

(e) germ cell mutagenicity; No data available

(f) carcinogenicity; No data available

There are no known carcinogenic chemicals in this product

(g) reproductive toxicity; No data available

(h) STOT-single exposure; No data available

(i) STOT-repeated exposure; No data available

Target Organs No information available.

(j) aspiration hazard; Not applicable
Solid

Other Adverse Effects The toxicological properties have not been fully investigated.

Symptoms / effects,both acute and delayed No information available.

11.2. Information on other hazards

Endocrine Disrupting Properties Assess endocrine disrupting properties for human health. This product does not contain any known or suspected endocrine disruptors.

SECTION 12: ECOLOGICAL INFORMATION

12.1. Toxicity

Ecotoxicity effects Do not empty into drains. .

12.2. Persistence and degradability

Persistence Insoluble in water.

12.3. Bioaccumulative potential May have some potential to bioaccumulate

12.4. Mobility in soil Spillage unlikely to penetrate soil Is not likely mobile in the environment due its low water solubility.

12.5. Results of PBT and vPvB assessment No data available for assessment.

12.6. Endocrine disrupting properties

Endocrine Disruptor Information This product does not contain any known or suspected endocrine disruptors

12.7. Other adverse effects

Persistent Organic Pollutant This product does not contain any known or suspected substance

Ozone Depletion Potential This product does not contain any known or suspected substance

SECTION 13: DISPOSAL CONSIDERATIONS

13.1. Waste treatment methods

Waste from Residues/Unused Products Chemical waste generators must determine whether a discarded chemical is classified as a hazardous waste. Consult local, regional, and national hazardous waste regulations to ensure complete and accurate classification.

Contaminated Packaging Empty remaining contents. Dispose of in accordance with local regulations. Do not re-use empty containers.

European Waste Catalogue (EWC) According to the European Waste Catalog, Waste Codes are not product specific, but application specific.

Other Information Waste codes should be assigned by the user based on the application for which the product was used.

SECTION 14: TRANSPORT INFORMATION

IMDG/IMO Not regulated

14.1. UN number
14.2. UN proper shipping name
14.3. Transport hazard class(es)
14.4. Packing group

ADR Not regulated

14.1. UN number
14.2. UN proper shipping name
14.3. Transport hazard class(es)
14.4. Packing group

IATA Not regulated

14.1. UN number
14.2. UN proper shipping name
14.3. Transport hazard class(es)
14.4. Packing group

14.5. Environmental hazards No hazards identified

14.6. Special precautions for user No special precautions required.

14.7. Maritime transport in bulk according to IMO instruments Not applicable, packaged goods

SECTION 15: REGULATORY INFORMATION

15.1. Safety, health and environmental regulations/legislation specific for the substance or mixture

International Inventories
Europe (EINECS/ELINCS/NLP), China (IECSC), Taiwan (TCSI), Korea (KECL), Japan (ENCS), Japan (ISHL), Canada (DSL/NDSL), Australia (AICS), New Zealand (NZIoC), Philippines (PICCS). US EPA (TSCA) - Toxic Substances Control Act, (40 CFR Part 710)

Component	CAS No	EINECS	ELINCS	NLP	IECSC	TCSI	KECL	ENCS	ISHL
Soybean lecithin	8002-43-5	232-307-2	-	-	X	X	KE-21956	-	-

Component	CAS No	TSCA	TSCA Inventory notification - Active-Inactive	DSL	NDSL	AICS	NZIoC	PICCS
Soybean lecithin	8002-43-5	X	ACTIVE	X	-	X	X	X

Legend: X - Listed '-' - Not Listed **KECL** - NIER number or KE number (http://ncis.nier.go.kr/en/main.do)

Authorisation/Restrictions according to EU REACH Not applicable

Component	CAS No	REACH (1907/2006) - Annex XIV - Substances Subject to Authorization	REACH (1907/2006) - Annex XVII - Restrictions on Certain Dangerous Substances	REACH Regulation (EC 1907/2006) article 59 - Candidate List of Substances of Very High Concern (SVHC)
Soybean lecithin	8002-43-5	-	-	-

Seveso III Directive (2012/18/EC)

Component	CAS No	Seveso III Directive (2012/18/EC) - Qualifying Quantities for Major Accident Notification	Seveso III Directive (2012/18/EC) - Qualifying Quantities for Safety Report Requirements
Soybean lecithin	8002-43-5	Not applicable	Not applicable

Regulation (EC) No 649/2012 of the European Parliament and of the Council of 4 July 2012 concerning the export and import of dangerous chemicals
Not applicable

Contains component(s) that meet a 'definition' of per & poly fluoroalkyl substance (PFAS)?
Not applicable

Take note of Directive 98/24/EC on the protection of the health and safety of workers from the risks related to chemical agents at work .

National Regulations

UK - Take note of Control of Substances Hazardous to Health Regulations (COSHH) 2002 and 2005 Amendment

WGK Classification See table for values

Component	Germany - Water Classification (AwSV)	Germany - TA-Luft Class
Soybean lecithin	WGK1	

15.2. Chemical safety assessment

A Chemical Safety Assessment/Report (CSA/CSR) has not been conducted

SECTION 16: OTHER INFORMATION

Full text of H-Statements referred to under sections 2 and 3

Legend

CAS - Chemical Abstracts Service

EINECS/ELINCS - European Inventory of Existing Commercial Chemical Substances/EU List of Notified Chemical Substances
PICCS - Philippines Inventory of Chemicals and Chemical Substances
IECSC - Chinese Inventory of Existing Chemical Substances
KECL - Korean Existing and Evaluated Chemical Substances

TSCA - United States Toxic Substances Control Act Section 8(b) Inventory
DSL/NDSL - Canadian Domestic Substances List/Non-Domestic Substances List
ENCS - Japanese Existing and New Chemical Substances
AICS - Australian Inventory of Chemical Substances
NZIoC - New Zealand Inventory of Chemicals

WEL - Workplace Exposure Limit
ACGIH - American Conference of Governmental Industrial Hygienists
DNEL - Derived No Effect Level
RPE - Respiratory Protective Equipment
LC50 - Lethal Concentration 50%
NOEC - No Observed Effect Concentration
PBT - Persistent, Bioaccumulative, Toxic

TWA - Time Weighted Average
IARC - International Agency for Research on Cancer
Predicted No Effect Concentration (PNEC)
LD50 - Lethal Dose 50%
EC50 - Effective Concentration 50%
POW - Partition coefficient Octanol:Water
vPvB - very Persistent, very Bioaccumulative

ADR - European Agreement Concerning the International Carriage of Dangerous Goods by Road
IMO/IMDG - International Maritime Organization/International Maritime Dangerous Goods Code
OECD - Organisation for Economic Co-operation and Development
BCF - Bioconcentration factor

ICAO/IATA - International Civil Aviation Organization/International Air Transport Association
MARPOL - International Convention for the Prevention of Pollution from Ships
ATE - Acute Toxicity Estimate
VOC - (Volatile Organic Compound)

Key literature references and sources for data
https://echa.europa.eu/information-on-chemicals
Suppliers safety data sheet, Chemadvisor - LOLI, Merck index, RTECS

Training Advice
Chemical hazard awareness training, incorporating labelling, Safety Data Sheets (SDS), Personal Protective Equipment (PPE) and hygiene.

Prepared By	Health, Safety and Environmental Department
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Revision Summary	New emergency telephone response service provider.

This safety data sheet complies with Regulation UK SI 2019/758 and UK SI 2020/1577 as amended. .

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Disclaimer

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End of Safety Data Sheet