Advances in Analytic Science (2022) Volume 3 Issue 1
doi: 10.54517/aas.v3i1.1977

Asia Pacific Academy of Science Pte. Ltd.

ORIGINAL RESEARCH ARTICLE

Determination of persistent organic pollutants in cow's milk from national production by means of gas chromatography coupled to mass spectrometry (GC/MS)

Camila Marranzini[1,*], Lizaira Bello[2], Alejandro Vallejo Degaudenzi[3], Aris Mendis Gomez[1], Enmanuel Echavarría[1]

[1] *Laboratory of the General Directorate of Customs, Santo Domingo 10602, Dominican Republic*
[2] *Santo Domingo Institute of Technology (INTEC), Santo Domingo 10602, Dominican Republic*
[3] *Institute of Tropical Medicine and Global Health, Santo Domingo 10602, Dominican Republic*

*** Corresponding author:** Camila Marranzini, cmmarranzini@gmail.com

ABSTRACT

Persistent organic pollutants (POPs) are organic chemicals capable of persisting in the environment, being transported over long distances, bioaccumulating and biomagnifying in ecosystems. The harmful effects of these compounds on the environment and the health of living beings are of concern. In particular, humans can come into contact with POPs when consuming contaminated food of animal origin with a high fat content. In the Dominican Republic these compounds have been widely used and generated. However, the levels of POP exposure to which the population is exposed are unknown. Therefore, the objective of this study was to determine the presence of 34 POPs in five main brands of nationally produced cow's milk. The samples were prepared using the QuEChERS extraction method and the analytical technique used was GC/MS. The results obtained indicated that no POPs were present in the samples. The results obtained indicated that there is no presence of the POPs evaluated in any of the cow's milk samples, which suggests that their consumption does not represent a threat to the health of consumers. In addition, this study contributes to the knowledge on the evaluation of POPs in the Dominican Republic.

Keywords: contaminant; milk; Dominican Republic; chromatographic analysis; agricultural chemistry

1. Introduction

Chemicals known as persistent organic pollutants (pops) are the focus of international attention because of growing scientific evidence that they can cause conditions such as cancer, damage to the central and peripheral nervous systems, immune system diseases, reproductive disorders, metabolic disorders and alterations in the normal development of infants and children[1]. In addition, these compounds are capable of persisting in the environment, long-range transport through the atmosphere, bioaccumulate in human and animal tissues and biomagnify their concentrations in food chains[2]. Pops have been widely used in various human activities; as pesticides, in different pharmaceutical processes, and in the generation of chemical

ARTICLE INFO
Received: 6 April 2022 | Accepted: 30 May 2022 | Available online: 18 June 2022

CITATION
Marranzini C, Bello L, Vallejo Degaudenzi A, et al. Determination of persistent organic pollutants in cow's milk from national production by means of gas chromatography coupled to mass spectrometry (GC/MS). *Advances in Analytic Science* 2022; 3(1): 1977. doi: 10.54517/aas.v3i1.1977

products and by-products in several industries. In particular, pops include different types of chemical species such as, organochlorine pesticides, polychlorinated dibenzo-dioxins (pcdds), polychlorinated dibenzo-furans (pcdfs) and polychlorinated biphenyls (pcbs)[1].

As a result, sustained exposure of many species, including humans, has been generated over periods of time spanning generations. In order to mitigate the adverse effects produced by these substances, a global treaty known as the Stockholm Convention was created in which the participating countries, including the Dominican Republic, agreed to eliminate or reduce the production, use and release of pops. Different activities, such as the use of organochlorine pesticides, waste burning and the use of electrical transformers and capacitors have contributed to the dispersion of pops in the country[3].

Specifically, human exposure to pops occurs mainly through contaminated foods, especially those of animal origin with a high fat content[4]. Consequently, international organizations such as the United States Environmental Protection Agency (USEPA), the European Union (EU) and the Codex Alimentarius of the Food and Agriculture Organization of the United Nations (FAO) have created standards establishing maximum residue limits (mrls) of pops allowed in food[5–7]. Due to the need to study the adverse effects in humans produced by the consumption of food contaminated with such substances, several analytical techniques have been used for their detection and identification[8–11]. However, the fast, easy, inexpensive, efficient, effective, robust and safe extraction method (quechers) and the analytical technique of gas chromatography coupled to mass spectrometry (GC/MS) are the most widely employed tools at present due to their outstanding advantages of flexibility, speed, economy, ease, efficiency and robustness, high sensitivity, accuracy and versatility[12–15]. In particular, abundant scientific studies evaluate the pops content in dairy products such as milk and even concentrations above internationally established mrls have been detected[12,13,15–21].

