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Determination of the Residue Levels of Some Commonly Used Organophosphorus Pesticides in Breast Milk

Bazı Sık Kullanılan Organofosfatlı Pestisitlerin Anne Sütünde Kalıntılarının Belirlenmesi

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Abstract
Objectives: There is limited data on levels of organophosphorus pesticides (OPPs) in humans, but no previous reports from Turkey on chemically determined levels of OPP residues in human milk are present. The aim of this study is to determinate and to report the presence of residues of OPPs, which are frequently used in fruit production in the study area (chlorpyrifos, diazinon, ethion, parathion methyl and dichlorvos) in breast milk.
Materials and Methods: In this cross-sectional study, breast milk samples were collected from 100 women living in intense fruit production area (agricultural area, n:50), and resided in provincial center (urban area, n:50), selected by random sampling method. High resolution analyses were performed by a gas chromatography (GC).
Results: Dichlorvos residue was detected in breast milk of 17% of study group. The rate of pesticide residue detection was especially high in breast milk of women with a farmer spouse; always/frequently applying pesticides to the plants, actively working in agriculture pesticide preparation and pesticide application ($p<0.05$).
Conclusion: Pesticide residues were detected in breast milk and this showed the necessity of raising consciousness among women living in the study area and near areas of pesticide application about avoiding exposure to pesticides, especially during pregnancy and breast feeding periods.
Key words: Pesticide residues, breast milk, organophosphorus

Öz
Amaç: İnsanlarda organofosfatlı (OPP) pestisitlerin kalıntı seviyeleri ile ilgili sınırlı veri vardır, Türkiye'de de anne sütündeki kalıntı seviyeleri ile ilgili olarak çalışmalar oldukça azdır. Bu çalışmanın amacı, Isparta bölgesinde meyve üretiminde sık kullanılan OPP'lı pestisitlerin (Klorpyrifos, diazinon, etiyon, paratiyon metil ve diklorvos) anne sütünde var olup olmadıklarının tespit edilmesidir.
Materyal ve Metot: Bu kesitsel çalışmada, yoğun meyve üretimi yapılan alanda (kırsal alan, n=50) ve şehir merkezinde yaşayan (kentsel alan n=50) rastgele örnekleme yöntemi ile seçilen toplam 100 kadından anne sütü numuneleri alındı. Analizler yüksek çözünürlüklü gaz kromatografi cihazında yapıldı.
Bulgular: Çalışma grubunun % 17'sinde diklorvos kalıntıları tespit edildi. Pestisit kalıntılarının tespit düzeyi çiftçi eşlerinde, her zaman/sık pestisit uygulayıcılarında ve pestisitlerin hazırlanmasında aktif rol alanlarda daha yüksekti ($p<0,05$).
Sonuç: Anne sütünde pestisit kalıntıları tespit edilmesi, çalışma alanında ve pestisit uygulamalarının yapıldığı bölgelere yakın alanlarda yaşayan kadınların özellikle hamilelik ve emzirme dönemlerinde maruziyetten kaçınmaları konusunda bilinçlendirilme gerekliliğini göstermiştir.
Anahtar kelimeler: Pestisit kalıntıları, anne sütü, organofosfat

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Introduction

The term "pesticide" is defined as a chemical designed to combat the attacks of various pests and vectors on agricultural crops, domestic animals, and human beings. The definition above implies that pesticides are toxic chemical agents (mainly organic compounds) that are deliberately released into the environment to combat crop pests and disease vectors.[1] Pesticides could harm people in the course of their application and as a result of consumption of foods containing pesticides.[2-4] Agricultural combat should be performed in accordance with recommendations in order to safely transport foods from fields to forks. However, using unlicensed pesticides or using them against the recommended use can result in residue problems primarily, as well as other problems for human and environment health.[5,6]

