UNIVERSITY OF SWAZILAND
BACHELOR OF SCIENCE

MAIN EXAMINATION 2018

TITLE OF PAPER (COURSE CODE)	:	RESEARCH METHODS (CHE303)
TIME	:	2 HOURS
INSTRUCTIONS	:	THIS EXAMINATION PAPER HAS THREE QUESTIONS
	:	ANSWER ALL THREE QUESTIONS
	:	BEGIN THE ANSWER TO EACH QUESTION ON A SEPARATE SHEET OF PAPER
	:	DATA SHEETS ARE PROVIDED WITH THIS EXAMINATION PAPER

DO NOT OPEN THIS PAPER UNTIL THE INVIGILATOR INSTRUCTS YOU TO DO SO.

Question 1(25 marks)

a) It is said that a scientific method of research uses deductive and inductive methods of enquiry. Using examples of your choice explain the meaning of this statement. (10)

b) Using a flow diagram Outline and explain the steps taken in a scientific research method. (15)

QUESTION 2 [50 marks]

a) Define and explain the major sections in a scientific research proposal. (10)

b) Define and explain with examples the meaning of the phrase "data validation" (6)

c) You are to perform research on environmental water pollution status of little Usuthu River across the Matsapha area. Describe briefly your research design stating your sampling, analytical and data analysis methods you would use. (20)

d) (i) Define the term Hypothesis as applied to scientific research. (4)

(ii) You measured the level of Cadmium in ppm in water using Atomic Absorption Spectrometry and validated the data by sending samples to an accredited laboratory that used Graphite Furnace Atomic Absorption Spectrometry to obtain the data below.

Atomic Absorption Spectrometry	Graphite Furnace Atomic Absorption Spectrometry
84.63	83.15
84.38	83.72
84.08	83.84
84.41	84.20
83.82	83.92
83.55	84.16
83.92	84.02
83.69	83.60
84.06	84.13
84.03	84.24

Using an appropriate hypothesis determine whether or not the results obtained confirm a significant similarity in the two methods at 95% confidence level ? (10)

Useful equation: $t = \dfrac{\bar{d} - \Delta_0}{s_d / \sqrt{n}}$

Question 3 (25 Marks)

To answer this question refer to the research paper attached

(a) Define the research problem highlighted in this paper. (3)

(b) Define and explain the objectives of the article. (3)

(c) Give the factors that you will consider in order to make a critical evaluation of the paper. Explain clearly why the factors you have mentioned are important. (5)

(d) Write down the main findings of the research. (4)

(e) Describe the general types of literature and attributes of a good literature review in a paper or report/thesis whilst making a critical evaluation of the literature cited in this article. (10)

Appendix 2: Statistical tables

The following tables are presented for the convenience of the reader, and for use with the simple statistical tests, examples and exercises in this book. They are presented in a format that is compatible with the needs of analytical chemists: the significance level $P = 0.05$ has been used in most cases, and it has been assumed that the number of measurements available is fairly small. Most of these abbreviated tables have been taken, with permission, from *Elementary Statistics Tables* by Henry R. Neave, published by Routledge (Tables A.2–A.4, A.7, A.8, A.12–A.14). The reader requiring statistical data corresponding to significance levels and/or numbers of measurements not covered in the tables is referred to these sources.

Table A.1 $F(z)$, the standard normal cumulative distribution function

z	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
−3.4	0.0003	0.0003	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0005	0.0005
−3.3	0.0005	0.0005	0.0005	0.0005	0.0006	0.0006	0.0006	0.0006	0.0006	0.0007
−3.2	0.0007	0.0007	0.0007	0.0008	0.0008	0.0008	0.0008	0.0009	0.0009	0.0009
−3.1	0.0010	0.0010	0.0010	0.0011	0.0011	0.0011	0.0012	0.0012	0.0013	0.0013
−3.0	0.0013	0.0014	0.0014	0.0015	0.0015	0.0016	0.0016	0.0017	0.0018	0.0018
−2.9	0.0019	0.0019	0.0020	0.0021	0.0021	0.0022	0.0023	0.0023	0.0024	0.0025
−2.8	0.0026	0.0026	0.0027	0.0028	0.0029	0.0030	0.0031	0.0032	0.0033	0.0034
−2.7	0.0035	0.0036	0.0037	0.0038	0.0039	0.0040	0.0041	0.0043	0.0044	0.0045
−2.6	0.0047	0.0048	0.0049	0.0051	0.0052	0.0054	0.0055	0.0057	0.0059	0.0060
−2.5	0.0062	0.0064	0.0066	0.0068	0.0069	0.0071	0.0073	0.0075	0.0078	0.0080
−2.4	0.0082	0.0084	0.0087	0.0089	0.0091	0.0094	0.0096	0.0099	0.0102	0.0104
−2.3	0.0107	0.0110	0.0113	0.0116	0.0119	0.0122	0.0125	0.0129	0.0132	0.0136
−2.2	0.0139	0.0143	0.0146	0.0150	0.0154	0.0158	0.0162	0.0166	0.0170	0.0174
−2.1	0.0179	0.0183	0.0188	0.0192	0.0197	0.0202	0.0207	0.0212	0.0217	0.0222
−2.0	0.0228	0.0233	0.0239	0.0244	0.0250	0.0256	0.0262	0.0268	0.0274	0.0281
−1.9	0.0287	0.0294	0.0301	0.0307	0.0314	0.0322	0.0329	0.0336	0.0344	0.0351
−1.8	0.0359	0.0367	0.0375	0.0384	0.0392	0.0401	0.0409	0.0418	0.0427	0.0436
−1.7	0.0446	0.0455	0.0465	0.0475	0.0485	0.0495	0.0505	0.0516	0.0526	0.0537
−1.6	0.0548	0.0559	0.0571	0.0582	0.0594	0.0606	0.0618	0.0630	0.0643	0.0655
−1.5	0.0668	0.0681	0.0694	0.0708	0.0721	0.0735	0.0749	0.0764	0.0778	0.0793

Table A.1 Continued

z	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
−1.4	0.0808	0.0823	0.0838	0.0853	0.0869	0.0885	0.0901	0.0918	0.0934	0.0951
−1.3	0.0968	0.0985	0.1003	0.1020	0.1038	0.1056	0.1075	0.1093	0.1112	0.1131
−1.2	0.1151	0.1170	0.1190	0.1210	0.1230	0.1251	0.1271	0.1292	0.1314	0.1335
−1.1	0.1357	0.1379	0.1401	0.1423	0.1446	0.1469	0.1492	0.1515	0.1539	0.1562
−1.0	0.1587	0.1611	0.1635	0.1660	0.1685	0.1711	0.1736	0.1762	0.1788	0.1814
−0.9	0.1841	0.1867	0.1894	0.1922	0.1949	0.1977	0.2005	0.2033	0.2061	0.2090
−0.8	0.2119	0.2148	0.2177	0.2206	0.2236	0.2266	0.2296	0.2327	0.2358	0.2389
−0.7	0.2420	0.2451	0.2483	0.2514	0.2546	0.2578	0.2611	0.2643	0.2676	0.2709
−0.6	0.2743	0.2776	0.2810	0.2843	0.2877	0.2912	0.2946	0.2981	0.3015	0.3050
−0.5	0.3085	0.3121	0.3156	0.3192	0.3228	0.3264	0.3300	0.3336	0.3372	0.3409
−0.4	0.3446	0.3483	0.3520	0.3557	0.3594	0.3632	0.3669	0.3707	0.3745	0.3783
−0.3	0.3821	0.3859	0.3897	0.3936	0.3974	0.4013	0.4052	0.4090	0.4129	0.4168
−0.2	0.4207	0.4247	0.4286	0.4325	0.4364	0.4404	0.4443	0.4483	0.4522	0.4562
−0.1	0.4602	0.4641	0.4681	0.4721	0.4761	0.4801	0.4840	0.4880	0.4920	0.4960
0.0	0.5000	0.5040	0.5080	0.5120	0.5160	0.5199	0.5239	0.5279	0.5319	0.5359
0.1	0.5398	0.5438	0.5478	0.5517	0.5557	0.5596	0.5636	0.5675	0.5714	0.5753
0.2	0.5793	0.5832	0.5871	0.5910	0.5948	0.5987	0.6026	0.6064	0.6103	0.6141
0.3	0.6179	0.6217	0.6255	0.6293	0.6331	0.6368	0.6406	0.6443	0.6480	0.6517
0.4	0.6554	0.6591	0.6628	0.6664	0.6700	0.6736	0.6772	0.6808	0.6844	0.6579
0.5	0.6915	0.6950	0.6965	0.7019	0.7054	0.7088	0.7123	0.7157	0.7190	0.7224
0.6	0.7257	0.7291	0.7324	0.7357	0.7389	0.7422	0.7454	0.7486	0.7517	0.7549
0.7	0.7580	0.7611	0.7642	0.7673	0.7704	0.7734	0.7764	0.7794	0.7823	0.7852
0.8	0.7881	0.7910	0.7939	0.7967	0.7995	0.8023	0.8051	0.8078	0.8106	0.8133
0.9	0.8159	0.8186	0.8212	0.8238	0.8264	0.8289	0.8315	0.8340	0.8365	0.8389
1.0	0.8413	0.8438	0.8461	0.8485	0.8508	0.8531	0.8554	0.8577	0.8599	0.8621
1.1	0.8643	0.8665	0.8686	0.8708	0.8729	0.8749	0.8770	0.8790	0.8810	0.8830
1.2	0.8849	0.8869	0.8888	0.8907	0.8925	0.8944	0.8962	0.8980	0.8997	0.9015
1.3	0.9032	0.9049	0.9066	0.9082	0.9099	0.9115	0.9131	0.9147	0.9162	0.9177
1.4	0.9192	0.9207	0.9222	0.9236	0.9251	0.9265	0.9279	0.9292	0.9306	0.9319
1.5	0.9332	0.9345	0.9357	0.9370	0.9382	0.9394	0.9406	0.9418	0.9429	0.9441
1.6	0.9452	0.9463	0.9474	0.9484	0.9495	0.9505	0.9515	0.9525	0.9535	0.9545
1.7	0.9554	0.9564	0.9573	0.9582	0.9591	0.9599	0.9608	0.9616	0.9625	0.9633
1.8	0.9641	0.9649	0.9656	0.9664	0.9671	0.9678	0.9686	0.9693	0.9699	0.9706
1.9	0.9713	0.9719	0.9726	0.9732	0.9738	0.9744	0.9750	0.9756	0.9761	0.9767
2.0	0.9772	0.9778	0.9783	0.9788	0.9793	0.9798	0.9803	0.9808	0.9812	0.9817
2.1	0.9821	0.9826	0.9830	0.9834	0.9838	0.9842	0.9846	0.9850	0.9854	0.9857
2.2	0.9861	0.9864	0.9868	0.9871	0.9875	0.9878	0.9881	0.9884	0.9887	0.9890
2.3	0.9893	0.9896	0.9898	0.9901	0.9904	0.9906	0.9909	0.9911	0.9913	0.9916
2.4	0.9918	0.9920	0.9922	0.9925	0.9927	0.9929	0.9931	0.9932	0.9934	0.9936
2.5	0.9938	0.9940	0.9941	0.9943	0.9945	0.9946	0.9948	0.9949	0.9951	0.9952
2.6	0.9953	0.9955	0.9956	0.9957	0.9959	0.9960	0.9961	0.9962	0.9963	0.9964
2.7	0.9965	0.9966	0.9967	0.9968	0.9969	0.9970	0.9971	0.9972	0.9973	0.9974
2.8	0.9974	0.9975	0.9976	0.9977	0.9977	0.9978	0.9979	0.9979	0.9980	0.9981
2.9	0.9981	0.9982	0.9982	0.9983	0.9984	0.9984	0.9985	0.9985	0.9986	0.9986
3.0	0.9987	0.9987	0.9987	0.9988	0.9988	0.9989	0.9989	0.9989	0.9990	0.9990
3.1	0.9990	0.9991	0.9991	0.9991	0.9992	0.9992	0.9992	0.9992	0.9993	0.9993
3.2	0.9993	0.9993	0.9994	0.9994	0.9994	0.9994	0.9994	0.9995	0.9995	0.9995
3.3	0.9995	0.9995	0.9995	0.9996	0.9996	0.9996	0.9996	0.9996	0.9996	0.9997
3.4	0.9997	0.9997	0.9997	0.9997	0.9997	0.9997	0.9997	0.9997	0.9997	0.9998