In contrast, in the Dominican Republic, the number of investigations on the presence of pops and their effects on the population are very scarce and old[3,22]. It is believed that the release and use of these toxic substances has decreased over the years due to the implementation of regulations governing the management of pops. However, the real situation of pops in the country is unknown. For this reason, the objective of this study was to determine the presence of 34 pops in five brands of cow's milk produced nationally in order to obtain accurate and reliable information through the application of sensitive and effective analytical techniques on the degree of exposure to pops in foodstuffs in Dominican society.

2. Materials and methods

2.1. Reagents and samples

This research determined in the samples of Cow's milk the presence of the following pops: aldrin, chlordane, decabromobiphenyl, dichlorodiphenyldichloroethane (DDD), dichlorodiphenyldichloroethylene (DDE), dichlorodiphenyltrichloroethane (DDT), dieldrin, endosulfan, endrin, heptachlor, hexabromobiphenyl, hexabromocyclododecane (HBCD), hexachlorobenzene, hexachlorocyclohexane (HCH), lindane (gamma-HCH), mirex, toxaphene and the following PCB congeners: 2,3,3,3',4,5'-pentachlorobiphenyl (PCB 122), 2,2',3,3',4,4,4',5-heptachlorobiphenyl (PCB 170), 2,3',4,4',6-pentachlorobiphenyl (PCB 119), 3,3',4,4'-tetrachlorobiphenyl (PCB 77), 2,3,4,4',5-pentachlorobiphenol(PCB114),2,2',3,4,4',5'-hexachlorobiphenyl (PCB 138), 2,2',4,5,5'-pentachlorobiphenyl (PCB 101), 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153), 2,2',5,5'-tetrachlorobiphenyl (PCB 52), 2,2',3,4,4,4'5,5'-heptachlorobiphenyl (PCB 180), 2,4,4'-trichlorobiphenyl (PCB 28), 2,3,3',4,4',5-hexachlorobiphenyl (PCB 156), 2,3',4,4,4',5,5'-hexachlorobiphenyl(PCB167),2,3,3',4,4'-pentachlorobiphenyl (PCB 105), 2,3,3',4,4,4'5,5'-heptachlorobiphenyl (PCB 189), 3,4,4',5-tetracyclobiphenyl (PCB 81) and 3,3',4,4',5,5'-hexachlorobiphenyl

(PCB 169). The method created by the Association of Official Analytical Chemists (AOAC) called AOAC 2007.01 was used as the basis for the present experimentation (Association of Official Analytical Chemists, 2007). All reagents and materials used were certified and of high purity, LC/MS grade, optimum and Fisher brand pesticide grade. In addition, five brands of cow's milk with a fat content of 3% (whole milk) produced in the Dominican Republic were selected as samples for the development of this study. The methodology was carried out through the following stages: sample collection, sample preparation and analytical technique.

During the months of May to July 2019, five different brands of nationally produced whole cow's milk marketed in the main supermarkets of the National District were selected. Three lots or productions of each whole milk brand were randomly selected. The analyses of each of the 15 lots were repeated three times, with a total of 45 samples (see **Figure 1**).

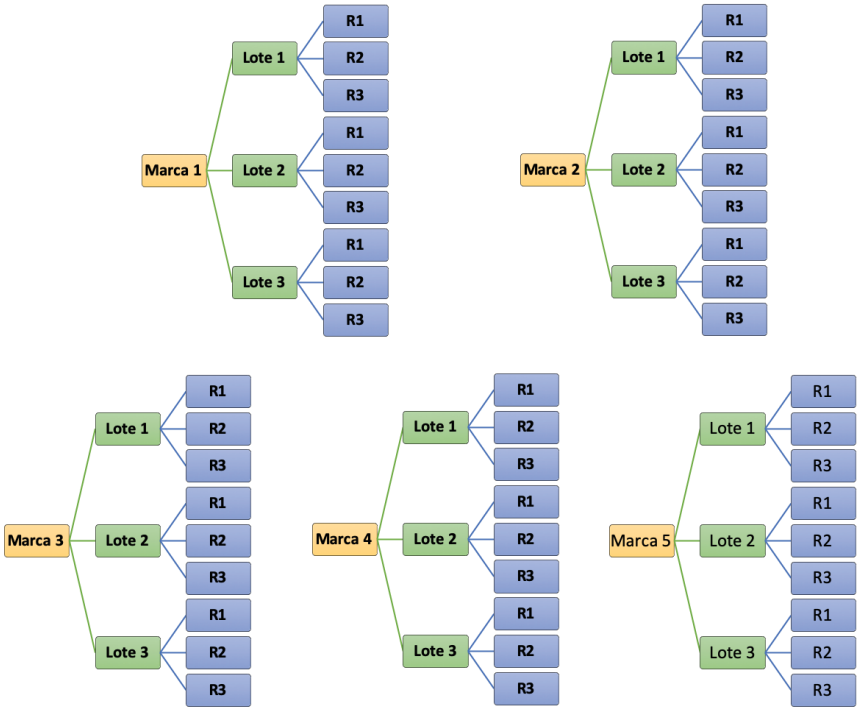

Figure 1. Schematic of the experimental design (Source: own elaboration).