The pesticides used in agricultural areas, forests and gardens undergo a transformation by permeating into the air, water and soil, and to the organisms living in the surrounding environment. They may reach other non-targeted organisms and plants, and they may cause residues and toxicity in them. Furthermore, their use may cause adverse effects on humans and animals nearby.[7] Children are at a higher risk than adults. Exposure may result in either acute or chronic effects. Acute effects range from irritation, dermatitis, and death, depending on systemic absorption. Approximately 355,000 people worldwide lose their lives due to acute exposure (unintentional intoxication) every year.[8] Consequences of chronic exposure include cancer, birth defects, neurotoxicity, neurobehavioral disorders, neurophysiological changes, and adverse effects on reproduction and fertility.[9]

In the study region (Isparta, Turkey) for this paper, the most important fruit produced is the apple. It was reported that in each of the past ten years, 2 million tons of apples were produced in this area. This amount ranked first in Turkey and accounted for 25% of the total apple production of Turkey. [10] The high production amount in the area indicates a great likelihood of high pesticide use, and indeed, such did appreciably increase during the study period.[11] The study area, including Isparta and its vicinity, has intense agricultural production activity. In particular, the intense apple and cherry production leads to intense pesticide use. However, previous studies in the study area revealed the inadequacy of the information level about pesticide use.[12,13] Therefore, it becomes even more important to determine how the people in the area are affected by pesticides. Pesticides and their residues can be deposited in tissues with high fat content in people who are subject to pesticides. Breast milk for example, is a fluid in which pesticides and their residues can be deposited and be distributed to babies, and it can also serve as a study material for investigating pesticide residue.

Breast milk has an important role in the healthiness and development of babies and children. Breast milk is not only important for its adequate and balanced nutrition, but it is also important for protection and development of the immune system. It is an indispensable and indisputable baby food with its ideal protein, carbohydrate, and fat contents and its immune factors.[14, 15]

The health effects of pesticides in humans can be acute (short-term) and / or chronic (long-term). [16] Acute exposure can result in irritation, dermatitis, and death, depending on systemic absorption. The symptoms are nonspecific and can be confused with diseases such as gastroenteritis, colds. Chronic exposure is more related to occupation

and especially those who work or live in agricultural areas where chemicals are used or stored are at risk.[17,18] Exposure to long-term pesticides pose a risk for the development of cancer and diseases associated with reproductive system, immune system, endocrine and nervous system functions. Children are at higher risk than adults. Chronic effects can be categorized by cancer, birth defects, neurotoxicity, neurobehavioral disorders, neurophysiological changes, fertility effects.[19-20]

Because mothers who are subject to pesticides will cause secretions of breast milk containing pesticides and because babies should be fed only with breast milk in the first six months[15], many new-born babies will be exposed to pesticides.

The aim of this study is to investigate and to report the presence of residues of the organophosphorus pesticide (OPP) production (which is frequently used on fruit, and especially apples, in the study area (chlorpyrifos, diazinon, ethion, parathion methyl and dichlorvos), in breast milk of the breast feeding women in two separate groups living in the provincial centre (urban area) and in an agricultural area (Gelendost district) in Isparta, Turkey.

Materials and Methods

Study population and samples

In this cross sectional study, the study group consisted of 100 women who, at the time of the study, resided in Isparta province, an intense fruit production area located in south-western Turkey. The participants continued to breastfeed their children who were under one year old, and they agreed to participate in the study. This work was supported by the Scientific Research Council of S. D. University (Grant Number: 1566 YL 07). The sample size (n=100) was determined according to the project supporting amount (5000 Turkish Liras). According to the data of Isparta Health Directorate, the number of women with babies under one year old and living in the studied agricultural area was 197 (the agricultural area group population size). Half of the women (n=50) were selected by a random sampling method among the first group of women. The data was collected from women if they continued breastfeeding and if they have been living in the fruit production area (agricultural area, Gelendost) for five years. Because five women were not breastfeeding and two women have not lived in the study area for five years, they were not included in the study. In replacement of them, the first seven women were selected from the waiting list. The other half (n=50) were chosen among the women who have consulted the breastfeeding polyclinic of the university hospital and who have resided in the provincial centre for the past five years. All women in the study group were accepted for the study.