Table A.2 The *t*-distribution

Value of t for a confidence interval of Critical value of $\|t\|$ for P values of number of degrees of freedom	90% 0.10	95% 0.05	98% 0.02	99% 0.01
1	6.31	12.71	31.82	63.66
2	2.92	4.30	6.96	9.92
3	2.35	3.18	4.54	5.84
4	2.13	2.78	3.75	4.60
5	2.02	2.57	3.36	4.03
6	1.94	2.45	3.14	3.71
7	1.89	2.36	3.00	3.50
8	1.86	2.31	2.90	3.36
9	1.83	2.26	2.82	3.25
10	1.81	2.23	2.76	3.17
12	1.78	2.18	2.68	3.05
14	1.76	2.14	2.62	2.98
16	1.75	2.12	2.58	2.92
18	1.73	2.10	2.55	2.88
20	1.72	2.09	2.53	2.85
30	1.70	2.04	2.46	2.75
50	1.68	2.01	2.40	2.68
∞	1.64	1.96	2.33	2.58

The critical values of $|t|$ are appropriate for a *two*-tailed test. For a *one*-tailed test the value is taken from the column for *twice* the desired *P*-value, e.g. for a one-tailed test, $P = 0.05$, 5 degrees of freedom, the critical value is read from the $P = 0.10$ column and is equal to 2.02.

Table A.3 Critical values of F for a one-tailed test ($P = 0.05$)

v_2	v_1												
	1	2	3	4	5	6	7	8	9	10	12	15	20
1	161.4	199.5	215.7	224.6	230.2	234.0	236.8	238.9	240.5	241.9	243.9	245.9	248.0
2	18.51	19.00	19.16	19.25	19.30	19.33	19.35	19.37	19.38	19.40	19.41	19.43	19.45
3	10.13	9.552	9.277	9.117	9.013	8.941	8.887	8.845	8.812	8.786	8.745	8.703	8.660
4	7.709	6.944	6.591	6.388	6.256	6.163	6.094	6.041	5.999	5.964	5.912	5.858	5.803
5	6.608	5.786	5.409	5.192	5.050	4.950	4.876	4.818	4.772	4.735	4.678	4.619	4.558
6	5.987	5.143	4.757	4.534	4.387	4.284	4.207	4.147	4.099	4.060	4.000	3.938	3.874
7	5.591	4.737	4.347	4.120	3.972	3.866	3.787	3.726	3.677	3.637	3.575	3.511	3.445
8	5.318	4.459	4.066	3.838	3.687	3.581	3.500	3.438	3.388	3.347	3.284	3.218	3.150
9	5.117	4.256	3.863	3.633	3.482	3.374	3.293	3.230	3.179	3.137	3.073	3.006	2.936
10	4.965	4.103	3.708	3.478	3.326	3.217	3.135	3.072	3.020	2.978	2.913	2.845	2.774
11	4.844	3.982	3.587	3.357	3.204	3.095	3.012	2.948	2.896	2.854	2.788	2.719	2.646
12	4.747	3.885	3.490	3.259	3.106	2.996	2.913	2.849	2.796	2.753	2.687	2.617	2.544
13	4.667	3.806	3.411	3.179	3.025	2.915	2.832	2.767	2.714	2.671	2.604	2.533	2.459
14	4.600	3.739	3.344	3.112	2.958	2.848	2.764	2.699	2.646	2.602	2.534	2.463	2.388
15	4.543	3.682	3.287	3.056	2.901	2.790	2.707	2.641	2.588	2.544	2.475	2.403	2.328
16	4.494	3.634	3.239	3.007	2.852	2.741	2.657	2.591	2.538	2.494	2.425	2.352	2.276
17	4.451	3.592	3.197	2.965	2.810	2.699	2.614	2.548	2.494	2.450	2.381	2.308	2.230
18	4.414	3.555	3.160	2.928	2.773	2.661	2.577	2.510	2.456	2.412	2.342	2.269	2.191
19	4.381	3.522	3.127	2.895	2.740	2.628	2.544	2.477	2.423	2.378	2.308	2.234	2.155
20	4.351	3.493	3.098	2.866	2.711	2.599	2.514	2.447	2.393	2.348	2.278	2.203	2.124

v_1 = number of degrees of freedom of the numerator; v_2 = number of degrees of freedom of the denominator.

Table A.4 Critical values of F for a two-tailed test ($P = 0.05$)

v_2	v_1												
	1	2	3	4	5	6	7	8	9	10	12	15	20
1	647.8	799.5	864.2	899.6	921.8	937.1	948.2	956.7	963.3	968.6	976.7	984.9	993.1
2	38.51	39.00	39.17	39.25	39.30	39.33	39.36	39.37	39.39	39.40	39.41	39.43	39.45
3	17.44	16.04	15.44	15.10	14.88	14.73	14.62	14.54	14.47	14.42	14.34	14.25	14.17
4	12.22	10.65	9.979	9.605	9.364	9.197	9.074	8.980	8.905	8.844	8.751	8.657	8.560
5	10.01	8.434	7.764	7.388	7.146	6.978	6.853	6.757	6.681	6.619	6.525	6.428	6.329
6	8.813	7.260	6.599	6.227	5.988	5.820	5.695	5.600	5.523	5.461	5.366	5.269	5.168
7	8.073	6.542	5.890	5.523	5.285	5.119	4.995	4.899	4.823	4.761	4.666	4.568	4.467
8	7.571	6.059	5.416	5.053	4.817	4.652	4.529	4.433	4.357	4.295	4.200	4.101	3.999
9	7.209	5.715	5.078	4.718	4.484	4.320	4.197	4.102	4.026	3.964	3.868	3.769	3.667
10	6.937	5.456	4.826	4.468	4.236	4.072	3.950	3.855	3.779	3.717	3.621	3.522	3.419
11	6.724	5.256	4.630	4.275	4.044	3.881	3.759	3.664	3.588	3.526	3.430	3.330	3.226
12	6.554	5.096	4.474	4.121	3.891	3.728	3.607	3.512	3.436	3.374	3.277	3.177	3.073
13	6.414	4.965	4.347	3.996	3.767	3.604	3.483	3.388	3.312	3.250	3.153	3.053	2.948
14	6.298	4.857	4.242	3.892	3.663	3.501	3.380	3.285	3.209	3.147	3.050	2.949	2.844
15	6.200	4.765	4.153	3.804	3.576	3.415	3.293	3.199	3.123	3.060	2.963	2.862	2.756
16	6.115	4.687	4.077	3.729	3.502	3.341	3.219	3.125	3.049	2.986	2.889	2.788	2.681
17	6.042	4.619	4.011	3.665	3.438	3.277	3.156	3.061	2.985	2.922	2.825	2.723	2.616
18	5.978	4.560	3.954	3.608	3.382	3.221	3.100	3.005	2.929	2.866	2.769	2.667	2.559
19	5.922	4.508	3.903	3.559	3.333	3.172	3.051	2.956	2.880	2.817	2.720	2.617	2.509
20	5.871	4.461	3.859	3.515	3.289	3.128	3.007	2.913	2.837	2.774	2.676	2.573	2.464

v_1 = number of degrees of freedom of the numerator; v_2 = number of degrees of freedom of the denominator.

Table A.5 Critical values of G ($P = 0.05$) for a two-sided test

Sample size	Critical value
3	1.155
4	1.481
5	1.715
6	1.887
7	2.020
8	2.126
9	2.215
10	2.290

Taken from Barnett, V. and Lewis, T., 1984, *Outliers in Statistical Data*, 2nd edn, John Wiley & Sons Limited.

Table A.13 The Spearman rank correlation coefficient. Critical values for ρ at $P = 0.05$

n	One-tailed test	Two-tailed test
5	0.900	1.000
6	0.829	0.886
7	0.714	0.786
8	0.643	0.738
9	0.600	0.700
10	0.564	0.649
11	0.536	0.618
12	0.504	0.587
13	0.483	0.560
14	0.464	0.538
15	0.446	0.521
16	0.429	0.503
17	0.414	0.488
18	0.401	0.472
19	0.391	0.460
20	0.380	0.447

Table A.14 The Kolmogorov test for normality. Critical two-tailed values at $P = 0.05$

n	Critical values
3	0.376
4	0.375
5	0.343
6	0.323
7	0.304
8	0.288
9	0.274
10	0.262
11	0.251
12	0.242
13	0.234
14	0.226
15	0.219
16	0.213
17	0.207
18	0.202
19	0.197
20	0.192

The appropriate value is compared with the maximum difference between the hypothetical and sample functions as described in the text.

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Review

Extraction and clean-up methods for organochlorine pesticides determination in milk

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HIGHLIGHTS

- This review discusses extraction and clean-up methods used to monitor OCP in milk.
- Conventional extraction methods are still the most used, despite its disadvantages.
- New extraction methods have not been evaluated in detail for OCP analysis in milk.
- More research is needed to obtain the ideal method for OCPs determination in milk.

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ABSTRACT

Organochlorine pesticides (OCPs) can cause environmental damage and human health risks since they are lipophilic compounds with high resistance to degradation and long half-lives in humans. As most persistent OCPs have been banned years ago, it is expected to find these compounds at trace levels in environment. Therefore, increasingly sensitive and reliable analytical techniques are required to ensure effective monitoring of these compounds. The aim of this review is to discuss extraction and clean-up methods used to monitor OCP residues in milk, reported in the last 20 years. To carry out this review, an exhaustive bibliographic review was conducted. Despite the disadvantages of conventional extraction and clean-up methods, such as liquid–liquid, solid-phase or Soxhlet extractions, these procedures are still used due to their reliability. New extraction methods, like solid-phase microextraction, matrix solid-phase dispersion or QuEChERS, have not been thoroughly evaluated for OCP determination in milk. Almost all the methodologies analyzed in this review presented good performance characteristics according to the performance acceptability criteria set in SANCO's procedure. Comparison between limits of quantification (LOQ) and detection (LOD), for the reported methodologies, is not always possible due to the heterogeneity of the units. Thus, researchers should take into account an homogenization of LOD and LOQ units, according to the international regulations and MRLs established. Finally, more research is necessary to obtain the ideal methodology for OCPs determination in milk, which comprises the environmentally friendly characteristics of the new techniques and the reliability of the traditional methodologies.

