Journal of Environmental Science and Health, Part B (2015) **50**, 1–7

ISSN: 0360-1234 (Print); 1532-4109 (Online)
DOI: 10.1080/03601234.2015.964128

Comparison of DDT and its metabolites concentrations in cow milk from agricultural and industrial areas

JAROSŁAW KUBA[1], AGNIESZKA TOMZA-MARCINIAK[1], BOGUMIŁA PILARCZYK[1], NATALIA TARASEWICZ[1], RENATA PILARCZYK[2] and MAREK LIGOCKI[3]

[1]*Department of Animal Reproduction Biotechnology and Environmental Hygiene, West Pomeranian University of Technology in Szczecin, Szczecin, Poland*
[2]*Department of Ruminant Science, West Pomeranian University of Technology in Szczecin, Szczecin, Poland*
[3]*Department of Poultry and Ornamental Bird Breeding, West Pomeranian University of Technology in Szczecin, Szczecin, Poland*

The risk of pesticidal intoxication in humans is severe, especially because of the strongly negative impact on human health. The consequences of the exposure to these substances may include cancerogenesis or endocrine abnormalities resulting for example in decreased fertility. Therefore, the aim of our study was to evaluate the content of dichlorodiphenyltrichloroethane (DDT) and its metabolites in cow milk from two regions of Poland, varying by level of industrialization. Samples were collected from agricultural ($n = 25$) and industrial ($n = 25$) areas, and the concentrations of DDT and its metabolites were evaluated by gas chromatography. Residues of DDT were detected in all the milk samples tested, mostly in the samples from the agricultural area, where a total DDT median concentration reached 0.336 μg L^{-1}. In the milk samples from the industrial area, the median concentration was lower, at 0.131 μg L^{-1}. 4,4′-DDT was the main metabolite, constituting 83% of total DDT metabolites. Although none of the samples exceeded the level above which they should be considered dangerous, the results showed that the problem of DDT had not diminished and so should be constantly monitored.

Keywords: DDT, pesticides, organochlorines, cow milk.

Introduction

The necessity of increasing plant growing efficiency over the last decades has resulted in numerous studies on more effective insecticides. Pesticides in which the active substances paralyze the nervous system of an insect have gained considerable popularity. An example of those is dichlorodiphenyltrichloroethane (DDT) which was and is still used in some countries to combat malaria, typhus and biting flies.[1] It is a white tasteless and almost odorless crystal-structured powder, whose insecticidal activity was discovered after World War II.[2] The need to reconstruct industries, economies and populations in many countries, depleted due to the global conflict, had resulted in a huge demand for pesticides based on DDT. It acted twofold: as a substance directly increasing crops, and as a tool to control diseases spread by insects, especially in the subtropics.

Over the years, however, studies on pesticides have shown that the active substances underpinning them are not species selective and show harmful effect on humans and other animals.[3,4] DDT has an estrogenic activity, affecting the functioning of the reproductive system, and the resulting imbalances in hormonal stability may have a mutagenic or cancerogenic effect, for example, increasing the rate of breast cancer in women chronically exposed to DDT.[5–7] DDT is known to cause disruptions in the antioxidant system and abnormalities in the central nervous system.[8] There is also a relationship between exposure to DDT and the frequency of general development disorders (regarding autism) in children.[9,10]

Given the aforementioned facts, many countries introduced a ban on using pesticides containing DDT or its derivatives; for example, in Poland the usage of DDT was prohibited in 1975. However, DDT is still widely administered in many regions of the world, with the consequences noticeable on a global scale.[11,12] For example, African countries still use DDT as an insecticide to prevent the breeding of insects carrying malaria.[13] As a result, large amounts of DDT have accumulated in tropical and subtropical levels, from where they can move on an intercontinental scale and reach Europe by processes of evaporation and air circulation in the atmosphere.[14] Therefore, the

Address correspondence to Jarosław Kuba, Department of Animal Reproduction Biotechnology and Environmental Hygiene, West Pomeranian University of Technology in Szczecin, Judyma 6, 71-466 Szczecin, Poland; Email: jarekkuba@gmail.com
Received May 23, 2014.

percentage of people endangered by the harmful activity of DDT is a global problem, and is not related only to permission or prohibition of its usage in a selected country. Despite the withdrawal of DDT itself, it is still one of the intermediate substrates in the production process of dicofol, which is based on DDT and was used worldwide, including in numerous European countries.[15]

The main source of DDT in humans is food of animal origin, which contains large amounts of fat. DDT obtained with food accumulates in fat tissues, and in favorable circumstances is released into the bloodstream. Exposure to this substance starts in fetal life and continues throughout the whole life.[16] One method to control DDT is to analyze its content in food products. Milk, as a widely available and eagerly consumed food product with a large fat content may provide valuable information about the threat of DDT to humans.[17] Milk is consumed by adults, youths and infants and as such allows monitoring of all age groups within the population for organochlorine pesticides (OCPs), including DDT. Therefore, the aim of our study was to evaluate the content of DDT and its metabolites in cow milk from two regions of Poland, with different levels of industrialization.