a. Collection method of data

Data was collected in the summer period (June-August 2007), when pesticide application was intense in the area, as we found through a questionnaire form that was designed to investigate socio-demographic properties and exposure to pesticides by a face-to-face interview method. Breast milk samples were collected at the same day of the interviews.

b. Laboratory analysis of samples

A total of 100 milk samples, 50 from breastfeeding mothers living in the agricultural area and 50 from mothers living the urban area, were analysed in a specialized

laboratory designed for analysing pesticide residue. Breast milk samples were collected by finger milking and/or electrical/battery-operated milking pumps. The collected samples were put in sterile milk storage bags and kept at -20° C until laboratory analyses.

Analyses were made with a gas chromatography (GC) (Shimadzu™ GC Models 17A (SPL-17 Injector), Kyoto, Japan) device in a central laboratory of the university (Suleyman Demirel University, Isparta, Turkey) located in the provincial centre. In the collected breast milk samples, OPP residues were investigated.

In the sample preparation, 10g of breast milk was extracted with 50mL chloroform/hexane (2:1), and then the extract was evaporated and the residue was dissolved in 1ml toluene, and then finally given to the system. The milk lipids were extracted following the protocol of the Environmental Protection Agency Protocol.[20] In the study, the injection block was set to 300° C, detection was set to 300° C, and the flow rate was arranged to be 10ml/min. The samples were kept at 80° C in a GC device for one minute and then it was heated to 160° C by a 10° C increase per minute. It was kept at 160° C for 5 minutes and then heated to 240° C by a 3° C increase per minute. The samples were kept at this temperature level and immediately heated to 300° C by a 25° C increase per minute, and they were kept at this temperature for 20 minutes before chemical analyses were performed.

c. Statistical analysis

The study data was evaluated with SPSS 15.0 package software (Statistical Package for Social Science, Inc., Illinois, USA). In the statistical analyses, descriptive statistics, chi-square, and Fisher's Exact Test were used. Variables found related to the presence of residue in univariate analyses were subject to logistic regression analysis. Independent variables were as follows: residing in an agricultural area, having a farmer spouse, always or frequently applying pesticides to plants in the house or the garden, actively working in pesticide preparation/application, staying in a pesticide preparation environment, staying in an environment where pesticides are applied and not wearing or rarely wearing gloves during contact with chemical substances. The dependent variable was residue detection (residue positive and residue negative). The significance level was set to $p<0.05$. The coherence of the model in logistic regression analysis was tested with the Hosmer-Lemeshow test.

The sample size of 50 achieves 89% power to detect an effect size (W) of 0,45 using a 1 degree of freedom Chi-Square Test with a significance level (alpha) of 0.05.

d. Ethics

This study was conducted according to the ethical standards of the Declaration of Helsinki, which promotes respect for all human beings and protects their health and rights. After informing the women about the purpose of the trial (investigation, research, study) and where and how the collected data would be used, their written consents were obtained. In addition, the approval of the Ethical Committee of the Medicine Faculty of Suleyman Demirel University was taken.

In addition, mothers who had residues detected in their breast milk were informed of this finding at the end of the study, and information was given to them about avoiding exposure to pesticides and the rules that should be considered in the use of pesticides.

Results

The descriptive characteristics of the study group are given in Table 1. The mean age of mothers in the study group was 26.5±4.9 years (min:17, max:39). The mean age of the children and breastfeeding times were the same and 5.4±3.0 months (min=1, max=11).

In the study group, dichlorvos residue was detected in the breast milk of 17 (17%) mothers. All of these women resided in the agricultural area (p<0.001). The mean quantity of dichlorvos residue (SD) was 0.10 (0.08) µg/kg (min=0.02, max=0.33, median=0.11). The rate of residue detection in the breast milk of mothers who had farmer spouses was (29.2%), which was higher than the others (13.2%) (p:0.047). The residue detection rate did not demonstrate any difference according to other socio-demographic properties of the mothers in the study group (Table 1).

