Persistent Organic Pollutants (POPs) are bioaccumulative toxic chemicals that long-lasting in the environment and accumulate in the food chain during movement. The organochlorine pesticides (OCP) effect on birth defects, neurological, behavioral effects, affect reproductive processes, alter the immunological response, endocrine disruptors and cancer. In this study was measured the content of organochlorine pesticides in 40 samples of milk, baby oil, lactose, whey protein and infant milk formulas produced in the Pegah dairy plants Iran in 2018 to 2019. The determination was done using gas chromatography in accordance with the instructions delineated by the International Dairy Federation. The result showed, the whey protein, lactose, baby oil and infant formula samples that all mean residual values of chlorinated pesticides in all samples were lower than LOD detection limits. The results of infant formula analysis showed that residual concentration of chlorinated pesticides was lower than the maximum residual value recommended by the FAO/WHO.

Keywords: Organochlorine Pesticides, Baby oil, Milk, Whey protein, Infant Milk Formulas, Gas Chromatography.
(PCBs) which have the effects on birth defects, neurological, behavioral effects, contribute to reproduction, serve as endocrine disruptors and change the immunological response [5-7].

The organochlorine pesticides have a substantially persistent nature because of their lipophilic structure and chemical stability, so that they are mainly stored in adipose tissue rich in fat and therefore it is translocated and excreted through fat of milk. This depends on a variety of factors including the amounts of milk produced per day, the amount and type of used pesticides, the species, and length of past exposure [2, 6].

Milk and dairy products play a very important part in the nutrition of elderly and children, including infants. As a fatty food, milk serves as an source of organochlorine pesticides and also an appropriate food to measure persistent OCPs [8].

Pesticide residues present in milk and dairy products are likely to derive from water, air or soil, pesticides used for cattle or their immediate living environment to kill disease vectors, as well as animal feeds that may contain some contamination [9].

The infant formulas are recommended to have adequate nutrients and high quality, and not to contain any OCP that are potentially harmful for them. The Codex Alimentarius seeks to ensure that the amounts of OCP residues present in food products are lower than the suggested tolerance levels so that their risk to human health are as low as possible [10].

Compounds are considered a serious health hazard, especially for infants whose metabolic and enzymatic systems have not yet been fully activated [11, 12]. Although the use of all organochlorine pesticides has been banned in Iran since the late 1990s, due to the low cost, traditional use and non-enforcement of organic organochlorine pesticides such as DDT, lindane, aldrin and dieldrin, the usual form is used in Iran. Given the toxicity of organochlorine pesticides and the role of milk and infant formula, especially in infants nutrition, the current research was done to investigate pesticides levels present in whey protein, milk, infant formula, lactose, and baby oil produced by Pegah Dairy Plants, IRAN.

**Materials and Methods**

**Sample collection**

Infant formulas (0–6 months) were randomly selected from the infant formulas (IMF) produced in the studied plant in 2018-2019. A total of 40 samples of lactose, raw cow’s milk, whey protein, baby oil, infant formula (immediate, whole and skim) samples were randomly collected during 2018 to 2019 were collected weekly. The samples were immediately cooled down and kept at -18°C until analyzed.

**The Gas Chromatograph condition**

The gas chromatograph was a Hewlett Packard® model 6890 Series II (US), with electron capture detector (ECD 63Ni) with model 3396 integrator and capillary column SPB-5 (phenylmethylsilicon at 5%) of 30 m × 0.25 mm DI and 0.25 μm of layer thickness of stationary phase.

