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An assessment of the extent of bovine milk pollution by DDT used in public Health in Zimbabwe

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ABSTRACT

Indoor residual spraying of dichlorodiphenyltrichloroethane (DDT) has been widely used in the control of malaria, and has been appreciated for its low cost and high effectiveness. Prolonged use, however, has adverse effects as DDT and its metabolites persist for long in the environment, resulting in human and animal contamination. Contamination in animals such as cattle may result from consuming contaminated water, feed and fodder. The aim of the study was to evaluate the extent of contamination of DDT and its metabolites in cow's milk from five towns in Zimbabwe. Samples were collected from farms around Bulawayo, Chiredzi, Esigodini, Harare and Mutare with a total of n=24 samples. The pesticide residues were extracted using the quick, easy, cheap, rugged and safe (Quechers®) method and solid phase extraction and analysed using gas chromatography with electron capture detector. Total DDT was detected in all samples at levels higher than the maximum allowable residue limit. Harare and Mutare samples had the highest DDT levels of 0.38 µg/ml and 0.26 µg/ml respectively, as compared with samples from other regions (0.08-0.13 µg/ml). The DDT metabolite residues,1,1-dichloro-2,2-bis(p-chlorophenyl)ethane (DDD) and 1,1-dichloro-2,2-bis(p-dichlorodiphenyl)ethylene (DDE), were also detected at varying levels in the samples. The results show the presence of DDT and its metabolite contaminants in bovine milk, posing a serious health risk to consumers. Alternatives to DDT may therefore need to be employed in the control of the malaria vector.

INTRODUCTION

Organochlorine pesticides such as 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT) have been used over the years in the elimination of malaria and other deadly human disease vectors, through indoor residual spraying (IRS) (Nag and Raikwar, 2008, Roberts, 2010). The implementation of IRS programs in many countries around the world brought about spectacular reductions in malaria and equally remarkable improvements in health (Roberts, 2010). It was later discovered that DDT was of high toxicity to wildlife, humans and the environment which led to the Stockholm Convention negotiating a treaty for a global ban on the use of DDT (UNEP, 2002). The World Health Organisation in 2006, however, declared its support for IRS of DDT in African countries where malaria remains a major problem, citing that benefits of the pesticide outweigh the health and environmental risks (WHO, 2006). In Zimbabwe, the minister of health, David Parirenyatwa, also backed the use of DDT citing its efficacy, cost effectiveness and longer residual killing power

(Hecht, 2004).

The DDT compound has a high persistence nature brought about by its chemical stability (Cerkvenik et al., 2000). In temperate soils the half-life of DDT was found to be 2.8 years and the compound breaks down to 1,1-dichloro-2,2-bis(p-chlorophenyl)ethane (DDD) and 1,1-dichloro-2,2-bis(p-dichlorodiphenyl)ethylene (DDE) metabolites which are extremely stable and resistant to further environmental breakdown or metabolism by organisms. The DDE form is usually found in high concentrations in animal tissue, particularly in areas where there has been no recent use of the parent compound (Rogan and Chen, 2005, Wandiga, 2001). Studies have revealed that despite a decrease in the use of DDT, levels of DDE metabolite tend to remain constant, as people continue to ingest the DDE that is present in some species of fish and other DDE containing foodstuffs (Friis, 2007).

As a result of the persistence of organochlorines in the environment and their presence in animal products, constatnt monitoring of these pesticides is of necessity (Cerkvenik et al., 2000). Livestock such as cattle may be exposed to DDT through consuming contaminated water, feed and fodder (Kampire et al., 2011, Nag and Raikwar, 2008). Due to DDT's lipophilic nature, it accumulates in fat containing tissues and can only be excreted through milk (Cerkvenik et al., 2000, Kampire et al., 2011, Nag and Raikwar, 2008). Using bovine milk as an index of environmental pollution, the objective of this study was to determine the levels of DDT and its metabolite residues present in milk obtained from farms around Zimbabwe. The farms were from the Bulawayo, Chiredzi, Esigodini, Harare and Mutare, towns/cities with high prevalence of malaria.

MATERIALS AND METHODS

Chemicals

Sep-Pak SPE C18 cartridges were purchased from Waters Corporation, Milford. Standards were purchased from Sigma Aldrich Chemical Company, Germany. All other laboratory reagents were of analytical grade.

Sampling

Twenty four raw milk samples were randomly collected from farms around Bulawayo, Harare, Chiredzi, Esigodini and Mutare and stored in 1L containers. Samples were kept at -20°C prior to analysis.