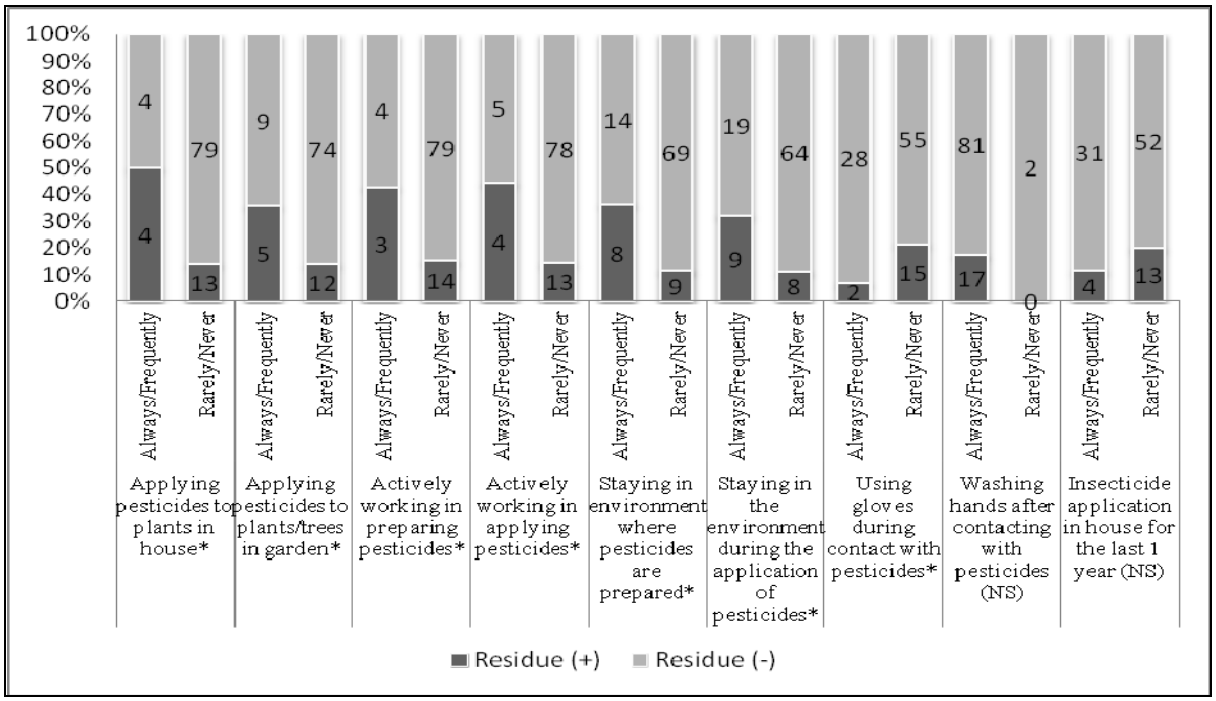
Table 1. Distributions of mothers by detection of pesticides in breast milk and socio-demographic properties

Properties	n	Residue (+)		Residue (-)		p
		n	%	n	%	
Residential area						
Agricultural area	50	17	34.0	33	66.0	
Urban area	50	0	0.0	50	100.0	**<0.001***
Duration of residence						
<10 years	37	6	16.2	31	83.8	
≥10 years	63	11	17.5	52	82.5	0.477*
Age						
17-25	45	9	20.0	36	80.0	
>25	55	8	14.5	47	85.5	0.674*
Education status						
≤8 years	69	10	14.5	59	85.5	
>8 years	31	7	22.6	24	77.4	0.674*
Employment Status						
Employed**	85	16	18.8	69	81.2	
Unemployed	15	1	6.7	14	93.3	0.248*
Profession of spouse						
Farmer	24	7	29.2	17	70.8	
Other	76	10	13.2	66	86.8	**0.047***
Number of children						
1	49	9	18.4	40	81.6	
2	29	6	20.7	23	79.3	
3 and higher	22	2	9.1	20	90.9	0.790
Birth order of breastfeeding children						
First	49	10	20.4	39	79.6	
Second	30	5	16.7	25	83.3	
Third and higher	21	2	9.5	19	90.5	0.915
Total	100	17	17.0	83	83.0	

