The distribution of environmental contaminants and pharmaceuticals among skim milk, milk fat, curd, whey, and milk protein fractions through milk processing

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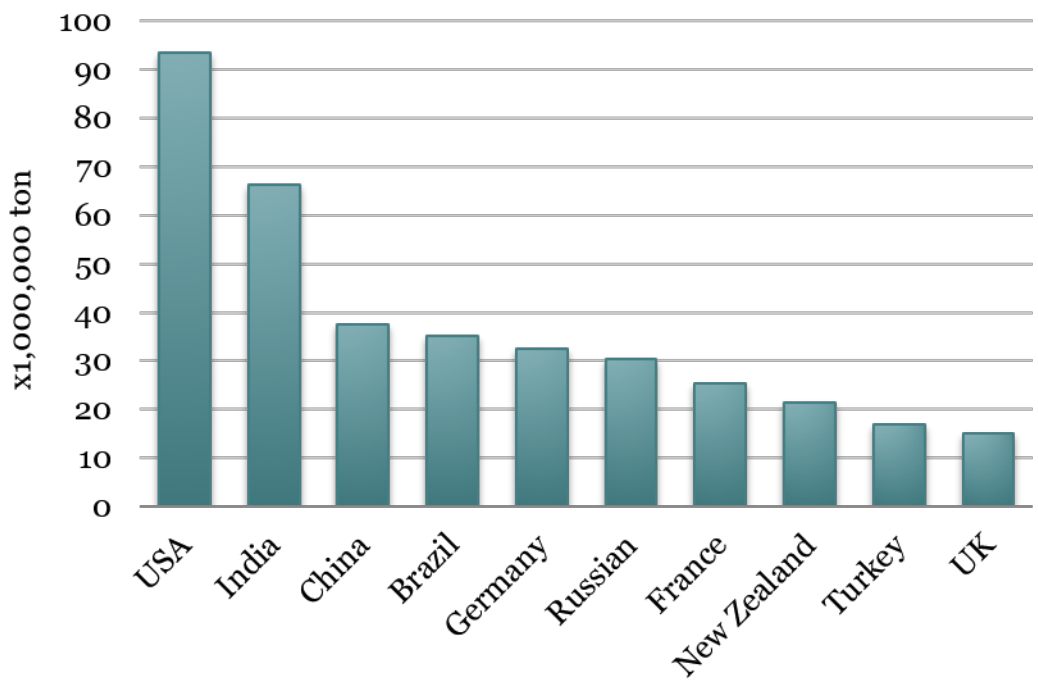
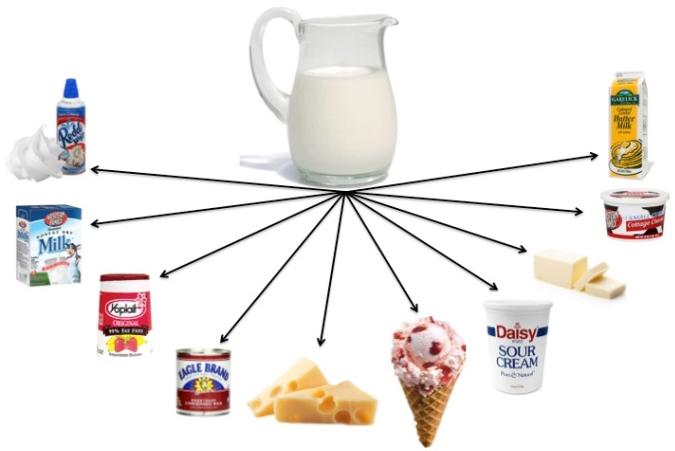
Milk



Top 10 cow milk production nations in 2014

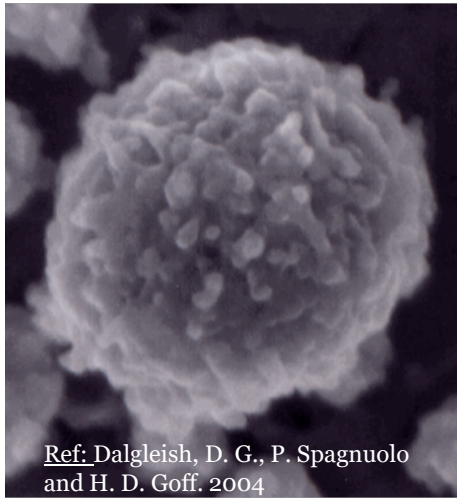


- Milk consumption > 150 kg/capita/year in Europe and North America
- More than 6 billion people worldwide consume milk and milk products
- Milk products provide 11-14% of dietary energy in Europe and North America

Image: https://www.agclassroom.org/teacher/matrix/lessonplan_print.cfm?lpid=246
Data source: Food and Agriculture Organization of the United Nations

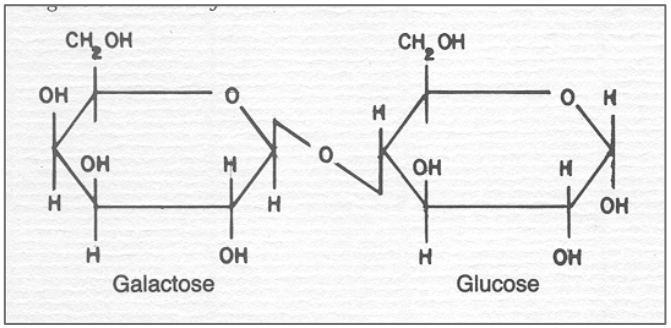
Milk Constituents

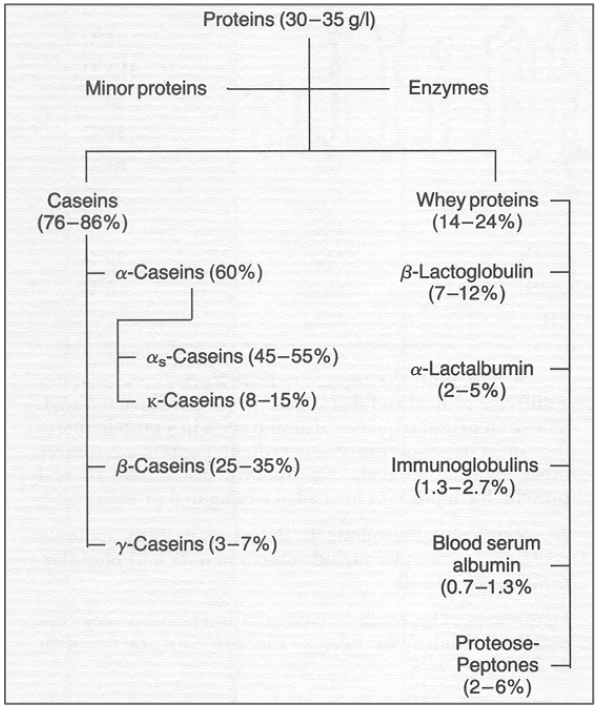
- Milk is a complex emulsion or colloid within a water-based fluid
  - Proteins (~3%, casein micelles, soluble whey)
  - Lipid (~3.5%, fat globules)
  - Sugars (lactose)
  - Minerals, vitamins, enzymes, etc…..



Ref: Dalgleish, D. G., P. Spagnuolo and H. D. Goff. 2004

Lactose





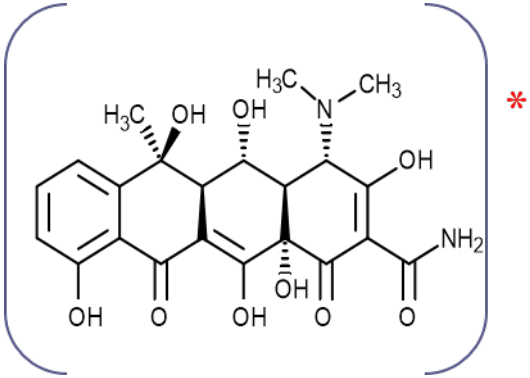
US FDA Risk Assessment for Milk

- National Conference on Interstate Milk Shipments
  - Every tanker truck tested for β-lactam antibiotics
    - ~0.05% test positive from > 3 million tankers per year
  - July 1, 2017 Pilot study- one out of every 15 tanker trucks tested for tetracyclines
- Multi-criteria based ranking system on over 500 animal drugs
  - Likelihood of a drug's use in lactating dairy cows
  - Likelihood of its presence in milk
  - Human exposure to drug residues through consumption of milk products
  - Potential to be a human health hazard
- Identified data gap

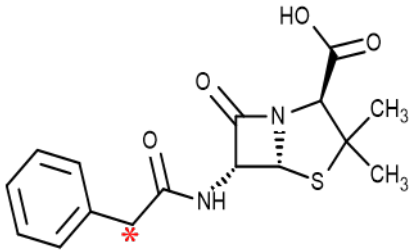
Compound Selection Criteria

- Drugs (or close analogs) were evaluated in the FDA 2015 ranking model
- Widespread environmental contaminants with potential to be present in cow milk
- Selected chemicals ensure the sample set spans a wide range of lipophilicity
- Commercial or in-house availability of radiolabeled test compounds