1. Introduction

Contamination by persistent chemicals is potentially harmful to organisms at higher trophic levels in the food chain. Humans are principally exposed to these chemicals through ingestion, since diet is the most important source of chronic exposure to low doses of these substances (LeDoux, 2011). Of the 24 chemicals targeted by the Stockholm Convention, listed in the annexes of the convention text, 15 are organochlorine pesticides (OCPs): aldrin, endrin, dieldrin, chlordane, chlordecone, dichlorodiphenyltrichloroethanes (DDTs), heptachlor, mirex, toxaphene, endosulfan and isomers, hexachlorobenzene (HCB), alpha-hexachlorocycloheaxane (α-HCH), beta-hexachlorocyclohexane (β-HCH), lindane, and pentachlorobenzene (Stockholm Convention, 2009). Studies on the concentration of OCPs in the environmental showed that emission sources of these compounds (such as DDT) in the last 20 years have moved from industrialized countries to developing countries, due either to the late production ban in these countries or to the use in agriculture and control of diseases such as malaria, typhus and cholera (Choi et al., 2009). Today, OCPs have been banned for agricultural or domestic uses in Europe, North America, and many countries of South America in agreement with the Stockholm Convention. Nevertheless, some of these compounds are still applied.

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An example is the pesticide DDT which is used to control the growth of mosquitoes that spread malaria, as mentioned above, or as an antifouling agent in some developing countries. In addition, dicofol, the most frequently used acaricide, which is made of DDT and its formulated products, always contains a small amount of DDT (Chung and Chen, 2011).

OCPs can cause environmental damage and human health risks since they are lipophilic compounds with high resistance to degradation and long half-lives in humans (Chao et al., 2006; Padrón et al., 2006). The half-life of most organochlorine pesticides can range from a few years to more than 10 (Padrón et al., 2006). Several studies have reported that OCPs have endocrine-disrupting activity. It has been well established that these compounds can accumulate in human tissue and can cause chronic toxicity after long-term exposure. Many organochlorine pesticides have been found to be carcinogenic in rodent studies. In addition, they can cause non-Hodgkin's lymphoma, hepatotoxicity, immunotoxicity, developmental abnormalities, neurobehavioral effects and population declines (Qu et al., 2010).

Although most of the OCPs are no longer used, these persistent chemicals can be transferred and magnified to higher trophic levels through the food chain due to their relative stability and bioaccumulation property (Chung and Chen, 2011). These compounds have been confirmed to bioaccumulate in blood (Greizerstein et al., 1999; Jaraczewska et al., 2006), breast milk (Chao et al., 2006; Romero and Dorea, 2000; Mueller et al., 2008), and adipose tissues of humans through dietary intake (Waliszewski et al., 1995; Chao et al., 2006). The presence of these compounds in milk from other mammals has also been reported (Waliszewski et al., 1997; Real et al., 2005; Prado et al., 2007; Ashnagar et al., 2009; Kampire et al., 2011). Therefore, pesticide residue analysis in environmental samples has received increasing attention in the last few decades, resulting in many environmental monitoring programs for a broad range of pesticides (Padrón et al., 2006). Residues of OCPs (including DDT, HCB and HCH isomers) have been determined in samples from areas where these compounds were or are used (such as Africa, Asia, Latin America) (Allé et al., 2009). Nevertheless, the presence of these substances has been also detected in zones where OCPs were never used, such as the Artic. Within the circumpolar studies, several populations living in Artic and sub-Artic areas were determined to be highly exposed to persistent organic pollutants (POPs) due to their local dietary habits. Long-range atmospheric transport and deposition of POPs in the Artic have been studied through the Artic Monitoring and Assessment Program (AMAP) (Polder et al., 2003).

Among the biological matrices mentioned above, milk is a convenient sampling matrix for measuring residue concentrations of persistent OCPs. Cow's milk is considered a nearly complete food since it is a good source of protein, fat and major minerals. Additionally, it is the main constituent of the daily diet, principally for vulnerable groups such as infants, school age children and the elderly. On the other hand, milk is an ideal liquid to dissolve environmental contaminants such as pesticides because most of them are fat-soluble (Kampire et al., 2011). Due to their lipophilic properties, OCPs are primarily stored in fat-rich tissues and subsequently translocated and excreted through milk fat (Waliszewski et al., 1997). Cow's milk may contain high levels of pesticides as a result of residue accumulation in the tissues following the cattle's exposure from feeding on contaminated feedstocks or from drinking contaminated water (Kampire et al., 2011). Thus, knowledge of cow's milk contamination by OCPs provides important information about human exposure to these contaminants, through the ingestion of dairy products.

Human milk is the most complete source of nutrients (proteins, carbohydrates, fat and vitamins), immune factors and other important constituents for infants (Azeredo et al., 2008). It is a convenient matrix for monitoring POPs, such as OCPs, in humans because of the non-invasive sample collection and the suitability for determination of these lipophilic compounds due to the relatively rich lipid content (Tue et al., 2010). Additionally and not less important, the concentration of OCPs in human breast milk is a key factor for evaluating the toxic potential of contaminants in breastfeeding infants (Mihn et al., 2004); who are at the early stage of development and vulnerable to toxic contaminants (Tue et al., 2010). Infants and small children do not have fully developed detoxification mechanisms. Their immune systems are immature and their organs are in the process of rapid growth (Yu et al., 2006.). Human milk offers a unique opportunity for estimation of total chemical intake by infants during breast-feeding (Romero and Dorea, 2000).

When breast milk is employed for human biomonitoring, it is important to take into account the process of depuration, that is, the reduction of chemicals in milk during lactation (Esteban and Castaño, 2009). Since most of the organochlorine pesticides, considered POPs, have been banned years ago, it is expected that these compounds will be found at trace levels in the environment. Thus, increasingly sensitive and reliable analytical techniques are required to ensure effective monitoring of OCPs.

In order to protect health for consumers, Maximum Residue Levels (MRLs) for pesticides and different commodities have been regulated internationally. In the case of the European Union, since 1 September 2008, a new legislative framework (Regulation (EC) No. 396/2005) for pesticide residues is applicable. This regulation completes the harmonization and simplification of pesticide MRLs, while ensuring better consumer protection throughout the EU. The Codex Alimentarius Commission, established by the Food and Agriculture Organization (FAO) and World Health Organization (WHO) in 1963, develops harmonized international food standards, guidelines and codes of practice to protect the consumers' health and ensure fair trade practices in the food trade. The Commission also promotes coordination of all food standards work undertaken by international governmental and non-governmental organizations. The Codex pesticide residues in food online database contain Codex Maximum Residue Limits for Pesticides (MRLs) and Extraneous Maximum Residue Limits (EMRLs) adopted by the Codex Alimentarius Commission up to and including its 34th Session (July 2011) (Codex Alimentarius, 2012).

Table 1 summarizes the OCP MRLs established by different international regulations, the European Union (EU) and the Codex Alimentarius regulations. In the case of the EU, MRLs are established for milk and cream (not concentrated, nor containing added sugar or sweetening matter), butter and other fats derived from milk, cheese and curd (Regulation (EC) No. 396/2005). Regarding the Codex Alimentarius, MRLs are established for milks in general (Codex Alimentarius, 2012). There are no MRLs specifically established for OCPs in human milk. However, due to the similarity of the matrix and the dairy intake of both commodities, MRL values established for OCPs in milks (in general) are taken as reference for the information analysis reported in this work.

In general, food and environmental samples cannot be analyzed without some preliminary sample preparation, since contaminants are too diluted and the matrix is rather complex. Due to the low levels of detection required by regulatory bodies and the complex nature of the matrix, the efficiency of the sample preparation is very important, as well as the low level detection and identification of the target compounds (Picó et al., 2007). In the case of milk samples, one of the main difficulties related to the determination of these analytes is its high fat and protein content that can often interfere in the analytical determination. For this reason, sample extraction can be long and tedious, involving several clean-up steps to remove the co-extracted material from the matrix (Aguilera-Luiz et al., 2011).

Table 1
EU and Codex MRLs established for different OCPs and for milks.

Pesticide (residue definition)	MRL (mg kg^{-1})	
	EU	CODEX
Aldrin and Dieldrin (Aldrin and Dieldrin combined expressed as Dieldrin)	0.006	0.006
Chlordane (sum of *cis*- and *trans*-chlordane and oxychlordane)	0.002[a]	0.002
Chlordecone	0.02	n.e.
DDT (sum of p,p′-DDT, o,p′-DDT, p-p′-DDE and p,p′-TDE (DDD) expressed as DDT)	0.04	0.02
Endosulfan (sum of alpha- and beta-isomers and endosulfan-sulfate expresses as endosulfan)	0.05[a]	0.01
Endrin	0.001	n.e.
Heptachlor (sum of heptachlor and heptachlor epoxide expressed as heptachlor)	0.004	0.006
Hexachlorobenzene	0.01	n.e.
Hexachlorociclohexane (HCH), alpha-isomer	0.004	n.e.
Hexachlorociclohexane (HCH), beta-isomer	0.003	n.e.
Lindane (gamma-isomer of hexachlorociclohexane (HCH))	0.001[a]	0.01
Methoxychlor	0.01[a]	n.e.
Quintozene (sum of quintozene and pentachloro-aniline expressed as quintozene)	0.01[a]	n.e.

n.e. – Not established.
[a] Lower limits of analytical determination.

There is some information that has been reported about methodologies for organochlorine pesticide determination in fatty foods (Gilbert-López et al., 2009; Chung and Chen, 2011) but we have not found reviews wherein analytical aspects of OCPs determination in milk are specifically discussed. This review is intended to discuss extraction and clean-up methods used to monitor OCP residues in milk, reported in the last 20 years.

2. Extraction methods

Generally, analytical methods involve processes such as sampling, sample preparation, separation, detection and data analysis. More than 80% of the analysis time is consumed in sampling and sample preparation steps, such as extraction, concentration, fractionation and isolation of analytes. Thus, it is easy to conclude that the choice of an appropriate sample preparation method influences considerably the reliability and accuracy of food analysis (Kataoka et al., 2000). Most pesticide residue determination methods include two key steps: extraction of target analytes from the bulk of the matrix and clean-up of the analytes from the matrix coextractives (Bennett et al., 1997).

In general, traditional procedures are still used nevertheless are time-consuming, labor-intensive, complicated, expensive and produce considerable quantities of wastes (Wilkowska and Biziuk, 2011). In recent years, several new analytical techniques to prepare food and environmental samples for extraction and determination of pesticide residues have been developed (Picó et al., 2007). The trends in recent years have been towards the ability to use smaller initial sample sizes (even for trace analysis), while obtaining greater specificity, greater selectivity in extraction, increased potential for automation or for on-line methods that reduce manual operations, and a more environmental friendly approach (green chemistry) with less waste and the use of small volumes or no organic solvents (Smith, 2003). The ideal methodology of sample preparation should also be fast, precise and accurate. Additionally, it should be easy to apply and use low cost materials (Picó et al., 2007).