* Fisher's Exact Test

** 2 of them are nurses, 5 of them are workers, 8 of them are officers.

The distribution of residue detection rates in the breast milk of the study group according to the situations of exposure to pesticides is given in Figure 1. The residue detection rate in breast milk was higher in women who always or frequently applied pesticides to plants in their houses and gardens (50.0% and 35.7%, respectively), who actively worked on pesticide preparation (42.9%), who actively worked in pesticide application (44.4%), who stayed in an environment where pesticides are prepared (36.4%), and who did not use gloves during contact with chemical substances (17.3%) than others (respectively, p:0.003, p:0.013, p:0.015, p:0.007, p:0.002, p:0.003, p:0,072). The residue rates did not demonstrate differences in other situations of exposure to pesticides.



NS: Not Significant, respectively, p:0.687, p:0.373 (Fisher's Exact Test) and * respectively, p:0.003, p:0.013, p:0.015, p:0.007, p:0.002, p:0.003, p:0,072 (Fisher's Exact Test)

Figure 1. Pesticide residues in breast milk by situations where study group could be exposed to pesticides

In the study group, the rate of those with a stillbirth history who also had residue in their breast milk (11.8%) was considerably higher than the rate for those with no residue in their breast milk (2.4%). In the study group, the rate of mothers with a physically handicapped child and residue in their breast milk (5.9%) was higher than it was for those with no residue in their breast milk (1.2%). However, neither of these differences was statistically significant (p=0.133 and p=0.313, respectively). The rate of self-induced miscarriages was similar among mothers in whose breast milk residue was detected and not detected (Table 2).

The results of the logistic regression analysis and the p values are shown in Table 3. In the evaluation of residue detection (dependent variable) in breast milk in the univariate analyses and the relevant variables with the logistic regression analysis, no significant relation was detected between any variable or with the presence of residue in breast milk.

Table 2. Distribution of study group by reproduction properties and residue detection

Properties	n	Residue (+)		Residue (-)		p
		n	%*	n	%*	
Self-miscarriage						
Yes	19	3	17.6	16	19.3	
No	81	14	82.4	67	80.7	0.590**
History of stillbirth						
Yes	4	2	11.8	2	2.4	
No	96	15	88.2	81	97.6	0.133**
History of disabled child birth						
Yes	2	1	5.9	1	1.2	
No	98	16	94.1	82	98.8	0.313**
Total	100	17	100.0	83	100.0	

* Percentage of column ** Fisher's Exact Test

Table 3. Logistic regression analyses on residue detection (residue positive and residue negative; dependent variable) and variables found related to the presence of residue in univariate analyses (independent variables)

Independent Variables	**Exp(B)**	**CI (95%)**	***p***
Residing in agricultural area	0.01	0.00-0.01	0.997
Having farmer spouse	0.78	0.18-3.25	0.727
Always/frequently applying pesticides to plants in house	3.45	0.18-65.37	0.409
Always/frequently applying pesticides to plants in garden	0.52	0.04-7.81	0.642
Actively working in pesticide preparation	1.07	0.08-14.63	0.957
Actively working in pesticide application	1.17	0.12-11.56	0.892
Staying in pesticide preparation environment	1.24	0.19-7.94	0.820
Staying in environment where pesticide is applied	0.79	0.13-4.68	0.796
Not/ rarely wearing gloves during contact with chemical substances	1.12	0.16-7.69	0.911
Constant	0.01		0.997

CI: Confidence interval

Discussion

In the present study, breast milk samples were evaluated for OPPs (chlorpyrifos, diazinon, ethion, parathion methyl ve dichlorvos) levels.