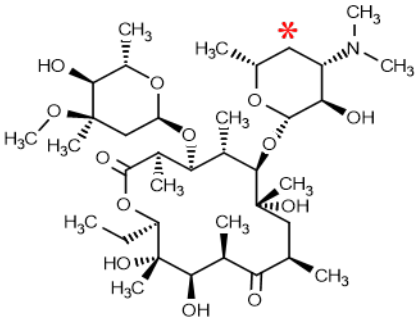
Drugs



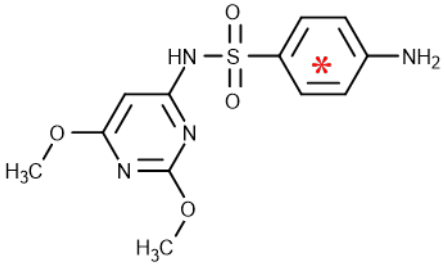
Oxytetracycline



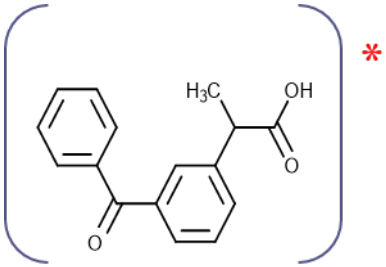
Penicillin G



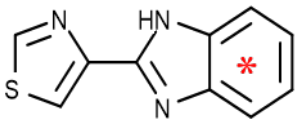
Erythromycin A



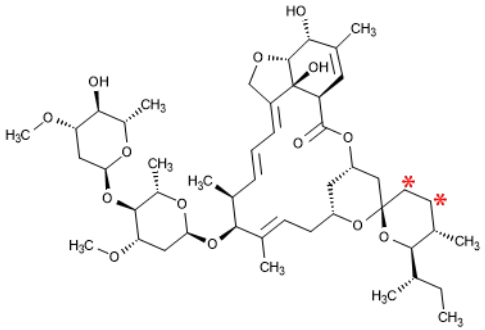
Sulfadimethoxine



Ketoprofen

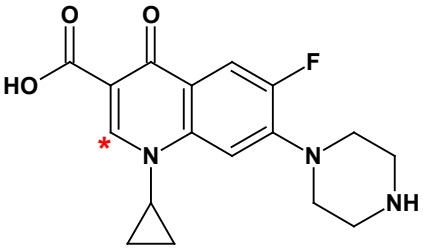


Thiabendazole

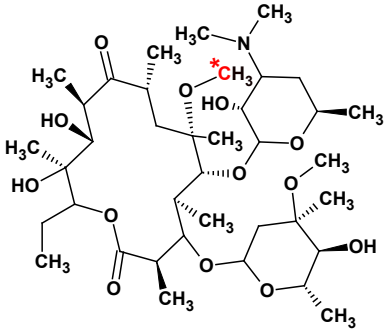


Ivermectin B1a

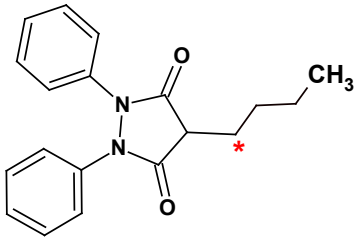
Drugs



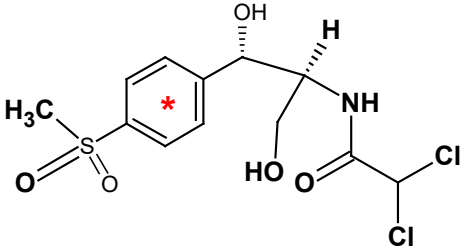
Ciprofloxacin



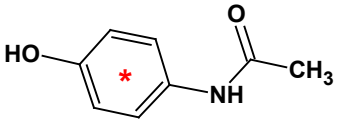
Clarithromycin



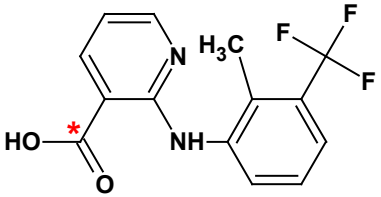
Phenylbutazone



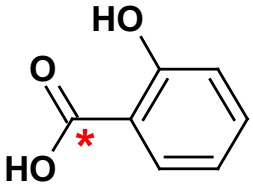
Thiamphenicol



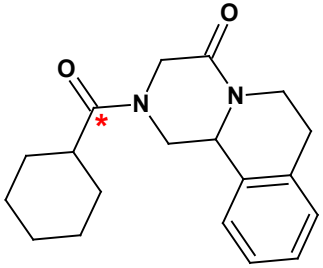
Acetaminophen



Flunixin

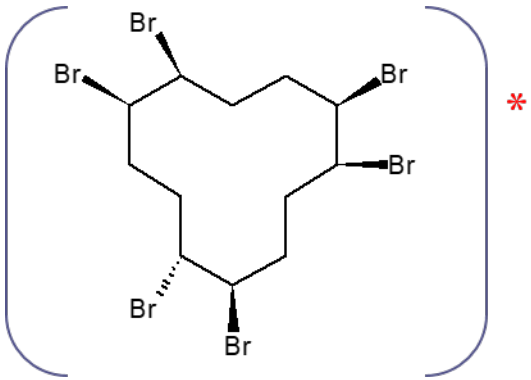


Salicylic acid

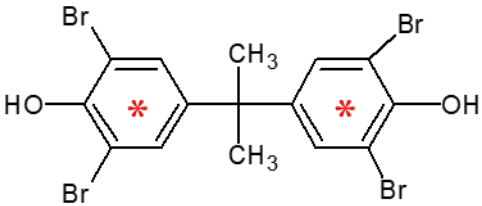


Praziquantel

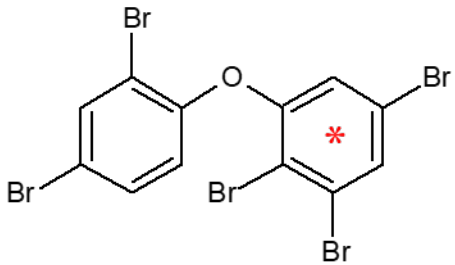
Environmental Contaminants



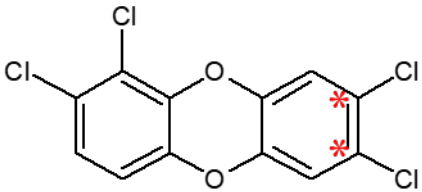
β-HBCD



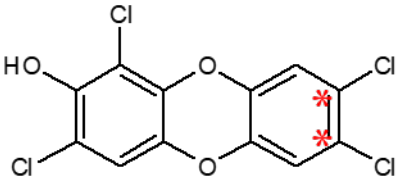
TBBPA



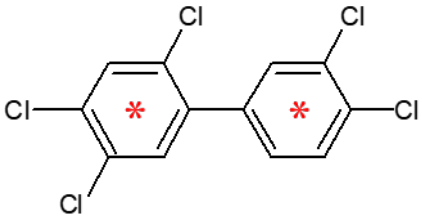
BDE-99



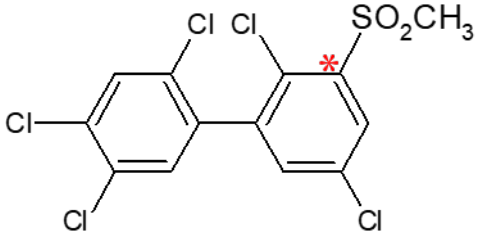
1278-TCDD



2-OH 1378-TCDD

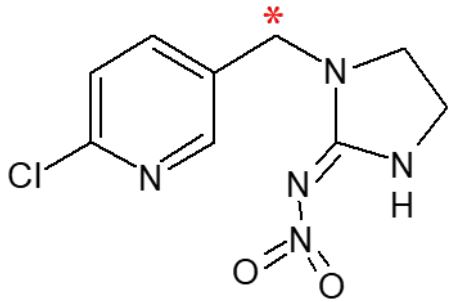


PCB 118

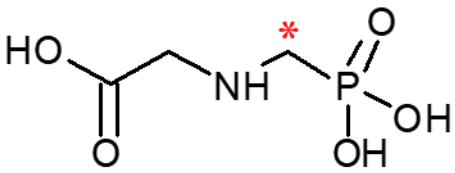


3’MeSO2 PCB 101

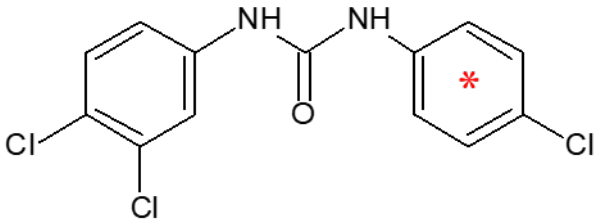