Even though conventional techniques such as liquid–liquid extraction (LLE) and solid-phase extraction (SPE) are still used for the extraction of pesticides from milk, other alternatives, such as matrix solid-phase dispersion (MSPD) (Focant et al., 2004), the QuEChERS method (acronymic name from quick, easy, cheap, effective, rugged and safe) (Aguilera-Luiz et al., 2011), pressurized liquid extraction (PLE) (Mezcua et al., 2007) and solid-phase microextraction (SPME) (Röhrig and Meisch, 2000) have been proposed.

2.1. Liquid–liquid extraction

For liquid milk, liquid–liquid extraction (LLE) is still the preferred method for extracting OCPs (Chung and Chen, 2011; LeDoux, 2011). This procedure consists of shaking liquid milk samples several times in selected organic solvents for extracting pesticide residues from the bulk of the milk (LeDoux, 2011).

When water-miscible extraction systems are used, it is also necessary to include a water-removal or partitioning step. Analysis of liquid milk for pesticides in a low range provides numerous analytical challenges due to the complexity of the matrix. The milk matrix comprises high moisture, lipids, sugars and proteins, which must be removed to achieve the required sensitivity. Milk tends to form emulsions during liquid–liquid partitioning (Bennett et al., 1997).

In the case of OCPs determination, the selection of appropriate solvents and extraction methods is critical in order to achieve a satisfactory recovery from the matrix of interest. Owing to their lipophilicity, organic solvents normally can extract OCPs from food efficiently but lipids are also co-extracted. Solvents such as ethanol, methanol, ethyl acetate, hexane and their mixtures like ethanol/ethyl acetate (Bennett et al., 1997), acetone/hexane (Romero and Dorea, 2000; Mueller et al., 2008; Bulut et al., 2011), ethyl acetate/acetone/methanol (Azeredo et al., 2008), hexane/dichloromethane (Qu et al., 2010), petroleum ether (Kampire et al., 2011) have been used to perform LLE. In some cases sonication and/or vortex are also applied to improve the extraction efficiency and recoveries (Stuetz et al., 2001; Azeredo et al., 2008). During liquid–liquid extraction, it is relatively common to add salts to the solution so that the organic phase can be separated from the aqueous phase more easily (Chung and Chen, 2011).

In some cases, prior to the LLE, an acid may be added to the samples since it allows the determination of free and conjugated pesticides and the degradation of phthalates that overlap the pesticides during gas chromatography (Waliszewski et al., 2008). The most common acids used are chloridric acid (HCl) (Qu et al., 2010), formic acid (Behrooz et al., 2009) and sulfuric acid (Waliszewski et al., 1997). On the other hand, this procedure may limit the determination of some OCPs which are sensitive to acid treatment (Chung and Chen, 2011).

Analyzing the literature, it is possible to observe that most of the reported works for OCPs determination in different kind of milks (human, cow's, buffalo's, and sheep's milks) are based on LLE techniques, as mentioned above. Some of these methodologies allowed the simultaneous determination of other pesticide groups or other types of pollutants such as organophosphorus (Schenck

and Casanova, 1999; Ciscato et al., 2002), carbamates, pyrethroids (Ciscato et al., 2002), PCBs (Behrooz et al., 2009), with good recoveries and acceptable quantification (LOQs) and detection limits (LODs).

Nevertheless, these classical methods of analyte extraction using sample homogenization and liquid–liquid partitioning are time consuming, require large amounts of expensive and hazardous organic solvents, multistep procedures associated with the high risk of analyte losses (Ojeda and Rojas, 2011) and cannot be automated (LeDoux, 2011; Chung and Chen, 2011). Furthermore, the evaporation of large solvent volumes is a source of atmospheric and environmental pollution (LeDoux, 2011). Recent studies still report the use of LLE for the OCPs extraction from milk (Qu et al., 2010; Bulut et al., 2011; Bergkvist et al., 2012; Hassine et al., 2012). Qu et al. (2010) reported a study of the exposure of young mothers and newborns to OCPs, in China, in which sample extraction was carried out by LLE. The samples of concern were extracted with hexane/dichloromethane mixture, following the addition of chloridric acid (HCl) and 2-propanol. In this study, 21 OCPs were analyzed. Nevertheless, only 10 compounds were reported because the other OCPs determined were detected in less than 15% of all samples. Endosulfan (II) and endrin ketone were not detected in any sample. Good limits of detection were achieved for the reported organochlorine pesticides. The low frequency of some OCPs such as heptachlor, heptachlor epoxide, aldrin, dieldrin, endosulfan (I), endosulfan sulfate, endrin, endrin aldehyde and methoxychlor, in the analyzed sample may be due to the addition of chloridric acid since some of these compounds are not resistant to acid treatment (as mentioned above). To achieve an accurate analysis it would be necessary to evaluate the effect of the acid addition on the extraction of these OCPs. Bulut et al. (2011) determined the OCPs' residues in buffalo, cow and sheep milk from Turkey. The extraction of all milk samples was performed by liquid–liquid extraction with hexane/acetone and without addition of acid. The developed methodology was used for monitoring purposes and data obtained showed that some pesticides in all milk analysed exceeded the acceptable Maximum Residue Level (MRL) when compared to Codex MRLs.

2.2. Solid–liquid extraction (SLE)

Liquid–solid extraction is based on the distribution ratio of the analyte between solid and fluid (Żwir-Ferenc and Biziuk, 2004). In the case of milk samples, it may be applied to powdered milk. This procedure has been applied to liquid milk after the milk samples were mixed with anhydrous sodium sulfate, obtaining a coarse powder (Waliszewski et al., 1997; Kumar et al., 2006).

As LLE, the selection of an appropriate solvent is a crucial factor to achieve good results in a solid liquid extraction. Dichloromethane (Kumar et al., 2006) and petroleum ether (Waliszewski et al., 1997) are some of the solvents for the SLE of OCPs from milk.

2.3. Soxhlet extraction

The traditional Soxhlet extraction method has also been applied to the extraction of organochlorine pesticides from milk (Brunetto et al., 1996; Prado et al., 2004; Zhou et al., 2011). Usually, n-hexane is used; however other solvent systems such as n-hexane/dichloromethane (Zhou et al., 2011) can also be used.

Brunetto et al. (1996) applied this methodology for extraction of DDT residues from human milk of Venezuelan women. Good results were obtained, with a recovery rate that ranged between 99% and 107% and a limit of detection (LOD) of 0.5 $\mu g\,L^{-1}$. Some years later, the same extraction technique was applied for determination of OCPs in human milk of women from Mexico City (Prado et al., 2004).

Soxhlet usually performs efficient extractions for a large range of pesticides. However, performing this technique under unfavorable conditions can result in poor recovery rates. In any case, Soxhlet extraction is time-consuming, expensive in terms of energy (heating), analyst time (much handling) and solvent use (large volumes) (LeDoux, 2011).

Zhou et al. (2011) reported a methodology, adopted for determination of OCPs, based on Soxhlet extraction, gel permeation chromatography and gas chromatography–negative chemical ionization–mass spectrometry (GC–NCI–MS) detection. Although the developed methodology presented good performance characteristics, the Soxhlet extraction time was 24 h.

2.4. Solid-phase extraction (SPE)

Solid-phase extraction (SPE) is widely accepted as an alternative extraction/clean-up method to LLE for extraction of pollutants in liquid samples (Lambropoulou and Albanis, 2007).

Conventional solid-phase extraction (SPE) is based on adsorption of analytes onto solid sorbents. This extraction technique is commonly used for enriching analytes from liquid and gaseous matrices. Many food matrices have been cleaned up by SPE for determination of several analytes. The solid-phase extraction or purification is performed in four steps: conditioning (the functional groups of the sorbent bed are solvated in order to make them interact with the sample), retention (the analytes are bound to the bed surface), selective washing (undesired species are removed) and elution (the analytes are desorbed and collected) (Żwir-Ferenc and Biziuk, 2004).

The selection of an appropriate sorbent depends on the interaction mechanisms between the sorbent and the analytes. Alumina, magnesium silicate and graphitized carbon are commercially available sorbents (Żwir-Ferenc and Biziuk, 2004). Alawi et al. (1992) and Sharaf et al. (2008) reported different methodologies for the determination of organochlorine pesticides in human milk, including SPE extraction and Florisil (activated magnesium silicate) as sorbent. Nevertheless, the most common material is silica since it is sufficiently reactive to allow its surface to be modified by chemical reaction and stable enough to permit its use with a wide range of solutions (Żwir-Ferenc and Biziuk, 2004). Diatomaceous earth, a natural material constituted primarily of silica, has been reported as a possible sorbent for SPE extraction in the case of OCPs determination in human milk (Mihn et al., 2004; Tsydenova et al., 2007). Polymer-based sorbents are also common for SPE. Sorbents in general, used in solid-phase extraction belong to three principal classes: nonpolar, polar and ion exchange. The choice of these materials depends on the food matrix, analytes of interest and their interferents (Żwir-Ferenc and Biziuk, 2004).

An innovation of the SPE method has been the introduction of a disk format which, in comparison with a packed cartridge, offers larger flow area and lower bed mass (Żwir-Ferenc and Biziuk, 2004). Covaci and Schepens (2001), developed and evaluated solid-phase disk extraction (SPDE) for the isolation and concentration of persistent organochlorine pollutants (including some organchlorine pesticides) from human body fluids (serum, cord blood, milk, between others). The authors concluded that solid-phase disk extraction provides an effective method for monitoring of selected organochlorine pollutants in different human body fluids.

Principal advantages of SPE are: the analytical procedure is simpler, small volumes of solvents are used and cleaner extracts and greater recoveries are usually obtained. SPE also allows avoidance of the emulsion formation often encountered in LLE. Automation is also possible (Lambropoulou and Albanis, 2007).

Although the potential of SPE for extraction and clean-up of pesticides from food samples is recognized, some characteristics must still be improved. The main problems that researchers need

to overcome include difficulties in: (1) choosing an adsorbent and elution solvent for multiresidue analysis of compounds with a very wide range of physicochemical characteristics; (2) substantial variation in the performance of the products offered by different manufacturers; and (3) the small sample volume that can be extracted with some SPE adsorbents (Lambropoulou and Albanis, 2007).

Together with liquid–liquid extraction, solid-phase extraction is one of the most frequently used extraction techniques for the determination of OCPs in milk.