The injected volume was 1 μL. of milk, lactose, whey protein, baby oil and infant formula were heated in a water bath and dissolved in 5 ml of petroleum ether. The sample was cooled at room temperature and the extract was washed with 5 ml hexane in a glass column filled 3 g activated Florisil in between two layers of anhydrous sodium sulphate. The detergent was concentrated to about 0.5 ml using nitrogen gas, boiled through the solution and evaporated to dryness. The volume of isoacetan was added to the flask and thoroughly mixed with 100 µL and transferred to vial for gas chromatography. 2 mL of each sample (milk, dry milk, lactose, whey and baby oil) were mixed with 5 mL acetonitrile and shaken for 10 minutes. The mixture was filtered through filter paper. The column was adjusted with 5 ml of methanol. Methanol was removed by 5 ml of SPE
5 mL of acetonitrile. The processed sample was then repeatedly aspirated into the column for 30 s SPE and the SPE column dried for a minute by aspirating ambient air. The analytes were washed with 5 mL ethyl acetate in a SPE vial column. The extract was concentrated by gentle nitrogen vapor to dryness. The residues were diluted in 100 μL of isoacetone and analyzed by GC-ECD. Recovery experiment was conducted by spiking the milk samples with all the pesticide standards taken for analysis at 0.5 and 1.0 mg/kg level to see the efficiency of extraction and analytical procedure. The limit of detection of the chromatographic system was evaluated in 0.001 ng/kg fat basis. The pesticides determined were α-HCH, β-HCH, γ-HCH, d-HCH, o,p′ DDE, p,p′ DDE, p,p′ DDD, o,p′ DDT, p,p′ DDT, 2,4,5,6-Tetrachloro-m-xylene, 2-Phenylphenol, Alachlor, Atrazine, Azinphos-ethyl, Benalaxyl, Chlordane-alpha, gamma and total, Chlorpyrifos, Chlorpyrifos-methyl, Cyhalothrin-lambda, Cyprodinil, Aldrin, heptachlor, α-endosulfan, β-endosulfan, endosulfan sulfate and dicofol.

Statistical analysis

In this study, statistical analysis was conducted using the software Statistical Package for the Social Sciences (SPSS), version 19. ANOVA was used to determine the differences among the means. P value of p<0.05 was considered as statistically significant. All samples were analysed in triplicate.

Result

Table 1 shows the findings regarding mean values and frequencies of pesticides concentrations in the studied milk samples, whey protein, lactose, oil baby and IMF, from October 2018 to September 2019, expressed in ppb (ng/kg) fat basis. The result it does not show a significant differences (<0.05) among the means of pesticides of the GC of the Pegah plant. Analysis of whey protein, lactose, baby oil and infant formula samples from Pegah plant during 2018 to 2019 showed that all mean residual values of chlorinated pesticides in all samples were lower than LOD detection limits (Table 1).

The results of infant formula analysis showed that residual concentration of chlorinated pesticides was lower than the maximum residual value recommended by the FAO/WHO. Out of the four HCH different isomers, α-isomer was detected in none of the 40 samples. None of samples contained these isomers in amounts above the recommended MRL of 0.05 mg/kg.

The isomer of HCH, i.e. cisomer was prescribed MRL of 0.01 mg/kg [10] and any sample was not higher than this concentration. The endosulfan residues, which is an insecticide belonging to the cyclodienes from organochlorines, were also noted as the toxic metabolite endosulfan sulphate and two of its isomers. In terms of total endosulfan, no samples were contaminated, the MRL of endosulfan in milk and dairy product are only 0.004 mg/kg [10], so any samples wasn’t above the MRL.

Total DDT residues comprising of o,p′ and p,p′ isomers of Dichlorodiphenyltrichloroethane, dichlorodiphenyldichloroethane and dichlorodiphenyl dichloroethylene were not detected in milk, whey protein, lactose, baby oil, infant formula. The allowed maximum residue level of 0.05 mg/kg on whole milk basis.

In this time, there was no presence of any pesticides in milk, whey protein, lactose, oil baby and infant milk formulas. The pesticides had lower occurrence and none of the pesticides exceeded the MRI for pesticides allowed via the CA (0.006 - 0.020 μg/kg).