Environmental Contaminants



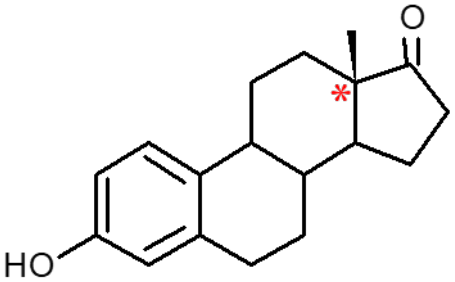
Imidacloprid



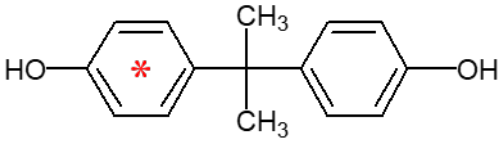
Glyphosate



Triclocarban

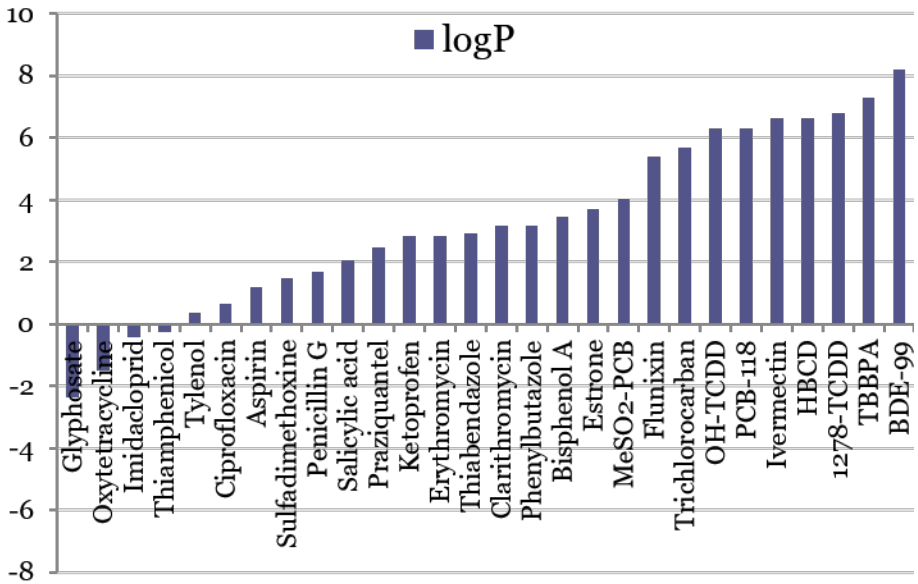


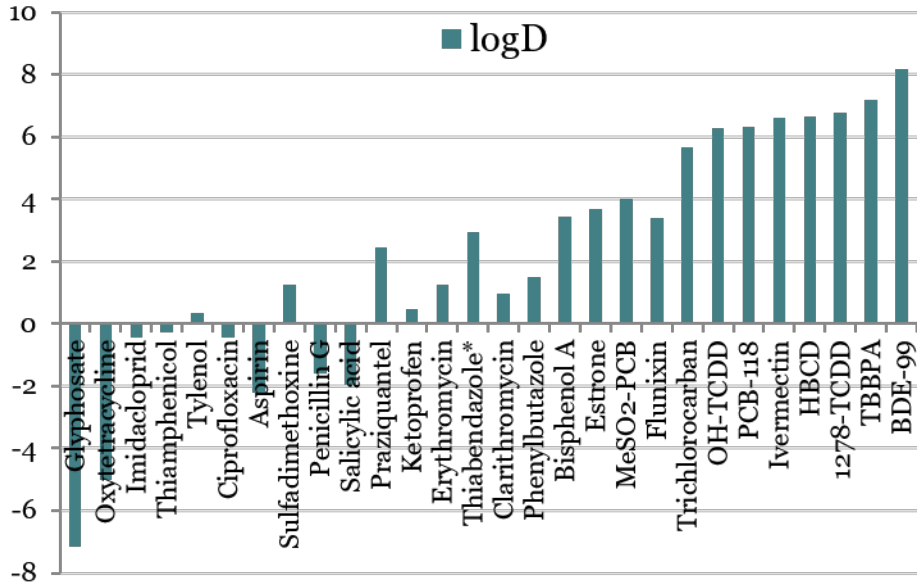
Estrone



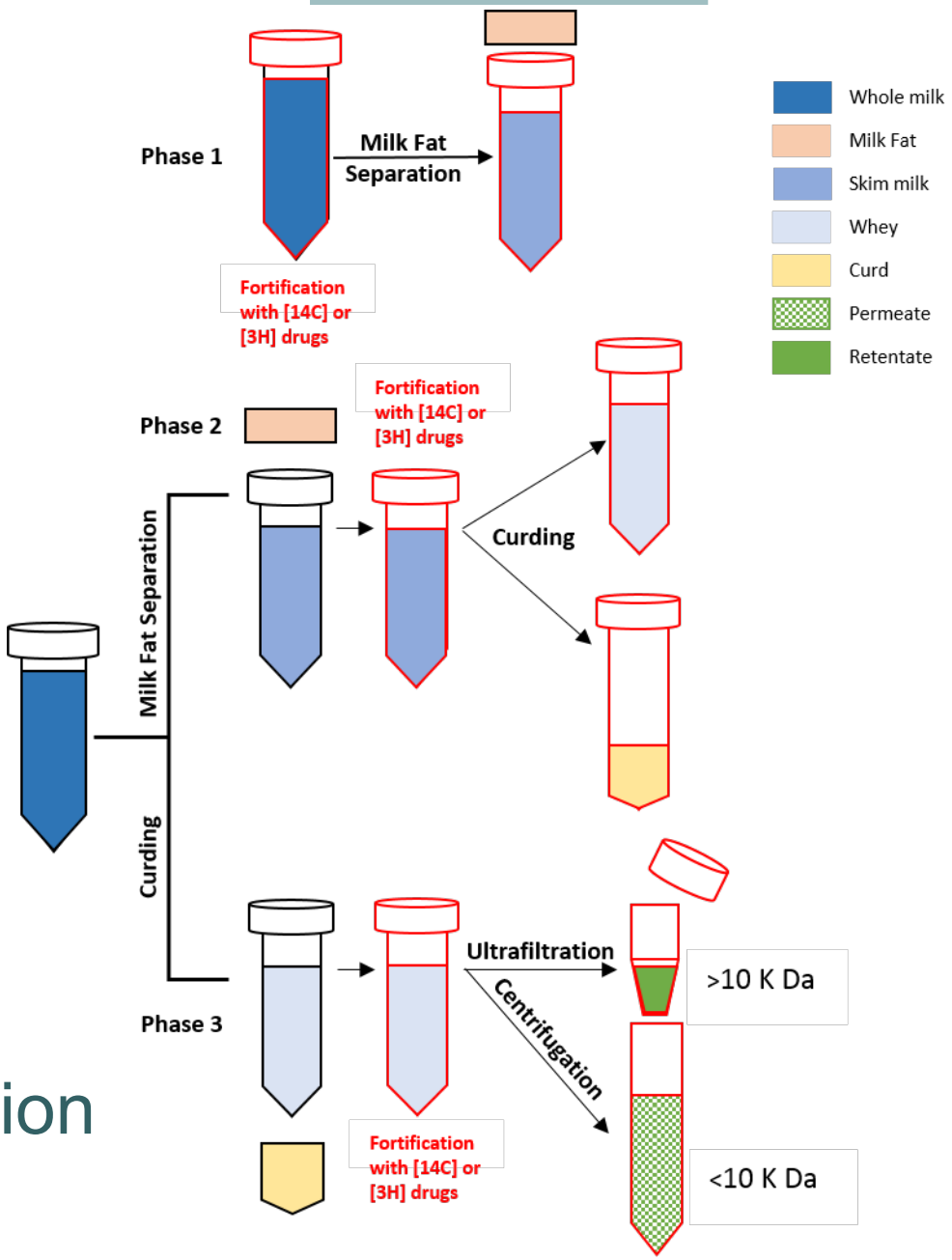
Bisphenol A

Compound Polarity





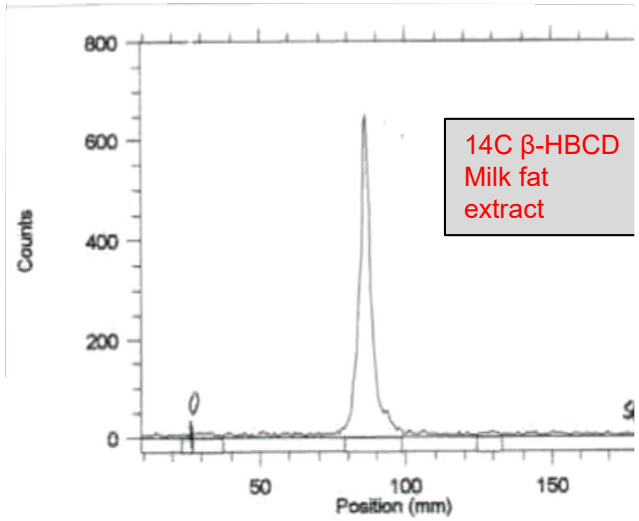
Milk Processing

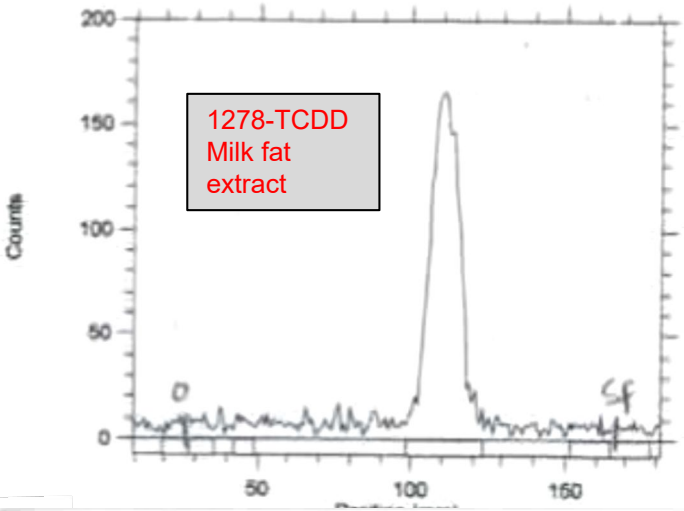


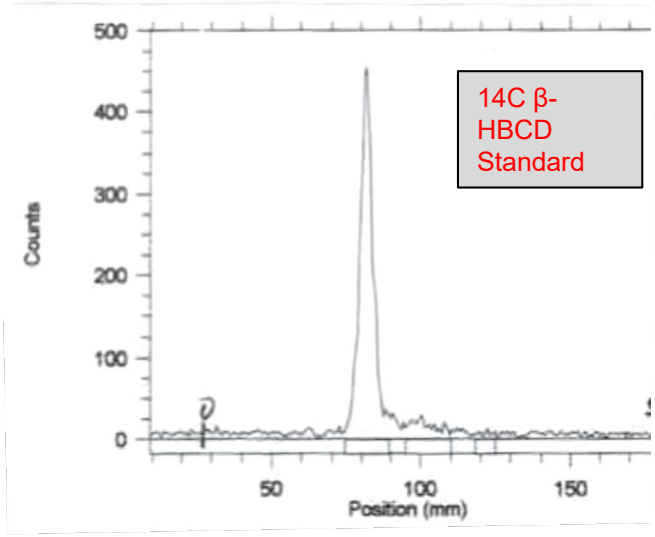
3 concentrations
3 replicates/concentration
(20 – 2000 nM)

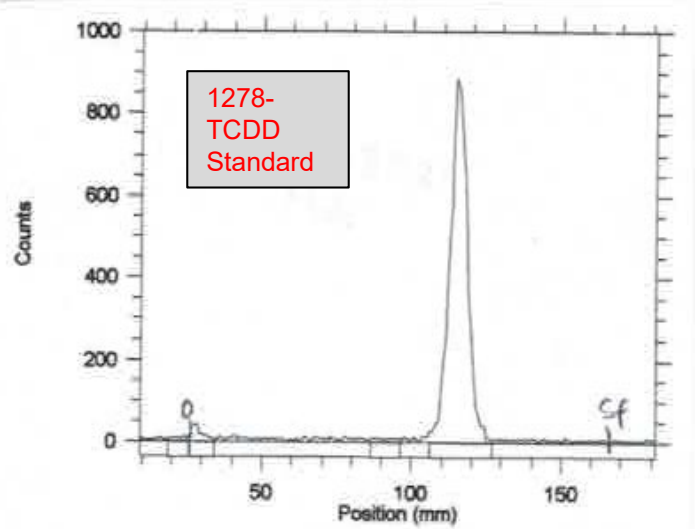
TLC Chromatograms

-Silica gel
-1:1 Hex:CH_2Cl_2
-Imaging Scanner





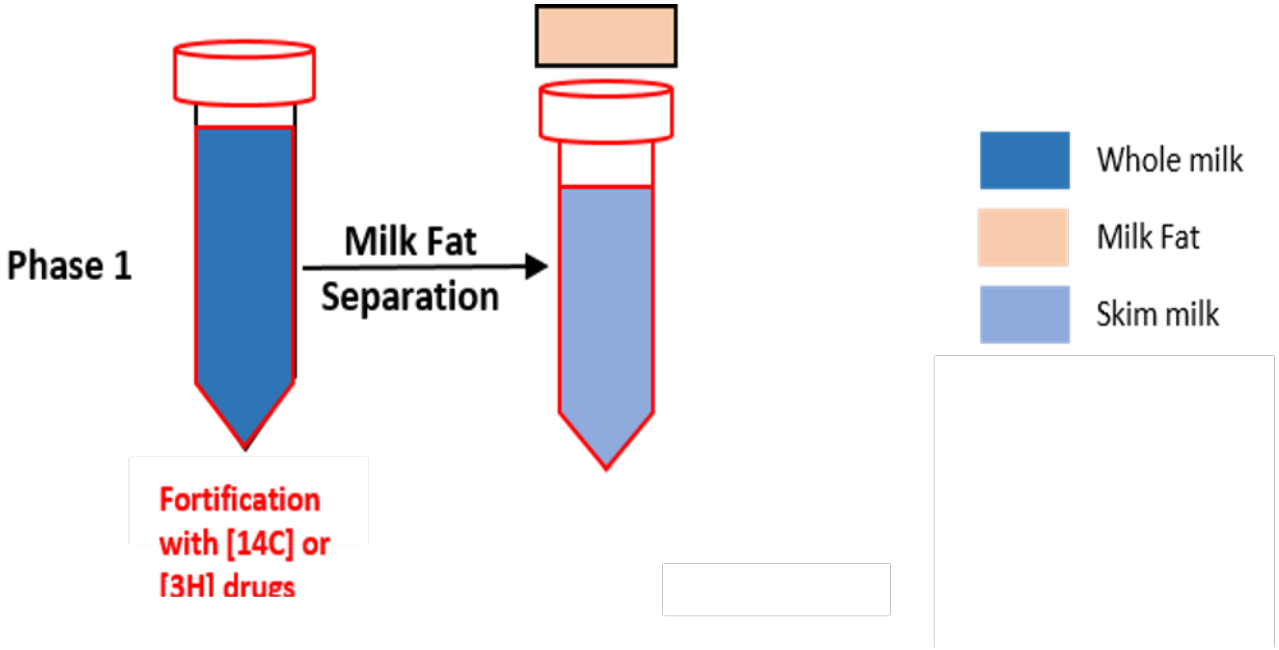




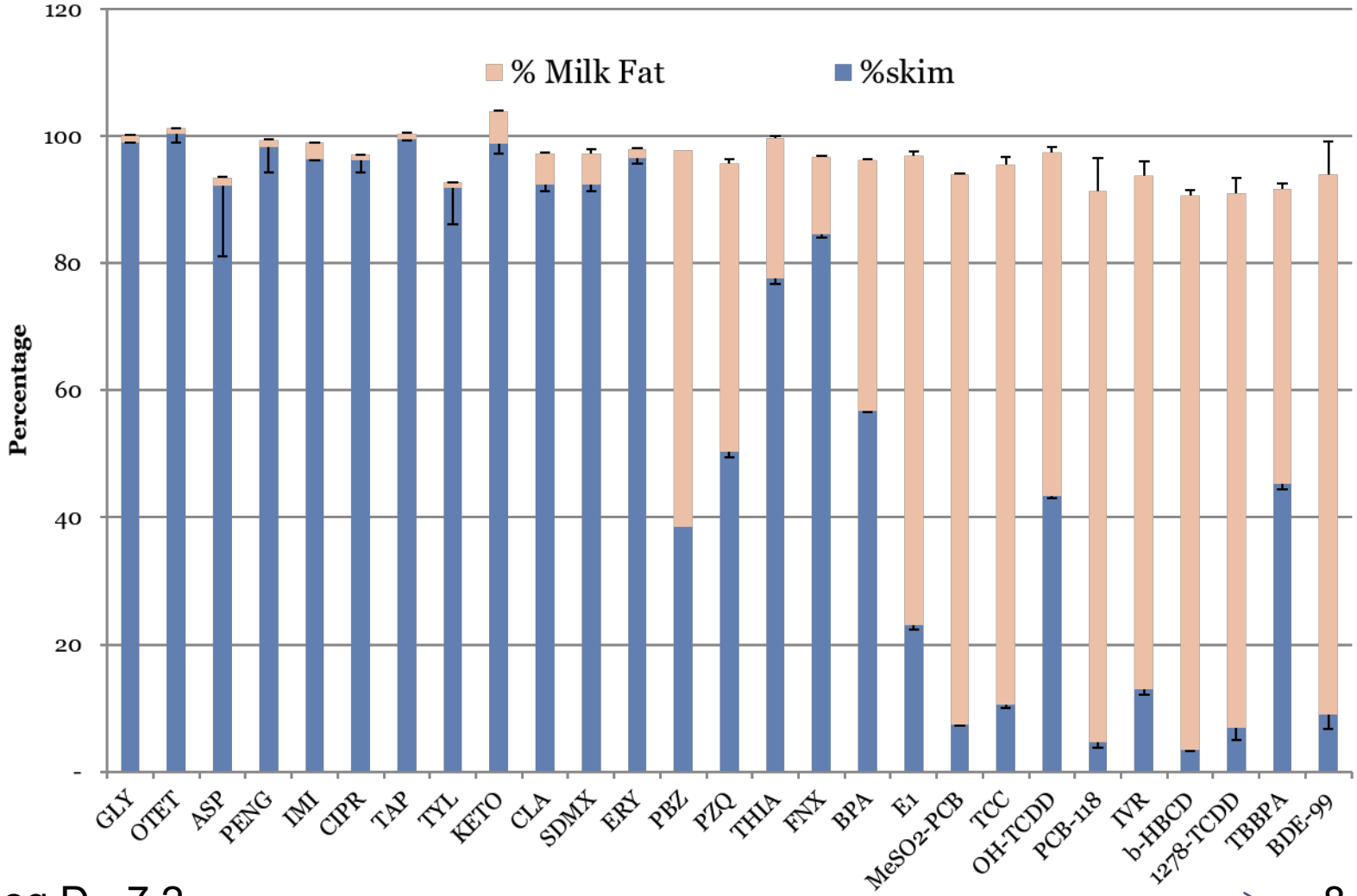
Compositional Analysis of Milk

Milk Fraction Compositional Data		Total Solids %		Lipid %		Protein %	
		Mean ± SD	**%RSD**	**Mean ± SD**	**%RSD**	**Mean ± SD**	**%RSD**
Phase 1	**Whole Milk**	12.29 ± 0.54	4.4	3.86 ± 0.20	5.2	3.13 ± 0.09	2.9
	Skim Milk	8.86 ± 0.44	5.0	0.24 ± 0.08	32.4	3.13 ± 0.10	3.3
	Milk Fat	83.59 ± 3.58	4.3	82.10 ± 2.61	3.2	1.02 ± 0.13	13.2
Phase 2	**Whey**	6.46 ± 0.18	2.8	0.08 ± 0.06	82.8	0.89 ± 0.03	3.0
	Curd	29.84 ± 2.39	8.0	1.95 ± 0.90	46.2	22.53 ± 1.92	8.5
Phase 3	**Retentate**	8.56 ± 0.54	6.3	0.33 ± 0.33	100.8	2.47 ± 0.36	14.7
	Permeate	5.71 ± 0.4	7.0	0.19 ± 0.19	96.2	0.19 ± 0.06	31.6