Butter fat test

The butter fat test was performed according to the Babcock method as documented by Lucas (1948). Briefly, 17.5 ml of raw milk and an equal volume of 91-95% sulphuric acid were added into 50 ml centrifuge tubes. The tubes were capped and the contents mixed by gentle inversion, before being centrifuged at 247 x g, for 5 min at 40°C. The percentage fat content of the milk was measured as a volume of the total liquid content of the tube.

Sample preparation for gas chromatography analysis

Pesticide extraction

Extraction was performed using the QuEcheRs method described by Payá et al. (2007), with modifications. Briefly, 10 ml of acetonitrile were added to an equal volume of milk sample in a 50 ml centrifuge tube and vigorously shaken by hand for 1 min. A salt mixture of 4 g of magnesium sulphate and 1 g of sodium chloride was added to the centrifuge tube and the contents were vigorously shaken by hand for 1 min, then vortexed for 30s. The mixture was then centrifuged at 8

000 x g for 12 min and the upper organic layer, containing the pesticide, was transferred to test tubes. The contents in the tubes were then evaporated to 1 ml, to remove the acetonitrile, and the volume made up to 10 ml with distilled water. The samples were then cleaned up before analysis.

Sample clean up

The clean-up was done by solid phase extraction (SPE) using Sepak waters SPE C18 cartridges, according to the manufacturer's instructions. Briefly, the cartridges were first preconditioned with 5 ml of hexane and 10 ml of distilled water. Using a syringe, 10 ml of the extracted pesticide sample was then loaded drop wise onto the cartridge bed. After isolation, the cartridge was rinsed twice with 5 ml of distilled water to remove unwanted components. Finally, elution of the analytes from the cartridge was done by passing through 5 ml of hexane in a stepwise manner (2 ml, 2 ml, 1 ml) and eluents were collected in the same collection tube. Samples were then analysed by gas **chromatography.**

Gas chromatography analysis

Total DDT and DDT residues were detected using the Agilent 6820 Gas chromatograph (GC). Standards used were 2,4' DDT, 4,4' DDT, 2,4' DDD, 4,4' DDD and 4,4' DDE. Two µl of sample or standard were injected and separated on an Agilent J&W 15 m x 530 mm x 0.50 mm column. Temperature programming used for the detection, was as follows:

	°C/min	Next °C	Hold (min)	Run Time (min)
Initial		110	1	1
Ramp 1	21	285	1	10.35
Ramp 2	30	300	2	12.83

Statistical analysis

One way analysis of variance (ANOVA) was performed using graph pad prism and Tukey's multiple comparison test was performed at $p<0.05$ significance level to determine any significant difference between the mean total DDT values between regions.

RESULT AND DISCUSSION

Butter fat test

The butter fat content in the samples ranged between 2.0 – 7.4%. The Bulawayo and Mutare samples contained 4.4-6.2% and 4.4-7.4% respectively. Chiredzi samples had 2.0-4.4%, while those from Harare had 3.0% to 6.7%. Samples from Esigodini contained 4.6-7.4% butter fat.

Gas chromatography analysis

Total DDT

Total DDT was found in the milk samples analysed. Harare samples had the highest levels of total DDT (0.38 µg/ml) followed by Mutare (0.26 µg/ml) (Figure 1). Chiredzi and Bulawayo samples had 0.13 µg/ml, 0.10 µg/ml respectively and Esigodini had the lowest of 0.08 µg/ml (Figure 1).