Materials and methods

Material

Samples of raw milk collected from cows in two areas with different levels of industrialization were analyzed. 25 samples were collected from Polish Holstein–Friesian Red–White variety cows from a farm located in Śląskie voivodeship, which is a strongly industrialized region. The cows were aged from 2 to 10 years. The herd was fed under a conventional system based on farm feed. In summer, feeding was based on pasturing. Samples were collected during the summer grazing period. In addition, 25 samples were collected from the northern part of Zachodniopomorskie voivodeship, a typically agricultural area. Milk was collected from Montbeliarde cows aged from 2 to 9 years, housed in a free-stall cowshed. Nutrition was based on a TMR (Total Mix Ration) system. During the day the animals stayed out on the pasture.

Milk was collected in 100 mL polypropylene containers. Until the laboratory procedures, samples were stored at –20°C. Data concerning fat content in particular samples were obtained from the farmers responsible for either herd.

Chemicals

Reagents used in the analysis were: *n*-hexane, acetone, sulfuric acid from Merck (KGaA, Darmstadt, Germany), anhydrous sodium sulfate, sodium chloride from Chempur (Piekary Śląskie, Poland). Reagents such as *n*-hexane and acetone were of HPLC grade. Sulfuric acid (95–97%) and anhydrous sodium sulphate were *pro analysi* grade. Internal standard Pesticide Surrogate Spike Mix (decachlorobiphenyl and 2,4,5,6-tetrachloro-m-xylene in acetone) was obtained from Sigma-Aldrich (St. Louis, MO, USA). The standards for gas chromatography were obtained from AccuStandard Inc. (New Haven, CT, USA).

Chemical analysis

The samples were prepared according to a modified procedure of Skrajnowska.[12] Milk samples (10 mL each) were poured into conical flasks with glass wool. Then 20 mL of acetone was added and the mixture was trickled into a separating funnel. The flask and the wool were washed twice with acetone (2×5 mL) and twice with *n*-hexane (2×10 mL). Then 60 mL of distilled water and 3 mL of saturated sodium chloride solution were placed into the funnel. This mixture was shaken for 5 min after which the water layer was removed while the organic layer was transferred into a new flask. This extract was concentrated in a rotary vacuum evaporator to a volume of 2 mL and then transferred to stoppered glass tubes.

The extract was purified with 8 mL of concentrated sulfuric acid. After separation of the layers, the *n*-hexane layer was trickled through a glass column with a filter and anhydrous sodium sulfate into the tubes. Eluted samples were concentrated on a water bath under vacuum using a rotary evaporator to 0.5 mL. Later the samples were separated using capillary gas chromatography with mass spectrometry GC/MS. Analyzed chemicals were separated on an ELITE-5MS column (60 m $\times$ 0.25 mm $\times$ 0.5 μm). Chromatographic analysis was performed under the following conditions: column oven programme: 140°C (1 min), increased by 10°C min^{-1} to 200°C (5 min), by 10°C min^{-1} to 280°C (5 min) and by 10°C min^{-1} to 300°C (15 min). The flow rate (splitless) was 1 cm^3 min^{-1}, injection volume was 2.5 μL.

Standard solutions were prepared at six different concentrations: blank, 0.24, 0.48, 2.4, 24 and 240 ng μL^{-1} in n-hexane. The accuracy of analyses was verified through the addition of known amounts of internal standard solution, Pesticide Surrogate Spike Mix, containing decachlorobiphenyl (50 μL of concentration 80 μg L^{-1}). The recoveries gained were in the range of 80%–91%. The limit of detection (S/Ns = 3) was 0.01 ng ml^{-1}. The quality of the analytical procedure was checked by analysis of blank samples. No cross-contamination was found.

Statistical analysis of the data

Statistical analysis of the data was performed using Statistica software (Statsoft Inc., ver. 10 Statsoft, Tulsa, OK, USA). Prior to analyses, data were investigated to determine their distribution using Shapiro–Wilk W tests. The data was not normally distributed. Differences in DDT and its metabolites concentrations in milk between industrial and agriculture areas and between conventional and TMR (Total Mix Ration) feeding systems were compared by Mann–Whitney U test. Differences were considered significant at $P < 0.05$.

Results and discussion

Results concerning DDT and metabolite concentrations in milk samples from agricultural and industrial areas are shown in Table 1. For comparison of all the results, median values were chosen as they illustrate the most frequent values, excluding the effect of maxima and minima.