Milk Processing

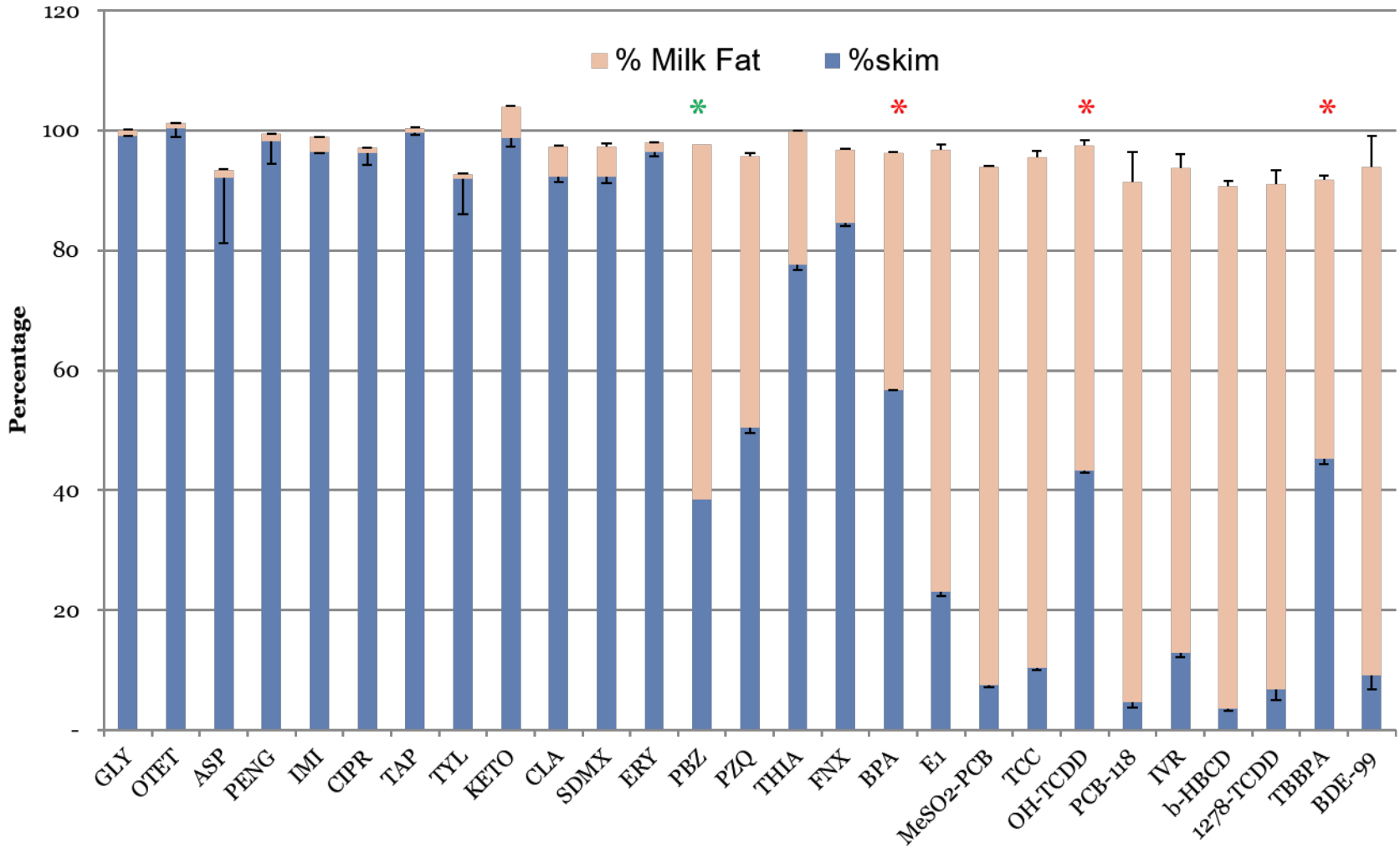


Distribution between Skim Milk and Milk Fat



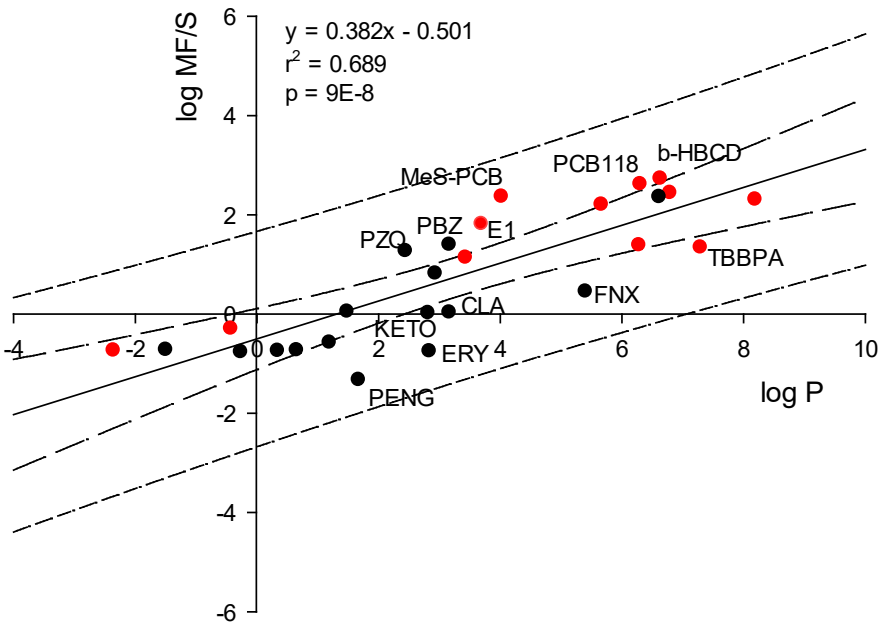
log D -7.2 → 8.2

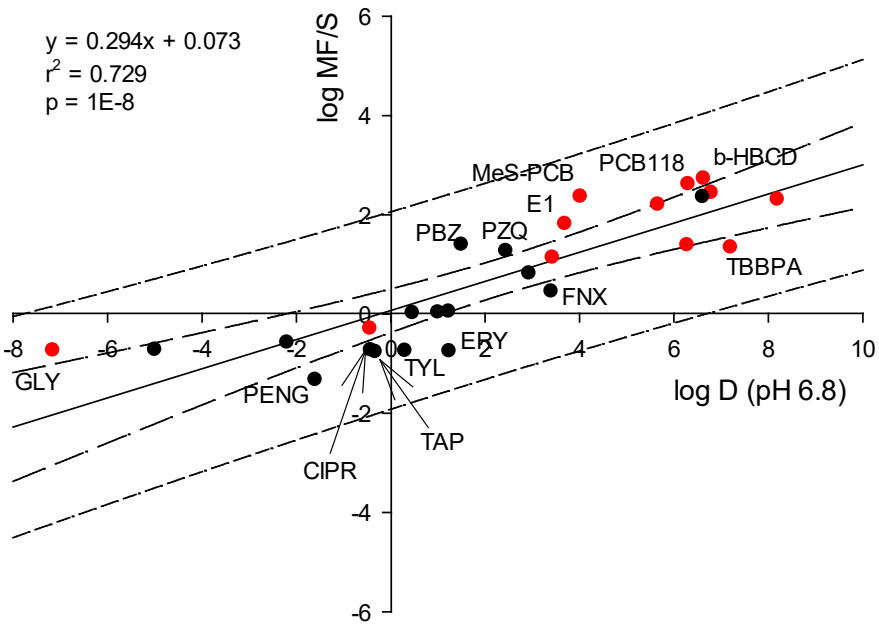
Distribution between Skim Milk and Milk Fat



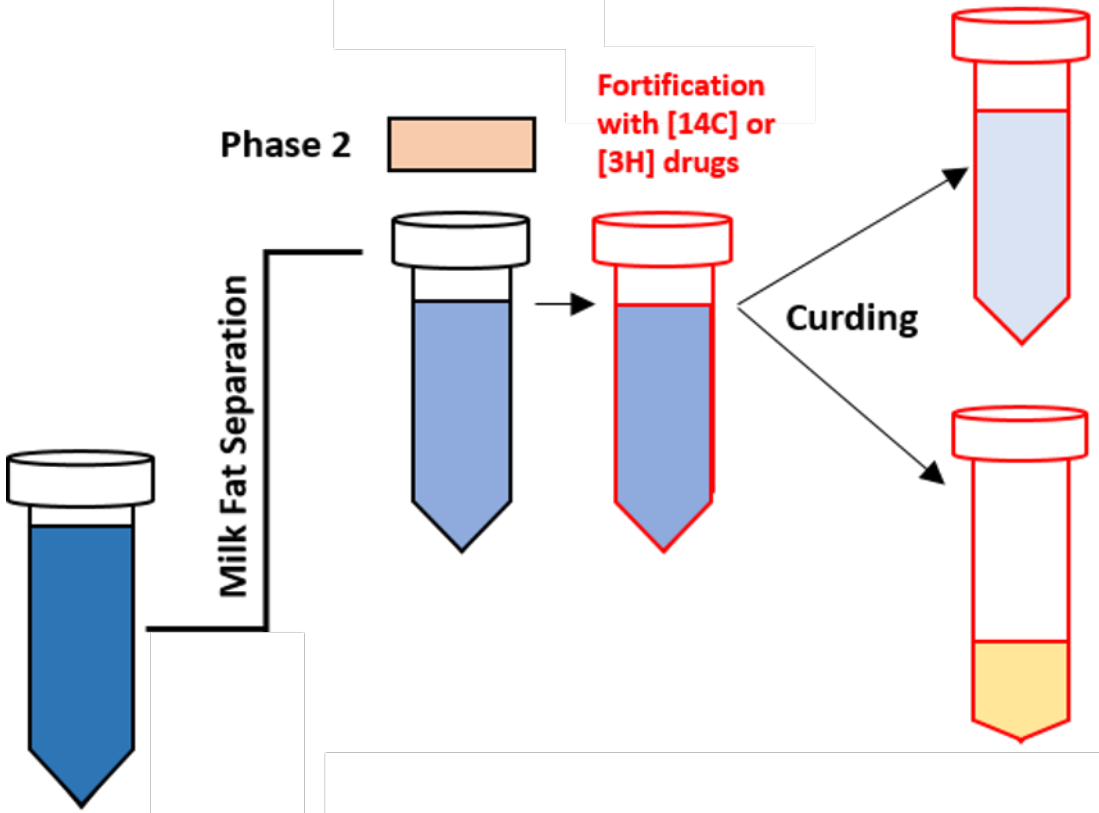
log D -7.2 → 8.2

Correlation of log P or log D vs log [Milk Fat]/[Skim Milk]