2.5. Solid-phase microextraction (SPME)

Solid-phase microextraction (SPME) was introduced several years ago by Pawliszyn and co-workers and represents a further advance as a complete solvent-free alternative technique (Arthur and Pawliszyn, 1990; Miège and Dugay, 1998; Balasubramanian and Panigrahi, 2011), reducing the laboratory-generated waste and time for sample preparation (Fernandez-Alvarez et al., 2008). This procedure also allows for achieving lower detection limits and good reproducibility (Wang et al., 2008). SPME can integrate sampling, extraction, concentration and sample introduction into a single uninterrupted process, resulting in high sample yield (Fernandez-Alvarez et al., 2008). Organic compounds are simply extracted by dipping the solid phase coating of a silica fiber support into an aqueous solution. Then, fibers are transferred into the heated injector of a gas chromatograph where the extracted analytes are thermally desorbed and analyzed. Contrary to SPE, the total amount of extracted sample is used for the determination by GC. SPME requires small sample volumes and numerous fibers with different polarities are available, such as polydimethylsioloxane (PDMS), polydimethylsiloxane–divinylbenzene (PDMS–DVB), polyacrylate (PA), carbowax–divinylbenzene (CW–DVB), and carboxen–polydimethylsiloxane (CX–PDMS), among others. Furthermore, the technique is easy to automate by using a commercially available auto-sampler (Miège and Dugay, 1998). A clean-up step is not necessary with the SPME technique because of the selective nature of the coatings (Alpendurada, 2000). The process involving SPME is different from those involving SPE. It is based on a partition process. A first step is the extraction of the analytes according to their partition coefficients. Consequently, extraction recoveries of 100% cannot be achieved and the partition equilibrium may sometimes be required to be reached (Miège and Dugay, 1998). SPME may be operated in the headspace mode, immersed into the aqueous matrix or in membrane protection mode (Balasubramanian and Panigrahi, 2011). It depends on the volatility of the analytes and the matrix characteristics. Several factors can influence the SPME efficiency. During the extraction step, the ionic strength and the pH of the aqueous sample, the nature and the thickness of the fiber, the stirring conditions and the temperature of the extraction must be optimized. During the desorption step, the temperature of the GC injector and the length of desorption time must be considered (Miège and Dugay, 1998). The principal advantages of SPME are: good analytical performance, simplicity and low cost. SPME produces relatively clean and concentrated extracts and it is ideal for mass spectrometry (MS) applications. This procedure does not suffer from the plugging encountered with SPE. It also completely eliminates use of organic solvents, as mentioned above. A relatively long equilibration time is needed and sample stirring, sonication, fiber vibration and rotation have been used to reduce this absorption time. An inherent disadvantage is that quantitative work is still rather laborious because carry-over between samples may be considerable (Lambropoulou and Albanis, 2007).

This technique is of increasing interest in the field of pesticide residues analysis and has been applied for the determination of different classes of pesticides including organophosphorus, organochlorine, and pyrethroid in a large number of matrices, such as wine (Martins et al., 2011a, 2011b, 2012), urine, serum (López et al., 2001), environmental liquid samples (Padrón et al., 2006), cow milk (Rodrigues et al., 2011), and breast milk (Röhrig and Meisch, 2000; Fernandez-Alvarez et al., 2008).

During the application of SPME for the analysis of organochlorine pesticides in breast milk, perchloric acid can be added to break down analyte–matrix composites, and headspace sampling mode is an option to avoid damages in the fiber and interferences on the analysis by other milk components (Fernandez-Alvarez et al., 2008;Rodrigues et al., 2011).

Röhrig and Meisch (2000) reported on an application of solid-phase microextraction for the rapid analysis of chlorinated organic compounds, including some OCPs, in breast milk. After optimization, the authors achieved a very fast and sensitive extraction method. The linearity of the method was very good and the limits of detection and determination were low enough for biomonitoring purposes. Fernandez-Alvarez et al. (2008) developed a SPME extraction technique combined with gas chromatography and microelectron-capture detection for the determination of pesticide residues (including OCPs) in bovine milk. The authors obtained a reliable and linear method in the concentration range of interest, with satisfactory limits of detection and quantification.

2.6. Matrix solid-phase dispersion (MSPD)

Matrix solid-phase dispersion is a new-based extraction and clean-up technique developed for pesticide multiresidue analysis. The main difference between MSPD and SPE is that, in SPE, samples must be in liquid state before application to the column while MSPD can handle solid or viscous liquid samples directly. Interactions of the system components are greater in MSPD and different, in part, from those in SPE (Lambropoulou and Albanis, 2007).

MSPD performs sample disruption while dispersing its components into a solid support. This technique combines sample homogenization with preliminary clean-up of the analytes (Picó et al., 2007). In a first step, homogenized samples are ground with a solid sorbent in order to disrupt the structure of the raw material and achieve its homogeneous distribution around the sorbent particles. Classic applications of MSPD procedure employ reversed-phase sorbents as dispersants. Octadecyl-silica (C_{18}) is the most often used but C_8 and C_{30} materials have been considered too. Normal-phase, non-bonded sorbents, such as Florisil, alumina and silica, have also been proposed as dispersants in several MSPD applications. Replacement of reversed or normal-phase dispersants by sand, diatomaceous earth or Celite has been reported. Several authors have demonstrated that similar precision and recovery results can be obtained. Nevertheless, it does lead to cost-effective methods (Garcia-López et al., 2008). Mixtures, such as silica gel and charcoal are used as solid sorbents. The layer of sorbent with adsorbed analytes is placed in a column, on a layer of anhydrous sodium sulfate (to capture the water contained in the sample). Subsequently, they are washed out with an appropriately chosen solvent that guarantees a good separation of the analytes (Żwir-Ferenc and Biziuk, 2004). The nature of the elution solvent is also crucial for obtaining efficient desorption of pesticides from the adsorbent while retaining interferences on the column. Most adsorbents have been tested in combination with a large variety of solvents including, for example, acetonitrile, dichloromethane or mixtures of these with methanol or hexane (Lambropoulou and Albanis, 2007).

MSPD extraction is a technique wherein a sample extraction and clean-up may be carried out in the same step with good recoveries and reproducibility, reducing the analysis time and the amount of solvent employed. Principal advantages are that this procedure allows rapid sample turnover and improved access to timely data on residue levels present in the sample. It also requires a small sample size and it decreases considerably the amount of

solvent used compared to the classical methods, thus decreasing environmental contamination and improving work safety (Żwir-Ferenc and Biziuk, 2004). Additional advantages of MSPD are: low cost per extraction and no need for expensive instrumentation (García-López et al., 2008). MSPD has become a well-established sample-preparation technique in food analysis (Lambropoulou and Albanis, 2007). Nevertheless, works reporting MSPD extraction of organochlorine pesticides from milk are still limited. Solvent evaporation remains a problem with this extraction technique and literature reports of on-line coupling of MSPD to liquid chromatography (LC) or gas chromatography (GC) instruments are scarce (Lambropoulou and Albanis, 2007).

Focant et al. (2004) developed a methodology for measurement of selected polybrominated diphenyl ethers, polybrominated and polychlorinated biphenyls and organochlorine pesticides in human serum and milk using comprehensive two-dimensional gas chromatography isotope dilution time-of-flight mass spectrometry. The extraction step for the human milk samples was performed by MSPD, using diatomaceous earth as sorbent and dichloromethane as elution solvent. However, in this case a clean-up step was required.

2.7. Pressurized liquid extraction (PLE)

To avoid some of the main disadvantages associated with the application of clean-up steps, such as long sample treatment time and large volumes of organic solvents consumed, modern extraction techniques are applied, specifically, the pressurized liquid extraction (PLE) technique (Mezcua et al., 2007). This technique, also known as accelerated solvent extraction (ASE), is one of the most recent solid and semisolid sample extraction methodologies. The fundamental difference between SFE and PLE is that SFE uses solvents near or above their critical point (usually CO_2-based fluids), whereas PLE uses traditional aqueous and organic solvents (Raynie, 2006; Lambropoulou and Albanis, 2007). PLE uses high temperature and pressurized liquid extraction conditions to extract the analytes (Chung and Chen, 2011). At high temperature the rate of extraction increases because the viscosity and the surface tension of the solvent decrease whereas its solvent strength and rate of diffusion into the sample increase. Pressure keeps the solvent below its boiling point and forces its penetration into the pores of the sample. The combination of high temperature and pressure results in better extraction efficiency, thus minimizing solvent use and expediting the extraction process. The time required for extraction is almost independent of sample mass and the efficiency of extraction is mainly dependent on temperature. PLE has been successfully used for determination of pesticides in different food matrices, with a particular interest in application of the technique to analysis of lipid-containing foods (Lambropoulou and Albanis, 2007). The efficiency of PLE is normally higher when compared to other extraction techniques, especially Soxhlet. The optimization of the methodology includes parameters such as the effects of extraction temperature, number of extraction cycles and various extraction solvent mixture compositions on the extraction effectiveness and recoveries of certain OCPs from fish samples. Besides, clean-up sorbent materials can also be imbedded in the extraction. PLE has the advantages of low solvent consumption and a short extraction period. Nevertheless, the initial cost is high, as large amounts of unwanted matrix substances are co-extracted and some unstable OCPs including endrin, endrin aldehyde and pentachlorobenzene (PeCB) yield low recoveries (Chung and Chen, 2011).

Mezcua et al. (2007) developed a methodology for determination of 12 organophosphorus and organochlorine pesticides in milk-based infant formulas combining PLE with online clean-up followed by gas chromatography tandem mass spectrometry. The authors concluded that the developed PLE–GC–MS/MS multiresidue method is a selective, simple, rapid and suitable procedure for the accurate identification and quantification of the 12 studied pesticides in milk infant formulas. The use of an online clean-up utilizing alumina in the extraction cell during the PLE process and the optimization of the desorption temperature during the GC injection allows the avoidance of typical interferences caused by co-extraction of lipids present in samples.

2.8. QuEChERS

Recently, the QuEChERS (quick, easy, cheap, effective, robust and safe) procedure has become very popular since it has been shown to be a powerful methodology in pesticide residue analysis in foodstuffs. This extraction method allows the simultaneous extraction of polar and non-polar compounds with adequate recoveries, and thus it can be suitable for the extraction of a wide range of compounds (Vidal et al., 2009). QuEChERS is a fast and convenient replacement for LLE which supplies high-quality results in a minimum number of steps and with low consumption of solvent and glassware (Lambropoulou and Albanis, 2007). The QuEChERS method is primarily used for non-fatty food samples, including natural agricultural products (Jeong et al., 2012).The original procedure consists in the extraction of the homogenized sample by hand-shaking or vortex mixing with the same amount of acetonitrile to obtain a final extract sufficiently concentrated to avoid solvent evaporation. Gram quantities of salts (anhydrous magnesium sulfate, $MgSO_4$, and sodium chloride, NaCl) are then added to the sample, with mixing, to drive partitioning of the analytes between the aqueous residue and the solvent. After simple vortex mixing and centrifugation, clean-up and removal of residual water is performed simultaneously using a fast procedure, called dispersive solid-phase extraction (DSPE). In this, a primary–secondary amine (PSA) adsorbent and more anhydrous $MgSO_4$ are mixed with the sample extract. Acetonitrile is the solvent of choice for the QuEChERS method and the dispersing adsorbent most frequently used is PSA. Mixed-mode materials containing two adsorbents, for example C_{18} and PSA, have also been tested (Lambropoulou and Albanis, 2007). $MgSO_4$ is used to reduce the water phase and promote partitioning of pesticides into an organic layer and PSA can be used to remove compounds such as fatty acids, organic acids, and various sugars. Graphite carbon block (GCB) can also be used and it has strong affinity for planar molecules and can effectively remove pigments such as chlorophylls and carotenoids (Jeong et al., 2012). The QuEChERS procedure has the advantages of high recovery, accurate results, high sample throughput, low solvent and glassware usage, less labor and bench space, lower reagent costs and ruggedness. Organic acids and other potential contaminants are removed during clean-up (Lambropoulou and Albanis, 2007). This methodology simplifies the extraction of analytes and the extract clean-up without adversely affecting the magnitude of analyte recoveries (Wilkowska and Biziuk, 2011). The main disadvantage of this extraction method is that the concentration of the target compounds in the final extract is lower than in the concentrated extracts obtained by use of most traditional procedures. Consequently, the final extract must be concentrated to a greater extent to provide the necessary sensitivity and to achieve the limits of quantification (LOQ) desired (Lambropoulou and Albanis, 2007). Difficulties may also arise when the official methods are applied to emulsified fatty foods such as milk. Some lipophilic pesticides such as DDE and HCB have been shown to be poorly recovered from milk and avocado samples, and the recovery rate tended to decrease as the fat content increased (Jeong et al., 2012).