2.2. Sample preparation

Sample preparation was carried out in three parts: extraction, purification and concentration (see **Figure 2**). First, 15 mL of milk was poured into extraction tubes and 6 g of magnesium sulfate (mgso4) and 1.5 g of sodium acetate (naoac) were added, followed by the addition of 15 mL of a solution of acetonitrile (C_2H_3N) with 1% acetic acid (CH_3CO_2H). It was shaken vigorously for one minute, and centrifuged at 3900 rpm for 8 min.

Then, 8 mL of the supernatant was transferred to dispersive solid phase extraction (d-SPE) tubes containing 150 mg of magnesium sulfate (mgso4), 50 mg of ethylenediamine-N-propyl (primary/secondary amine, PSA), 50 mg of a C18 sorbent (octadecyl bonded silica) and 50 mg of graphitized activated carbon (GCB). It was then shaken for one minute and centrifuged at 3900 rpm for 8 min.

Subsequently, the separated upper phase was concentrated, and the solvent was exchanged for toluene (C6H5CH3). For this, the final extract was heated at a constant temperature of 60 °C until evaporation of the solvent and then 1 mL of toluene was added. Finally, that solution was transferred to 1.5 mL GC vials.

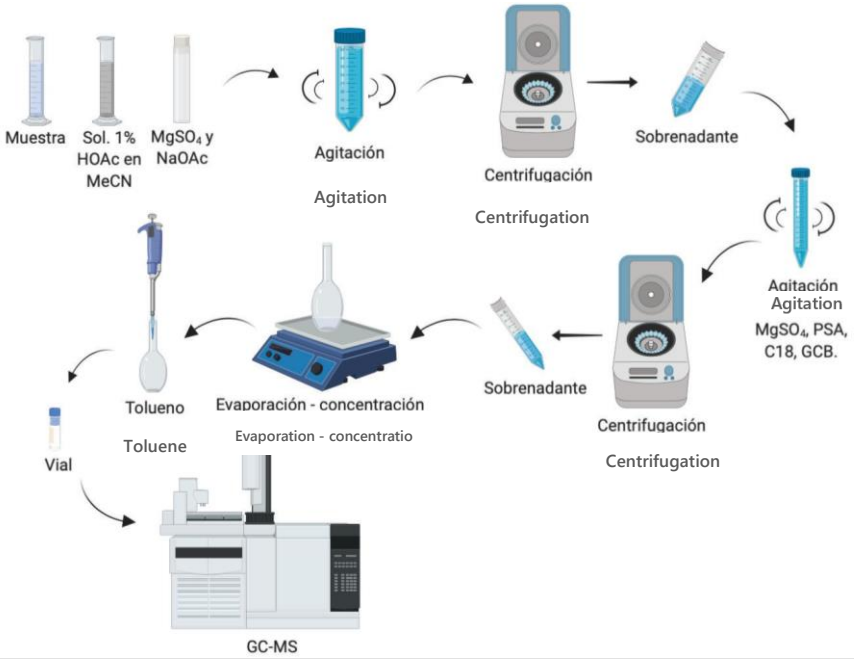

Figure 2. Workflow for sample preparation (Source: own elaboration).

2.3. Analytical technique

The determination of the presence of pops evaluated in the milk samples was carried out by the GC/MS technique which is the one recommended by the Codex Alimentarius international food standards, guidelines and codes of practice for the identification and confirmation of pops residues in food (Codex Committee on Pesticide Residues (CCPR), 2017). The equipment used was the Perkin Elmer brand Clarus SQ 8C GC/MS. 1 µL of the final extract was injected, in split mode, into the Perkin Elmer brand Elite 5MS column of film thickness 0.25 µm, inner diameter 0.25 mm, length 60 m and 1,4-bis(dimethylsiloxy)phenylene dimethylpolysiloxane phase. Hydrogen was used as a carrier gas and was maintained at a constant flow rate of 1.5 mL/min. The temperature ramp of the column oven was started from 90 °C with an increase of 3 °C/min until 320 °C was reached. The injector was maintained at a constant temperature of 250 °C, for a total run time of 75 min.

The MS detector was used in full scan mode to evaluate the range of possible cops in GC/MS, selecting a mass range from 50 to 550 m/z, with an electron collision energy of 70 ev. The ionization source used was the electron impact source. The transfer line and ionization source were maintained at a temperature of 300 °C and 320 °C, respectively.

2.4. Reference standards

The compounds naphthalene-d8 and acenaphthene-d10 were used as internal standard with a concentration of 40 ppm.