In accordance with European legislation, the maximum residue level (MRL) of DDT (sum of p,p′-DDT, o,p′-DDT, p-p′-DDE and p,p′-DDD) in milk is 40 μg kg^{-1}.[18] In this research the determined levels of this pesticide were much lower. The highest median concentration of total DDT of 0.336 μg L^{-1} was observed from the agricultural area. In samples from this region, 4,4′-DDE was the dominant metabolite representing 83% of total DDT. On the other hand the lowest median concentrations were observed in 2,4′-DDT and 4,4′-DDT, corresponding to only 2% each. The percentage of individual DDT metabolites in this area is shown in Figure 1. In mammalian bodies the biotransformation of DDT occurs in two ways. One is based on dehydrochlorination and leads directly to the formation of DDE, and the other involves the reductive dechlorination and leads to the formation of DDD. This compound is further metabolized, and the end product of these transformations is the DDA. While DDT, DDE and DDD accumulate in the adipose tissue, DDA is excreted in the urine.[19]

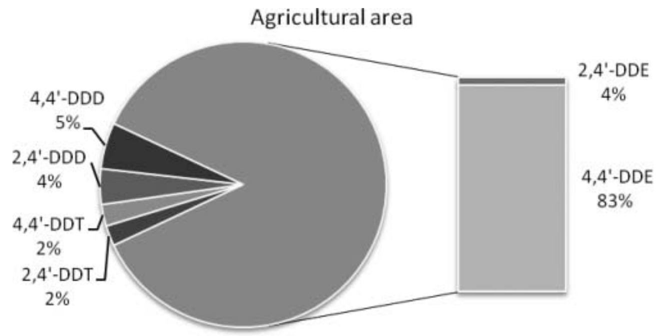

Fig. 1. Percentage of individual DDT metabolites (%) in the sum of DDTs in milk samples from the agricultural area.

In the industrial area total DDT median concentration was lower than in the agricultural area (0.131 vs. 0.336 μg L^{-1}). The highest median concentration in this region were similar to the agricultural area: 4,4′-DDE was also the dominant metabolite containing 83% of the sum of DDT, but a relatively elevated percentage of 4,4′-DDT in this region of 8% is noticeable. Possibly some chemical synthesis processes contribute to an unintentional formation of 4,4′-DDT. Several chemical facilities located in the southern regions of Poland have only in recent years improved technological lines to avoid or to utilize such harmful by-products, but they still produce substances chemically similar to DDT.[20] Many of those are not metabolized to DDE and therefore a DDT-like substance may still be present in the environment, while its transformation process is not expressed by an additional increase in DDE.[10] This partially explains the level of DDT observed in the industrial area, also given the fact that the use of dicofol was allowed in Poland until January 1,

Table 1. Concentration of DDT and its metabolites in milk samples with 4% fat content (μg/L).

	2,4′-DDT	*4,4′-DDT*	*2, 4′-DDD*	*4,4′-DDD*	*2,4′-DDE*	*4,4′-DDE*	*Σ DDT*
			Agricultural area ($n = 25$)				
Mean	0.020	0.008	0.016	0.020	0.019	0.304	0.387
Median	0.007	0.008	0.013	0.017	0.011	0.265	0.336
Sd	0.031	0.004	0.011	0.017	0.020	0.203	0.250
Min	0.002	0.003	>LoD	0.004	>LoD	0.054	0.074
Max	0.108	0.019	0.053	0.075	0.057	0.879	1.140
			Industrial area ($n = 25$)				
Mean	0.023	0.099	0.008	0.010	0.009	0.170	0.319
Median	0.001	0.009	0.002	0.006	0.001	0.097	0.131
Sd	0.059	0.374	0.014	0.016	0.017	0.183	0.444
Min	>LoD	>LoD	>LoD	>LoD	>LoD	0.009	0.014
Max	0.228	1.848	0.061	0.079	0.063	0.649	1.886
			Total milk samples ($n = 50$)				
Mean	0.022	0.053	0.012	0.015	0.014	0.238	0.354
Median	0.004	0.008	0.010	0.011	0.004	0.200	0.249
Sd	0.047	0.263	0.013	0.017	0.019	0.203	0.356
Min	>LoD	>LoD	>LoD	>LoD	>LoD	0,009	0.014
Max	0.228	1.848	0.061	0.079	0.064	0.879	1.886

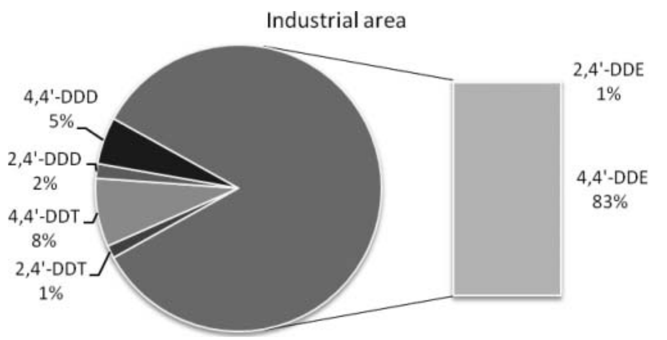

Fig. 2. Percentage of individual DDT metabolites (%) in the sum of DDTs in milk samples from the industrial area.