Jeong et al. (2012) developed a QuEChERS-based method by response surface methodology for the determination of pesticide residues (including some OCPs) in milk. According with the report

results, for the OCPs dieldrin and endosulfan sulfate, the QuEChERS method achieved acceptable results, with recoveries in the range of 82–99%. For the OCPs o,p-DDE, p,p′-DDE, o,p′-DDT and p,p′-DDT, it was possible to detect and quantify the molecules by the optimized method even though the recoveries were less than 80%.

3. Clean-up procedures

Even though the analytes of interest are isolated from the bulk matrix, several interfering compounds (fats, sugars, proteins, etc.) may also be co-extracted simultaneously with target compounds, which could interfere in the determination (Żwir-Ferenc and Biziuk, 2004; Gilbert-López et al., 2009). Moreover, co-extracted compounds, especially lipids, tend to adsorb in GC systems such as injection port and column, resulting in poor chromatographic performance (LeDoux, 2011). Consequently, a purification step is required before the determination (Żwir-Ferenc and Biziuk, 2004; Gilbert-López et al., 2009). This step is called the clean-up and allows for isolation of the target compounds from potential interfering co-extractives as well as discarding the extraction solvent (Gilbert-López et al., 2009). Several purification methods have been attempted to eliminate co-extracted interference from extracts including freezing centrifugation, liquid–liquid partitioning, gel permeation chromatography, and solid-phase extraction. Most of the published methods use anhydrous sodium sulfate at one or more steps in order to remove water traces from the extraction solvent system (LeDoux, 2011).

3.1. Freezing centrifugation

In the case of OCPs extraction from milk samples, lipids are one of the most important interferences. The simplest technique for lipids removal is by freezing centrifugation. Fatty substances have lower melting points than the solvent so that frozen lipids can be removed by centrifugation or filtering while OCPs remain dissolved in the solvent. Nevertheless, the solubility of lipids in solvent not only depends on the temperature but also the solubility product. Consequently, this technique can remove significant amount of lipids for some fatty matrix but not for every matrix (Chung and Chen, 2011).

3.2. Partitioning lipid extraction

Liquid–liquid partitioning has also been used, generally prior to additional clean-up procedures such as SPE, to eliminate co-extracted compounds from pesticide extracts. Nevertheless, solvent partitioning can lead to the loss of some analytes and thus to lower recoveries (LeDoux, 2011). Since organochlorine pesticides are also slightly soluble in polar solvent, loss of OCPs and thus lower recoveries are expected (Chung and Chen, 2011).

3.3. Adsorption chromatography

One of the most commonly used clean-up procedure is adsorption chromatography applying the SPE technique. With this technique, the analytes and interfering compounds are adsorbed on a solid sorbent, followed by elution of the target compounds from the substrate and arrest of the interfering substances. It is common to use, for this purpose, chromatographic columns that are factory-made and filled with modified silica gel, Florisil or alumina (Żwir-Ferenc and Biziuk, 2004). These packing materials are used for purification of the extracts from lipid fractions (Żwir-Ferenc and Biziuk, 2004) and may also be applied to remove lipids in sample preparation or the solid phase extraction step (as mentioned above) with minimal adverse effect on non-lipid chemicals (Chung and Chen, 2011).

Clean-up procedure using SPE technique is reported in several studies for OCP determination in milk (Brunetto et al., 1996; Bennett et al., 1997; Romero and Dorea, 2000; Behrooz et al., 2009; Prado et al., 2004; Kumar et al., 2006) and it can be combined with different extraction methodologies.

3.4. Gel permeation chromatography (GPC)

GPC was applied to the clean-up of OCP residues in late 1970s and became the most commonly employed and necessary step for lipids removal of fatty food. This method separates low molecular mass (up to several hundreds) compounds such as OCPs from high molecular mass compounds such as lipids of 600–1500 mass units (Żwir-Ferenc and Biziuk, 2004; Chung and Chen, 2011; LeDoux, 2011). Larger molecules are stopped in the deposit and smaller ones are washed out. Columns used in gel chromatography are usually filled with copolymers of styrene and divinylbenzene. The most common mobile phases used are cyclohexane or combinations of solvents with several elution forces (toluene/ethyl acetate; ethyl acetate/cyclohexane; acetone cyclohexane, etc.) (Żwir-Ferenc and Biziuk, 2004). If correctly used and without exceeding the maximum loading of the GPC, the residual lipids remaining after GPC clean-up normally fall less than 1% of the initial amount (Chung and Chen, 2011).

This clean-up procedure was used in some reported studies for OCPs determination in milk preceded by different extraction methods, such as SPE (Tsydenova et al., 2007), LL extraction (Ciscato et al., 2002; Mueller et al., 2008) and Soxhlet extraction (Zhou et al., 2011). Tsydenova et al. (2007) reported a clean-up stage which included two different procedures; a clean-up by GPC followed by SPE purification.

3.5. Sulfuric acid treatment

Another common purification procedure to remove fatty co-extracted interferences during the clean-up step is sulfuric acid treatment (Bouwman et al., 2006). Although some OCPs are classified as persistent organic chemicals, it is notable that some of them are not resistant to sulfuric acid treatment. These sulfuric acid-sensitive OCPs, such as dieldrin, endrin, endrin aldehyde, chlordecone, endosulfan I, endosulfan II, trans-heptachlor epoxide and dicofol, can be sulfonized completely in contact with concentrated sulfuric acid. In particular, dieldrin and endrin are the most sensitive and will degrade in a few minutes with this treatment. The other acid-labile OCPs will also degrade to different degrees in an hour (Chung and Chen, 2011). Nevertheless, this technique presents several advantages for the determination of organochlorine pesticides which are resistant to the sulfuric acid treatment. Apart from the determination of free and conjugated pesticides and the degradation of phthalates that overlap the pesticides during gas chromatography, leading to misinterpretations, the methodology allows for the replacement of expensive adsorbents and significant decrease in the volume of organic solvents used, (Waliszewski et al., 2008). Waliszewski et al. (1997) used the sulfuric acid treatment for the determination of organochlorine residues in milk and butter, obtaining good results for the analyzed pesticides, with the exception of dieldrin, endrin and methoxychlor, which are destroyed by the action of concentrated sulfuric acid (as stated above). These authors also obtained significant differences in recoveries of α and β-endosulfane due to partial conversion of β-endosulfane to α-endosulfane in the presence of concentrated sulfuric acid during clean-up.

Pandit et al. (2002) also used the sulfuric acid treatment as purification treatment, simultaneously with SPE. This study did not include the determination of sulfuric acid sensitive OCPs. Conse-

quently, the authors could achieve good results for all the analyzed compounds.

4. Quantification methods for OCPs

Most pesticides are volatile and thermally stable. In the case of OCPs, specifically, most of these compounds are non-polar and easily vaporized. Thus, gas chromatography (GC) is the most common technique for chromatographic separation.

Several selective detectors may be coupled with GC for OCP analysis such as electron-capture detection (ECD), nitrogen-phosphorus detection (NPD) and mass spectrometry (MS). GC–ECD is the most commonly used detection method with acceptable detection limits. However, even though the above mentioned detectors can be used for quantification, a GC–MS detector must be used for confirmation. To further increase confidence in confirmative analysis, a GC coupled with tandem MS is one of the suitable techniques (Chung and Chen, 2011).

Although liquid chromatography (LC) is not so common for OCP analysis, it is another option, principally in the case of compounds with poor volatility, high polarity and thermal instability (Sannino et al., 2004). A liquid chromatographic mass spectrometry (LC/MS) system is commonly used for polar, non-volatile and/or thermally labile pesticides. OCPs are mainly nonpolar compounds and are normally not ionized efficiently with atmospheric chemical (APCI) or electrospray ionization (ESI) mode of LC/MS. The development of atmospheric pressure photoionozation (APPI) technology has expanded the range of compounds amenable to LC–MS to include nonpolar compounds (Chung and Chen, 2011).

5. Reported methodologies for OCPs determination in milk samples

To carry out this review, a bibliographic survey of scientific databases was performed, followed by analysis of the collected information. It is important to note that the collected information included different classes of pesticides but that only OCPs data were analyzed. The most studied OCPs are aldrin, dieldrin, endrin, hexachlorocyclohexane and its isomers (HCHs), hexachlorobenzene (HCB), heptachlor, heptachlor epoxide, dichlorodiphenyltrichloroethane (DDT) and its isomers, and endosulfan and its isomers (information not shown).

Table 2 summarized the reported works found, published between 1992 and 2011. Analyzing the information obtained, it is possible to observe that LLE is the most common method for the extraction of OCPs from milk. SPE is also an usual OCP extraction procedure in milk samples. It is possible to observe that scarce information of recent extraction methods for OCPs determination in milk have been reported. New extraction techniques arose with the aim of decreasing the use of toxic solvents, extraction time, sample volume required, and analysis costs. However, methodologies such as PLE may not always be suitable for the determination of OCPs in fatty foods as those unstable compounds (such as endrin aldehyde and chlordecone) would be lost (Chung and Chen, 2011). The complexity of the milk matrix may be another obstacle for the application of faster and more recent extraction procedures for OCPs. Despite its disadvantages, traditional methods such as solid–liquid, liquid–liquid or Soxhlet extraction still provide promising approaches for the extraction of OCPs from milk.

Table 3 shows the main steps for different sample preparation techniques, where it is possible to observe that LLE is a multi-step procedure that often results in analyte losses and, on the other hand, SPME is a process with only two steps, making very fast sample preparation possible. Regarding sample clean-up methods, SPE is the most common procedure. Both conventional glass columns packed with sorbents and ready-to-use cartridges have been used with the most common phases already mentioned above. In the case of SPE clean-up the quality of the sorbents, the extraction solvents and all materials which are in contact with the sample are extremely important to achieving good results. Some extraction methods do not require an extra step for sample purification, such as SPME. As mentioned previously, SPME integrates sampling, extraction, concentration and sample introduction into a single uninterrupted process, avoids the requirement of a clean-up step and results in high sample yield. Methodologies which avoid the requirement of sample clean-up are the second most frequently reported. Sulfuric acid treatment and liquid–liquid partitioning are the purification procedures less reported for the OCPs determination in milk samples.