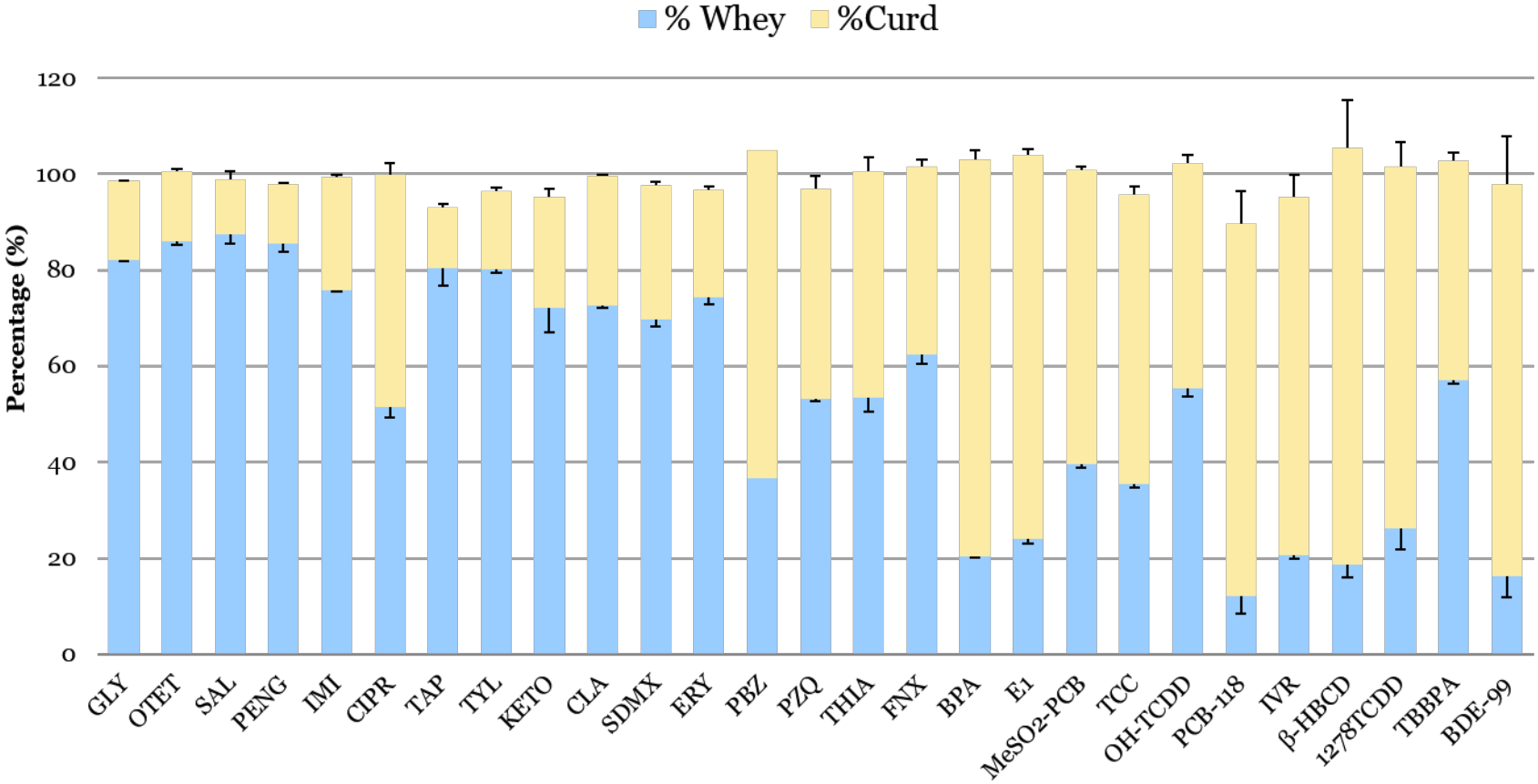




Milk Processing

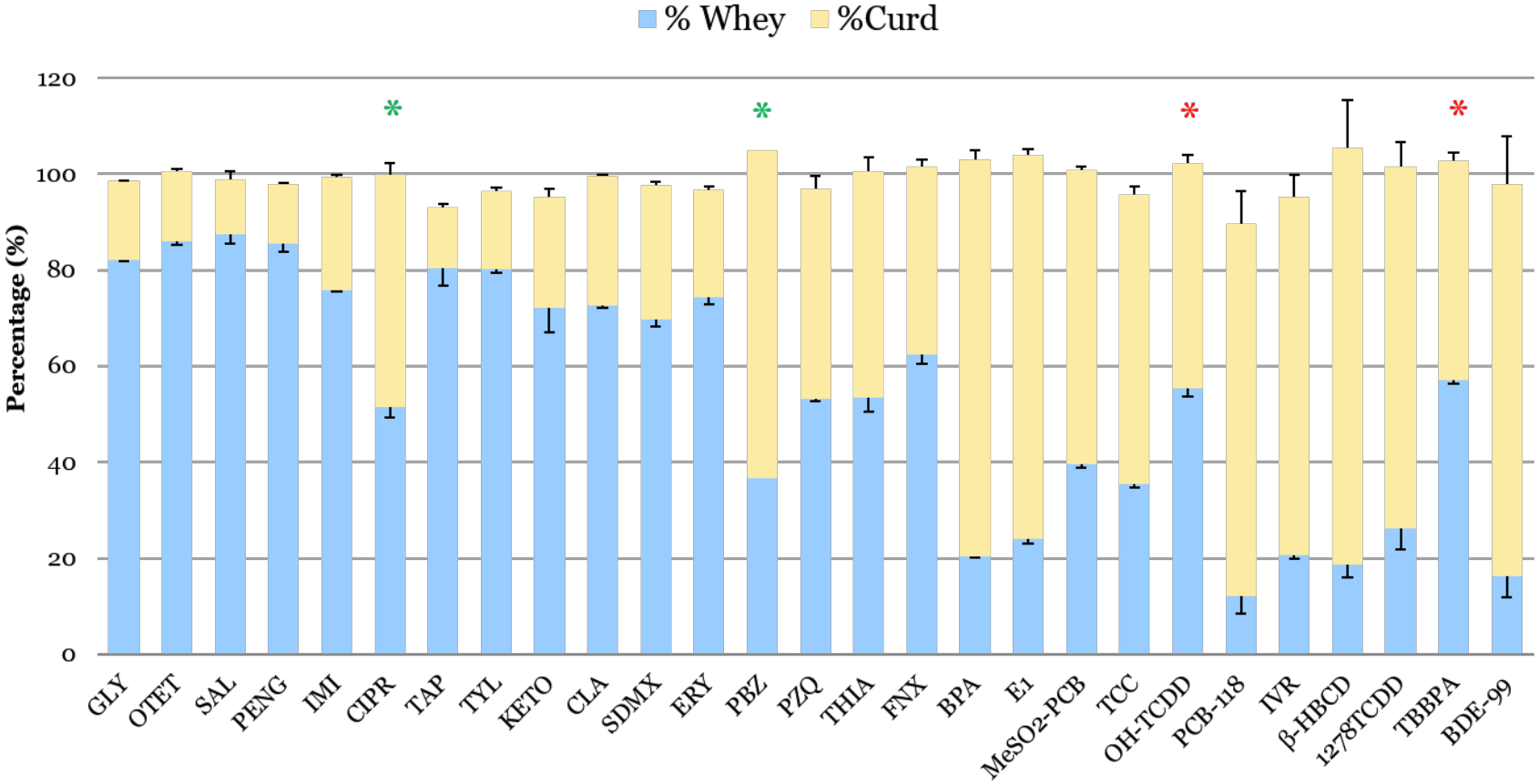


Distribution between Curd and Whey



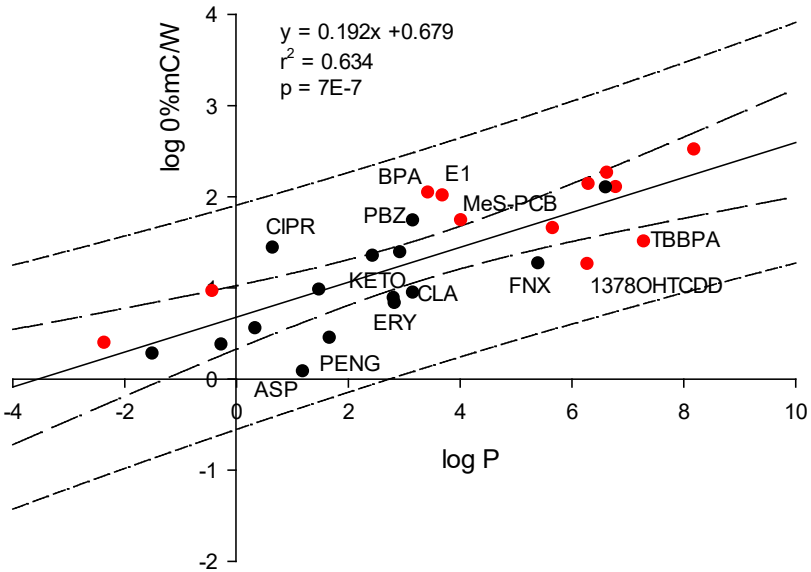
log D -7.2 → 8.2

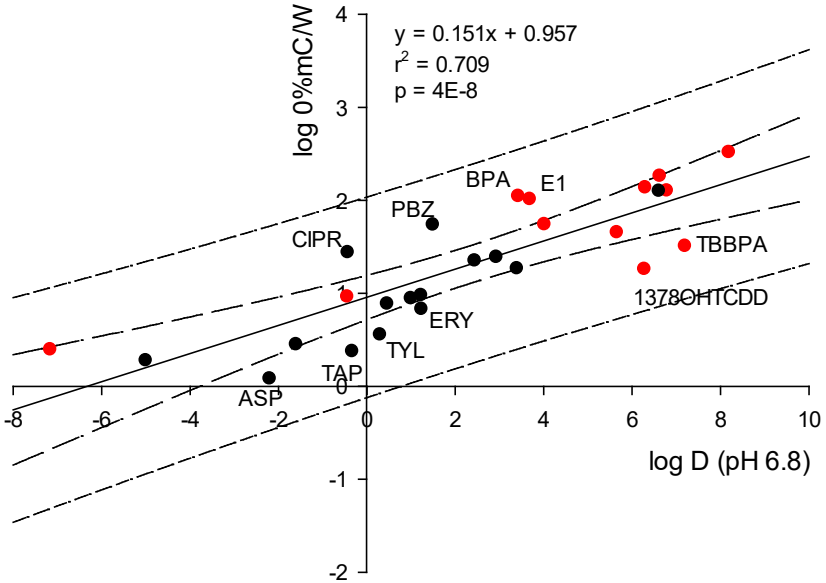
Distribution between Curd and Whey



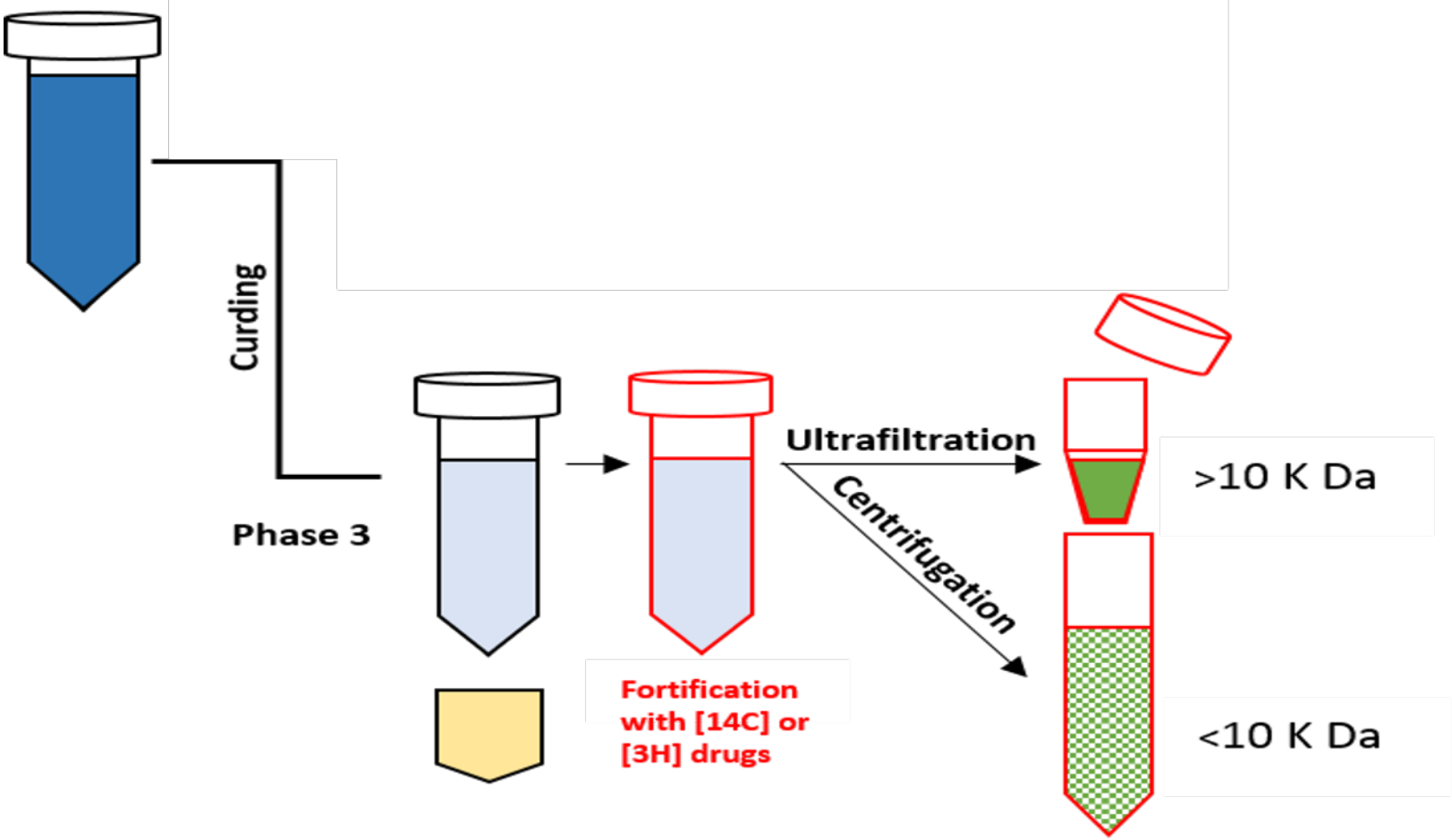
log D -7.2 → 8.2

Correlation of log P or log D vs log [curd]/[whey]