Table 2. Results of Mann–Whitney's U test analysis.

	P *value*	
	Agricultural vs. industrial area	*Conventional feeding vs. TMR system*
2,4′-DDT	0.02090	0.17711
4,4′-DDT	0.23013	0.65908
2,4′-DDD	0.00008	0.83624
4,4′-DDD	0.00126	0.65121
2,4′-DDE	0.00350	0.80550
4,4′-DDE	0.01331	0.70994
Σ DDT	0.01389	0.55180

Significant differences at $P < 0.05$.

2014[21] and milk samples were collected during 2013. On the other hand the lowest median concentrations were observed in 2,4′-DDT and 2,4′-DDE. This may be related to the fact that the 2,4′- isomer of DDT is less frequent and comprises 15%–21% while the abundant and much more persistent 4,4′–isomer comprises 65%–80% of total DDT.[22] The percentages of individual DDT metabolites in the industrial area are shown in Figure 2.

The median values of concentrations observed in both areas were estimated with Mann–Whitney U-test to (a) evaluate whether the regions vary in content of DDT bioaccumulation and (b) define if there is a difference in DDT intake according to the cattle feeding system, because in the agricultural area a TMR system was used while in the industrial region a conventional feeding system was used. In the first comparison the differences proved to be significant ($P < 0.05$) for all DDT and its metabolites excluding 4,4′-DDT, which means that in both regions the amounts of this substance were comparable (Table 2). Significantly higher levels of DDTs in the agricultural area can be explained by the greater number of roads and the pesticide input into the soil. In the agricultural area, soil contamination may have been due to the use of the pesticide directly onto the soil, spraying of the aerial parts of plants, as well as the introduction of DDT-treated grain into the soil. In the industrial areas the main sources of DDT are atmospheric deposition or chemical plants producing pesticides similar in structure to DDT.[19]

In the second comparison, however, no significant differences were observed between the two systems of cattle feeding, suggesting that neither type of cattle farming especially exposes animals to DDT intake. In the milk of both herds the concentration of DDT was much lower than the highest permissible level in milk and in the conventional and TMR feeding systems the observed DDT concentrations were 0.33% and 0.84% of MRL, respectively. The results of U tests in the first comparison suggest that the areas were unequally exposed to DDT bioaccumulation. A higher content of DDT was observed in the agricultural area, more exposed to DDT in the past which resulted in higher bioaccumulation of the described chemicals. In the industrial area, usage of DDT pesticides was minimal, so the chemical industry is the only possible source of environmental exposure to this substance. In every exposed region DDT undergoes metabolizing processes which include a transformation into DDD and then into DDE, the final metabolite. In this study samples collected in the agricultural area represent the highest levels of DDT metabolites (2,4′-DDD and 4,4′-DDD as well as 2,4′-DDE and 4,4′-DDE). Apart from a slightly elevated level of 4,4′-DDT a similar tendency in both isomers of DDD and DDE in the industrial area was noticeable. Thus, the highest median concentrations were noticed in 4,4′-DDE in both agricultural and industrial areas, suggesting that DDT had already been partially metabolized. It also indicates that in both areas a distant exposure to the studied substances was present.

Since the environmental presence of DDE results solely from the conversions of DDT, the ratio of DDE do DDT is used to determined the potential "fresh input" of DDT. A ratio higher than 1 generally indicates the past use of DDT, and lower than 1 suggests recent usage of DDT and fresh input.[23] To confirm the temporal range between DDT exposure and sample examination in both regions, a DDT:DDE ratio was calculated. In our study the ratio was 1:20 (0.05) in the agricultural area and about 1:7.1 (0.14) in the industrial area, which supports the hypothesis about both recent and historic usage in the studied regions. The total in milk samples reached 1:13.6 (0.07), which is shown in Figure 3. The estimated metabolizing time from DDT to DDE in the environment could take from 4 to 30 years, depending for example on soil type and other environmental factors.[24,25]

The most important factors influencing the metabolism of DDT in the soil include the physicochemical properties of the soil, its moisture and pH, as well as the climatic conditions (temperature), sunlight and microfauna soil composition. These factors determine the type and direction of conversions of a given substance; accordingly, under different environmental conditions, decomposition of a given substance may take place in different ways. In the case of DDT it was found that anaerobic conditions accelerated