In Turkey, most previous researches have aimed at determining the level of organochlorine pesticides (OCPs) and other organochlorine compounds.[21-31] These studies reported that samples includes OCPs residue were found. On the other hand,

very few papers exist that provide evidence of human exposure by reporting OPP contamination levels in human milk from Turkey.

In the currently presented study, pesticide residues were detected in the breast milk of women living in the agricultural area; however, such residues were not detected in the breast milk of women living in the urban area. It is an expected situation that women in an agricultural area are more exposed to pesticides. This finding is compatible with the information that the negative effects of pesticides on health are related to living in areas near agricultural fields and to the duration, dose, and medium of exposure to pesticides.[32,33]

In this study, the rate of pesticide residue detection was higher in the breast milk of women with farmer spouses, who always or frequently applied pesticides to plants in houses or gardens, who actively worked in pesticide preparation and application, who always or frequently stayed in an environment near pesticide preparation and application sites, and who did not or rarely used gloves during contact with chemical substances. Individuals and their families actively participating in pesticide application are among people who are most exposed to pesticides.[34-36] Together with this information in the relevant literature, these results strengthen the suspicion that women are more exposed to pesticides than are men.

Organic phosphorous compounds are distributed and accumulate in fatty tissue, in the liver and in the kidneys. Phosphorothioate (P=S) are more lipophilic than are phosphates (P=O); therefore, they accumulate more in fatty tissue. As they are stored in fatty tissue, their removal is slow and can take several days in lipophilic ones.[37-39] Only a limited number of studies have been conducted regarding the distribution and accumulation of organic phosphorus. However, some phosphorothioates like chlorpyrifos can remain in the body for weeks and can cause the reappearance of clinical effects after recovery in severe cases. This fact is explained by the mobilization of OPPs stored in adipose tissue.[40] As breast milk is rich in fat content, it is a suitable fluid for the accumulation and removal of pesticides. However, it is the best and an indispensable food for babies during the initial year of life. In the study, dichlorvos was detected in 17 out of 100 samples, and this finding should be investigated and emphasized in terms of mother and baby health in regions of dense agricultural activities, such as the study region.

In an Italian study carried out to determine the contamination level of Italy's most commonly used organophosphates that are in raw milk, residues were detected in 37 out of 135 raw milk samples collected during the spring-summer and fall-winter periods, and 10 of these samples contained between 5 and 18µg/kg organophosphates. The detected organophosphates included dichlorvos. The period when the highest residue level was reported was fall/winter, and the reason the authors attributed to the fact was that plants subject to pesticides in summer periods were given to animals during the winter period.[41] In the present study, data was collected during the summer period when pesticide application is more common. All of the mothers in whom breast milk residue was detected resided in the agricultural area. Therefore, it is possible to claim that women subject to pesticides are mostly close to agricultural activities.

In another study, performed by Salas et al. in Mexico, OPPs were detected in 8 out of 96 pasteurized milk samples collected from the supermarket; dichlorvos was detected

in five of these samples; and phorate, chlorpyrifos and chlorfenvinphos were detected in one sample each. The mean quantity of the detected dichlorvos was 0.0203 ppm. Due to the high toxicity of dichlorvos, the presence of this pesticide residue in milk, the main food of children, is of particular importance, and this issue has been emphasized for the protection of children.[42] In our present study, dichlorvos residue was detected in breast milk samples in quantities ranging between 0.02 and 0.33μg/kg. As it was detected in breast milk, which is the best food source of babies during the initial 6 months of life, its presence is noteworthy regardless of the quantity.

Babies have smaller body masses, and they are exposed to this toxic substance in a period when they are most sensitive and susceptible. In addition, considering the fact that babies are most often given breast milk in this period, the importance of this result increases significantly. In our study, women in whose breast milk residue was detected could have been exposed to pesticides in any period of their life, and it could have accumulated in their fatty tissue. Therefore, women should not only be protected from exposure to pesticides during pregnancy and during the breastfeeding period but also for their entire lives.