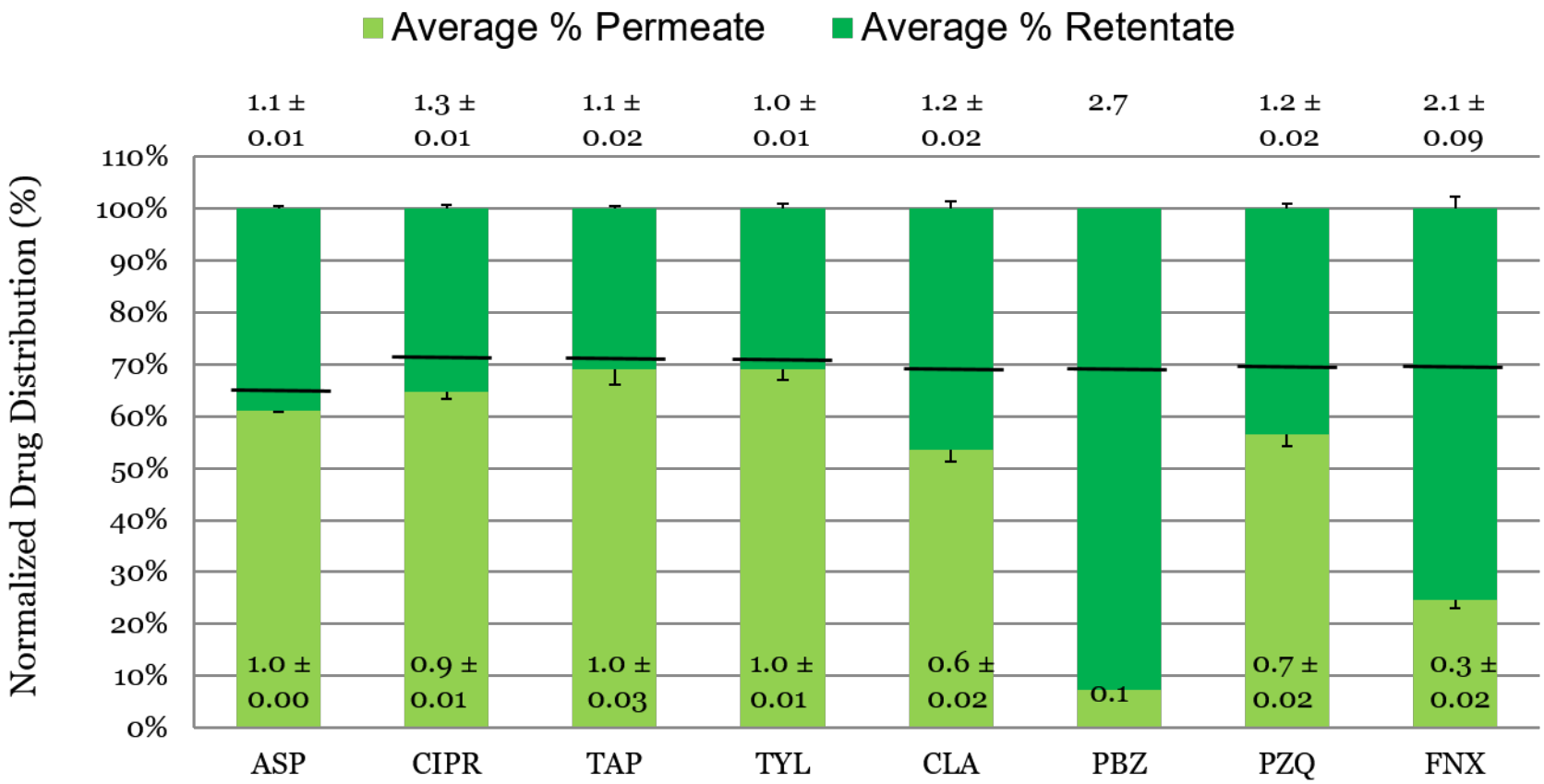




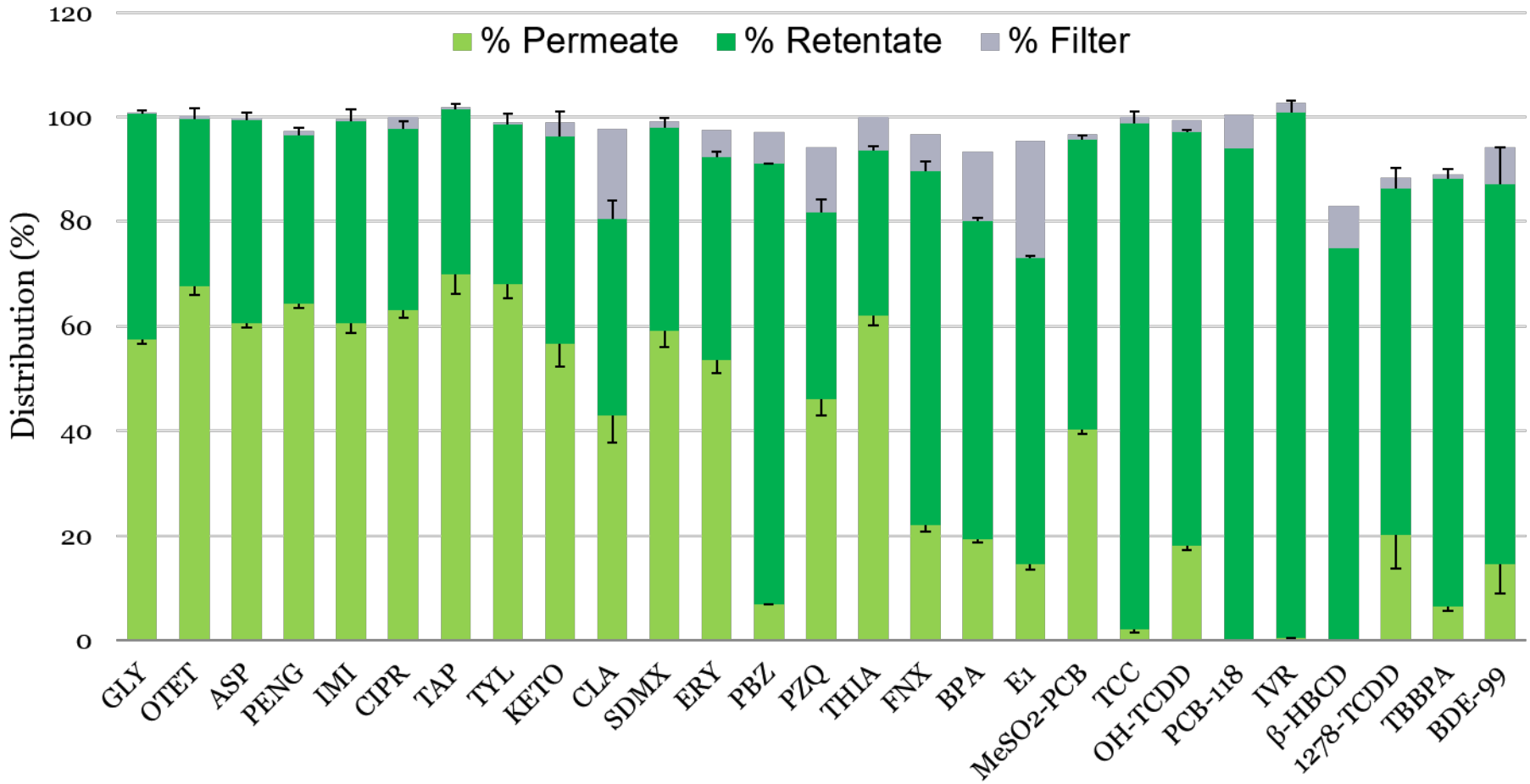
Milk Processing



Distribution between Whey Retentate and Permeate

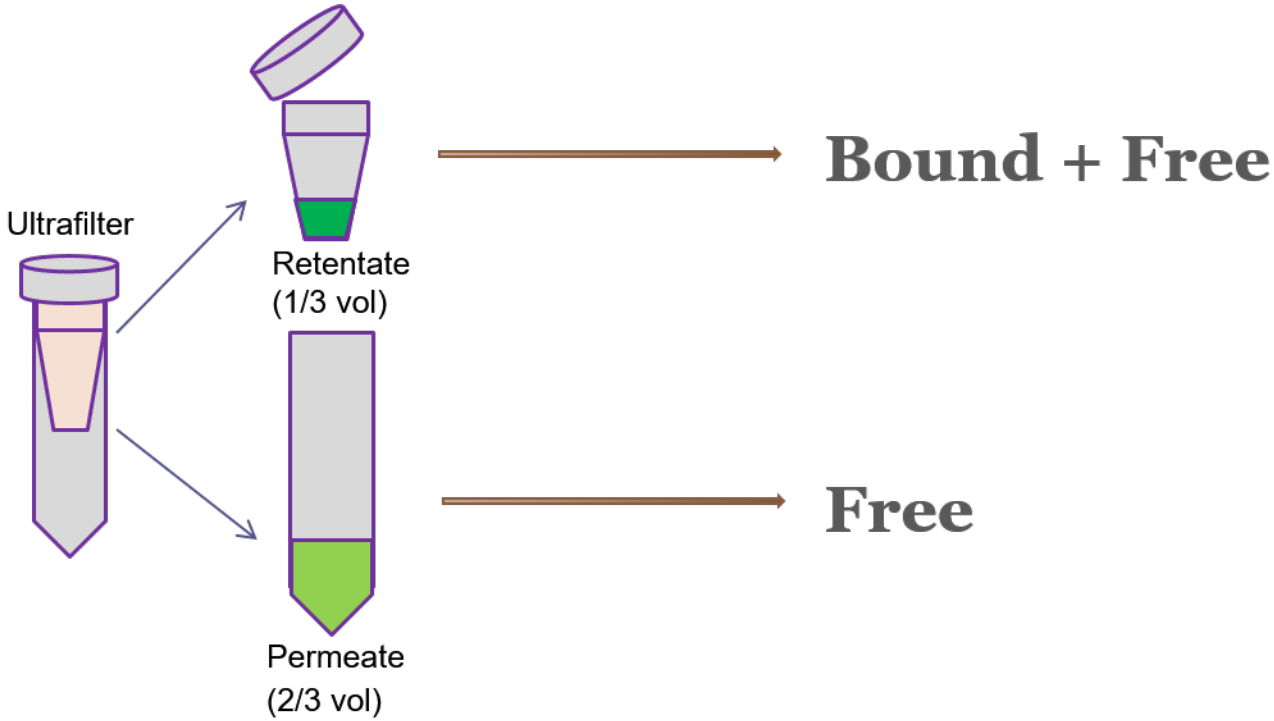


Distribution between Whey Retentate and Permeate

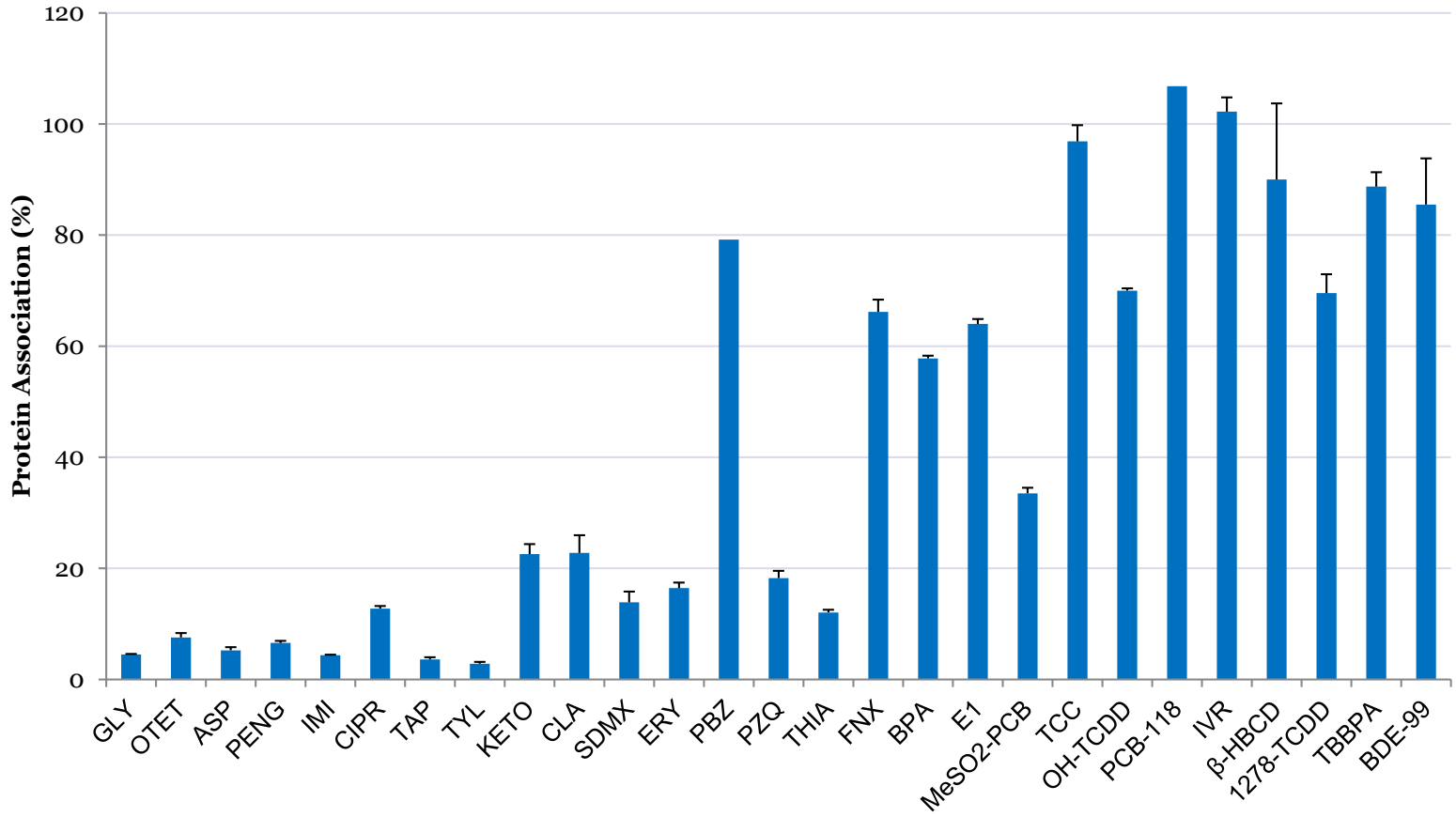


log D -7.2 → 8.2

Chemicals Associated with Whey

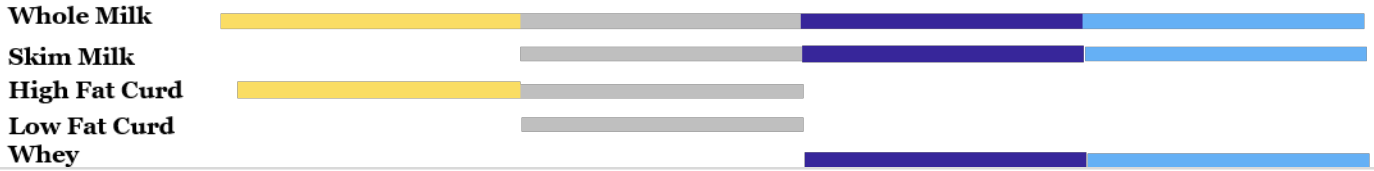


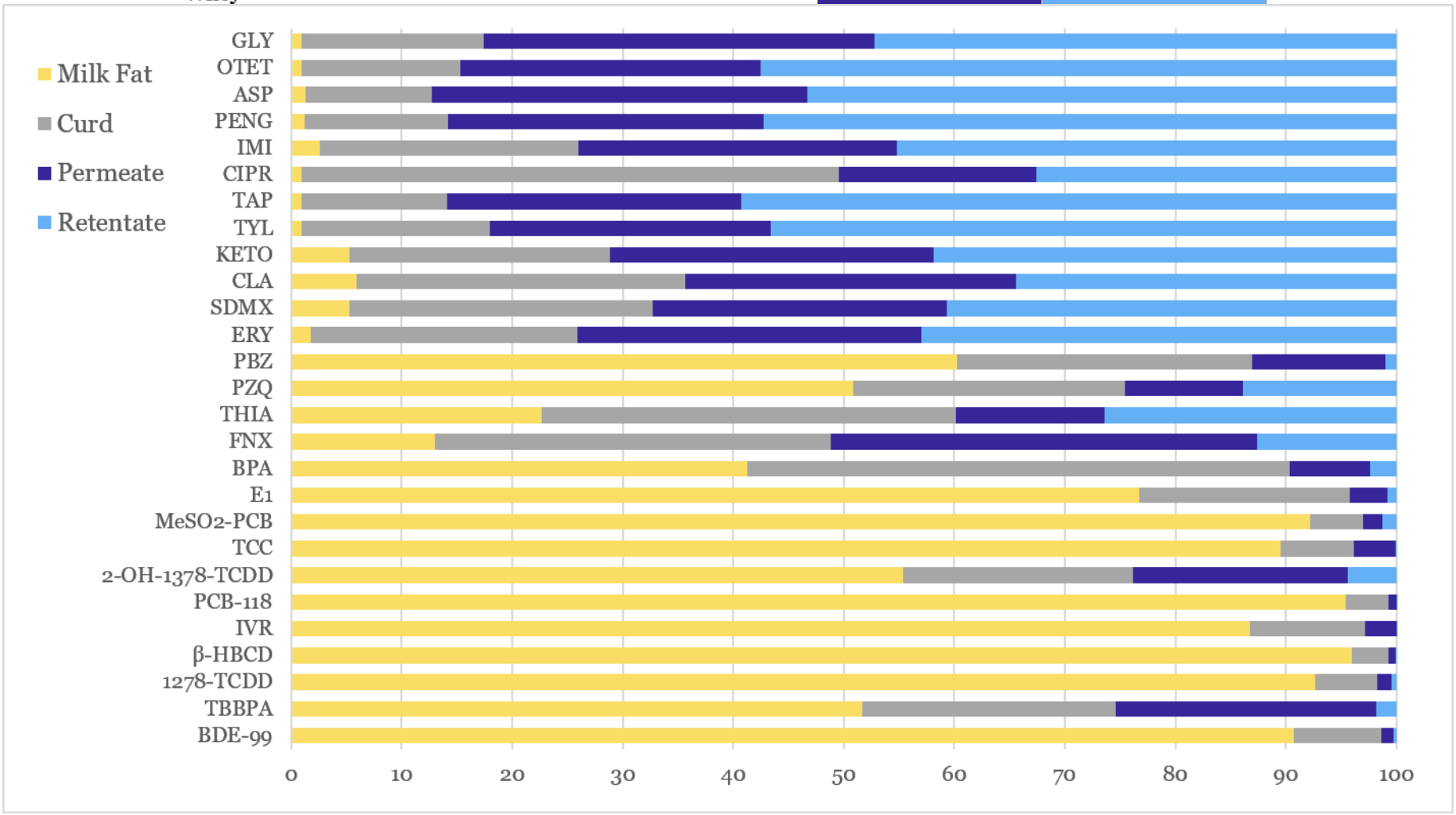
Percentage of Chemical in Whey Associated with Protein



log D -7.2 → 8.2

Overall Distribution





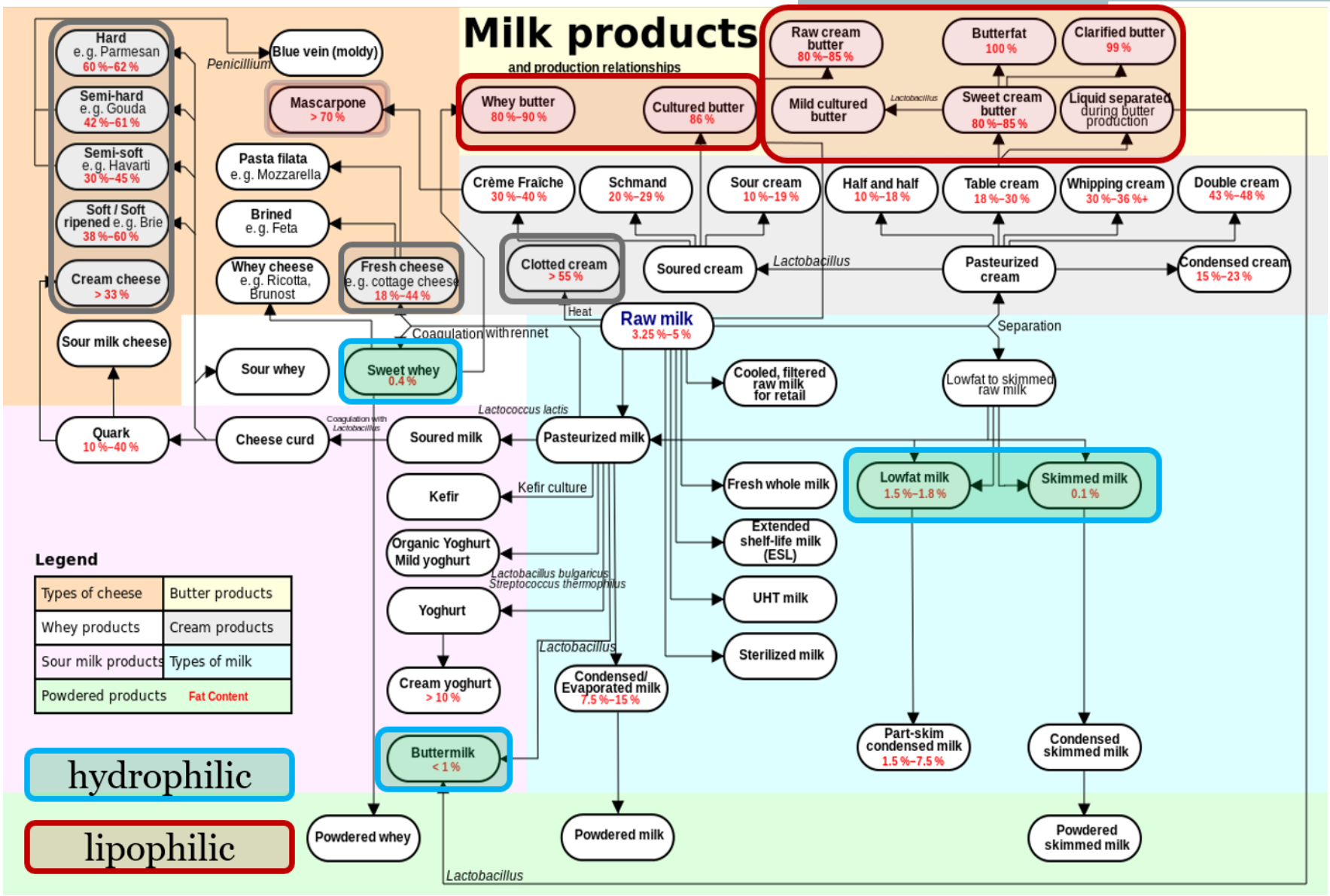


Figure adapted from https://en.wikipedia.org/wiki/Dairy_product#/media/File:Milkproducts_v2.svg

Conclusion

- Twenty-seven environmental contaminants and pharmaceuticals encompassing a wide range of physicochemical properties were utilized to determine the xenobiotic distribution among milk fractions.
- Log D values were better indictors than log P values for predicting the distribution of chemicals between fat and skim fractions and between insoluble (curd) and soluble (whey) proteins.
- Phenolic chemicals such as BPA, OH-TCDD and TBBPA are less predictable based on their lipophilicity to predict distribution into milk products.

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Thank you

Pergamon

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A SURVEY OF PCB CONGENERS IN U.K. COWS' MILK

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(Received in Germany 22 February 1996; accepted 14 March 1996)

Abstract

Samples of unpasteurised bulked milk, taken directly from ten herds of dairy cattle on rural and urban farms in the north west of England on five separate sampling occasions, were analysed for a range of PCB congeners. ΣPCB concentrations (sum of 37 congeners) ranged from 3.4-16.4 ng/g milk fat with a mean ΣPCB concent ng/g milk fat. The dominating congeners were 118, 153, 138 an contributed 15%, 20%, 17% and 9% of the ΣPCB load respectively

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Organochlorine pesticide distribution in an organic production system for cow's milk in Chiapas, Mexico

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he presence of organochlorine pesticides in samples of forage, nic production system for cow's milk (samples of forage, milk, co. The organochlorine pesticides were extracted from forage, guideline and from milk based on the IDF 1991 guideline. The by gas chromatography with electron capture detector (CG- entration of total pesticides was found in the samples of milk g^{-1} respectively). Although, the production systems analyzed detected in all environmental samples (forage, soil, water, and l the defined limits of Mexican and International regulation it is taminants in these production systems is continued.

ARTICLE HISTORY
Received 9 November 2015

KEYWORDS
Organochlorine compounds; environment; organic production; Chiapas

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A Survey of Organochlorine Pesticide Residues in Cheese Samples from Three Mexican Regions*

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Organochlorine pesticide residues were determined in cheese samples from three Mexican regions. All samples were found to be contaminated by these residues: p,p'-DDE was present in 100% of the samples. Other pesticides found with high frequencies were HCB, three of the BHC isomers, as well as heptachlor and its epoxide. Also found, although less frequently, were residues of p,p'-DDD, p,p'-DDT, aldrin, dieldrin and endrin. Samples from the region