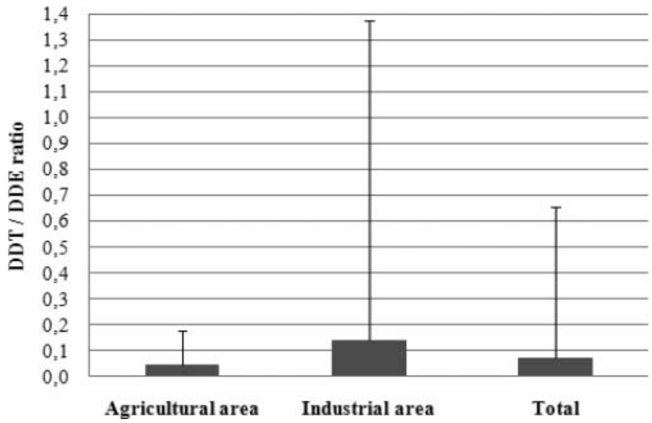

Fig. 3. The DDT/DDE ratios determined in milk samples from agricultural and industrial areas and in the total tested samples of milk.

the conversion of DDT to DDD, while aerobic conditions facilitated the conversion of DDT to DDE.[26,27] The basic reactions in the biodegradation of DDT and other organochlorine compounds include the removal of the chlorine atom from the molecule via hydrolysis, reductive dehalogenation and dehydrohalogenation.[28]

DDT's route starts in the soil and plants, and because of its physical features and long-lasting metabolizing process it may end up in cow milk.[20,29] DDT and its metabolites are strongly lipophylic and show an affinity to the fat tissue of animals and humans.[30] Fat-rich cow milk is a cheap and rich source of proteins, fats, minerals such as calcium, phosphorus or potassium and vitamins: A, B_2, B_3 and B_{12}.[31] Milk consumption is quite common in Poland and in general reaches 2.6 L per person per month. Milk consumption in agricultural areas is higher than average and according to statistical data reaches 4.0 L per person per month in farm households.[32] Therefore, the amount of DDT consumed with milk may be significant and requires consideration. The Polish Central Statistical Office does not specify milk consumption data in industrial areas, but assuming that it does not differ from the average, an average person's daily intake of DDT with milk is about 0.011 μg kg^{-1} body weight (b.w.). In the agricultural area this value was higher and reached 0.045 μg kg^{-1} b.w. Acceptable DDT daily intake according to FAO and WHO[33] is 10 μg kg^{-1} b.w. but these standards refer to a complete food ration which includes different food products like meat, bread, vegetables etc. Therefore it can be concluded that milk intake does not constitute a significant part of total DDT daily intake in either of the examined regions.

According to former studies on milk carried out in Poland in the 1970s which showed a concentration of 8.3 μg L^{-1} and in the 1990s of 1.4 μg L^{-1},[34] a decreasing trend is obvious. A decreasing trend in DDT is observed not only in the milk itself but also in other dairy products, such as cheese, butter and cream.[35–37] Studies performed in Spain in the 1990s showed a concentration of 10.9 μg L^{-1},[17] which was more than 7.5 times higher than in Poland in that time. Nevertheless concentrations observed in milk in Europe seem to be relatively low in relation to other continents where concentrations of DDT are much higher, and may be associated with some countries fighting malaria. Studies in India show a decreasing tendency since 1998 when a concentration of 90 μg L^{-1} was reported.[38] In 2007 it was lower at 36.7 μg L^{-1}[39] and then 0.7–30.7 μg L^{-1} in 2014.[40] On the other hand the situation in Latin America looks different. For example, in Mexico levels of DDT have remained stable, ranging from 139 to 159 μg L^{-1}.[41–43] Moreover, recent studies on DDT in milk in Columbia showed surprising levels from 290 to 810 μg L^{-1}.[44] However, according to a FAO report, the biggest burden of DDT was observed in the 1980s in Africa, reaching a level measured in tens of milligrams per liter of milk.[45] Studies from Ghana in 2008 showed a concentration of 13.95 μg L^{-1}.[13] As Ghana is located in a similar region to some countries with reported extreme values of DDT, it can be expected that the tendency may be downward in the future. However, these results clearly show that problem of DDT in the environment is severe especially that, by consuming dairy products, people are currently and will probably be in the future constantly exposed to organochlorine contaminants.[14,41,46] Most OCPs are relatively resistant to the technological processes that are used for milk and dairy products.[47]

In spite of the quite low levels of DDT shown in our study, accumulation in human fat tissue cannot be ignored. Despite the fact that over several years, there has been a marked decline in DDT and its metabolites in the environment and consequently in our food, it is expected that exposure to these compounds will last for many years due to the high stability of DDT and its continuous use in countries where malaria is found.