In a study carried out in another city (Van) of Turkey, the residual level of dichlorvos, which is less persistent than other pesticides and which has a higher water solubility, was observed to decrease under a tolerance value five days after its application. The duration between the last pesticide application date and the harvest was reported to be three to five days for dichlorvos in vegetables. However, it is reported to become more difficult to provide consumers with safe foods when pesticides exceeding harvest maturity are used for products (e.g. cucumbers) in places like greenhouses that require harvesting to be completed in two to three days and or with a 5 to 14 day waiting period, depending on the product.[43] In the multiple analyses, no significant relation was detected between any of the exposure situations and the residue detection in the breast milk investigated in the study. Therefore, women were possibly exposed to pesticides in other ways in addition to agricultural activities. People living in agricultural areas have the opportunity to consume their own agricultural products, e.g. oranges, apples, cherries, etc. directly from the trees (perhaps even before the completion of the waiting period), and therefore, they could have been exposed to fresh residue on products through the food chain. Therefore, in order to prevent exposures in this way, people living in agricultural areas should be informed about the possibility that they could be exposed to pesticides through the food chain.

Organic phosphorous pesticides are densely present in fields as they can more easily degrade and remain for shorter periods of time in contrast to organic chloric pesticides, which can remain in nature for long periods of time without degradation. OPPs are more easily biodegradable than are OCPs. The main reason for their distribution is their property of not accumulating in nature. OPPs residues were detected in deep water wells and surface waters, indicating that organic phosphorous pesticides can also accumulate in nature, which finding is contrary to popular belief.[44] Pesticides enter the human food chain as plants absorb them or their residues directly from the soil, and these plants are used as human food or as animal feed. It was reported that OPPs enter into the food chain through contamination of meat, fish, poultry products, and milk products.[45] More studies that include different analyses should be performed on products for pesticides that could be exposed to products like

meat and milk. There was no significant relation between any of the media of exposure to pesticides that were investigated in the present study nor to the residue quantities in breast milk. It is possible that people living in agricultural areas could have been exposed to pesticides as they more often consumed the pesticide-contaminated products of animals that were exposed to pesticides. Women living in agricultural areas are subject to other unidentified risks than are women residing in urban areas. Further detailed studies analysing pesticide residues in animal products in these areas based on the diet and diet contents of people in agricultural areas are required for establishing the situation with conclusive evidence.

Among the women in our study, the incidence of breast milk with pesticide residue detected was found to be higher than in the normal population probably because the samples in the study were collected from women living in a region of fruit production and who actively participate in activities such as preparing and applying pesticides.

Limitations

In this study, which was conducted as the thesis for a master of science in the public health field, the reporting period was delayed. However, in the study area (Isparta, Turkey), there is no study on this topic and on the particular material of breast milk. Therefore reporting the results of this study as an article would be beneficial for researchers studying in the field. On the grounds that in the past ten years, fruit production activities have continued as they have in the study area (Isparta, Gönen)[10], the results of this study are valid.

In this study, hence, the women who resided in the provincial centre (urban area) were selected among women who consulted at the breastfeeding polyclinic of the university hospital. Therefore, the results could be different from the general Isparta population and thus are not generalizable.

Conclusions

The fact that pesticide residues were detected in breast milk samples demonstrates that it is possible to be exposed to pesticides during any period of life, even in the initial days of life. Abandoning breast milk for baby nutrition cannot be considered an option, except in cases of certain indications. Therefore, breast milk should be prevented from being contaminated with toxic substances. Considering the fact that active contact with pesticides increases this risk, all people, but especially women, should avoid contact with pesticides, not only during pregnancy and breastfeeding, but in every period of life. If this is not possible, then protective measures should be taken during contact. People can become exposed to pesticides both during their application and through the food chain, and detailed studies should be conducted on the food-chain pesticide exposure of people living in agricultural areas. People who apply agricultural pesticides and who can be directly or indirectly exposed to agricultural pesticides should be given detailed training.

According to the results and limitations of this study, new studies on excessive pesticide use in the study (agricultural) area with biological monitoring and longitudinal study should be conducted.

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