Heptachlor was not observed higher than detection level of 0.002 mg/kg in milk, whey protein, lactose, baby oil and infant formula samples.
| Sample                                | N  | Mean + SD | LOD ng/g | LOQ Ng/g |
|---------------------------------------|----|-----------|----------|----------|
|                                       |    | Whey protein | lactose | Oil baby | milk | Infant |          |          |
| 2,4,5,6-Tetrachloro-m-xylene          | 40 | *         | *        | *        | *    | *      | 14       | 40       |
| 2-Phenylphenol                       | 40 | *         | *        | *        | *    | *      | 14       | 40       |
| Alachlor                              | 40 | *         | *        | *        | *    | *      | 14       | 40       |
| Aldrin-R                              | 40 | *         | *        | *        | *    | *      | 14       | 40       |
| Atrazine                              | 40 | *         | *        | *        | *    | *      | 14       | 40       |
| Azinphos-ethyl                        | 40 | *         | *        | *        | *    | *      | 14       | 40       |
| Benalaxyl                             | 40 | *         | *        | *        | *    | *      | 14       | 40       |
| Chlordane-alpha                       | 40 | *         | *        | *        | *    | *      | 14       | 40       |
| Chlordane-gamma                       | 40 | *         | *        | *        | *    | *      | 14       | 40       |
| Chlordane-Total                       | 40 | *         | *        | *        | *    | *      | 14       | 40       |
| Chlorpyrifos                          | 40 | *         | *        | *        | *    | *      | 14       | 40       |
| Chlorpyrifos-methyl                   | 40 | *         | *        | *        | *    | *      | 14       | 40       |
| Cyhalothrin-lambda                    | 40 | *         | *        | *        | *    | *      | 14       | 40       |
| Cyprodinil                            | 40 | *         | *        | *        | *    | *      | 14       | 40       |
| DDD-O,P                               | 40 | *         | *        | *        | *    | *      | 14       | 40       |
| DDD-P,P                               | 40 | *         | *        | *        | *    | *      | 14       | 40       |
| DDE-O,P                               | 40 | *         | *        | *        | *    | *      | 14       | 40       |
| DDE-P,P                               | 40 | *         | *        | *        | *    | *      | 14       | 40       |
| DDT-Total                             | 40 | *         | *        | *        | *    | *      | 14       | 40       |
| HCH-alpha                             | 40 | *         | *        | *        | *    | *      | 14       | 40       |
| HCH-beta                              | 40 | *         | *        | *        | *    | *      | 14       | 40       |
| HCH-Total                             | 40 | *         | *        | *        | *    | *      | 14       | 40       |
| Heptachlor-epoxide (Cis)              | 40 | *         | *        | *        | *    | *      | 14       | 40       |
| Heptachlor-epoxide (Trans)            | 40 | *         | *        | *        | *    | *      | 14       | 40       |
| Hexaconazole                          | 40 | *         | *        | *        | *    | *      | 14       | 40       |
| Cyhalothrin-lambda I                  | 40 | *         | *        | *        | *    | *      | 14       | 40       |
| Cyhalothrin-lambda II                 | 40 | *         | *        | *        | *    | *      | 14       | 40       |
| Cyhalothrin-lambda-Total              | 40 | *         | *        | *        | *    | *      | 14       | 40       |
| Cypermethrin-alpha I                  | 40 | *         | *        | *        | *    | *      | 14       | 40       |
| Cypermethrin-alpha-Total              | 40 | *         | *        | *        | *    | *      | 14       | 40       |
| Disulfoton                            | 40 | *         | *        | *        | *    | *      | 14       | 40       |
| Endosulfan-alpha                      | 40 | *         | *        | *        | *    | *      | 14       | 40       |
| Endosulfan-beta                       | 40 | *         | *        | *        | *    | *      | 14       | 40       |
| Endosulfan-Total                      | 40 | *         | *        | *        | *    | *      | 14       | 40       |
| Endrin                                | 40 | *         | *        | *        | *    | *      | 14       | 40       |
| Endrin keton                          | 40 | *         | *        | *        | *    | *      | 14       | 40       |

*ND=*
The concentration of 2,4,5,6-Tetrachloro-m-xylene, 2-Phenylphenol, Alachlor, Atrazine, Azinphos-ethyl, Benalaxyl, Chlordane-alpha, gamma and total, Chlorpyrifos, Chlorpyrifos-methyl, Cyhalothrin-lambda, Cyprodinil, aldrin pesticides (OCPs) was not on detection by GC-ECD in raw milk, baby oil, lactose, whey protein and infant formula samples.

**Discussion**

The mean level of organochlorine pesticide in the milk samples, whey protein, baby oil, lactose and infant formula of the study was similar or less than those reported in several other studies expressed in the other researches.

Aytenfsu et al. [5] evaluated the status of organochlorine pesticide (OCP) residues in cow’s milk of different region in Bundelkhand city of India. From the total of 325 samples 206 (63.38%) were contaminated with residues of different OCPs. The average concentration of total HCH was 0.162 mg/kg. Endosulfan was detected in 89 samples with mean concentration of 0.0492 mg/kg while total DDT comprising of DDT, DDE and DDD was present in 114 samples having mean concentration of 0.1724 mg/kg. Dicofol was detected in seventeen samples.