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(1) World's Number 1 Herbicide Discovered in U.S. Mothers' Breast Milk

ne Testing also Shows Levels over 10 Times Higher than in Europe

Water Testing shows 70% of American Household's Drinking Water Positive for Above Detectable Levels

first ever testing on glyphosate herbicide in the breast milk of American women, Moms Across ica and Sustainable Pulse have found 'high' levels in 3 out of the 10 samples tested. The ting results point to glyphosate levels building up in women's bodies over a period of time, has until now been refuted by both global regulatory authorities and the biotech industry.

Regulatory Toxicology and Pharmacology 73 (2015) 758–764

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Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

alysis of Moms Across America report suggesting bioaccumulation glyphosate in U.S. mother's breast milk: Implausibility based on onsistency with available body of glyphosate animal toxicokinetic, nan biomonitoring, and physico-chemical data

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Residual Content of Hexachlorobenzene in Spanish Cheeses

A. Bentabol,[1] M. D. Garrido,[2] M. Jodral[1]

Table 1. Concentration of HCB in groups of Spanish ch

	Number of positives	ng/g fat ba Mean
Industrially-made cheeses (n=64)	64	12.48
Farm-produced cheeses (n=82)	82	6.76
Total (n=146)	146	9.26

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(*Received 17 May 2013; accepted 18 August 2013*)

This study summarises the results of the levels of 21 perfluoroalkyl substances (PFASs) in 50 selected pooled samples representing 15 food commodities with the special focus on those of animal origin, as meat, seafood, fish, milk, dairy products and hen eggs, which are commonly consumed in various European markets, e.g. Czech, Italian, Belgian and Norwegian. A new, rapid sample preparation approach based on the QuEChERS extraction procedure was applied. Ultra-performance liquid chromatography (UHPLC) coupled to tandem mass spectrometry (MS/MS) employing electrospray ionisation (ESI) in negative mode was used for the quantification of target analytes. Method quantification limits (MQLs) were in the range of 1–10 ng kg^{-1} (ng l^{-1}) for fish, meat, hen eggs, cheese and milk, and in the range of 2.5–125 ng kg^{-1} for butter. Only 16 of the group of 21 PFASs were found in at least one analysed sample. From 16 PFASs, perfluorooctane sulfonate (PFOS) was the most frequently detected analyte present in approximately 50% of samples (in the range of 0.98–2600 ng kg^{-1}). PFCAs with a C8–C14 carbon chain were presented in approximately 20% of samples. The concentration ranges of individual compounds in the respective groups of PFASs were: 2.33–76.3 ng kg^{-1} for PFSAs (without PFOS), 4.99–961 ng kg^{-1} for PFCAs, 10.6–95.4 ng kg^{-1} for PFPAs, and 1.61–519 ng kg^{-1} for FOSA. The contamination level in the analysed food commodities decreased in the following order: seafood > pig/bovine liver >> freshwater/marine fish > hen egg > meat >> butter. When comparing the total contamination and profiles of PFASs in food commodities that originated from various sampling countries, differences were identified, and the contents decreased as follows: Belgium >> Norway, Italy > Czech Republic.