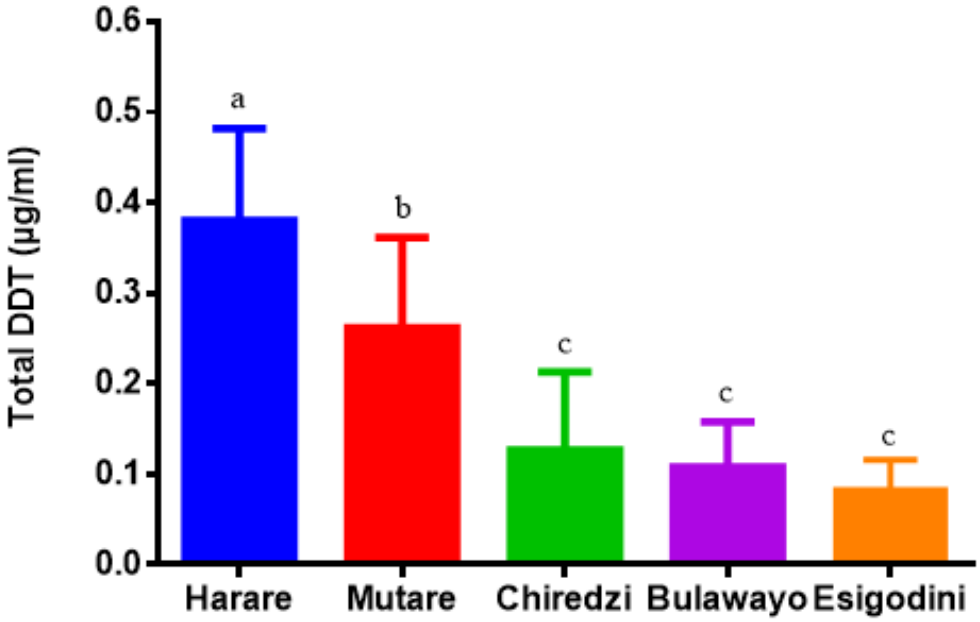

Figure 1. Total DDT (DDT, DDD and DDE) concentration (µg/ml) present in dairy milk from the selected regions. Values represent the average of samples per region and these are expressed as mean ± SD. Different letters between each group indicate significant differences ($p<0.05$).

Levels of DDT and individual metabolites.

Harare samples contained the 2,4 DDT and 2,4 DDD metabolites while the Mutare samples had the aforementioned metabolites, in addition to the 4,4 DDE metabolite (Figure 2). Chiredzi samples had the 2,4 DDT and 4,4 DDE metabolites, while Bulawayo samples had 2,4 DDD, 4,4 DDD and 4,4 DDT metabolite residues (Figure 2). From the Esigodini samples, 2,4 DDT, 2,4 DDD and 4,4 DDE metabolites were detected. The most predominant metabolite per town was: Harare and Bulawayo: 2,4 DDD; Chiredzi and Mutare: 4,4 DDE; Esigodini: 2,4 DDT (Figure 2).

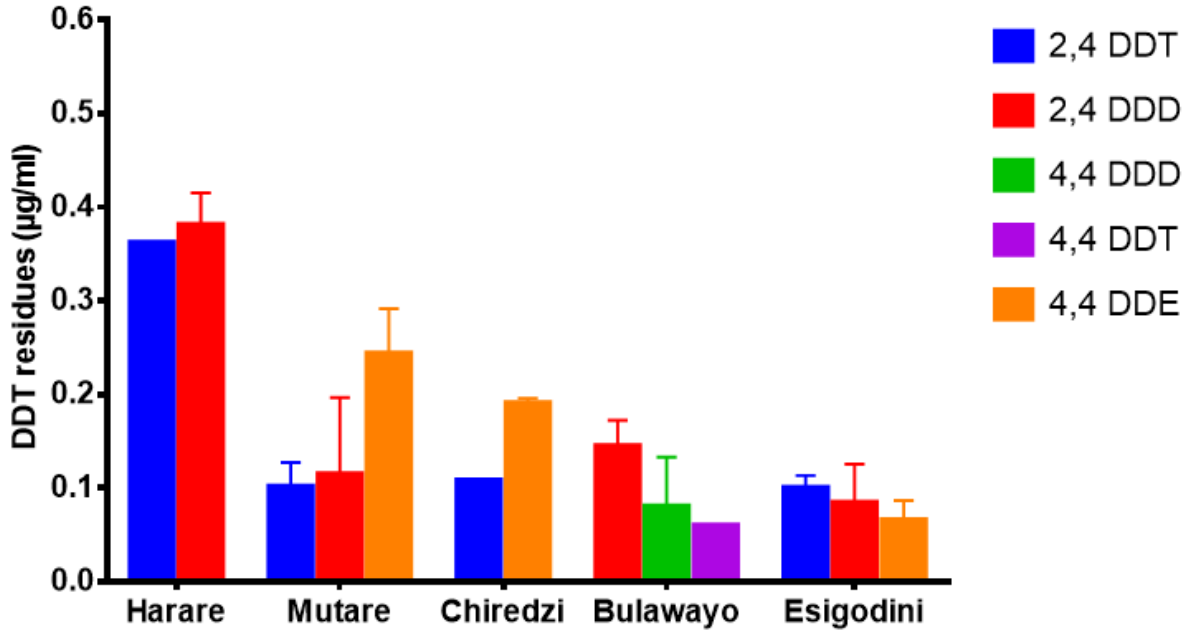

Figure 2. DDT metabolite residues (µg/ml) present in dairy milk from the selected regions. Values represent the average metabolite values ± SD for the samples within each region.