For quality control purposes, a solution was prepared containing a mixture of external reference standards of the evaluated Sigma Aldrich pops with a purity between 95.8% and 99.8%, and these were studied under the same chromatographic conditions described above. In addition, they presented a limit of detection (LOD) of 0.010 ppm. Subsequently, the chromatograms and mass spectra obtained from these external standards were compared with those obtained from the samples.

3. Results and discussion

3.1. Sample selection

The selection of processed milk as a sample for the development of this study was satisfactory, because the usual treatments in milk for human consumption such as pasteurization, sterilization and UHT processing do not cause any appreciable effect on the content of POP residues in this matrix[23]. In addition, evaporation and solvent exchange contributed to the reduction or elimination of interferences and concentration of target analytes.

3.2. Quechers extraction method

The use of magnesium sulfate (mgso4) and sodium acetate (naoac) in the quechers extraction method were successfully used to remove the water present in the samples. Also, the use of ethylenediamine-N-propyl (primary/secondary amine, PSA) was successful in removing fatty acids, other organic acids and sugars, as well as the use of C18 sorbent (octadecyl bonded silica) in order to remove non-polar interferences contained in the samples.

Determination of persistent organic pollutants in cow's milk of national production by means of gas chromatography coupled to mass spectrometry (GC/MS).

3.3. GC/MS analytical technique

The use of the GC/MS analytical technique allowed the complete separation and identification of all the components present in the samples, yielding characteristic chromatograms and mass spectra (see **Figures 3** and **4**). After performing the analyses by means of this technique, none of the pops evaluated in any of the cow's milk samples were detected in this study.

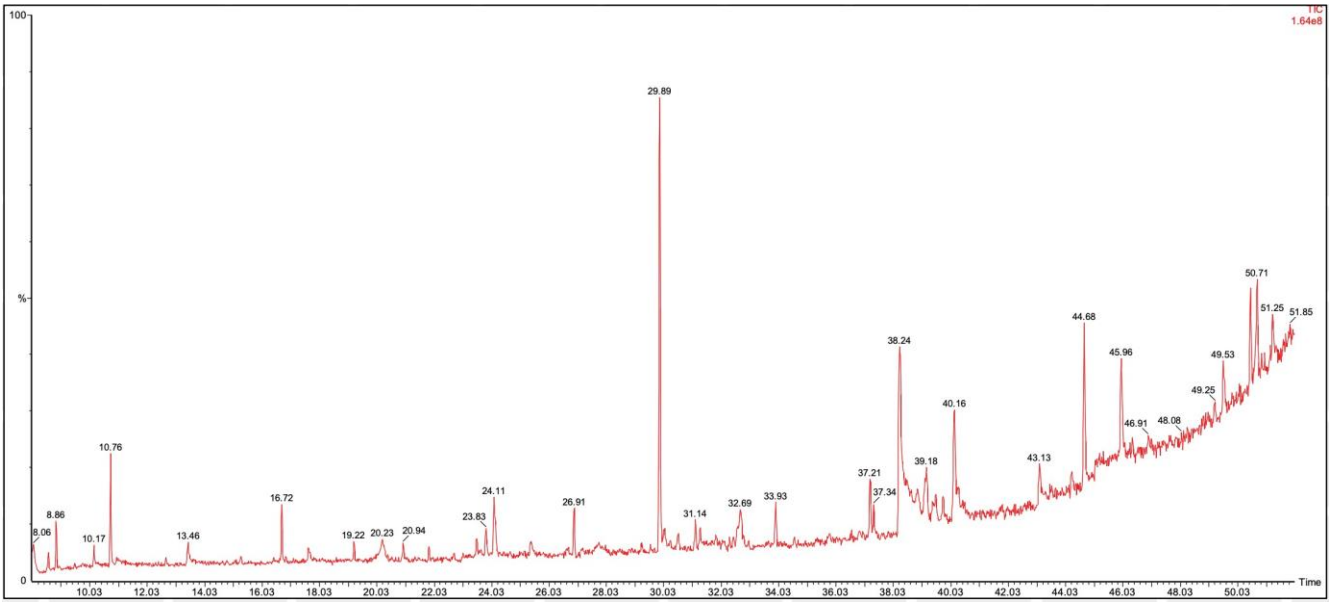

Figure 3. Chromatogram of a milk sample (Source: own elaboration).