Milk Processing

Phase 1

Pasteurized, whole milk
Fortify 20, 200, or 2000 nM POP
Incubate 30 min, 38°C
Centrifuge@ 4000xg, 45 min, 35°C
Separate milk fat/skim milk
- 48 g skim milk, 2 g milk fat
- Assay fractions for radioactivity

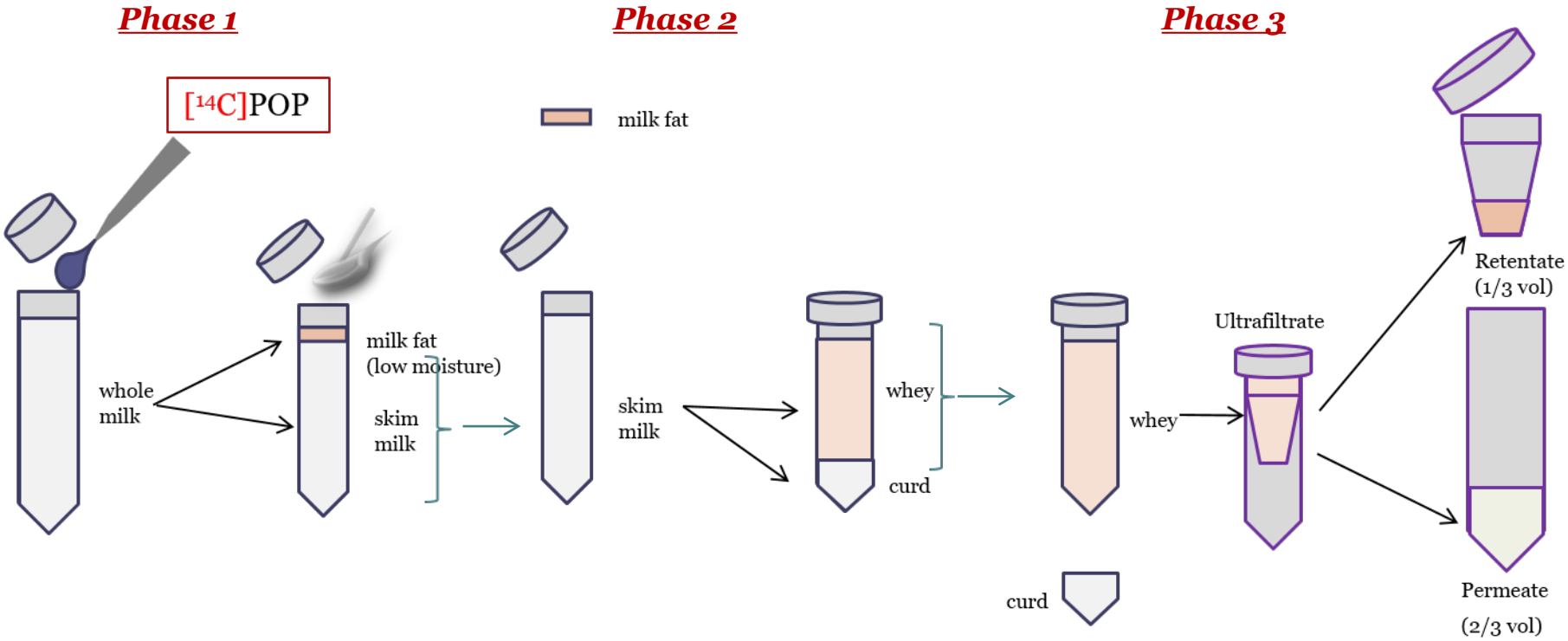
Phase 2

Skim milk from (1)
- Add rennet, 1 hr, 38°C
- Centrifuge @ 3000xg, separate whey/curd
- Assay fractions for radioactivity

Phase 3

Whey from (2)
- Ultrafiltration @ 4000xg
- Separation of retentate and permeate
- Assay fraction for radioactivity

Milk Processing



Partition Coefficient, Distribution Coefficient

- $\log D_{acids} = \log P + \log [1/(1+10^{(pH-pKa)})]$
- $\log D_{bases} = \log P + \log[1/1+10^{(pKa-pH)}]$
- acids $(pH - pKa) > 1$ $\log D_{acid} = \log P + pKa - pH$
- bases $(pKa - pH) > 1$ $\log D_{bases} = \log P - pKa + pH$
- Non-ionizables $\log D \sim \log P$
- Sources of log P, mainly Chemspider
- Sources of pKa, mainly Drug Bank

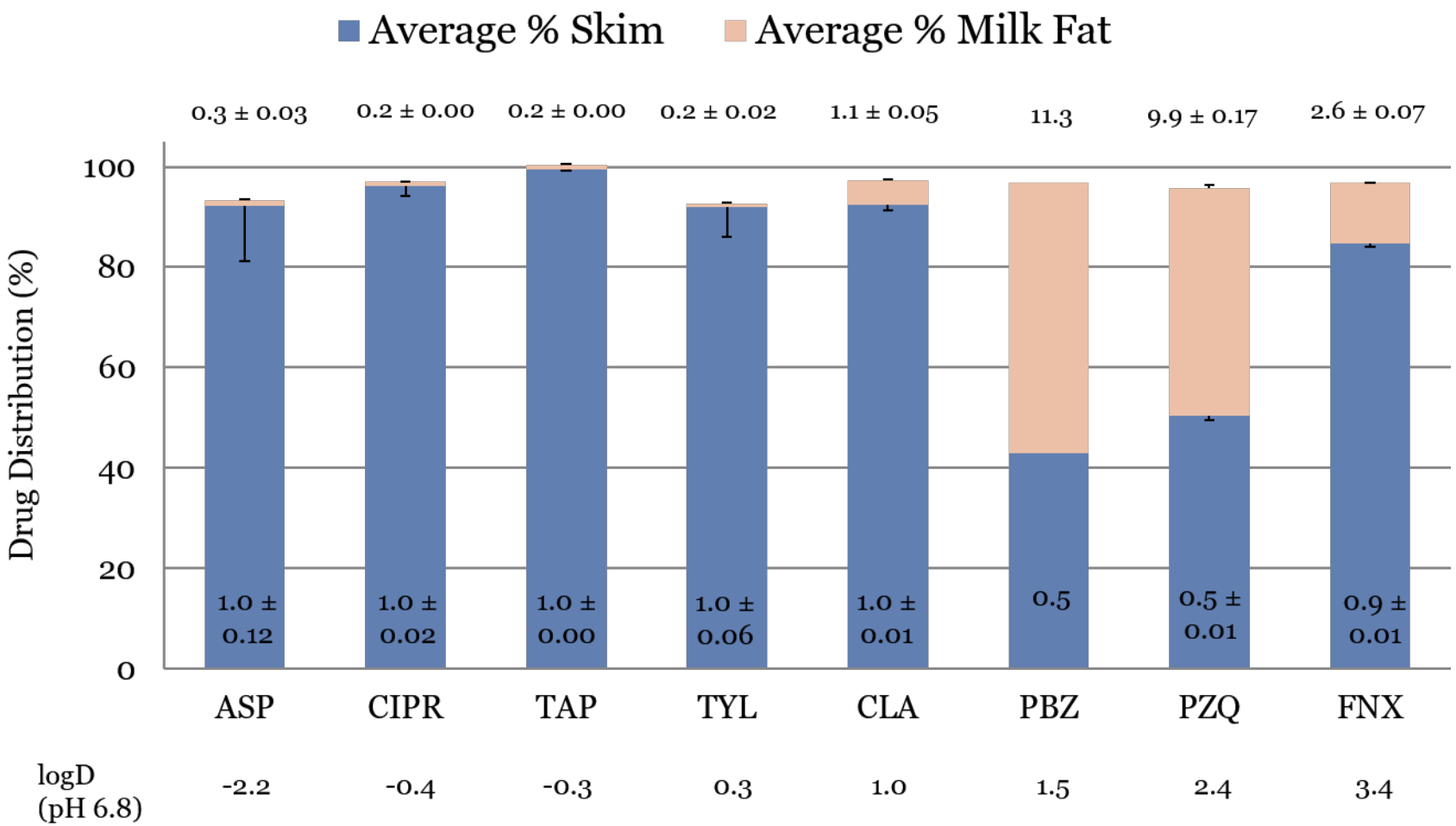
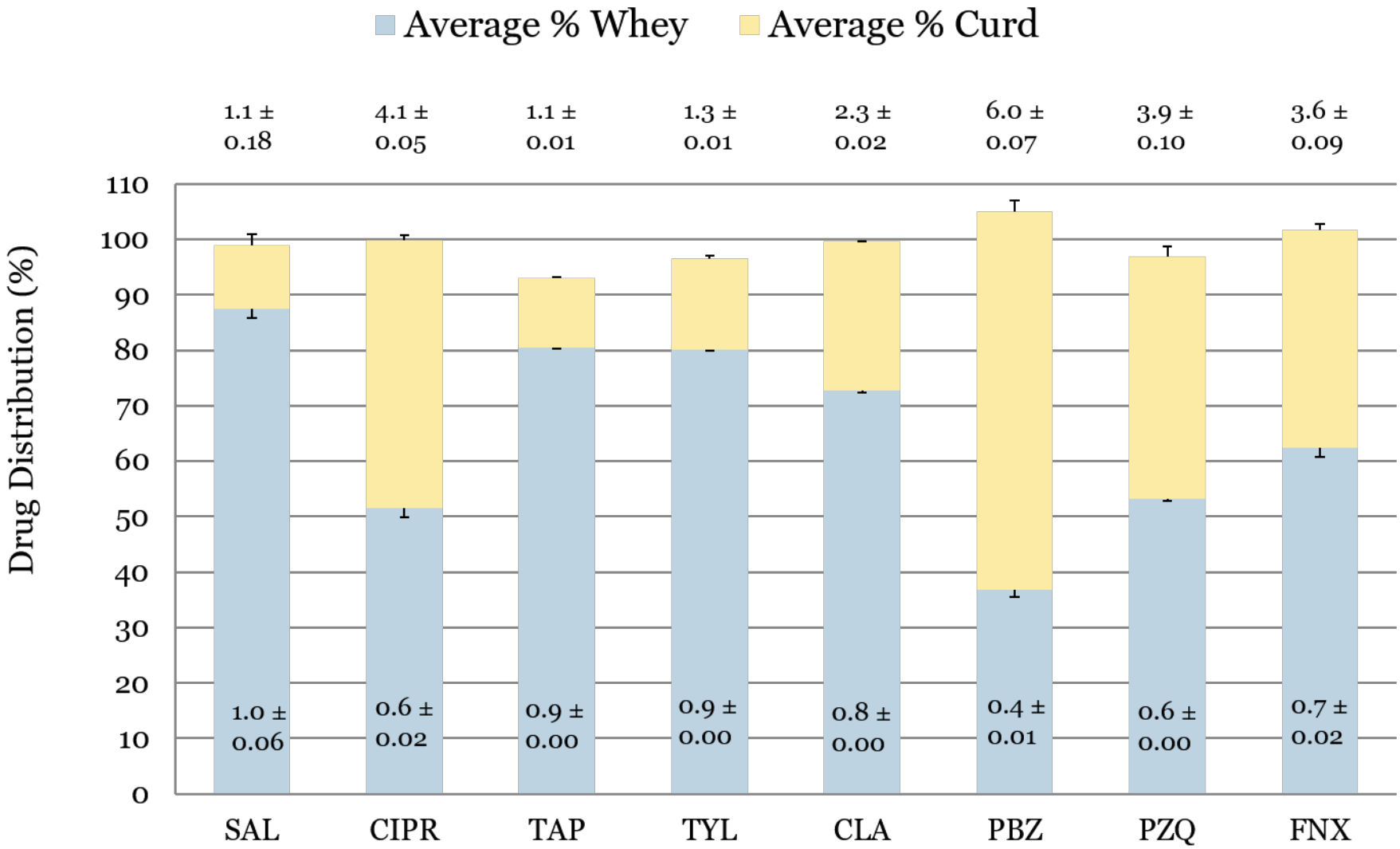
Polarities of Studied Compounds

Compound	log P	log D	Compound	log P	log D
Glyphosate	-2.36	-7.16	Clarithromycin	3.16	0.97
Oxytetracycline	-1.5	-5.00	Bisphenol A	3.43	3.43
Imidacloprid	-0.43	-0.44	Estrone	3.69	3.69
Thiamphenicol	-0.27	-0.27	MeSO2-PCB	4.02	4.02
Tylenol	0.34	0.34	Flunixin	5.40	3.40
Ciprofloxacin	0.65	-0.43	Triclocarban	5.66	5.66
Aspirin	1.19	-2.20	OH-TCDD	6.28	6.28
Sulfadimethoxine	1.48	1.23	PCB-118	6.30	6.30
Penicillin G	1.67	-1.60	Ivermectin	6.61	6.61
Salicylic acid	2.06	-1.95	HBCD	6.63	6.63
Praziquantel	2.44	2.44	1278-TCDD	6.79	6.79
Ketoprofen	2.81	0.46	TBBPA	7.29	7.29
Erythromycin	2.83	1.24	BDE-99	8.19	8.19
Thiabendazole	2.93	2.93			

Chemicals Associated with Whey

Compound	% Association	log D	Compound	% Association	log D
Glyphosate	0.6	-7.16	Thiabendazole	12.0	2.93
Oxytetracycline	7.6	-5.00	Flunixin	66.2	3.40
Aspirin	5.2	-2.20	Bisphenol A	81.9	3.43
Penicillin G	6.6	-1.60	Estrone	81.9	3.69
Imidacloprid	4.4	-0.44	MeSO2-PCB	55.6	4.02
Ciprofloxacin	12.8	-0.43	Triclocarban	96.9	5.66
Thiamphenicol	3.6	-0.27	OH-TCDD	79.5	6.28
Tylenol	2.8	0.34	PCB-118	96.4	6.30
Ketoprofen	22.6	0.46	Ivermectin	102.2	6.61
Clarithromycin	22.8	0.97	HBCD	101.8	6.63
Sulfadimethoxine	13.9	1.23	1278-TCDD	69.5	6.79
Erythromycin	16.5	1.24	TBBPA	88.7	7.29
Praziquantel	79.2	2.44	BDE-99	8.19	8.19

Distribution between skim milk and milk fat



Distribution between whey and curd