In an analytical method, several extraction and clean-up steps are combined to achieve maximum analyte recovery with minimal matrix interference. In the pesticide analysis field, recovery rates in the range of 70–120% are considered to be acceptable and can be extended to routine analysis, as recommended by the Codex Alimentarius guidelines (LeDoux, 2011) as well as by the EU Commission guidelines, set in SANCO's procedure "Method Validation and Quality Control Procedures for Pesticide residues analysis in food and feed" (SANCO/12495/2011).

It is possible to observe that almost all the methodologies reported presented good performance characteristics in accordance with the performance acceptability criteria set in SANCO's procedure (SANCO/12495/2011). Even some of the analyzed methodologies presented a deviation $\leqslant$10% from the recovery value of 100% (Brunetto et al., 1996; Waliszewski et al., 1997; Mihn et al., 2004; Kumar et al., 2006; Behrooz et al., 2009). Some examples are: the methodology reported by Brunetto et al. (1996) based on Soxhlet extraction and SPE clean-up with recovery values in the range of 99–107%; two methodologies grounded in SLE presenting very good recovery values, in the range of 90–99% (Waliszewski et al., 1997) and 86–99% (Kumar et al., 2006), respectively; a liquid–liquid based extraction technique and SPE clean-up, reported by Behrooz et al. (2009), with recoveries in the range of 90–110%; and an SPE methodology with GPC and SPE clean-up, described by Mihn et al. (2004), reporting recoveries that ranged between 90% and 108%. Obviously, the characteristics of each OCP and the number of pesticides included in the same analysis play an important role in the recovery results for an analytical methodology. It is difficult to compare the performance characteristics of different analytical methodologies when all or some of the target compounds are different. Furthermore, in most of the analyzed works, authors do not publish performance characteristics of the analytical methods for each individual pesticide. Comparison between limits of quantification (LOQ) and detection (LOD) for the different methodologies reported is not always possible due to the heterogeneity of the units. Some authors present LOD or LOQ values in a lipid basis while others present them on a whole milk basis. In this sense, researchers should take into account a homogenization of the LOD and LOQ units, according to the international regulations and MRLs established. Comparing the OCP MRLs presented in Table 1, with the LOD and LOQ values (Table 2) expressed in the same units (LOD and LOQ for each pesticide not shown), it is possible to observe that values of LOD and LOQ reported are acceptable, ensuring compliance with the MRLs established by European Commission and Codex Alimentarius, for OCPs residues in milk. Considering results expressed in $\mu g\ L^{-1}$ (Table 2), the lowest limits of detection were reported by Fernandez-Alvarez et al. (2008), Azeredo et al. (2008), and Castilla-Pinedo et al. (2010), with values ranging between 0.003–0.016 $\mu g\ L^{-1}$, 0.004–0.034 $\mu g\ L^{-1}$ and in the range of 0.0001 $\mu g\ L^{-1}$, respectively. Azeredo et al. (2008) and Castilla-Pinedo et al. (2010) also present good recovery results while Fernandez-Alvarez et al. (2008) present a maximum recovery value greater than the maxi-

Table 2
Methodologies reported for OCPs determination in milk samples and respective performance characteristics (works published between 1992 and 2012).

Refs.	Matrix	OCPs tested	Extraction procedure	Clean up procedure	Separation technique	Recovery (%)	Anal. features
Alawi et al. (1992)	HM, PcowM, PoM	15	SPE: Florisil	–	GC–ECD (external standard)		
			Eluent: petroleum ether/DCM Reconst.: hexane		GC–MS (confirmation)	n.s.	n.s.
Picó et al., (1995)	HM, IF	26	SPE: octadecylsilica	–	GC–ECD	44–106 (for HM)	LOD range 0.14–6.36 μg L^{-1} (ECD) and 0.25–10.85 μg L^{-1} (MS)
			Eluent: hexane		GC–MS		
Brunetto et al. (1996)	HM	2	Soxhlet: n-hexane	SPE: Florisil	GC–ECD (external standard)	99–107	LOD: 0.5 μg L^{-1}
					LC-DAD (confirmation)		
Bennett et al. (1997)	LWM	30	LLE ethanol:ethyl acetate	SPE: C_{18} cartridges (eluent: acetonitrile)	GC–ECD (external standard)	45–111	LOD (ECD) range 0.6–58.6 μg kg^{-1}
				SPE: aminopropyl column (methanol:methylene chloride)	GC–MS (internal standard for MS detection: anthracene-d_{10}, pyrene-d_{10}, chrysene-d_{12})		
				Reconst.: acetone			LOD (MS) range 9–102 μg kg^{-1}
Waliszewski et al. (1997)	CowM, Bt	14	SLE: petroleum ether	Conc. sulfuric acid	GC–ECD (external standards)	91–99	LOD range 0.001–0.003 mg kg^{-1}
				Reconst.: petroleum ether			
Schenck and Casanova (1999)	RM	6	LLE: acetonitrile, methanol and acetone	SPE: C_{18} column	GC–ECD	68–120	–
				SPE: GCB column (isooctane), for OC pesticides			
Romero and Dorea (2000)	HM	13	LLE: acetone:hexane	SPE: Florisil (hexane:diethylether) and secondly with hexane:diethylether)	GC–ECD (internal standard: hexachorobenzene)	75–120	LOD (lipid basis) range 0.01–0.355 mg kg^{-1}
			Reconstitution in n-hexane				
Röhrig and Meisch (2000)	HM	10	SPME	–	GC–ECD (external standard calibration)	–	LOD range 0.31–3.41 μg L^{-1}; LOQ range 1.17–12.92 μg L^{-1}
Schinas et al. (2000)	HM	7	LLE: ethyl acetate, methanol and acetone	SPE: C_{18} cartridges (isooctane)	GC–ECD (internal standard procedure)	80–113	–
Campoy et al. (2001)	HM	17	Addition of methanol and sodium oxalate	Conc. sulfuric acid	GC–ECD	n.s.	n.s.
			LLE: ethyl ether:hexane (extraction procedure repeated twice)	SPE: silica Sep-Pak (hexane) and hexane:methanol:isopropanol	GC–MS (confirmation)		
Covaci and Schepens (2001)	HS, UCS, HM, FF, SF	6	SPE: C_{18} extraction disk cartridges	SPE: acid silica cartridge (hexane followed by isooctane)	GC–ECD (internal standard)	70–108 (for OCPs in milk)	LOD range 10–100 ng L^{-1}
			Eluent: hexane and hexane:DCM				
Stuetz et al. (2001)	HM	7	LLE: ethyl acetate:acetone:methanol	SPE: octadecyl (C_{18})-bonded silica cartridges Conc. sulfuric acid Reconst.: isooctane	GC–ECD (internal standard: aldrin)	83–110	LOD range 0.5–2.5 μg L^{-1}
Bates et al. (2002)	HM	6	LLE: hexane:acetone	Partitioning with acetonitrile	HRGC–HRMS	–	–
				SPE: Florisil			
Pandit et al.	M, DP	7	LLE: hexane and acetone	Conc. sulfuric acid	GC–ECD	83–97	LOD range

(continued on next page)

Table 2 (*continued*)

Refs.	Matrix	OCPs tested	Extraction procedure	Clean up procedure	Separation technique	Recovery (%)	Anal. features
(2002)			(for milk)				0.001–0.003 mg kg^{-1}
			Soxhlet (for powder milk)	SPE: Florisil (for milk and powder milk)			
Ciscato et al. (2002)	CowM	13	LLE: acetone (partitioning with DCM)	GPC: cyclohexane and ethyl acetate	GC–ECD; GC–NPD; GC–FPD	70–105 (for OCPs)	LOD range 0.002–0.01 mg kg^{-1}
Prado et al. (2004)	HM	12	Soxhlet: n-hexane (for 8 h)	SPE: chromatographic column packed with Florisil (hexane:DCM)	GC–ECD (external standard)	⩾80	LOD (lipid basis) range 0.010–0.038 mg kg^{-1}
				Reconst.: isooctane			
Mihn et al., (2004)	HM	8	SPE: column packed with diatomite earth (diethyl ether)	GPC: hexane:DCM	GC–MS (for TCPMe quantification)	90–108	LOD (lipid basis) range 0.1–1 μg kg^{-1}
				SPE: Florisil (PCBs and OCPs)	GC–ECD (for the quantification of the others OCPs)		
Focant et al. (2004)	HS; M	11	MSPD: diatomaceous earth	SPE: silica and sulfuric acid silica (hexane)	GC X GC–IDTOFMS	–	LOD range 1–15 μg L^{-1}
			Eluent: dichloromethane (for human milk)		GC–IDHRMS		
Ghidini et al. (2005)	OM, CM, MP	24	SPE: ready-to-use Chem Elut CE 1005 (for milk ssmples)	SPE: Florisil (for milk samples)	GC–ECD; GC–MS (for confirmation)	–	LOD: 0.2 μg L^{-1} (for milk); LOQ: 0.4 μg L^{-1} (for milk)
Poon et al. (2005)	AT, HM	17	LLE: acetane:hexane	GPC: preswollen (dichloromethane)	GC–MS	89 (for DDTs)	n.s.
Jaraczewska et al. (2006)	UCS, HS, HM	10	SPE: Oasis™ SPE cartridge	SPE: acid silica (DCM)	GC–MS (ECNI mode, internal standard, for OCPs and PCBs analysis)	70–102 (milk)	LOQ (lipid basis) range 0.5–4 μg kg^{-1} (for PCBs, OCPs and PBDEs)
			Eluent: DCM (for milk samples)	Reconst.: isooctane (for milk samples)			
Kumar et al. (2006)	HM	7	SLE: DCM	SPE: Florisil (n-hexane/DCM)	GC–ECD (external standard)	86–99	LOD: 0.001 mg L^{-1}; LOQ: 0.01 mg L^{-1}
				Reconst.: n-hexane			
Yu et al. (2006)	HM	6	LLE: hexane and acetone	Conc sulfuric acid	n.s.	80–120	n.s.
Yu et al. (2007)	HM (colostrum and mature milk)	4	LLE: 5% natrium oxalate solution, ethanol, diethyl ether and finally hexane	SPE: Florisil-silica gel column (DCM:hexane)	GC–ECD (external standard)	n.s.	LOD (lipid basis): 0.055–0.747 μg kg^{-1} (for colostrum)
			The resulting aqueous phase was extract with hexane		HRGC/LRMS–SIM (for confirmation in problematic cases)		LOD (lipid basis): 0.101–0.683 μg kg^{-1} (for mature milk)
Tsydenova et al. (2007)	HM	13	SPE: diatomaceous earth (diethyl ether)	GPC: Bio-Bead S-X 3	GC–ECD (for OCPs quantification, external standard	60–120 (for $^{13}C_{12}$-labeled BDEs)	n.s.
				SPE: Florisil (first fraction eluted with hexane and second fraction eluted with DCM:hexane)	GC–MS(for TCPMe quantification)		
Mueller et al. (2008)	HM	17	LLE (acetone:hexane)	GPC SPE: Florisil	HRGC/HRMS (internal standard: $^{13}C_x$ surrogate standards – ES-5021 labeled compound surrogate solution)	n.s.	n.s.
Fernandez-Alvarez et al., (2008)	CowM	19	SPME	–	GC–μECD (external standard)	76–139 (corresponding only to OCP values)	LOD range 0.003–0.16 μg L^{-1}; LOQ range 0.010–0.52 μg L^{-1}
Azeredo	HM	3	LLE: ethyl	SPE: C_{18} solid SPE cartridge	GC–ECD (internal	82–103	LOD range