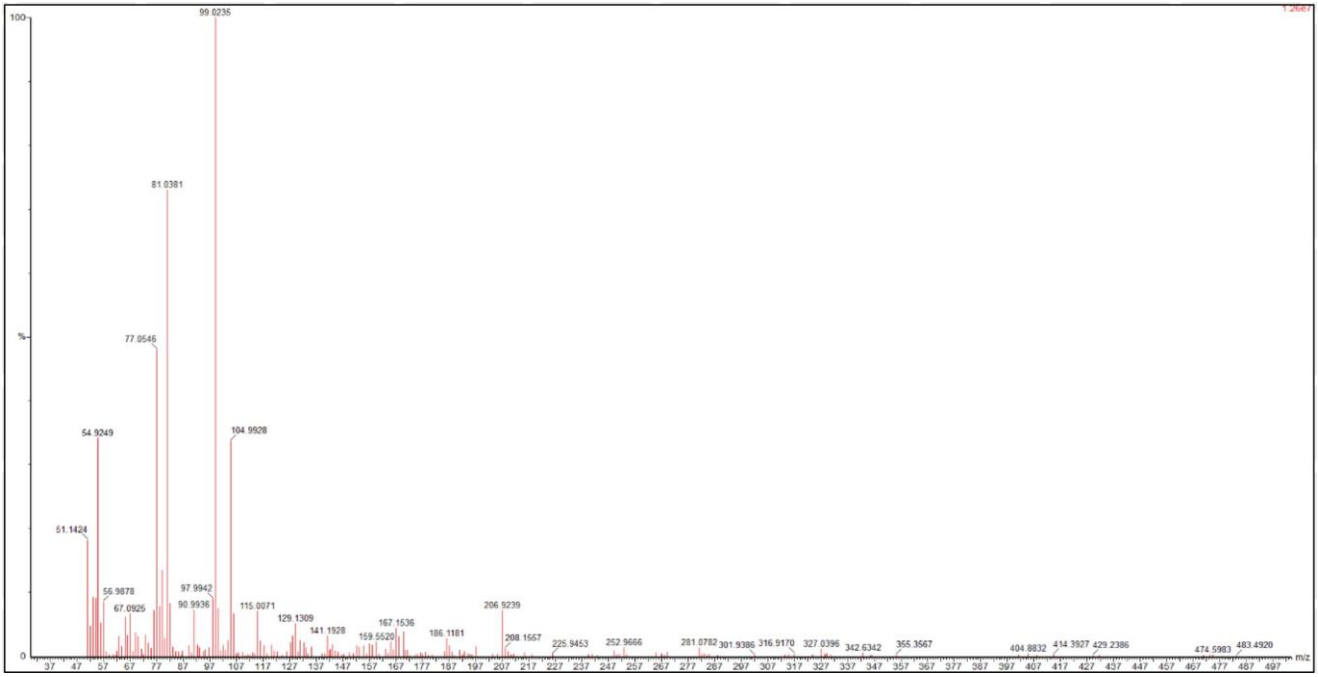

Figure 4. Mass spectrum of a component of a milk sample (Source: own elaboration).

3.4. Confirmation of results

The obtained mass spectra were corroborated with the NIST mass spectral library and the Wiley mass spectral data registry[24,25], confirming the absence of the evaluated pops in the samples.

In addition, the solution of the reference standard mixture of the pops evaluated was analyzed under the same conditions as the samples and showed retention times (see **Figure 5**), mass spectra (see **Figure 6**) and fragmentation ions (see **Table 1**) specific for each compound. These data were compared with the respective chromatograms and mass spectra obtained from the samples and these did not coincide, confirming that none of the pops evaluated were present in the cow's milk samples.

Table 1. Ion fragmentation mass/charge (m/z) values of external standards (Source: own elaboration).

Compound	Molecular ion (m/z)	Primary fragmentation ion (m/z)	Secondary fragmentation ion (m/z)	Tertiary fragmentation ion (m/z)
Lindane	288	181	219	109
Hexachlorocyclohexane	288	183	181	219
2,4,4'-trichlorodiphenyl	256	256	258	186
Heptachlor	370	100	272	274
2,2',3,6-tetrachlorodiphenyl	290	292	220	290
Aldrin	362	66	263	265
Heptachlor-epoxide	386	81	353	355
2,2',4,5',6-pentachlorobiphenyl	324	326	324	328
2,4,4,4',6-tetrachlorobiphenyl	290	292	290	220
Dieldrin	378	79	81	82
2,3,3,3',4,5'-pentachlorobiphenyl	324	326	324	328
2,2',3,4,4,4',5-hexachlorobiphenyl	358	360	362	290
2,3,3,3',4',5-pentachlorobiphenyl	324	326	324	254
2,2',4,4,4',6,6'-hexachlorobiphenyl	360	360	362	145

2,2',3,3',6,6'-hexachlorobiphenyl	360	360	362	358
2,3,4,5,6,2',5'-heptachlorobiphenyl	392	394	396	324
2,2',3,3',4,5,5'-Heptachlorobiphenyl	392	396	394	324