DISCUSSION

Owing to its richness in fat, milk has been listed as an important source of lipophilic organochlorine pesticide accumulation, thus convenient for measuring persistent OCPs (Nag and Raikwar, 2008). In the present study, total DDT was detected in all milk samples, with the detected values higher than the 0.02 µg/ml maximum allowable residue level for milk, recommended by the Codex Alimentarius Commission (www.fao.org/fao-who-codexalimentarius/standards/pestres/pesticide-detail/en/?p_id=21 [Accessed 2 August 2016]). Samples from the Harare and Mutare regions had the highest levels as compared to the other regions. According to an aide memoire from the Zimbabwe Ministry of Health and Child Welfare, malaria is described as mainly seasonal, with a potential for epidemics during the rainy seasons (GOZ, 2011). When rainfall reaches its peak, there is an increase in the prevalence of mosquito breeding sites and high humidity which favours mosquito survival (GOZ, 2011, Taylor and Mutambu, 1986). Annual rainfall patterns show that the Harare and Mutare regions receive more rainfall (> 800 mm) as compared to the other regions in this study and reports have stated that most malaria cases are recorded in Mashonaland central and Manicaland (Cimas 2015, http://en.climate-data.org/country/228/ [Accessed 24 June 2016]). Such a scenario may then lead to an increased use of DDT as a means of malaria vector control resulting in subsequent livestock contamination, as observed in the bovine milk samples in the present study.

Dichlorodiphenyltrichloroethane (DDT) metabolites were detected in all samples. The presence of the 4,4 DDE metabolite detected in the Chiredzi, Mutare and Esigodini regions may be attributed to two factors, the presence of DDE in the environment or DDT metabolism to DDE in organism tissues. In the environment, DDT degrades to DDE, which can easily be taken up by living organisms (Deti et al., 2014). The DDE metabolite persists longer in the environment, as it can be found in areas where the DDT parent compound has not been applied over a period of time (Rogan and Chen, 2005). Metabolism of DDT may occur in tissues, producing DDE as observed by Chikuni and colleagues (1997). They observed an inverse relationship where a decrease in the level

of DDT in tissue resulted in an increase in the DDE metabolite. Concerning the DDD metabolite, studies by Miskus and co-workers (1965) showed that bacteria present in bovine rumen fluid have the ability to convert DDT to DDD. Experiments of C14 labelled DDT incubated with bovine rumen fluid showed partial conversion to C14 DDD. Rumen microorganisms are also said to convert the 2,4 DDT isomer (which constitutes 15-20% of technical grade DDT) to 2,4 DDD and 4,4 DDT at the same rate (Fries et al., 1969). Microorganisms are said to achieve these conversions via two major pathways: reductive dechlorination, which is favoured under anaerobic environments and dehydrochlorination, which occurs in the presence of oxygen (Krishnamoorthy and Lal, 2012). This could therefore account for the metabolites detected in the samples of the present study.

Milk is an important part of the human diet and thus the presence of DDT and its metabolites pose a health risk to consumers. To alleviate the pesticide load in milk, studies have shown that processes such as skimming (removal of fat in milk) reduce DDT levels (Abou-Arab, 1991). Also, heat treatments such as pasteurisation and ultra-heat treatment (UHT) have shown to be effective in reducing total DDT levels in milk (Heck et al., 2007). Consumption of heat treated milk is therefore much safer than taking in raw milk. There are, however, challenges to treating milk, particularly for the Zimbabwe rural population that occasionally consumes traditionally fermented milk (sour milk) from fresh unpasteurized cow's milk. Technologies would therefore need to be put in place, to facilitate heat treatment processes for rural communities and produce quality milk, with lower pesticide content, much similar to their urban counterparts (Feresu, 1992).

CONCLUSION

The present study shows that there is contamination of DDT and its metabolites in bovine milk collected from farms around Bulawayo, Chiredzi, Esigodini, Harare and Mutare. Relevant authorities are therefore encouraged to embark on awareness campaigns, educating farmers and the public on the use of DDT and the risk of animal and human contamination. Less harmful DDD alternatives may be adopted in an effort to build an eco-friendly environment.

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