Table 2 (*continued*)

Refs.	Matrix	OCPs tested	Extraction procedure	Clean up procedure	Separation technique	Recovery (%)	Anal. features
et al. (2008)			acetate:acetone:methanol	(n-hexane) SPE: Florisil	standard: DCN)		0.0040–0.0340 μg L^{-1}
Der Parsehian, et al., (2008)	HM	23	SPE: Florisil (methylene chloride and petroleum ether)	–	GC–ECD	n.s.	LOD range 0.3–1.3 μg L^{-1}
Sharaf et al. (2008)	HM, HS, UCS, AT	10	SPE: Florisil (diethyl ether:petroleum ether)	–	GC–ECD (external standard)	83–91	LOQ range 0.003–0.017 μg kg^{-1}
Rodas-Ortíz et al., (2008)	HM	26	LLE: hexane	SPE: Florisil (first fraction: hexane; second fraction: hexane:DCM)	GC–ECD	n.s.	n.s.
Allé et al. (2009)	HM, AT	10	SPE: Florisil Eluent: petroleum benzene:DCM Reconst.: hexane	–	GC–ECD	n.s.	LOD (lipid basis): 5 ng kg^{-1}
Ashnagar et al. (2009)	CowM	7	LLE (addition of aqueous potassium oxalate solution followed by methanol, diethyl ether and finally n-hexane)	LLE: Petroleum:acetonitrile	HPLC–UV	n.s.	n.s.
Behrooz et al. (2009)	HM	10	LLE: n-hexane:DCM after addition of formic acid	SPE: silica, impregnated with concentrated sulfuric acid (44%, w/w) (eluted with n-hexane followed by DCM) Reconst.: isooctane	GC–ECD	90–110	LOQ (lipid basis) range 0.01–0.2 μg kg^{-1}
Haraguchi et al. (2009)	HM	9	Addition of calcium oxalate solution and ethanol:diethylether LLE: n-hexane	GPC: Bio-Beads S-X3 SPE: silica gel (n-hexane:DCM)	GC–MS	n.s.	LOQ (lipid basis): 0.1–2.5 μg g^{-1}
Qu et al. (2010)	B, UCB, S, HM	10	HCl and 2-propanol were added to the sample LLE: hexane:DCM	Conc. sulfuric acid SPE: silica/sulfuric acid column (eluted with hexane:DCM) Reconst.: hexane	GC–MS (internal standard: PCNB)	76 (for surrogate standards)	LOD (lipid basis) range 0.5–0.8 μg kg^{-1}
Castilla-Pinedo et al. (2010)	PM	20	SPE: C_{18} cartridge(hexane:acetone)	–	GC–μECD (external standard) GC–MS(for confirmation)	⩾80	LOD in the range of 0.0001 μg L^{-1}; LOQ: 0.02 μg L^{-1}
Fujii et al. (2011)	HM	5	Addition of potassium oxalate solution and ethanol:diethylether LLE: n-hexane	GPC: Bio-Beads S-X3	GC–MS	84–94	LOD (lipid basis): 0.005–0.05 μg kg^{-1} LOQ (lipid basis): 0.01–0.10 μg kg^{-1}
Zhou et al. (2011)	HM	23	Soxhlet: n-hexane:DCM (24 h)	GPC: low pressure column SPE: Florisil Reconst.: hexane	GC–NCI–MS (internal standard: $^{13}C_{12}$-labeled)	85–130	LOD (lipid basis) range 0.05–3.00 μg kg^{-1}
Kampire et al. (2011)	FCowM, PCowM	8	LLE: petroleum ether Reconst.: hexane	– SPE: Florisil (n-hexane) – Reconst.: cyclohexane	GC–ECD (external standard) GC–MS (for confirmation)	78–95 (fresh milk) 78–94 (pasteurized milk)	LOD (lipid basis) range 0.01–0.04 μg kg^{-1}; LOQ range 0.01–0.04 μg kg^{-1}
Mezcua et al. (2007)	M-basedIF	4	PLE: acetonitrile	–	GC–MS/MS	87–110 (for OC pesticides)	LOD range 0.03–

(*continued on next page*)

Table 2 (*continued*)

Refs.	Matrix	OCPs tested	Extraction procedure	Clean up procedure	Separation technique	Recovery (%)	Anal. features
							0.21 μg kg^{-1}; LOQ range 0.10–2.60 μg kg^{-1}
Bulut et al. (2011)	CowM, BuM, ShM	19	LLE: hexane/acetone	– SPE: Florisil (diethyl ether:petroleum ether) – Reconst.: hexane	GC–ECD	71–98	n.s.
Jeong et al. (2012)	M	6	QuEChERS	–	GC–ECD	72–90, for OC pesticides	LOD range 0.52–1.50 μg kg^{-1}; LOQ range 1.72–5.00 μg kg^{-1}
Hassine et al. (2012)	HM	7	LLE: n-hexane:acetonitrile:ethanol	SPE: Florisil (DCM:n-hexane)	HRGC–ECD	79–100	LOD ranged 0.5–1 μg kg^{-1} (lipid basis)
Bergkvist et al. (2012)	HM	8	LLE: methyl tert-buthyl ether/hexane	SPE: acid silica	GC–MS	79–106	LOQ ranged 0.010–0.300 μg kg^{-1}
Luzardo et al. (2012)	CM, OM	21	SPE: C_{18} cartridge	GPC: fluorinated divinylbenzene (DCM)	GC–MS	n.s.	LOQ in the range of 10 μg L^{-1}
			Eluent: hexane	SPE: silica gel (DCM:hexane and then methanol:DCM)			

Abbreviations: AT – adipose tissue; B – blood; Bt – butter; BuM – buffalo's milk; CM – conventional milk; CowM – Cow's milk; MS – maternal serum; DB – dolphin blubber; DCM – dichloromethane; DCN – dichloronaphthalene; DP – dairy products; ECD – electron capture detection; ECNI – electron-capture negative ionization; FcowM – fresh cow's milk; FF – follicular fluid; FPD – GC – gas chromatography; GCB – graphitized carbon black; GPC – gel permeation chromatography; HCl – hychloridric acid; HL – hake liver; HM –human milk; HMT – herring muscle tissue; HPLC – high performance liquid chromatography; HRGC – high resolution gas chromatography; HRMS – high resolution mass spectrometry; HS – human serum; IF – infant formulas; LC – liquid chromatography; LLE – liquid–liquid extraction; LOD – limit of detection; LOQ – limit of quantification; LWM – liquid whole milk; M – milk; M-basedIF – milk-based infant formulas; MP – meat products; MS – mass spectrometry; n.s. – not specified; NCI – negative chemical ionization; NPD – nitrogen phosphorus detection; OM – organic milk; PCBs – polychlorinated biphenyls; PcowM – pasteurized cow's milk; PCNB – pentachloronitrobenzene; PM – pasteurized milk; PoM – powder milk; Reconst. – reconstitution; RM – raw milk; S – serum; SF – seminal fluid; ShM – sheep's milk; SIM – selective ion monitoring; SLE – solid–liquid extraction; SPE – solid-phase extraction; SPME – solid-phase microextraction; TCPMe – tris(4-chlorophenyl)methane; UCB – umbilical cord blood; UCS – umbilical cord serum; UV – ultraviolet.

Table 3
Protocols used in different sample preparation techniques: LLE, SPE, and SPME (Alpendurada, 2000).

LLE	SPE	SPME
Addition of organic solvents to the sample	Conditioning of cartridges or membranes	Exposing SPME fiber to the sample
Agitation in a separatory funnel	Sample elution	Desorption of analytes in the analytical instrument
Separation of aqueous and organic phases	Solvent elution to remove interferences and analyte desorption	
Removal of organic phase	Evaporation/concentration of the organic phase	
Evaporation/concentration of the organic phase	Injection in the analytical instrument	
Injection in the analytical instrument		

mum recovery value acceptable by the Codex Alimentarius and EU commission guidelines. Regarding limits of detection expressed in μg kg^{-1} or mg kg^{-1} (Table 2), Mezcua et al. (2007) reported a methodology based on PLE which presented the lowest LOD values (0.030–0.210 μg kg^{-1}), with also good recovery values (87–110%). The lowest LOQ value was obtained with SPE based methodology reported by Sharaf et al. (2008), in the range of 0.003–0.017 μg kg^{-1}. LOD values were not published and good recovery values were likewise obtained with this methodology. As in the case of the recovery values, the comparison of the LOD and LOQ values between different methodologies is difficult, since analytical methods are not always developed for the determination of the same target compounds, as stated above.

6. Conclusions

The determination of POPs such as organochlorine pesticides is extremely important to protect environment and human health, since they are lipophilic compounds with high resistance to degradation and long half-lives in humans. Consequently, reliable methods with sufficiently low detection limits are required to support monitoring and regulatory enforcement.

One analytical challenge in this field is to present consistent results, following official guidelines, as fast as possible and considering method characteristics such as recovery, accuracy, sensitivity and specificity. Conventional extraction methodologies, such as LLE, Soxhlet and clean-up procedures are tedious, time consuming, require considerable handling and use large volumes of solvents. However, these methodologies are still used due to their reliability.

Increasing attention, nowadays, is pointed to techniques which are environmentally friendly by minimizing the use of organic solvents and hazardous waste production. Advances in sample preparation are intended to save employee labor and time, reduce the cost per sample and at the same time, improve the efficiency of the analyte isolation. Recent researches have focused on the development of efficient, economical and miniaturized sample preparation methods. Microextraction methods have attracted much attention in the recent years as alternatives for classic extraction

procedures. Although these analytical trends, new extraction methods have not been thoroughly evaluated for OCPs determination in milk. Traditional methods are still providing promising approaches for the OCPs extraction in milk samples.

According to the current situation, extraction methods should be methodically evaluated to find one that is fast and effective for the determination of OCPs in milk.

Concerning clean-up methods, the methodologies that do not require a clean-up step are preferred since they are faster and less laborious. Nevertheless, they are not always effective. SPE is the most common procedure. It is an effective clean-up method but is dependent on the quality of the sorbents, the extraction solvents and all materials that enter into contact with the sample.

Economic issues for each method were not evaluated. The choice of one of them and especially of an extraction procedure will depend on each specific case as well as on the ultimate aim. The properties of the OCPs and the matrix are the decisive factor for the selection of the appropriate technique for pesticide determination. Nevertheless, the properties and concentrations of interfering compounds, the time required for analysis and sample preparation simplicity of an extraction technique are also important. Finally, the laboratory equipment, the economic support for the investigation, and the experience of the researcher are other factors which play an important role in the choice of the methodologies for determination of OCPs in milk. In conclusion, more research is needed to achieve the ideal method for OCPs determination in milk samples.

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