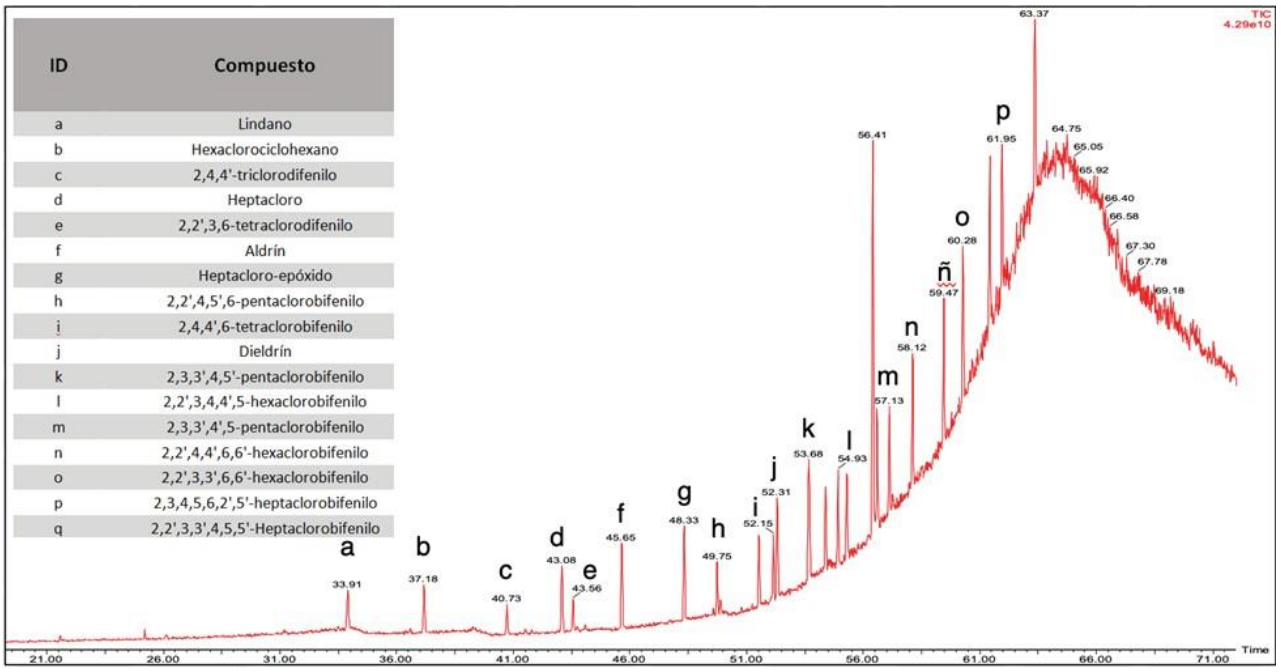

Figure 5. Chromatogram of a mixture of external reference standards of some of the pops evaluated (Source: own elaboration).

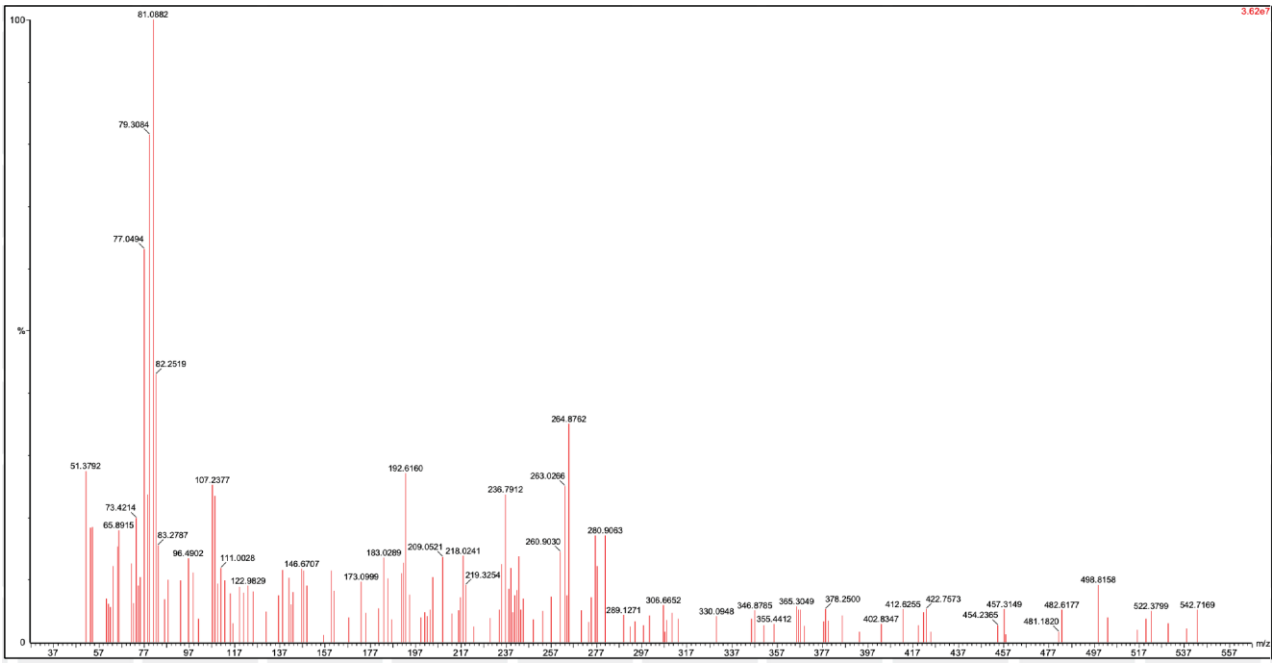

Figure 6. Mass spectra of the external reference standard dieldrin (Source: own elaboration).