Maternal milk is considered the last element of the food chain; therefore, infants are exposed to the highest concentrations of OCPs.[48] Women's milk contains several times higher levels of DDT in comparison to animal milk.[34] The highest amounts of hazardous compounds are observed in the first stage of lactation.[49] Infants and children belong to a special group at risk by consuming much greater quantities of milk than adults.[50] Importantly, some of the effects may not be detected until puberty or even later in life.[51] Exposure to p,p´-DDT early in life may increase breast cancer risk in adult life.[6] There are also reports on exposure to DDTs contributing to an increased risk of developing hypothyroidism and other disorders of the secretory activity of the thyroid.[52] In addition, maternal serum DDE is associated with reduced psychomotor development in 6-month-old infants, and mental development in two year olds.[53] In another study it was also found that an elevated maternal serum DDE concentration is associated with an increased risk of being overweight in children at 14 months of age.[54]

Conclusion

To conclude, in this study we detected residues of DDT in all tested milk samples. None of them exceeded a level above which the concentrations should be considered as dangerous. On the other hand, the results show that the problem of DDT has not ceased and therefore the presence of this pesticide in milk remains a potential health risk and should be monitored regularly.

Acknowledgment

The authors would like to thank Klaudia Zielińska, M.Sc., for her technical help in sample collections.

References

[1] Bouwman, H.; van den Berg, H.; Kylin, H. DDT and malaria prevention: addressing the paradox. Environ. Health Persp. **2011**, *119*(6), 744–747.
[2] Agency for the Toxic Substances and Disease Registry. Toxicological profile for DDT, DDE and DDD; Federal Register on November 17, 1997 (62 FR61332). Available at http://www.atsdr.cdc.gov/ToxProfiles/tp.asp?id=81&tid=20 (accessed Sep 2002).
[3] Yoon, K.; Kwack, S.J.; Kim, H.S.; Lee, B.M. Estrogenic endocrine-disrupting chemicals: Molecular mechanisms of actions on putative human diseases. J. Toxicol. Env. Heal. B. **2014**, *17*(3), 127–174.
[4] Savitz, D.A.; Klebanoff, M.A.; Wellenius, G.A.; Jensen, E.T.; Longnecker, M.P. Persistent organochlorines and hypertensive disorders of pregnancy. Environ. Res. **2014**, *132*, 1–5.
[5] Li, F.; Wang, X.; Zhou, S.; Liu, C. Reviews on abiotic transformation of organochlorines on the interface of iron oxides and water in red soil colloids. Ecol. Environ. **2006**, *15*, 1343–1351.
[6] Cohn, B.A.; Wolff, M.S.; Cirillo, P.; Sholtz, R.I. DDT and breast cancer in young women: New data on the significance of age at exposure. Environ. Health Perspect. **2007**, *115*(10), 1406–1414.
[7] Soto, A.M.; Sonnenschein, C. Environmental causes of cancer: Endocrine disruptors as carcinogens. Nat. Rev. Endocrinol. **2010**, *6*(7), 363–370.
[8] Castilla-Pinedo, Y.; Alvis, E.L.; Alvis-Guzmán, N. Estimating exposure to organochlorine compounds from consuming pasteurised milk in Cartagena, Colombia. Rev. Salud. Publica **2010**, *12*(1), 14–26.
[9] Guillette, E.A.; Meza, M.M.; Aquilar, M.G.; Soto, A.D.; Garcia, I.E. An anthropological approach to the evaluation of preschool children exposed to pesticides in Mexico. Environ. Health Perspect. **1998**, *106*(6), 347–353.
[10] Roberts, E.M.; English, P.B.; Grether, J.K.; Windham, G.C.; Somberg, L.; Wolff, C. Maternal residence near agricultural pesticide applications and autism spectrum disorders among children in the California Central Valley. Environ. Health Perspect. **2007**, *115*(10), 1482–1489.