Tolentino et al. [4] evaluated the content of in 21 samples of infant milk formulas marketed in the south of Mexico City (2010). The result showed the majority presence was found 100% for α-HCH, 95.2% for β-HCH, 90.5% for γ-HCH, aldrin (85.7%), heptachlor (80.9%) and heptachlor epoxide (80.9%) with mean amount of 0.24, 0.13, 0.32, 0.62, 0.92 and 0.18 μg/kg of fat, respectively; all samples are below the levels permitted by the CA. With null or lower recurrence and in lower quantities, the family of Dichlorodiphenyltrichloroethane, C₁₂H₈Cl₆O, endrin aldehyde and the endosulphanes done.

Da Silva et al. [13] were investigated organochlorine pesticides in Brazil in feedstuff, water and dairy milk. Total 30 milk samples were analyzed out of which 17% samples were contaminated with OPPS.

Barman et al. [14] the milk samples which seasonally collected, and remaining pesticide were measured by GC with an electron capture detector. The results indicated the milk samples were contaminated with DDT and its metabolites (DDE and DDD). Samples collected during winter season were found to contain higher residue levels as compared to other seasons.

In Brazil, Avancini et al. [11] evaluated the presence of organochlorine substances in 100 samples of cow’s milk in the region of Mato Grosso do Sul. A total of 90% of the samples had some type of organochlorine pesticides. Among the contaminated samples (% in parenthesis) were contaminated with chlordane, aldrin DDT, endosulfan, dicofol [14], heptachlor [11], mirex, and dieldrin [11]. Among these samples, 47% presented levels of chlordane above the maximum allowable limit (2.0 ng g⁻¹). Of the total samples contaminated with aldrin/dieldrin, 14% had levels above the maximum permitted limits (6.0 ng g⁻¹). The samples contaminated with heptachlor, 30% presented levels greater than 6.0 ng g⁻¹.

Lans-Ceballos et al. [12] evaluated the existence of OCPs in samples of pasteurized cow milk in Montería, Colombia. Of the 144 analyzed samples, all had OCP content above the Maximum Residual Limit established by the Codex Alimentarius (FAO/WHO, 2014). Concentration of α-HCH/β-HCH was 0.53 mg/kg, concentration of γ-HCH 0.15 mg/kg, concentration of δ-HCH 0.57 mg/kg, concentration of aldrin/dieldrin 0.40 mg/kg, concentration of heptachlor/heptachlor epoxide 0.22 mg/kg, concentration of eldrin 0.20 mg/kg, concentration of α-chlordane 0.014 mg/kg, concentration of γ-chlordane 0.002 mg/kg and that of endosulfan 0.028 mg/kg.

Sharma et al. [6] analysed one hundred forty seven samples of bovine milk for the presence of OCPs resides. Their result showed 8% of...
samples contained ΣHCH at concentrations above the MRL of 0.10 mg/kg according to the WHO recommendation, 4% samples had α-HCH concentrations of above 0.05 mg/kg, 5% samples had γ-HCH in amounts exceeding 0.01 mg/kg, 26% samples contained β-HCH of higher than 0.02 mg/kg per recommendation of PFAA and 24% samples had ΣDDT of higher than 0.05 mg/kg based on FAO recommendation.

The results showed that organochlorine pesticide residues in milk and infant ingredients (lactose, whey protein, baby oil, lactose) and final product were lower than concentrations of most residual levels. Although these residues were present in the samples in infinitesimal amounts, their concentrations could increase due to accumulation in the body. This study served as a preliminary investigation of the concentrations of OCPs existence in milk and infant formula in the Shahrekord city. These results will contribute to a scientific assessment of the possible residual effects of pesticides on human hazards in Iran.

Conclusion

Organochlorine pesticide residues could not be found in samples because this ingredient is used in small amounts and for a limited number of crops. Therefore, animal feeds did not normally contain organochlorine contamination. Our study showed that because of milk contaminants, whey protein, lactose, baby oil and infant formula with OC residues that no samples were exceeding the tolerance level of allowed by FAO; however, milk dairy products specially infant formula should be constantly monitored for pesticide residues from the viewpoint of safety.

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Conflict of interest

The authors declare no conflict of interest.

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