4. Conclusions

In this work, it was determined that there is no presence of the pops evaluated in any of the cow's milk samples of the main brands of national production. These results may be due to the reduction in the use and generation of pops in the country, as a result of the national and international legalizations that have been

implemented in order to regulate the application of these compounds.

In addition, unlike other countries in the region such as the United States[3], Brazil[19], Argentina[15], Colombia[13] and Chile[17] cow's milk samples produced in the Dominican Republic do not represent a source of exposure to the pops evaluated for people who consume this product. Similarly, this study plays a very important role in contributing to research on the current levels of pops in the Dominican Republic, as it is the first research study on the determination of pops in cow's milk produced and consumed in the Dominican Republic.

5. Recommendations

Based on the results obtained in this research, it is recommended that studies with similar objectives be carried out to evaluate other substances classified as pops, in order to have a better estimate of the POP content of cow's milk produced in the country.

It is also suggested that the number of samples be expanded to include other brands of milk produced nationally and with different fat content in order to obtain more complete and representative results.

Finally, it is recommended to carry out studies on other foods and to evaluate the presence of the different

Pops, in order to know the real exposure of consumers to pops-contaminated foods produced in the Dominican Republic.

Conflict of interest

The authors declare no conflict of interest.

References

1. Secretariat of the Stockholm Convention. Listing of pops in the Stockholm Convention. Available online: http://chm.pops.int/theconvention/ thepops/allpops/tabid/2509/Default.aspx (accessed on 27 May 2019).
2. World Health Organization (WHO) Persistent organic pollutants (pops). WHO. Available online: https://www.who.int/foodsafety/areas_work/chemical-risks/pops/en (accessed on 2 June 2021).
3. Sbriz L, Aquino MR, Alberto De Rodriguez NM, et al. Levels of chlorinated hydrocarbons and trace metals in bivalves and nearshore sediments from the Dominican Republic. Marine Pollution Bulletin. 1998; 36(12): 971-979. doi: 10.1016/S0025-326X(98)00097-6
4. Lee DH, Lee IK, Song K, et al. A Strong Dose-Response Relation Between Serum Concentrations of Persistent Organic Pollutants and Diabetes. Diabetes Care. 2006; 29(7): 1638-1644. doi: 10.2337/dc06-0543
5. Codex Alimentarius of Food and Agriculture Organization of the United Nations/World Health Organization FAO/WHO. Maximum Residue Limit (MRL). Available online: http://www.fao.org/fao-who-codexalimentarius/codex-texts/dbs/pestres/glossary/en/ (accessed on 26 January 2020).
6. European Commission. EU Pesticides database. Available online: https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?Event=homepage&language=EN (accessed on 26 January 2020).
7. United States Environmental Protection Agency (US EPA). Regulation of Pesticide Residues on Food. Available online: https:// www.epa.gov/pesticide-tolerances (accessed on 2 June 2021).
8. Fernández-Alba AR, García-Reyes JF. Large-scale multi-residue methods for pesticides and their degradation products in food by advanced LC-MS. TrAC Trends in Analytical Chemistry. 2008; 27(11): 973-990. doi: 10.1016/j.trac.2008.09.009
9. Hernández F, Grimalt S, Pozo ÓJ, Sancho JV. Use of ultra-high-pressure liquid chromatography-quadrupole time-offlight MS to discover the presence of pesticide metabolites in food samples. Journal of Separation Science. 2009; 32(13): 2245-2261. doi: 10.1002/jssc. 200900093
10. Lacina O, Urbanova J, Poustka J, Hajslova J. Identification/quantification of multiple pesticide residues in food plants by ultra-high-performance liquid chromatography-time-of-flight mass spectrometry. Journal of Chromatography A. 2010; 1217(5): 648-659. doi: 10.1016/J.CHROMA. 2009.11.098
11. Polgár L, García-Reyes JF, Fodor P, et al. Retrospective screening of relevant pesticide metabolites in food using liquid chromatography high resolution mass spectrometry and accurate-mass databases of parent molecules and diagnostic fragment ions. Journal of Chromatography A. 2012; 1249: 83-91. doi: 10.1016/j.chroma.2012.05.097