[11] Żurek, J.; Sadowski, M. Krajowy program wdrażania Konwencji Sztokholmskiej, GF/POL/01/004; Ministerstwo Środowiska RP: Warszawa, 2004; 47–56.
[12] Skrajnowska, D.; Strzelczyk, E.; Kotlińska, A.; Tokarz, A. Contamination with DDT and metabolites in breast milk. Bromat. Chem. Toksykol. **2009**, *42*(3), 532–536.
[13] Darko, G.; Acquaah, S.O. Levels of organochlorine pesticides residues in dairy products in Kumasi, Ghana. Chemosphere **2008**, *71*(2), 294–298.
[14] Gutiérez, J.; Ruiz, J.L.; Ortiz, R.; Vega, S.; Schettino, B.; Yamazaki, A.; de Lourdes Ramirez, M. Organochlorine pesticide residues in bovine milk from organic farms in Chiapas, Mexico. Bull. Environ. Contam. Toxicol. **2012**, *89*(4), 882–887.
[15] Skotak, K. Substancje kandydujące do protokołu w sprawie trwałych zanieczyszczeń organicznych – aspekty zdrowotne. In *Konferencja Konwencja w Sprawie Transgranicznego Zanieczyszczenia Powietrza na Dalekie Odległości*; Szczotko, M., Eds.; National Research Institute: Warsaw, 2011; 1–13.
[16] Vall, O.; Gomez-Culebras, M.; Puig, C.; Rodriguez-Carrasco, E.; Arelis Gomez, B.; Canchucaja, L.; Joya, X.; Garcia-Algar, O. Prenatal and postnatal exposure to DDT by breast milk analysis in Canary Islands. PLoS ONE **2014**, *9*(1), 1–7.
[17] Losada, A.; Fernández, N.; Diez, M.J.; Terán, M.T.; García, J.J.; Sierra, M. Organochlorine pesticides residues in bovine milk from Leon (Spain). Sci. Total Environ **1996**, *181*(2), 133–135.
[18] COMMISSION REGULATION (EU) No 212/2013 of 11 March 2013 replacing Annex I to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards additions and modifications with respect to the products covered by that Annex.
[19] Kilanowicz, A. Pestycydy. In *Podstawy Toksykologii*; Piotrowski J., Eds.; Wydawnictwo Naukowo-Techniczne: Warszawa, 2008; 326–327.
[20] Kołsut, W. *Wyniki inwentaryzacji produkcji, importu i eksportu TZO objętych konwencją sztokholmską*, GF/POL/SEM.3/R.1; Instytut Ochrony Środowiska: Warszawa, 2003; 1–8.
[21] Czarnomski, K. Trwałe zanieczyszczenia organiczne (TZO)–definicja i właściwości. In. *Trwałe zanieczyszczenia organiczne–gospodarka odpadami*; Wydawnictwo Naukowe Gabriel Borowski: Warszawa, 2008; 7–8.
[22] Lane, J.M. Extent of exposure. In *NIOSH Special Occupational Hazard Review: DDT*; May, J.R.; Wills, J.H.; Hassett, C.C., Eds.; U.S. Department of health, education and welfare: Rockville, 1978; 1–27.
[23] Tavares, T.; Beretta, M.; Costa, M. Ratio of DDT/DDE in the all Saints Bay, Brazil and its use in environmental management. Chemosphere **1999**, *38*(6), 1445–1452.
[24] Makles, Z.; Domański, W. Ślady pestycydów– niebezpieczne dla człowieka i środowiska. Bezpieczeństwo Pracy **2008**, *1*(436) 5–9.
[25] Bulut, S.; Akkaya, L.; Gök, V.; Konuk, M. Organochlorine pesticide (OCP) residues in cow's, buffalo's, and sheep's milk from Afyonkarahisar region, Turkey. Environ. Monit. Assess. **2011**, *181*(1–4), 555–562.
[26] Boul, H.L.; Garnham, M.L.; Hucker, D.; Baird, D.; Aislable, J. Influence of agricultural practices on the levels of DDT and its residues in soil. Environ. Sci. Technol. **1994**, *28*(8), 1397–1402.
[27] Nadeau, L.J.; Menn, F.M.; Breen, A.; Sayler, G.S. Aerobic degradation of 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) by *Alcaligenes eutrophus* A5. Appl. Environ. Microb., **1994**, *60*(1), 51–55.
[28] Struciński, P.; Górlaczyk, K.; Ludwicki, J.K. Abiotic and biotic transformations of persistent organochlorine compounds in the environment. Roczn. PZH, **1995**, *3*, 279–292.
[29] Snedeker, S. Pesticides and breast cancer risk: A review of DDT, DDE and dieldrin. Environ. Health Perspect., **2001**, *109*(1), 35–47.