12. Chen X, Lin Y, Dang K, Puschner B. Quantification of Polychlorinated Biphenyls and Polybrominated Diphenyl Ethers in Commercial Cows' Milk from California by Gas Chromatography-Triple Quadruple Mass Spectrometry. Plos One. 2017; 12(1): e0170129. doi: 10.1371/journal.pone. 0170129
13. Jawaid S, Talpur FN, Nizamani SM, et al. Multipesticide residue levels in UHT and raw milk samples by GC-μECD after QuEChER extraction method. Environmental Monitoring and Assessment. 2016; 188(4). doi: 10.1007/s10661-016-5222-6
14. Rahman MM, Lee HS, Abd El-Aty AM, et al. Determination of endrin and δ-keto endrin in five food products of animal origin using GC-μECD: A modified quechers approach to traditional detection. Food Chemistry. 2018; 263, 59-66. doi: 10.1016/J.FOODCHEM. 2018.04.099
15. Selvi C, Paramasivam M, Rajathi DS, Chandrasekaran S. Multiresidue Analysis of Organochlorine Pesticides in Milk, Egg and Meat by GC-ECD and Confirmation by GC-MS. Bulletin of Environmental Contamination and Toxicology. 2012; 89(5): 1051-1056.
16. Battu RS, Singh B, Kang BK. Contamination of liquid milk and butter with pesticide residues in the Ludhiana district of Punjab state, India. Ecotoxicology and Environmental Safety. 2004; 59(3): 324-331. doi: 10.1016/j.ecoenv.2003.08.017.
17. Dagnac T, Garcia-Chao M, Pulleiro P, et al. Dispersive solid-phase extraction followed by liquid chromatography–tandem mass spectrometry for the multi-residue analysis of pesticides in raw bovine milk. Journal of Chromatography A. 2009; 1216(18): 3702-3709. doi: 10.1016/j.chroma.2009.02.048
18. Fromberg A, Granby K, Højgård A, et al. Estimation of dietary intake of PCB and organochlorine pesticides for children and adults. Food Chemistry. 2011; 125(4): 1179-1187. doi: 10.1016/J. FOODCHEM.2010.10.025
19. Martins JG, Amaya Chávez A, Waliszewski SM, et al. Extraction and clean-up methods for organochlorine pesticides determination in milk. Chemosphere. 2013; 92(3): 233-246. doi: 10.1016/j.chemosphere. 2013.04.008
20. Rahman MM, Lee HS, Abd El-Aty AM, et al. Determination of endrin and δ-keto endrin in five food products of animal origin using GC-μECD: A modified quechers approach to traditional detection. Food Chemistry. 2018; 263, 59-66. doi: 10.1016/J.FOODCHEM. 2018.04.099
21. Rawash ES, Mohamed G, Souaya E, et al. Distribution and Health Hazards of Polycyclic Aromatic Hydrocarbons in Egyptian Milk and Dairy-Based Products. Beverages. 2018; 4(3): 63. doi: 10.3390/beverages4030063
22. Rodríguez M, De Pratt C, Peña C, Beltré B. Determination of organochlorine pesticides in vegetables: broccoli, lettuce and celery from the Constanza Valley. Available online: https://docplayer.En/67500237-Base-de-datos-de-compuestos-organicos-persistentes-cop-s-en-la-republica-dominicana.html (accessed on 26 January 2020).
23. Deiana P, Fatichenti F. Pesticide residues in milk processing. Italian Journal of Food Science (Italy). 1992; 4(4): 229-245.
24. National Institute of Standards and Technology. NIST Standard Reference Database 1A v17|NIST. Available online: https://www.nist.gov/srd/nist-standard-reference-database-1a-v17 (accessed on 26 January 2020).
25. Wiley. Wiley Registry of Mass Spectral Data, 11th Edition. Available online: https://www.wiley.com/en-us/Wiley+Registry+of+Mass+Spectral+Data%2C+11th+Edition-p-9781119171010 (accessed on 2 June 2021).
26. Pastor Ciscato CH, Gebara AB, de Souza Spinosa H. Pesticide residues in cow milk consumed in são paulo city (BRAZIL). Journal of Environmental Science and Health, Part B. 2002; 37(4): 323-330. doi: 10.1081/pfc-120004473
27. Lorenzatti EA, Maitre MI, Lenardon A. Evaluation of pesticide contamination in dairy products. Revista FAVE Ciencias Veterinarias. 2003; 2(1): 56.
28. Lans-Ceballos E, Lombana Gomez M, Pinedo-Hernández J. Residuos de pesticidas organoclorados en leche pasteurizada distribuida en Montería, Colombia. Revista de Salud Pública. 2018; 20(2): 208-214. doi: 10.15446/rsap.v20n2.51175
29. Muñoz C. Residue Levels of Organochlorine Pesticides in UHT Pasteurized Milk from the IX and X Regions of Chile (Universidad Austral de Chile). Available online: http://cybertesis.uach.cl/tesis/uach/2005/ fam828n/doc/fam828n.pdf (accessed on 26 January 2020).