[30] Kampire, E.; Kiremire, B.T.; Nyanzi, S.A.; Kishimba, M. Organochlorine pesticide in fresh and pasteurized cow's milk from Kampala markets. Chemosphere, **2011**, *84*(7), 923–927.
[31] Ahmed, N.S.; Zaki, E.M.S. Detection of some organochlorine pesticides in raw milk in Giza governorate. J. Appl. Sci. Res., **2009**, *5*(12), 2520–2523.
[32] Barlik, M.; Brochowicz-Lewińska, A.; Jacyków, D.; Lewandowska, B.; Mirosław, J.; Siwiak, K.; Wągrowska, U.; Łysoń, P. Medium monthly per capita consumption of selected foodstuffs in households By socio-economic groups. In *Household Budget Survey in 2012*; Statistical Publishing Establishment, Central Statistical Office: Warsaw, 2013; 143–145.
[33] Food and Agriculture Organization/World Health Organization. Pesticite Residues in Food and Feed- Codex Alimentarius norms. Available at http://www.codexalimentarius.net/pestres/data/pesticides/details.html?id=21 (accessed Oct 2013).
[34] Pietrzak-Fiećko, R.; Tomczyński, R.; Smoczyński, S.S. Residues of chlorinated carbohydrates in animal and human milk. Med. Wet. **2000**, *56*, 715–717.
[35] Góralczyk, K.; Ludwicki, J.K.; Czaja, K.; Struciński, P. Monitoring of pesticide residues in food products in Poland. Roczn. PZH. **1998**, *49*, 331–339.
[36] Radzymińska, M.; Smoczyński, S.S.; Kopeć, M. Persistent organochlorine pesticide, lead, Cadmium, Nitrate (V) and Nitrate (III) in polish milk and dairy products. Pol. J. Environ. Stud. **2008**, *17*(1), 95–100.
[37] Niewiadowska, A.; Semeniuk, S.; Żmudzki, J. Residues of chlorinated hydrocarbons in cheeses. Roczn. PZH **1996**, *47*(4), 371–376.
[38] Surendranath, B.; Unnikrishnan, V.; Gayatri, V.; Chitra, P.; Priya, C.; Ramamurthy, M.K. Organochlorine pesticides in animal tissues and their excretion through milk. J. Food Sci. Technol. **1998**, *35*(6), 547–548.
[39] Sharma, H.R.; Kaushik, A.; Kaushik, C.P; Pesticide in bovine milk from a predominantly agricultural state of Haryana, India. Environ. Monit. Assess. **2007**, *129*(1–3), 349–357.
[40] Kaushik, C.P.; Kaushik, A.; Sharma, H.R. Seasonal trends in organochlorine pesticide residues in raw bovine milk from rural areas of Haryana, India. Bull. Environ. Contam. Toxicol. **2014**, *92*(1), 15–22.
[41] Waliszewski, S.M.; Pardio, V.T.; Waliszewski, K.N.; Chantiri, J. N.; Aguirre, A.A.; Infanzhn, R.M.; Rivera, J. Organochlorine pesticide residues in cow's milk and butter in México. Sci. Total Environ. **1997**, *208*(1–2), 127–132.
[42] Waliszewski, S.M.; Villalobos-Pietrinir, R.; Gomez-Arroyo, S.; Infanzon, R.M. Persistent organochlorine pesticides in Mexican butter. Food Addit. Contam. **2003**, *20*(4), 361–367.
[43] Pardio, V.T.; Waliszewski, K.N.; Landin, L.A.; Bautista, R.G. Organochlorine pesticides residues in cow's milk from a tropical region of Mexico. Food Addit. Contam. **2003**, *20*(3), 259–269.
[44] Hernández, M.; Vidal, J.V.; Marrugo, J.L. Organochlorine pesticides in cows' milk supplemented with cotton waste in San Pedro, Colombia. Rev. Salud Pública **2010**, *12*(6), 982–989.
[45] Food and Agriculture Organization. International Code of Conduct on the Distribution and Use of Pesticides. Available at http://www.fao.org/docrep/005/y4544e/y4544e00.htm (accessed Nov 2002).
[46] Salem, N.M.; Ahmad, R.; Estaiteh, H. Organochlorine pesticide residues in dairy products in Jordan. Chemosphere **2009**, *77*(5), 673–678.
[47] Iftikhar, B.; Siddiqui, S.; Rehman, S. Assessment of the dietary transfer of pesticides to dairy milk and its effect on human health. Afr. J. Biotechnol. **2013**, *13*(3), 476–485.
[48] Hernik, A.; Góralczyk, K.; Struciński, P.; Czaja, K.; Korcz, W.; Minorczyk, M.; Łyczewska, M.; Ludwicki, J.K. Characterizing the individual health risk in infants exposed to organochlorine pesticides via breast milk by applying appropriate margins of safety derived from estimated daily intakes. Chemosphere **2014**, *94*, 158–163.
[49] Pawlicki, L.; Jaworski, J.; Smoczyński, S. Pozostałości chlorowanych węglowodorów w tłuszczu mleka kobiecego w okresie laktacji. Probl. Lek. **1985**, *24*, 173–178.
[50] Landrigan, P.J. Risk assessment for children and other sensitive populations. Ann. NY Acad. Sci. **1999**, *895*, 1–9.
[51] Massart, F.; Harrell, J.C.; Federico, G.; Saggese, G. Human breast milk and xenoestrogen exposure: A possible impact on human health. J. Perinatol. **2005**, *25*(4), 282–288.
[52] Goldner, W.; Sandler, D.; Yu, F.; Shostrom, V.; Hoppin, J.; Kamel, F.; LeVan, T. Hypothyroidism and pesticide use among male private pesticide applicators in the agricultural health study. J. Occup. Environ. Med. **2013**, 55(10), 1171–1178.
[53] Roberts, J.; Karr, C. Pesticide Exposure in children. Pediatrics. **2012**, *130*(6), 1765–1788.
[54] Mendez, M.A.; Garcia-Esteban, R.; Guxens, M.; Vrijheid, M.; Kogevinas, M.; Goñi, F.; Fochs, S.; Sunyer, J. Prenatal organochlorine compound exposure, rapid weight gain, and overweight in infancy. Environ. Health Perspect. **2011**, *119*(2), 272–278.